Aldehydogenic Lipids

by
N. A. PREOBRAZHENSKII
and
G. A. PARFENOV

Sugar Orthoesters and their Synthetic Applications

by
N. K. KOCHETKOV
and
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Thalictrum Alkaloids

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N. M. MOLLOV
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and
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AKADÉMIAI KIADÓ, BUDAPEST

RECENT DEVELOPMENTS IN THE CHEMISTRY OF NATURAL CARBON COMPOUNDS

Volume IV

The present volume of the series contains three monographs, written by distinguished researchers of the organic compounds treated.

The study by *N. A. Preobrazhenskii* and *G. A. Parfenov* deals with the different types of natural aldehydogenic lipids, such as the natural plasmalogens. The methods of isolation and analysis of plasmalogens and acetalphosphatides are discussed. Great emphasis is laid on the synthesis of this interesting class of compounds; the elucidation of the structure of these substances, their metabolism and the biological role of plasmalogens are described.

In the part by *N. K. Kochetkov* and *A. F. Bochkov*, after a brief historical survey, the types of sugar orthoesters, their systematization and nomenclature, chemical properties and reactions are discussed. This is followed by a description of the methods of synthesis and analysis of these sugar derivatives, as well as a detailed treatment of structural problems and structure elucidation is given. The great possibilities of applying these compounds in carbohydrate synthesis are pointed out,

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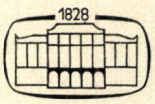

AKADÉMIAI KIADÓ
PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES
BUDAPEST, 1971

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BUDAPEST, 1971

Translation revised by

D. A. DURHAM PH. D.

Printed in Hungary

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ALDEHYDOGENIC LIPIDS

I. INTRODUCTION

It was first stated by R. Feulgen in 1924 that among lipids of animal origin there exists a material containing aldehyde [1, 2]. Since aldehydes could be detected only in cytoplasma of tissue sections not deprived of fat following a mercuric chloride treatment, they were called 'plasmals' and the substances bearing them 'plasmalogens'. In a relatively short period much experimental material has been accumulated clearly demonstrating that plasmalogens, which proved to be phosphatides, are just as characteristic in animal organisms as are phosphatides of a diacyl type; more recently the term has been extended to aldehydogenic lipids not containing phosphorus.

The study of plasmalogens can be considered a new topic in the chemistry and biochemistry of lipids, since it was only some ten years ago that the fundamental structural investigations on these unstable compounds were performed, while problems of chemical synthesis, biosynthesis and the biological role of this interesting type of lipid are the subjects of present-day studies.

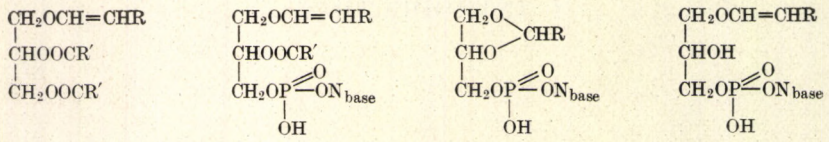
II. TYPES OF ALDEHYDOGENIC LIPIDS

Derivatives of glycerol containing one aldehyde-residue were the first to be recognized. They can be divided into three groups:

(a) α-O-1-alkenyl-β-α′-O-diacyl glycerols, or 1-O-(alk-1′-enyl)-2,3-diacyl glycerols (I);

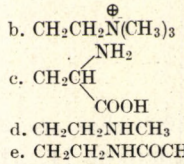
(b) α-O-1-alkenyl-β-O-acyl-glycerylphosphoryl ethanolamines, -cholines and -serines, or 1-O-(alk-1′-enyl)-2-O-acyl-3-glycerylphosphoryl ethanolamines, -cholines and -serines (IIa, b and c, resp.), or phosphatidal ethanolamines, cholines and serines;

(c) α, β-O-alkylidene-glycerylphosphoryl ethanolamines and -cholines, or 1,2-O-alkylidene-3-glycerylphosphoryl ethanolamines (IIIa) and -cholines (IIIb).



I II III IV

N_{base} in II–IV: a. $CH_2CH_2NH_2$



d. $CH_2CH_2NHCH_3$

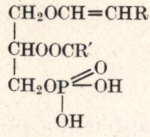
e. $CH_2CH_2NHCOCH_3$

Aldehydogenic lipids of the first type are also referred to as 'neutral plasmalogens'. Aldehydogenic lipids derived from lower diols are to be counted among them, too.

The second type of aldehydogenic lipids are the 'compound (phosphatidic) plasmalogens'. These may be subclassified into two groups. The first group contains the compound plasmalogens (IIa–c) mentioned above, the N-monomethyl-ethanolamine plasmalogen (IId) from bacterial sources [3–5] and the N-acetoethanolamine plasmalogen (IIe) recently detected by H. Debuch [6] in lipids of bovine brain and also in lipids of human brain and placenta. In this group we find also lysoplasmalogens (IV) analogous to the compounds mentioned (IIa–e), but lacking the fatty acid residue in the β-position. Small amounts of lysolecithin-plasmalogen (IVa) [7] and lysoethanolamine plasmalogen (IVb) [8] have been detected in natural sources.

The second group consists of nitrogen-free compound plasmalogens: phosphatidic acid (V) (mammalian liver [9] and brain [10–12], leech Ybathobdellida [13]), polymer phosphatidic acid ('infarction plasmalogen') detected by M. Hack in dogs suffering from experimental myocardial infarction [14, 15], phosphatidylglycerol (VI) (in lipids of *Clostridium butiricum* [5, 16]), cardiolipine (diphosphatidylglycerol, VII) [16–19], or a lipid similar to it, and inositolphosphatide (VIII) [10–12, 20–23].* Until now there have been no reports concerning diolic compound plasmalogens, perhaps since diolic phosphatides were detected later than neutral diolic lipids [24] and therefore they have not yet been thoroughly investigated.

* For some minor compound plasmalogens, the structural formulae of which are reported here, the location of the aldehyde-residue has not yet been proved; nevertheless on analogy with established structures it is assigned to the α-hydroxyl of glycerol.



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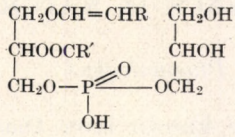
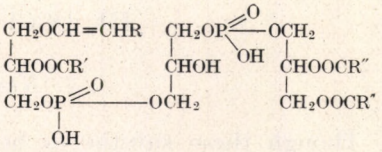
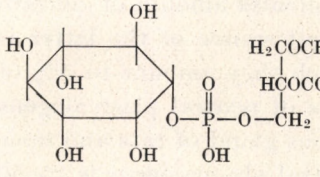


VI

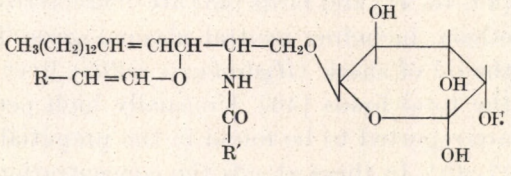


VII

The third type of aldehydogenic lipids (acetalphosphatides) includes compounds containing ethanolamine (IIIa) [25] and choline (IIIb) [26] residues; in acetalphosphatides the aldehyde is bound to the glycerol part of the molecule by a cyclic acetal group so as to form a dioxolane ring. Its occurrence in nature is questionable.



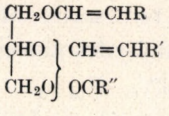
VIII



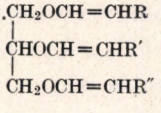
IX

Sphyngoplasmalogens (IX), detected in lipids of bull brain and lungs [27–29], form a special group. There is a report [23] on the determination of aldehyde in the sphyngomyeline fraction obtained from rat myocardium.

The molecule of aldehydogenic lipids may contain one or more aldehyde residues. According to M. Hack and F. Helmy [7], the major neutral plasmalogen (X) of the preputial sebaceous gland of mice contains two aldehyde residues, and in the same material these authors found a rather unusual sort of glyceride (XI) in which all three hydroxyls were etherified by aldehydes.



X



XI

Two aldehyde residues can occur in one molecule of compound plasmalogen [30].

It is to be expected that aldehydogenic lipids will gradually be detected as minor components of all classes of acyl lipids.

III. OCCURRENCE IN NATURE

1. Neutral Plasmalogens

Though these substances became known later than phosphatide plasmalogens [31, 32], their existence could be postulated on the basis of investigations of the 'non-saponifiable fraction' obtained from neutral lipids following alkaline treatment [33, 34].

Neutral plasmalogens (I) usually constitute 30–70% of the total amount of non-acyl glycerides (about 0·1% of the sum of lipids [35–37]) in mammalian tissues and organs [31, 35–39], among them human ones [38, 40–41], and they have been detected in milk fat [31] and egg-yolk [42] too. Fats of marine invertebrates [13, 25, 32, 34, 38, 43–45], of certain fish [40, 46, 47] and birds [48] are characterized by a greater amount of glycerol ethers, including neutral plasmalogens. The richest source of the latter is the oil of shark (*Hydrolagus colliei*) liver, in which they amount to 5% of the total lipids [46]. Unusually high percentages of neutral plasmalogens are reported to be found in the preputial sebaceous gland of rats and mice [7, 49]. In these glands the concentration of neutral plasmalogens is 25–50 times higher than in any other mammalian tissue [7]. Neutral diol plasmalogens [50–52] have been detected among neutral lipids in rat liver oil and in soil yeast *Lipomices sp.* [40].

As compared with triglycerides the neutral plasmalogens of human stock fats are richer in long-chain polyene acids, their aldehyde composition being:

$$C_{16:0} - 62{\cdot}1\%;\ C_{18:0} - 21{\cdot}3\%;\ C_{18:1} - 16{\cdot}6\%\ [41].$$

Neutral plasmalogens having a far more complex aldehyde composition are to be found in the oil of shark (*Hydrolagus colliei*) liver [37]; in this material 20 aldehydes have been identified, $C_{20:1}$ dominating among them (38·2%). The presence of uncommon aldehydes is worth mentioning: aldehydes with odd number C — 14·2%; two series of branched aldehydes — 16·4%. The sum of C_{19}, C_{20} and C_{22} aldehydes amounts to 50·3%. Among the twenty fatty acids $C_{18:1}$ — 42·3%, $C_{16:0}$ — 16·1% and $C_{20:1}$ — 15·0% predominate, the percentage of acids C_{22} and C_{24} both (saturated and monoene) amounting to 20·5%.

For the time being we have not sufficient experimental data concerning the extent of occurrence of neutral plasmalogens, as we have in the case of compound plasmalogens. Nevertheless, advances in separation and analysis techniques (e. g. chromatographic methods) [53] can be expected to ensure a considerable increase in our knowledge in the very near future.

2. Compound (Phosphatide) Plasmalogens (IIa–e)

These substances, called also simply plasmalogens, represent the most thoroughly investigated group of all aldehydogenic lipids [54, 64]. Their occurrence in plants is not certain. Although, according to some reports, they have been detected in the lipids of soybean and green pea [65, 66], arachis [67], *Acacia dialbata* [68] and olive oil [69], these early data should be assessed with caution since the determinations were effected by means of the Feulgen reaction with fuchsin-sulfurous acid, demanding the most careful preparation of the test material. Even if the reaction is positive it does not prove the presence of plasmalogens unless the absence of non-lipid aldehydes and autoxidation products is absolutely certain. Vegetable oils are particularly rich in unsaturated acids, and consequently it is always to be expected that they contain oxidized materials. In addition, fatty aldehydes have been detected in plants having nothing in common with plasmalogens (sugar cane [70, 71], grape [72], cotton-plants [73]). Besides, for the last ten years no reports have been published on the determination of plasmalogens in higher plants.

Until very recently plasmalogens were not supposed to be present in microorganisms. At present, on the strength of studies on lipids obtained from tubercle bacilli [74], *Bacillus abortus* Bang [75], *Clostridium butiricum* [3–5], and ruminants' gastric bacteria [76, 77], this point of view needs reconsidering, though perhaps certain microorganisms (e. g. *Escherichia coli*, [78]) actually do not contain plasmalogens. Results of comparative investigations on lipids of elementary organisms are interesting [16, 79]. While no plasmalogens could be detected in lipids of alga *Chlorella* with its immobile manner of life nor in flagellates classified as plants (*Chlamidomonas*, *Euglena*), on the other hand, in lipids of flagellates classified as animal organisms (*Crithidia*, *Herpetomonas*, *Leishmania Tripanosoma*) ethanolamine plasmalogen has been detected, and also traces of choline plasmalogen (*Leishmania*). These plasmalogens together with serine plasmalogen occur in the lipids of amoeba (*Acanthamoeba*). As shown by many experimental results, plasmalogens are charasteristic of the animal world. They can be detected in organs and tissues of all animals irrespective of their organization level: from various protozoa [80–82], sea-water, freshwater and terrestrial investebrata [13, 18, 21, 44, 83–89] up to fish [95, 96] and other vertebrata [97–101], and to man [102, 103].

Flora and fauna, the two worlds of animate nature, have entirely different methods for drawing their energy from environmental sources, and consequently quite dissimilar features of existence as well. In all probabil-

ity plasmalogens can be considered as substances of a comparatively late origin in the animal world, characteristic of this very type of living material. From this point of view it is to be expected that plasmalogens should be present in all vitally important systems of every animal organism irrespective of their multiformity, for, as emphasized by a number of biochemists [104, 106], if nature has once found some way for the successful chemical solution of a biological problem, she will adhere to it during subsequent evolution.

Table I

The Plasmalogen Content of Rat Organs

Organ	μM/g fresh tissue
Cardiac muscle	2·16– 3·16
Skeleton muscles	1·27– 1·73
Lungs	2·88– 3·51
Kidneys	2·52– 4·80
Liver	0·66– 1·18
Spleen	1·58
Myocardium	2·16– 3·16
Nerve-tissue	10·40–14·90

There are only a few body fluids in which plasmalogens have not been detected: the humoral fluid of eyes, urine [78] and gastric juice [107, 108]. In the lipids of all other tissues and organs plasmalogens are present in greater or smaller quantities (skin [109, 110], bone tissues, heart [111–116] and skeleton [39, 111–113, 115, 117–119], muscles, lungs [111–113, 115, 120], kidneys [111–113, 121–123], liver [111, 113, 115, 121], spleen [111, 115, 117], stomach and intestines [117], myocardium [111, 112, 124], bone marrow [125, 126], nerve tissue [111, 113, 117, 121, 125–166], genital organs [111, 117, 167, 168], salivary glands [169–171], pancreas [172, 173] and thyroid gland [174], adrenal glands [80, 81, 113, 172, 175, 176], conjunctive tissue [177], cornea [178], retina and retinal rods [179], white and coloured fat [180–182], plasma, serum [183–195] and corpuscles and platelets of blood [22, 193, 196–199], sperma [2, 200–207], cerebro-spinal fluid [17, 208–209], milk and amnion fluid [80, 81], tumours [210–214] and faeces [215]). As a basis of comparison we present a few data on plasmalogen concentration in rat and human organs (Tables I and II).

The greatest amounts of plasmalogens are to be found in brain, particularly in the white matter [129], cardial muscle and sperma [216–218].

The plasmalogen content of ram sperma is several times greater than its concentration in brain: 47·2 μM/g fresh tissue [204], i.e. almost 10^7 plasmalogen molecules per spermatozoid. In the sperma of invertebrata the plasmalogen level seems to be much lower [85].

Table II

The Plasmalogen Content of Human Organs [73]

Organ	μM/g fresh tissue	% of total phosphatides
Skeleton muscles	6·9	29·3
Uterus muscle	1·8	20·5
Adrenal glands	3·5	14·3
Testicles	1·8	11·3
Ovaria	1·6	17·3
Hypophysis	2·7	17·0

Phospholipids make up to 25–30% of dry weight of brain. Considering that plasmalogens form a considerable part of phospholipids (from 48 to 90% in different areas of brain) [121, 131, 138], the significance of these compounds in the activity of the central nervous system is obvious. Plasmalogens, like the rest of phospholipids in nervous cells are concentrated mostly in myelin [151, 219–223], their main representatives being plasmalogens containing ethanolamine [128, 134, 220], while lecithin- [135, 212, 224] and serine- [133, 225–227] plasmalogens detected in brain only in small amounts are principally connected with subcellular particles [147]. In the peripheral non-myelinated nerves of crustaceae, plasmalogens have been determined in the form of lecithins [162]. Apparently the reason for this specific distribution is the different functions of nerve cell membrane and its subparticles. A more systematic study seems to be necessary before we may draw a conclusion as to whether there exists a specific distribution of plasmalogens resulting from specialization of animal tissues and organs. According to the available data ethanolamine plasmalogen seems to be the most characteristic one in the animal world [13, 228]. Apart from brain tissues, this compound predominates among aldehydogenic lipids of mammalian bone marrow [229], blood corpuscles and platelets [199, 230–232], diaphragm [233], lungs and kidney [234, 235]; it is the dominating plasmalogen of meal-beetle (*Tenebrio molitor*) larvae [236], and the only plasmalogen in lymphosarcoma of rats [237]. Although lecithins constitute a considerable portion of plasmalogens in heart [33, 112, 238–240] and skeleton

[241–243] muscles, it is perhaps only in sperma [244–246] and blood plasma [247, 248] that they prevail to a greater extent. Pelagic silt [249] and chicken embryos [250] are relatively rich in plasmalogens containing serine. Nevertheless this group of compound plasmalogens is usually to be found in smaller amounts than ethanolamine and choline plasmalogens [13, 251].

Clinical physicians pay particular attention to aldehydogenic lipids of blood. The plasmalogen content of human serum is higher than that of any other mammal (6·69–11·75 μM/100 ml) [183–186, 190], except horned cattle, the concentration being 15% greater in women than in men. In the plasma, plasmalogens are not to be found in the free form but together with other types of lipids they constitute part of the lipoproteids of α- and β-globulines [188]. There is a marked difference between the plasmalogen contents of blood serum for different individuals; nevertheless for one person in normal condition it remains at the same level for a long time.

Human blood corpuscles and platelets contain more of the plasmalogens than plasma, the concentration of the latter in precipitated erythrocytes of men and women being 84·1 and 94·7 μM/100 g, respectively [189, 192, 247], and in thrombocytes 340·0 μM/100 g [22, 197].

Studies on the distribution of plasmalogens in living cells are very important from the point of view of understanding the role of these substances. Even the first hystochemical investigations [252–255] detected some differences. The amount of plasmalogens in one or other part of the cells is closely connected with the function of the tissue being investigated. While microsomes and mitochondria of brain and heart muscle cells are rich in plasmalogens [257–260] (up to 17% of the weight of mitochondria [259]), mitochondria of adrenal glands contain only a small amount [261]. The plasmalogen level in mitochondria of liver cells [203, 262, 263] is even lower (according to recent data they are completely lacking [264, 265]) in spite of the fact that this organ is abundant in phosphatides. Nuclei of liver cells, however, contain more plasmalogen than cell nuclei of other tissue [263]. In the mitochondria and other subparticles of brain there is a marked predominance of ethanolamine plasmalogens over other plasmalogen types [11, 148].

The above-mentioned facts seem to confirm the opinion that the distribution of plasmalogens in cells depends on the special features of cell behaviour in the given type of tissue, i.e. on the role of the latter.

The principles concerning aldehyde and fatty acid composition are not so clear. There is only a slight divergence among the aldehyde composition of plasmalogens from different brain areas. Aldehydes with non-branched C_{16} and C_{18} chains (palmitic, stearic and oleic aldehydes) make up to 98%

of the total aldehyde content [166, 220, 266, 267], the grey matter containing considerable more stearaldehyde than the white one [268]. The remaining 2% consists of saturated aldehydes, both normal and branched, of the even and odd number series C_{14}–C_{17} and of monoenic aldehyde C_{16} [269]. Among the fatty acids palmitic and oleic acids have been found to predominate [145, 163, 164, 268–270]. Analogous distribution of plasmalogens is characteristic of the peripherial nervous system, too [156, 161, 162, 271, 272].

Plasmalogens from any other source are also characterized by normal aldehydes with chain lengths of C_{16} and C_{18} [273, 276]; sometimes n-tetradecanal is also to be found in considerable amount (14% of the total aldehyde content in equine muscles [277]). C_{16} and C_{18} aldehydes usually constitute 90–98% of the aldehyde total [172, 277–279]. Another interesting point is their being represented mainly by saturated compounds. The single unsaturated aldehyde characteristic of plasmalogens — n-9-octadecenal — is rarely to be found in measurable quantities. In human, as opposed to all animal tissues, an extraordinarily high content of olealdehyde is to be found (20% of the sum of aldehydes in erythrocytes [274, 275, 279, 280], 50% in brain [278, 281], etc., cf. Table III). It must be noted that the amount of this aldehyde is several times smaller in the plasmalogens of bovine heart muscles (0·7%) [282]. An interesting fact is that, nothwithstanding the high concentration of polyene acids (C_{20} and C_{22}) in phospholipids of brain [270, 283] and of adrenal glands [284, 285], polyene aldehydes can be detected in them only as traces. Only traces of octadecyldienal are detectable in erythrocytes [102] and it is only in human testicles and ovarium that this aldehyde may amount to about 1% [103]. In human meningioma, however, the octadecyldienal concentration increases as much as 10 times (making up to 10%), and an unusual compound, icosatetraenal, is also to be found in an amount of 2·9% [286].

Aldehydes with hydrocarbon chains longer than C_{18} are just as infrequent as these earlier members. (In human adrenal glands [103] C_{20} — 4%, about 2% of this being monoene aldehyde; in tentacles of octopus [92] C_{21} — 8%.) Aldehydes with shorter chains (less than C_{14}) as well as odd number chains and branched ones are more frequent. Thus, plasmalogens of rat heart muscle are characterized by butyric aldehyde [287]; 16–23% of branched aldehydes have also been detected [203, 288]. Milk fat is rich in short-chain, odd number and branched aldehydes [31] (22·5% of branched C_{14} aldehyde). The aldehyde composition of lipids in marine invertebrates [92] and microorganisms [289–295] is even more varied (cyclopropane aldehydes in *Clostridium butyricum*, e.g. [294–296]). The presence of one aldehyde —

n-hexadecanal — in plasmalogens is rare (plasmalogens of ram [297] and bull [298] sperma).

Table III

Aldehyde Composition of Human Organs (in % of Total Aldehyde Content [103]*)*

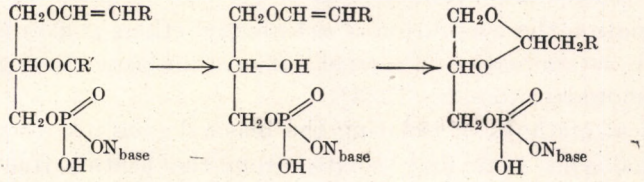
Organ	C_{16}	C_{18}	$C_{18}F_1$	ΣC_{18}
Skeletal muscles	56·9	21·7	19·4	41·1
Heart muscle	53·1	24·2	18·2	42·4
Uterus muscle	39·9	33·0	19·7	52·7
Adrenal glands	38·2	38·7	14·8	53·5
Testes	35·5	26·8	27·3	54·8
Ovaries	32·4	36·6	21·4	58·7
Hypophysis	35·1	40·2	18·7	58·9

The fatty acid composition of plasmalogens corresponds with that of diacyl phosphatides if determined in the same sources [90, 299–303]. This analogy becomes even more evident when the objects of comparison are fatty acids located at the β-position in diacyl phosphatides [304]. A particular feature of phosphatides of animal origin is that the unsaturated acid residue is usually located at the β-position while the end (α)-position is more frequently occupied by a saturated acid group. In the plasmalogens of most tissues there is a marked predominance of unsaturated fatty acids [99, 305, 306], while in ethanolamine plasmalogens of human brain saturated fatty acids cannot be detected at all [307]. The choline plasmalogens of pig lungs [99] can be mentioned as an exception, being characterized by saturated fatty acids; but cephalin plasmalogens from the same source contain more unsaturated than saturated fatty acids. Consequently, the structural principle of plasmalogens seems to be the same as that of diacyl phosphatides of animal origin: saturated hydrocarbon chains (belonging to fatty aldehydes) attached preferably at the α-position, and unsaturated ones (belonging to fatty acids) located mainly at the β-hydroxyl of glycerol.

3. Methods for Isolation and Analysis of Plasmalogens

When isolating plasmalogens from the fractions of compound lipids the first problem is the high lability of these natural compounds. This is the reason why the actual structural formula of the plasmalogens (II) remained

unknown for such a long time, and investigators tended to suggest a cyclic acetal structure (III) which is formed from plasmalogens if isolated without the necessary precautions [308, 309].



II IV III

Actually, the use of alkalies in extracting lipids from tissues results in formation of lysoplasmalogens (IV) which tend to cyclize on being acidified [10, 310–313] or at high temperatures [314, 315]. In tissues stored or prepared for the extraction of lipids a 'spontaneous' degradation of plasmalogens [13, 316–321] is possible too.

Compound lipids can be separated by means of column or thin layer adsorption chromatography into classes corresponding to the type of their aminoalcohols. In this way, the plasmalogens appear in the fractions of the corresponding diacyl analogues (of phosphatidylethanolamine, -choline or -serine) [99, 227, 265, 322]. In some cases, if significant differences exist in the degree of unsaturation of the plasmalogens, they can be isolated from these fractions by means of chromatography on silvered adsorbents [323]. Following the destruction of diacyl phosphatides the unchanged plasmalogens can be isolated. Thus, use of alkalies under mild conditions results in selective decomposition of diacyl phosphatides, and plasmalogens are easy to separate from the decomposition products by chromatography [194, 324–326]. M. A. Wells and J. C. Dittmer demonstrated the technique of artificial chemical degradation of lipids (a combination of alkaline and acid treatment) coupled with the chromatographic method [10]. They accomplished a quantitative analysis of a complex mixture of lipids belonging to 24 classes using only 150 mg of fresh brain tissue. Isolation of unchanged plasmalogens was carried out by anion-exchange chromatography, while cyclic acetals of glycerol were treated on silica-filled chromatography columns. Special attention should be paid to the quality of adsorbents and their preliminary preparation [327], to avoid losses during chromatographic treatment on silica or silica gel [92, 202]. Plasmalogens remain unchanged if the phospholipids are separated on columns filled with weakly basic phloridzin [328].

Treatment with alcoholic alkaline solutions may lead to formation of ethers [329, 330]. Hence, if alkyl ethers are to be determined parallel with plasmalogens, the modification using lithium aluminium hydride should be preferred [92, 331, 332]. The further separation of the hereby formed glycerol, alkyl ethers and cyclic acetals of glycerol is quite simple [92]. In another variation the cyclization of α-1-alkenyl ethers is avoided and their quantitative determination is carried out on thin-layer chromatograms by means of photodensitometry [333].

Biochemical methods of isolating the plasmalogens are very promising. Pure plasmalogens were first isolated from the lecithin fraction of bull heart by means of the selective hydrolysis of diacyl phosphatides by phospholipase A obtained from *Crotalis atrox* snake venom [334, 335]. The isolation was successfully carried out with phospholipase C [336] and phospholipase D [337] of cabbage as well, phospholipase D showing absolute specificity for diacyl phospholipids.

When investigating the composition of aldehydes and fatty acids in plasmalogens, the first step is, as a rule, a chemical reaction on the enol ether bond. There are a number of methods of specific hydrolysis using acetic acid [203, 299, 301, 338], trichloroacetic acid [226], salts of mercury [203, 339–341] and of other heavy metals [342]. Lyso-compounds are easy to isolate by means of adsorption chromatography on columns filled with alumina [343–346] or silica gel [300, 301, 347–349], or by counter current distribution methods [307, 350]. The individual separation may be well controlled using thin-layer [147, 351–359] or paper [21, 33, 225, 360–363] chromatography.

Employing combinations of two-dimensional thin-layer chromatography and chemical decomposition of plasmalogens by mercuric chloride three types of plasmalogens can be determined in mixtures of choline-, ethanolamine- and sulphur-containing phosphatides [364]. Acetates obtained from phosphatides following a treatment with a mixture of acetic anhydride and acetic acid [365, 366] are easy to determine by thin-layer chromatography. This method, combined with biochemical modification of lipids, allows the three classes of lipids to be determined according to the type of ether-linkage at the α-position of the glycerol group (esters, ethers and vinyl ethers), as shown by the determination of the lecithin fraction from bull brain [366]. Another method for determining these three classes consists in the conversion of compound lipids into dimethyl ethers of phosphatidic acids followed by a separation on thin-layer adsorbent; by this procedure methyl or dinitrophenyl ethers of cephalins can be separated too [367].

There are certain reactions employed in practice for the analytical deter-

mination of plasmalogens, the first of them being the Feulgen reaction [130, 308, 368–371] (a colour reaction of aldehydes with Schiff's reagent). This method often tends to give values too high [111] because of the sensitivity of the reagent to the autoxidation products of unsaturated fatty acids [372–374]. The procedures for preparing the solvents and extractants [375], and the test material too, should be accomplished with great care; determinations should be done in a darkened room [360]. There is another colour reaction based on the formation of yellow *p*-nitrophenylhydrazones [111, 376]. This method leads to too low values [335, 337]. The addition of iodine to the double bond is the most specific reaction of plasmalogens; moreover, the preliminary release of aldehydes from plasmalogens is not necessary. While the addition of bromine to plasmalogens [378] takes place with the same ease as to ordinary olefins, in the case of iodine, conditions can be chosen so that the double bond of plasmalogens which is in a conjugated position relative to the ether bond can display its greater reactivity compared to the isolated one. There are several publications describing modifications of the analytical determination of plasmalogens by this method [25, 113, 117, 335, 341, 379, 380]. The use of 2,4-dinitrophenylhydrazine provides data [381, 382] comparable with results of both latter methods.

Various methods are available for the determination of the aldehyde composition of plasmalogens. The formation of crystalline products: of thiosemicarbazones [200, 308, 383–384], of *p*-carboxyphenylhydrazones and of carboxymethoximes [385] is practicable only in the case of saturated aldehydes. That is the reason why octadecenal has been detected in plasmalogens only somewhat more recently, following the introduction of more sensitive methods. In one of the first procedures [286, 386] fatty acids and aldehydes obtained by saponification of plasmalogens are etherified with methanol; the mixture of methylethers and dimethyl acetals is treated with alkali, the salts of fatty acids separated, and the remaining dimethyl acetals transformed into oximes, the latter being separated into two groups, saturated and unsaturated. The final identification is carried out in the form of fatty acids. The number of steps may be reduced by oxidizing aldehydes directly to acids [277, 387].

The introduction of practical gas-liquid chromatographic methods made analytical determinations both easier and more accurate. In most cases mixtures of dimethyl acetals [275, 297, 308, 389] obtained by methanolysis of phosphatides in the presence of catalytic amounts of hydrochloric acid [172] or boron trifluoride [390] are investigated after separating them from methyl ethers of fatty acids by means of thin-layer chromatography. During the formation of dimethyl acetals [391] and during gas-liquid chromatog-

raphy [392–394] 1-alkenyl ethers can be formed. In view of this, the results of determinations of branched aldehydes in earlier investigations [268, 269, 275, 297] may be considered questionable [391]. Recently, the identification of aldehydes in the form of more stable products, namely cyclic acetals of ethylene- or trimethylene-glycol [395–397] and of dithioglycol [398] has been suggested. More infrequently, aldehydes are transformed into alcohols (chromatography in the same form [37, 275] or in the form of acetates [381]) or acids (chromatography in the form of methyl esters [288]). Aldehydes, in turn, can be separated from the accompanying fatty acids not only in the form of dimethyl acetals but also as 2,4-dinitrophenyl-hydrazones [381, 389, 399, 400] being subsequently regenerated from the latter by laevulic acid [381, 401, 402], by carbonyl compounds [396] or by dithioglycol [398]. A direct separation of dimethyl hydrazones of aldehydes is also practicable using gas-liquid chromatography [403].

Bearing in mind the fact that free aldehydes are present in animal tissues [404, 405] and can be formed during the storage of acetals and phospholipids [395, 406, 407], particular attention should be paid to specific determination procedures of plasmalogens in presence of aldehydes [408, 409], of aldehydes in presence of plasmalogens [376] and of aldehydes in presence of acetals [409–411].

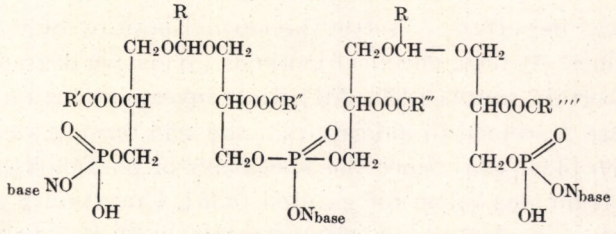
Hystochemical methods represent a special group.

4. Acetalphosphatides

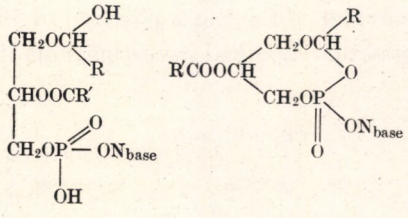
Substances with acetalphosphatide structure (III) were first isolated from horse muscles [308]. Later, however, they proved to be artefacts formed by decomposition of the original structure of compound plasmalogens (I) on alkaline treatment. Actually there has been only a single report describing a native aldehydogenic lipid with its aldehyde bound in the acetal form. Since isolation was carried out under mild conditions a conversion of the original structure of the lipid could be considered impossible. The authors of the report W. Bergmann and R. A. Landowne found this lipid in the sea anemone *Anthopleura elegantissima* and identified it using α, β-O-alkylidene glycerylphosphorylcholine (IIIb). Subsequently, repeated investigations [419] on lipids of this marine invertebrate failed to confirm the presence of an unusual aldehydogenic lipid with the acetal structure, while in special studies on lysocholine plasmalogen [420, 421] (IVb) it could be identified by the aid of W. Bergmann's and R. A. Landowne's reagent. Thus, in all probability the enol ether bond seems to be the only way by which aldehydes are connected to lipids from any natural source.

IV. DETERMINATION OF STRUCTURE

Acetalphosphatides (III) are insoluble in ether and 95% alcohol. Compound plasmalogens isolated later under milder conditions differ from acetalphosphatides in their solubility in ether [251] and in their reaction rate with Schiff's reagent [422]. On the strength of this the validity of the α, β-cyclic acetal structure (III) attributed to compound plasmalogens was considered questionable [423–425]. Later on a direct comparison between ethanolamine plasmalogen isolated from brain tissue and a sample of synthetic acetalphosphatide led to the conclusion that the aldehyde of plasmalogens plays no part in the formation of a cyclic acetal group [426]. On the basis of recent analytical determinations [223, 264, 321, 324–326], the presence of fatty acids in plasmalogen molecules could be taken as certain, this giving rise to the suggestion of 4 more structural formulae (II, XII–XIV) [307, 423, 427].

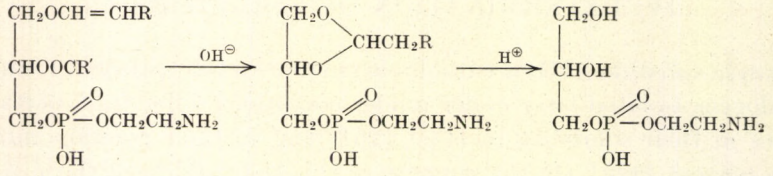


XII



XIII XIV

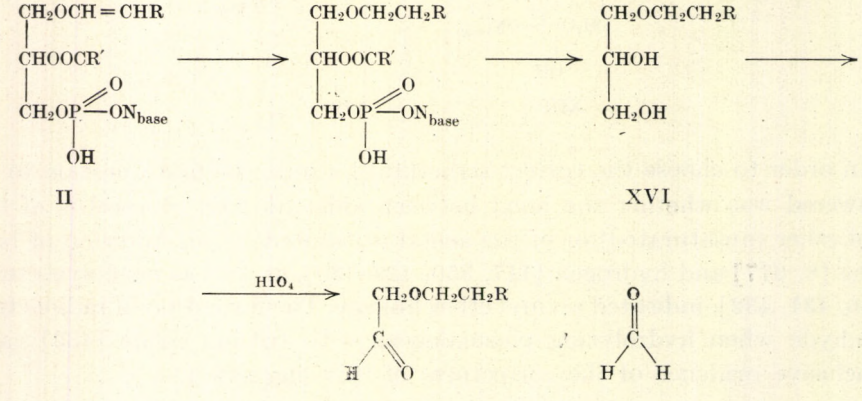
In order to choose the correct structural formula the first question to be answered was whether the bond between aldehyde and glycerol is of the enol ether (unsaturated) or of the acetal (saturated) type. Addition of bromine [8, 377] and hydrogen [117, 350, 428–430], as well as ozonolysis tests [266, 431, 432], indicated a vinyl ether linkage. The formation of radioactive aldehyde when hydrolyzing plasmalogens with tritium oxide [433] gave conclusive evidence of the correctness of this suggestion.



Ia IIIa XV

The saponification of acetalphosphatides (IIIa) (obtained by isolation of plasmalogen containing ethanolamine (IIa) [309]) under mild conditions (catalysis with mercuric chloride or 20% acetic acid at 37°C) is not accompanied by phosphate migration and leads to α-glycerylphosphorylethanolamine (XV) [339]. The attachment of phosphate to the α-hydroxyl of glycerol has been determined in a periodate oxidation test. Thus, the position of the phosphoric acid residue in the plasmalogen molecule could be stated.

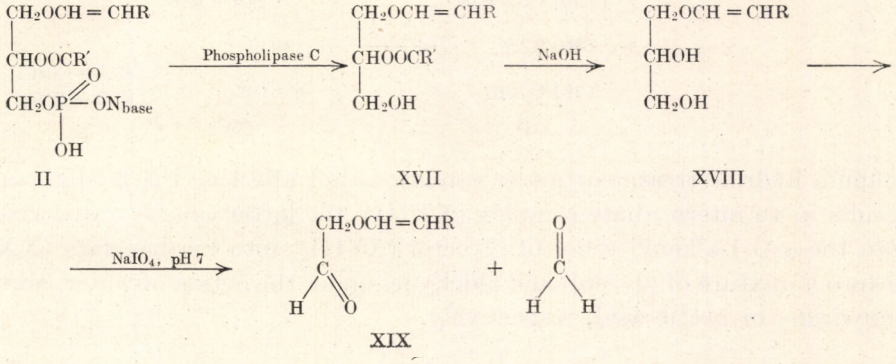
Further, it was important to settle the mutual position of aldehyde and fatty acid residues. At first, chemical methods (oxidative degradation tests etc.) led to uncertain results [299, 301] while investigations on hydrolysis by phospholipase A seemed to indicate that the acid residue was connected in the α-position [434, 435], since the specificity of phospholipase for the terminal ester bond was taken for granted [436]. Uncertainty arose again when, in a series of biochemical experiments, the specificity of snake venom phospholipase A proved questionable and it was found to be able to desacylate the β-linkage of phosphoglyceride at a similarly high rate [361, 437–441]. Complete evidence for the α-position of the aldehyde residue has been obtained by repeated chemical investigations including hydrogenation



II XVI

of plasmalogens (II) and examination by IR spectroscopy and periodate oxidation of a mixture of monoalkyl glycerol ethers (XVI) produced after subsequent transformations [117, 442–448].

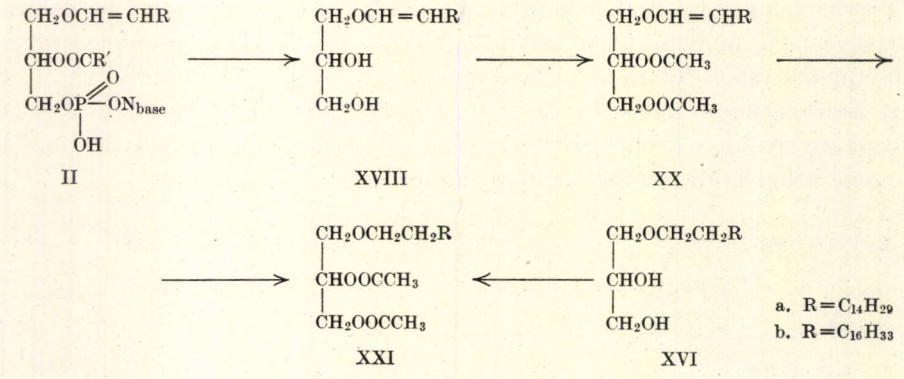
If degradation is carried out under milder conditions (cleavage of this phosphate group by phospholipase C, desacylation with alkali at 37°C) the enol ether bond can be kept unchanged:



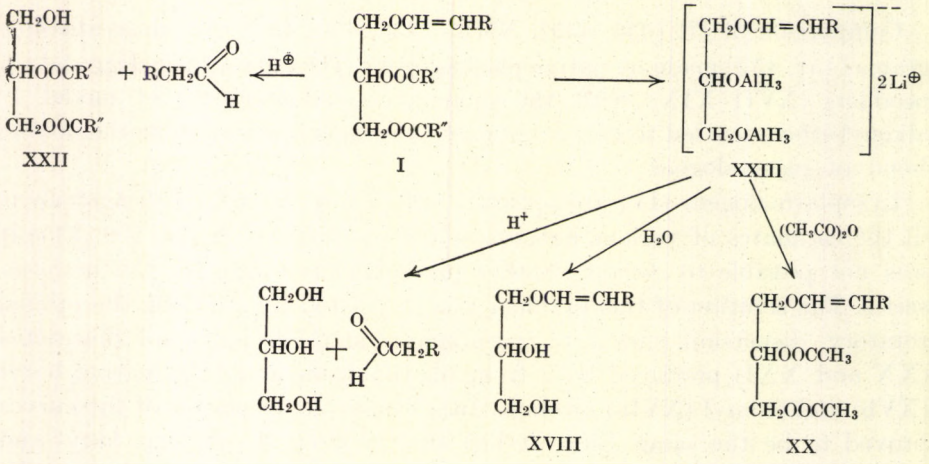
Comparison of IR [336, 449], NMR- [336, 450, 451], and mass-spectrograms [451, 452] made on native plasmalogens (II) and on their degradation products (XVII–XIX) with the analogous characteristics of model 1-alkenyl ethers has led to the assignment of a *cis*-configuration to the double bond of plasmalogens.

Lysoplasmalogens (IV) are optically active but because of disagreement in the estimates of various authors as to this activity [8, 26, 309, 335] it was not possible to decide whether plasmalogens belong to the D- or L-series. The solution of this problem was facilitated by studying the optical rotatory dispersion curves of α-1-alkenyl- and alkyl-glycerol diacetates (XX and XXI) produced both from plasmalogens and from native batyl (XVb) and chimyl (XVIa) alcohols. In all cases the character of the curves proved to be the same. Since it has already been shown that batyl and chimyl alcohols belong to the D-series [454–456], 1-alkenyl ethers of glycerol must be regarded too as members of this series. This also indicates that α-O-1-alkenyl-β-O-acylglycerol (XVII) previously produced by enzymatic hydrolysis of plasmalogen belongs to the D-series [457]. Thus, phosphatide plasmalogens, similar to their diacyl analogues, belong to the L-series. (see p. 28).

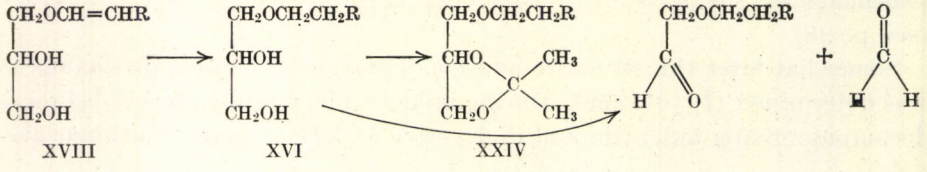
Somewhat later the structure and configuration of neutral plasmalogens was determined (I) [37, 458]. During mild acid hydrolysis these substances decompose giving aldehyde and diglyceride (XXII), whereas a lithium alu-



minium hydride treatment under conditions not affecting the double bond results in an intermediate complex (XXIII); the latter can be transformed into the α-O-1-alkenyl ether of glycerol (XVIII), into its diacetate (XX), or into a mixture of glycerol and aldehyde, under the action of water, acetic anhydride, or acetic acid, respectively.



After the hydrogenation of the α-O-1-alkenyl ether (XVIII) the resulting diol (XVI) is completely oxidized by periodate and with acetone it forms a ketal (XXIV).



The above-mentioned chemical characteristics and the optical activity of neutral plasmalogens seem to prove that the aldehyde chain is linked through the terminal hydroxyl of glycerol, and comparison of the signs of optical rotation of neutral plasmalogens and their hydrogenation products with those of synthetic α-alkyl ethers of diglycerides [36, 47] indicates that neutral plasmalogens belong to the D-series. The IR and NMR spectra of neutral plasmalogens and of 1-alkenyl ethers (XVIII) derived from the former indicate the *cis*-configuration of the double bond.

Can structural investigations on plasmalogens be considered as complete? Apparently the structures of the isolated substances are now completely solved. Nevertheless, this does not mean that in the living cell these established structures might not comprise parts of more complex groupings, as mentioned by R. Feulgen and T. Bersin [308]. For instance, hystochemical studies lead us to suppose that, in the cell, at least some part of the plasmalogens exists in a bound form unaccessible to detection by the normal qualitative reactions of the enol ether bonds [459, 460]. There might be another possibility of more complicated plasmalogen molecules: the formation of one more ether bond with carbohydrates or inositol at the free hydroxyl of the phosphate group [461, 462].

V. CHEMICAL SYNTHESIS

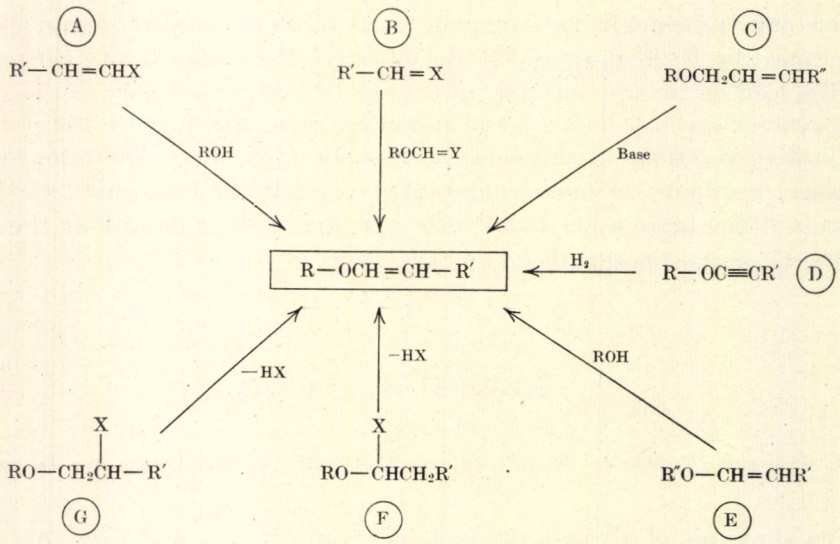
1. Possible Routes to Build up the Structure of Aldehydogenic Lipids

The synthesis of glycerol products containing cyclic acetal groups does not raise considerable experimental difficulties, since the well-known methods for obtaining substituted dioxolanes are completely suitable for constructing this type of aldehydogenic lipid.

In synthesizing plasmalogens there arose an entirely new problem — that of the selective introduction of the 1-alkenyl ether group into a polyalcohol molecule. The need for stereospecificity when performing the synthesis poses a further problem, as it is desirable that the methods used permit the preparation of optically active substances as well.

In view of the above limitations it seems reasonable first to discuss briefly the possible ways of synthesizing 1-alkenyl ethers of glycerol (and other polyalcohols) before evaluating the already realized schemes. Apparently we have a choice of not less than seven ways of producing 1-alkenyl ether bonds (Scheme 1, Courses A–G).

Course A. Alkylation of alcohols with 1-haloalkene-1 Although this was the very first way by which vinyl ethers were obtained [463–465], in the present case the chance of its realization is low. The reason is the chemical inertness of the vinyl halogen atom in nucleophilic substitution reactions. This leads to severe reaction conditions and low yields of vinyl ethers even in the simplest cases [466, 467]. There are, however, a few reports [468, 469] describing accomplishment of this reaction under relatively mild conditions. One of them has not proved reproducible [470]. Another, dealing with unusually easy formation of alkoxy sterols [468, 471], is of partial importance [472].

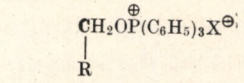


Scheme 1

Long-chain 1-haloalkene-1 compounds show even less tendency to undergo exchange reactions with alkoxides than do the simplest halogenated olefins and are even more apt to release halogen acids under these conditions [473]. The halogen atom can be activated by introducing an electron-acceptor group into a conjugated position. Thus, the activity of alkyl-chlorovinyl ketones is comparable to that of acid halides [474] and they easily form 1-alkenyl ethers [475]. But how should the carbonyl be selectively eliminated if it is conjugated with the enol ether bond, the latter being extremely reactive with regard to additon?

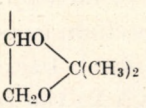
Course B. Among the methods of creating a double bond by means of condensation reactions the Wittig reaction seems to be the most promising

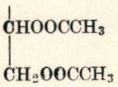
for synthesizing 1-alkenyl ethers. Recently some reports have been published on its successful application to produce 1-alkenyl ethers of mono-alcohols [476–478]. The possibility of its steric control has been discussed [479]. Unfortunately, ylides produced from phosphonium salts having various substituents in the β- and α'-positions of the glycerol residue (XXXVa–d) [480, 481] would not react with aldehyde. Similarly, the earlier attempts of Bohlmann and Herbst [482] met with little success in accomplishing reactions with phosphonium salts of slightly simpler structure (XXVI and XXVII). The origin of the failure of these attempts apparently lies in the dependence of Wittig's reaction on steric factors [483].

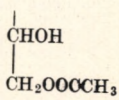


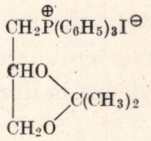
XXVa–d

a. R =  X = I

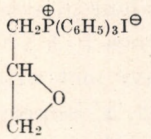
b. R =  X = Cl

c R =  X = Cl

d. R =  X = Cl

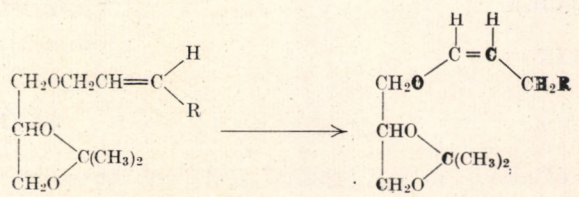


XXVI



XXVII

Course C. The isomerization of allyl ethers under the action of strong bases is strictly stereospecific, producing only *cis*-propenyl ethers [484–487]. This feature of the isomerization makes its use extremely promising in the synthesis of plasmalogens.

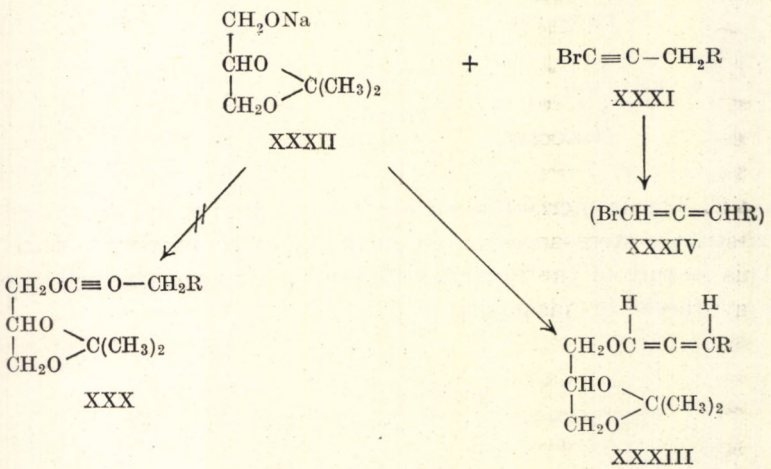


XXVIII XXIX

It has been found that isopropylidene-glyceryl allyl ether (XXVIII, R = H) completely isomerizes into *cis*-propenyl ether (XXIX, R = H) in dimethylsulphoxide (catalytic amount of potassium t-butoxide) in less than 50 minutes at 100°C [488] or in three days at 18–20°C [450–451]. Unfortunately, homologous ethers under the same conditions either do not change at all (XXVIII, $R = CH_3$) [450, 451, 489] or undergo decomposition (cleavage of the diene, when XXVIII, $R = C_{14}H_{29}$) [490]. Thus, introduction of electron-donor substituents into the methyl group of allyl ethers impedes the redistribution of electrons and leads to isomerization of the double bond.

Course D. In order to obtain 1-*cis*-alkenyl ethers one more method is feasible, namely the selective hydrogenation of a triple bond in acetylene ethers yielding a double bond. It is known that if the hydrogenation of dialkyl acetylenes is done under suitable conditions *cis*-olefins can be produced.

Though the methods of synthesizing acetylene ethers of mono-alcohols [491, 492] have been well elaborated, attempts to obtain 1-alkenyl ethers of isopropylidene glycerol (XXX) by means of nucleophilic substitution of 1-bromoalkynes-1 (XXXI) by reaction with sodium isopropylideneglycerate (XXXII) have met with little success [493–495]. Instead of the expected α-1-alkynyl-β, α′-isopropylidene glycerols (XXX) the corresponding allene ethers (XXXIII) are formed, the cause of this being that bromides with the allene structure (XXXIV) take part in the reaction; the latter are formed

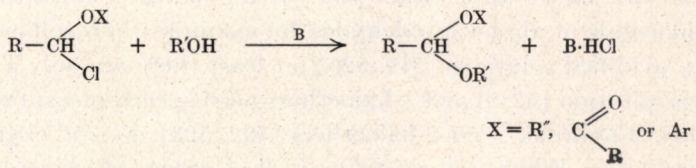


by the action of alkali on the original acetylene bromides (XXXI) [495] which are inactive in the nucleophilic substitution reaction. The high chemi-

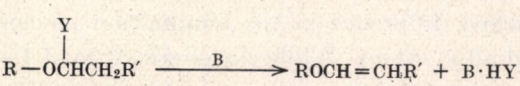
cal activity of acetylene ethers raises a considerable obstacle to their employment in the multi-step synthesis scheme of plasmalogens. Thus, for instance, acetylene ethers, unlike vinyl ethers, are ready to take part in additions under neutral conditions [491] or in the presence of alkalies [491] and easily undergo thermal conversion reactions at relatively low temperatures [491, 496]. This raises great difficulties in choosing a suitable blocking group for the selective introduction of the 1-alkynyl group into the α-position of the glycerol molecule.

Course E. Alcohols are apt to take part in cross-esterification reactions (cross-vinylation) with vinyl esters, or with vinyl ethers of phenols or of alcohols. The reaction is usually carried out in the presence of mercuric salts (to hinder the formation of acetals) under mild conditions (at negative or only moderate temperatures). The disadvantages of this method lie in the low yield of vinyl ether (usually not more than 25%) and in the impossibility of stereospecific control of the reaction.

Course F. The elimination of 1-substituted alkyl ethers. The method of obtaining vinyl ethers by decomposition of acetals or 1-haloalkyl ethers has been well-elaborated. It is not surprising that the first success in the synthesis of plasmalogens was connected with these reactions. It is to be expected that on using mixed acetals containing phenol [498, 499] or fatty acid residues [500, 501] a more controlled pyrolytic decomposition is practicable. The wider utilization of the readily available 1-haloethers [502, 503] seems to be promising too. During the synthesis of aldehydogenic lipids the use of 1-chloroethers has the advantage of rendering mineral acids unnecessary both in the stage of formation of mixed acetal [504–508]

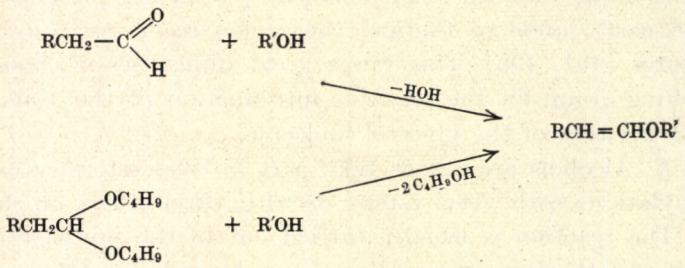


and during the elimination reaction [509–512]:



Both reactions take place under mild conditions: tertiary amines can be used as base; thus the possibility of acyl migration, otherwise always to be contended with in the synthesis of plasmalogens, is excluded.

Interesting methods for the synthesis of 1-alkenyl ethers have been reported by French authors, alcohols interacting directly with aldehydes [513] or with linear acetals [514] on ion-exchange resins:



A common disadvantage of the methods of preparation of 1-alkenyl ethers by means of conversion of higher fatty aldehydes, of various acetals or of 1-haloethers (Courses B, E and F) lies in the instability of these substances, this sometimes raising serious difficulties during the synthesis. A further problem is to ensure that the equilibrium reaction proceeds in the proper direction, it being extremely sensitive to minor changes in conditions (time, reagent ratio, amount and quality of catalyst, temperature). It must be borne in mind, too, that mixed acetals show an inclination to symmetrization [515] and acetals and vinyl ethers are ready to undergo self- and mutual condensation reactions during acid catalysis [516–518]. There are also complications involved in the presence of the residue of the polyfunctional alcohol, i.e. of glycerol.

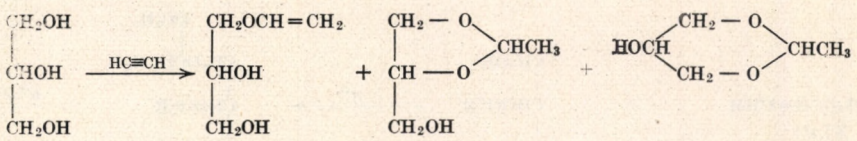
Course G. Elimination of 2-substituted alkyl ethers. The 2-substituted alkyl ethers are more stable than the corresponding 1-substituted ones. While hemiacetals of aliphatic aldehydes, for example, show sufficient stability only in acidified solutions [519–520] (at least there are only a few cases of their precipitation [521]) and 1-haloethers need special measures on being stored [470], 2-hydroxy- and 2-haloethers [522, 523] do not change under normal conditions. When α-2-substituted alkyl ethers of glycerol are synthesized, the use of long-chain aldehydes or intermediates containing them (vinyl ethers, haloethers, acetals) as raw materials can be avoided.

There is a marked difference in the elimination mechanisms of even 1- and 2-substituted alkyl ethers. While the elimination of 1-substituted ethers takes place as a unimolecular process via carbonium ion formation, in the case of 2-substituted ethers either a bimolecular reaction mechanism is to be expected or a unimolecular one with the formation of a carbanion (depending on the strength of the alkali). In addition, as has recently been stated, the elimination of tosylates of secondary alcohols [524–527] and of onium

compounds [528, 529], taking place as bimolecular reactions under certain circumstances, permits some degree of steric control. This leads to a predominance of the *cis*-olefin in the reaction product.

2. *Practically-realized Methods of Synthesis of Neutral Plasmalogens*

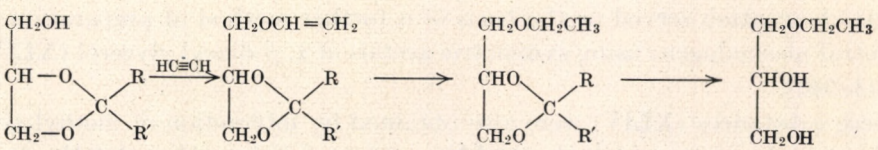
Addition reactions occur between alcohols and acetylene, in the presence of basic catalysts, under pressure [470, 530]. Addition of alcohols to alkylacetylenes results in formation of 1-alkylvinyl ethers [531]. M. F. Shostakovskii and E. P. Gratcheva [532] succeeded in preparing α-vinylglycerol (XXXV) by vinylation of glycerol; the former can be considered as the first member in the homologous series of α-O-1-alkenyl ethers of glycerol (XVIII) which are the intermediates in the synthesis of plasmalogens.



XXXV XXXVI XXXVII

A more detailed examination showed that monovinylglycerol was only a minor reaction product [533], the major products consisting of cyclic acetals (XXXVI and XXXVII) (the reaction takes place at temperatures above 100°C) together with the products of further vinylation. Acetalization accompanies the vinylation of other polyalcohols, too (e.g. of pentaerythritol) [534].

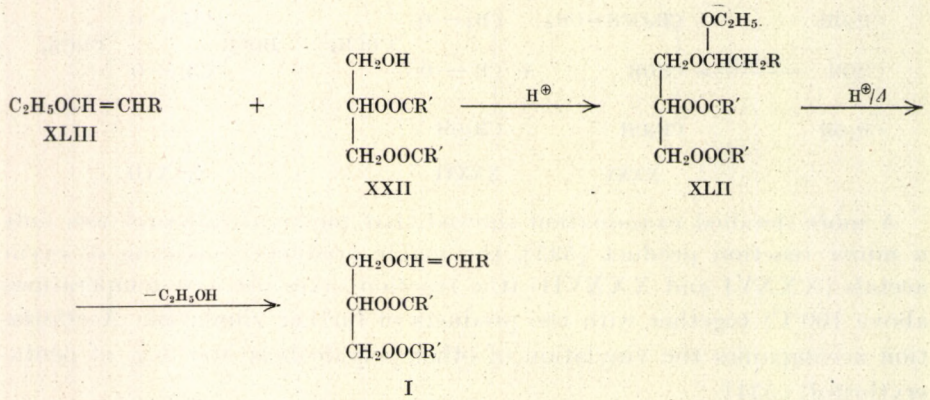
The controlled introduction of the vinyl group can be accomplished by employing the ketal protection of hydroxyls which are not desired to take part in the reaction. Thus, starting from α, β-O-alkylidene glycerols (XXXVIII) the corresponding vinyl ethers (XXXIX) have been produced, as confirmed by their hydrogenation and the subsequent acid hydrolysis of the intermediate ethyl ethers (XL) giving α-O-ethyl glycerol (XLI) [535, 536].



XXXVIII XXXIX XL XLI

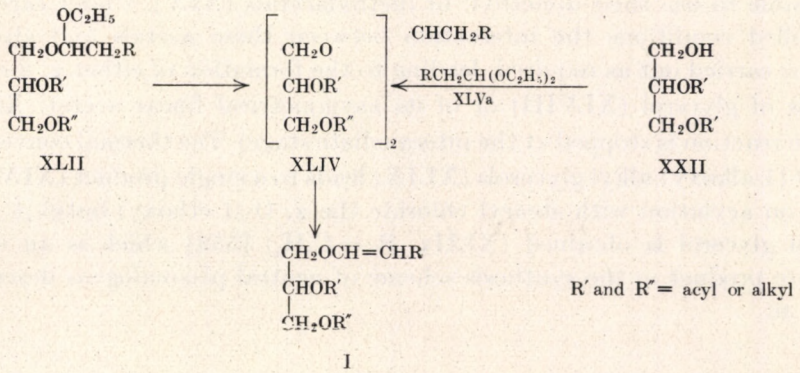
Selective elimination of the protecting ketal group in the presence of the vinyl ether group is impossible. It has been shown, on the contrary, that in a number of carbohydrates the vinyl ether group is hydrolyzed more rapidly with acid catalysis than the isopropylidene group [537, 538].

Substances of the neutral plasmalogen type (I) were synthesized for the first time (in the laboratory of N. A. Preobrazhenskii) by the use of asymmetric linear acetals of glycerol (XLII) [539, 540]. The formation of the latter is based on the ability of vinyl ethers to add alcohol thereby producing asymmetric acetals. α, β-Diglycerides (XXII) were employed as alcoholic components to be condensed with 1-alkenylethyl ethers (XLIII) [492, 541, 542]. Thus, after the pyrolytic cleavage of alcohol from the intermediate mixed acetal (XLII) an alkenyl ether of the neutral plasmalogen type (I) could immediately be produced (alkenyl analogues of tripalmitoine and tristearoine has been formed).



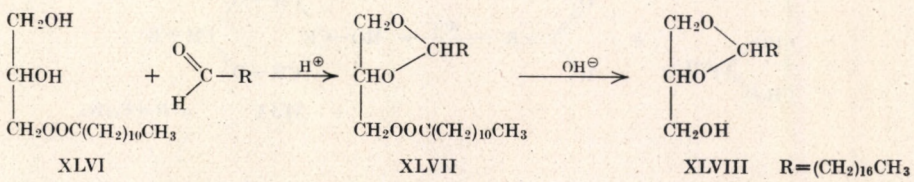
This reaction is accompanied by formation of the original 1-alkenyl ethyl ethers (XLIII). The yield of the final lipids (I) was about 25% (in the form of *cis-trans*-isomers). It is interesting that at higher temperatures (above 180°C) the pyrolytic reaction is stereospecific yielding only *trans*-neutral plasmalogen (I) [543]. A further side reaction accompanying the decomposition of asymmetric linear acetals of α, β-diacyl glycerol (XLII) is a symmetrization process leading to symmetric acetals (XLIV) [544]. This observation served as the basis of a further method of preparation of neutral plasmalogens using symmetric acetals of α, β-diacyl glycerol (XLIV) [544–546].

These substances (XLIV) were also obtained by interaction of diethyl acetals of higher fatty aldehydes (XLVa) and α, β-diglycerides (XXII). It is to be noted that, under the circumstances of the synthesis of neutral plasma-



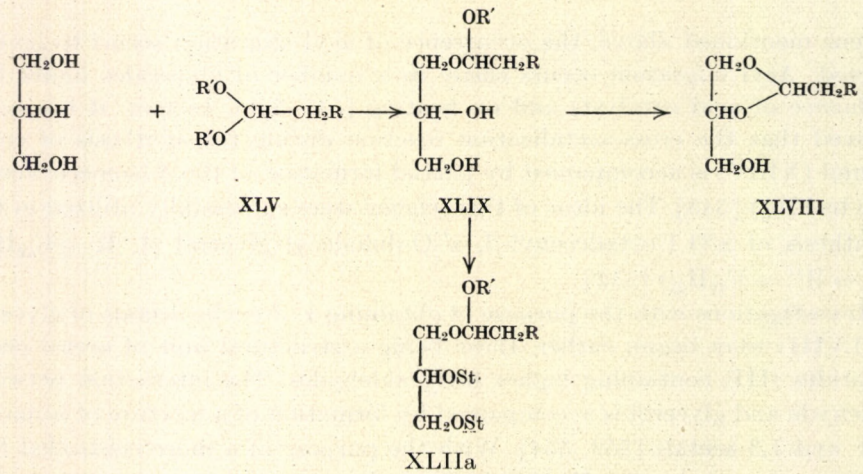
logens mentioned above, the occurrence of acyl migration seems to be expected. Acyl migration occurs easily in a number of glycerides under the influence of acid catalysts and on heating [547–551]. In fact, it has been proved that the cross-acetalization reaction during the synthesis of compound (XLIV) is accompanied by partial formation of the β-isomeric acetal (up to 17%) [545]. The ideas of this scheme were successfully utilized in the synthesis of α-O-1-octadecenyl-β, α'-O-didodecyl glycerol (I, R $= C_{16}H_{33}$, R′ $=$ R″ $= C_{12}H_{25}$) [552].

Investigations with the purpose of obtaining α, β-cyclic acetals of glycerol (XLVIII) were begun earlier, these being a structural unit of acetal-phosphatides (III) containing higher fatty aldehydes. The interaction between aldehyde and glycerol is accompanied by formation of a mixture of isomeric 1,2- and 1,3-acetals [553, 554]. With the purpose of a more controlled formation of α, β-acetals, T. Malkin [555] suggested that α-monoglycerides should be used. Though α-monoglycerides in the presence of acids tend to form equilibrium mixtures with the β-isomer [556], according to Malkin's data α-monolaurin (XLVI) seems to form only one acetal with n-octadecenal, namely α, β-O-octadecylidene-α'-lauroylglycerol (XLVII). If treated with alkali this compound gives α, β-O-octadecylidene glycerol.

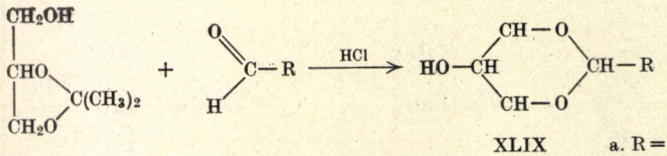


According to C. Piantadosi and co-workers [557–559] instead of the highest fatty aldehydes (which are sensitive to atmospheric oxygen [560]) it is

preferable to use their dimethyl- or diethylacetals (XLV). Under carefully controlled conditions the interaction between these acetals and glycerol may be carried out as required, leading to the formation of either α, β-cyclic acetals of glycerol (XLVIII) or of its asymmetrical linear acetal (XLIX, i.e. the reaction is stopped at the intermediate stage). The thermal conversion of α-O-(1-alkoxy)-alkyl glycerols (XLIX) leads to a single product (XLVIII), while on acylation with stearyl chloride the α, O-(1-ethoxy)-butyl-β, α-distearol glycerol is obtained (XLIIa, R $= C_2H_5$) [559] which is an intermediate product in the synthesis scheme of neutral plasmalogens described on p. 36.

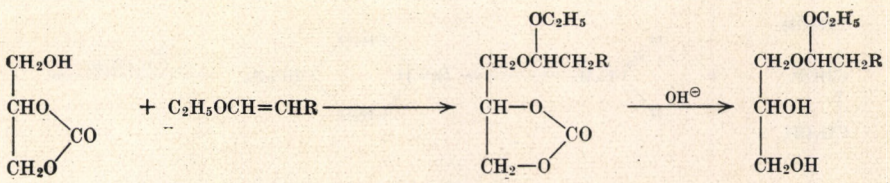


α, α'-O-hexadecylidene glycerol (XLIXa) has been obtained by passing a stream of hydrogen chloride through a cooled mixture of isopropylidene glycerol and palmitaldehyde in chloroform [561]:



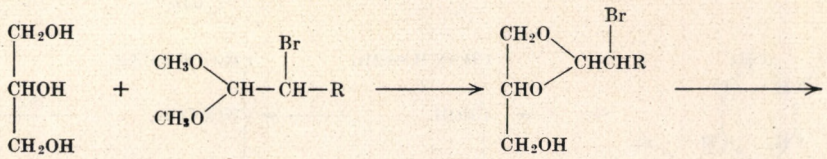
XLIX a. R = $C_{15}H_{31}$

A direct synthesis of asymmetrical linear acetals of glycerol (XLIX) has been carried out employing the carbonate protection of the α- and β-hydroxyl groups of glycerol [562]:

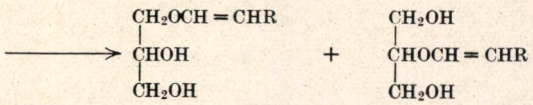


XLIXa

Acetalization of glycerol by the aid of α-bromodimethylacetals (L) [554] leads to the corresponding α-bromalkyl-substituted dioxolanes (LI):



L LI

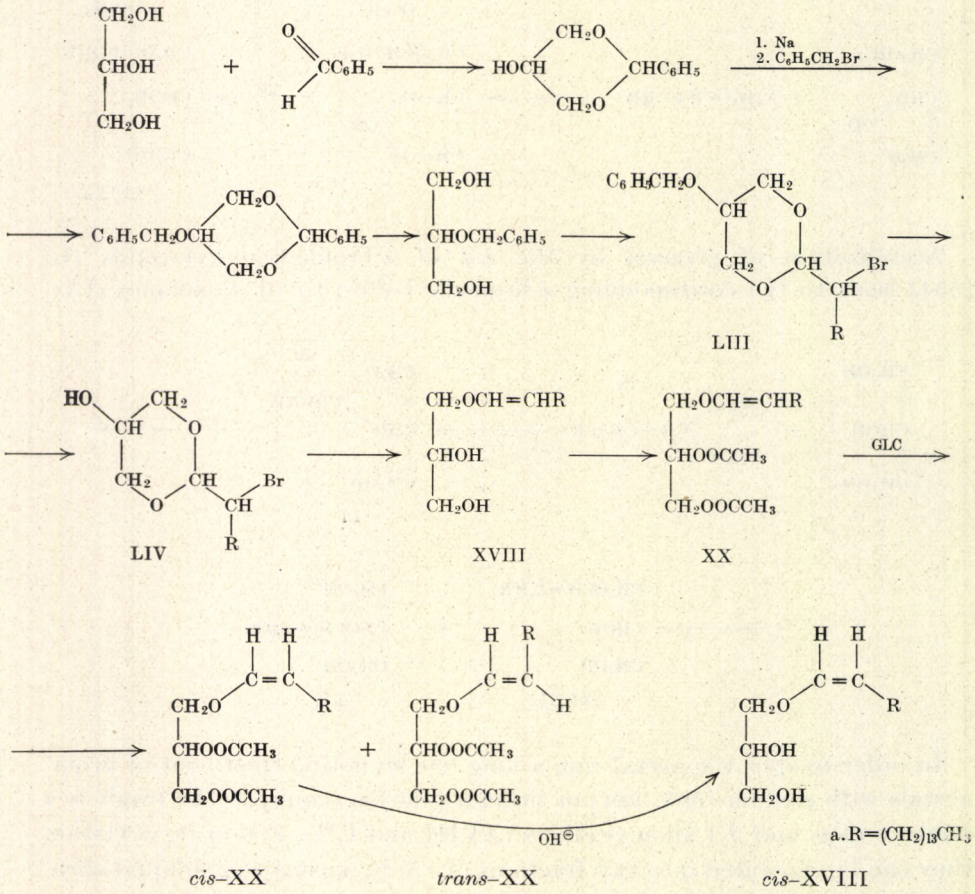


XVIII LII

In order to split the acetal ring a long (60–90 hours) treatment of bromacetals with sodium [563, 564] or lithium [480] is required. The result is a mixture of α- and β-1-alkenyl ethers (XVIII and LII). Following acylation they can be separated into two fractions (4 : 5) by means of gas-liquid chromatography. According to IR and NMR spectroscopic investigations these proved to be *cis*- and *trans*-isomers [480]. The investigation of each fraction using periodate oxidation methods (after hydrogenation and saponification) suggested that each of them in turn consisted of a mixture of α- and β-substituted glycerols (47 : 53).

The formation of the β-isomer (LII) could be prevented by means of an amplification of the synthesis scheme so as to obtain symmetrical cyclic bromacetals of glycerol (LIII and LIX) [450, 451] (see p. 40). In this case a mixture of *cis-trans* isomers of the olefin is obtained. However, the α-1-hexadecenyl ether of glycerol (XVIIIa) showing natural *cis*-configuration has been successfully isolated from a mixture of diacetate isomers (XXa) by means of preparative gas-liquid chromatography.

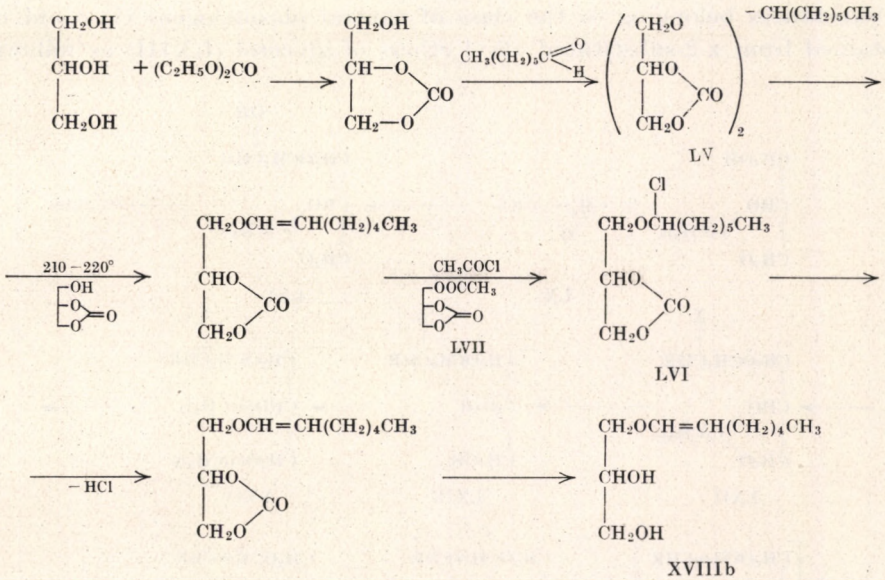
Starting from linear symmetrical acetals of glycerol, and employing a cyclic carbonate group [565] (stable in acid media, during the acetal formation,



but sensitive to alkalies) for the protection of the α- and β-hydroxyl groups, J. Cunningham and R. Gigg [490] synthesized α-O-1-heptenyl glycerol (XVIIIb) by two methods. By the first, the carbonate-glyceryl acetal of oenanthol (LV) is decomposed on heating at 210–230°C without catalyst; by the second, hydrogen chloride is eliminated from the chloroether (LVI) the latter being formed together with the acetate of carbonate-glycerol (LVII) by the action of acetyl chloride on the symmetrical acetal (LV).

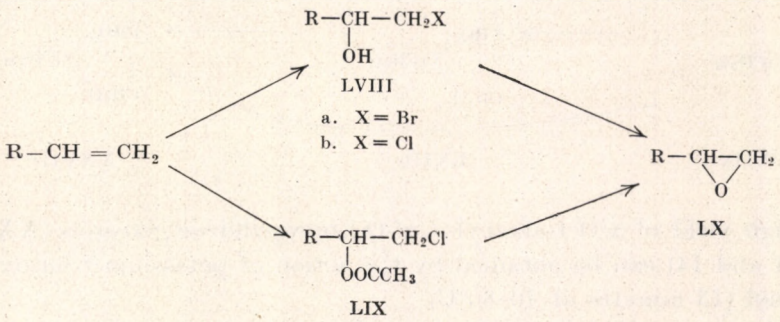
An interesting point is that with longer hydrocarbon chains the instability of α-1-chloroalkyl ethers of β, α′-substituted glycerols increases, and hence it is impossible to obtain α-O-1-hexadecenyl-β, α′-dipalmitoyl glycerol via the corresponding chloroether [543].

The synthesis of long-chain α-2-substituted alkyl ethers of glycerol (LXIII) has practically not been studied until recently. Their synthesis is

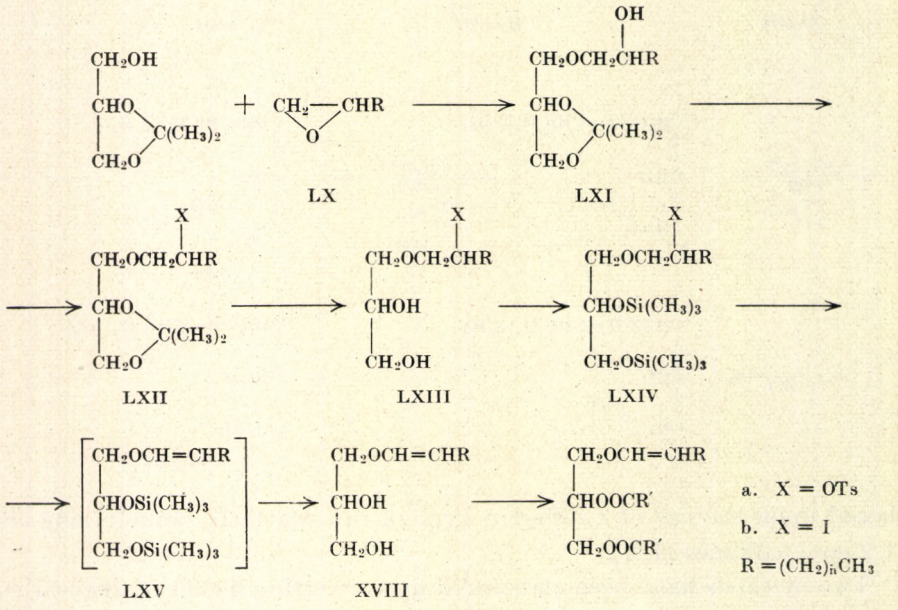


based upon the use of a different type of raw material, namely long-chain 1,2-epoxyalkanes (LX).

Two methods have been elaborated for converting higher n-alkene-1 compounds (on the basis of available higher fatty acids of the even number series, C_{14}–C_{18} corresponding to the normal composition of plasmalogenic aldehydes) into α-oxides [566]. One of these methods uses bromohydrins of alkenes-1 (LVIIIa) obtained from the corresponding olefins by the action of bromosuccinimide in aqueous-organic media [567, 568]. The corresponding chlorohydrins (LVIIIb) can be used too [569–572], but their acetates, which are easily obtainable and in high yield (up to 90%) by interaction of olefins with t-butylhypochlorite in acetic acid, seem more advantageous, since they can be converted much more easily into 1,2-epoxyalkanes (LX) [573].

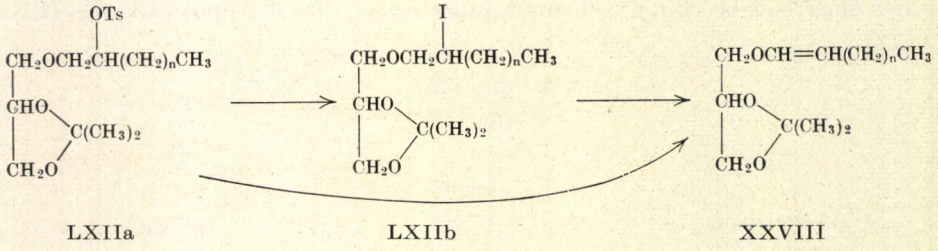


Substances belonging to the class of neutral plasmalogens (I) could be obtained from α-2-substituted alkyl ethers of glycerol (LXIII) as follows:



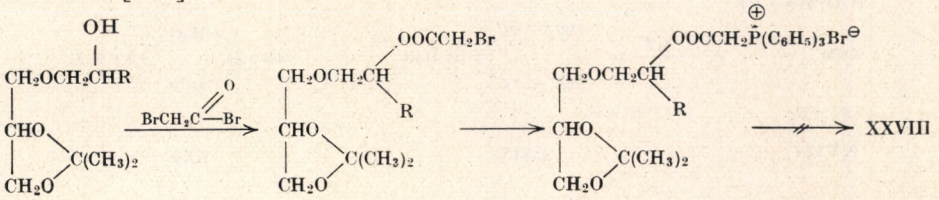
Scheme 2

The conditions of the elimination, which is the key reaction in this synthesis of neutral plasmalogens, have been determined in an experiment when model tosylates (LXIIa, n = 11 and 14) and the iodide (LXIIb, n = 14) obtained from one of them by the Finkelstein reaction [566, 568] underwent conversions leading to (XXVIII).



A high yield of α-O-1-alkenyl-β,α′-O-isopropylidene glycerols (XXVIII, n = 11 and 14) can be obtained by the action of potassium t-butoxide in t-butanol (15 minutes at 70–80°C).

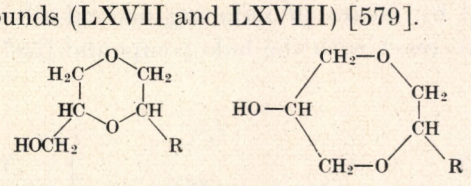
The olefin formation from alkoxycarbonylmethyl-triphenylphosphonium halides [574–576] is of some interest the latter being easy to synthesize from alcohols. However, though the pyrolysis of phosphonium salts (LXVI) is accompanied by release of gas, in the complex mixture of the reaction products α-O-1-octadecenyl-β,α-O-isopropylidene glycerol (XXVIII) could not be detected [566].



LXVI R = $(CH_2)_{15}CH_3$

The selective elimination of the isopropylidene group from 1-alkenyl ethers (XXVIII) is not practicable. Since the ether hydroxyl-protecting groups which are traditional in lipid chemistry are also eliminated either by hydrogenolysis or acid hydrolysis* a trimethylsilyl functional group has been chosen for blocking β- and α′-hydroxyl groups of glycerol during this synthesis. After elimination of α-O-(2-tosyloxy)-alkyl-β,α′-bis-O-trimethylsilyl glycerols (LXIVa) and following a water treatment of the reaction mixture a rapid hydrolysis of α-O-1-alkenyl-β,α′-bis-O-trimethylsilyl glycerols (LXV) takes place and finally α-O-1-alkenyl glycerols are formed (XVIII). The structure of these compounds has been confirmed by reactions of α-O-1-tetradecenyl glycerol (XVIIIc) which was quantitatively hydrogenated to the already known α-O-tetradecyl glycerol, or which by acid catalysis was completely hydrolyzed yielding myristaldehyde [579]. If the elimination is performed by the t-butoxide ion, the competing reaction of intermolecular nucleophilic substitution fails to occur; in all cases the formation of 1-alkenyl ethers having the *trans*-configuration is predominant [566].

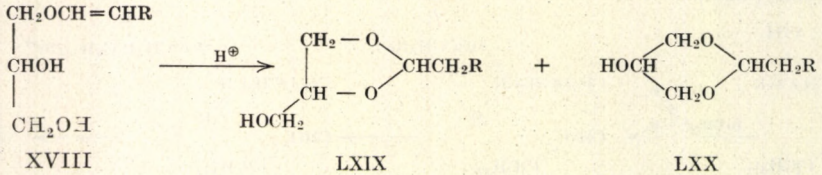
Simultaneously a competing reaction of intramolecular substitution takes place accompanied by formation of a mixture of seven- and six-membered heterocyclic compounds (LXVII and LXVIII) [579].



LXVII LXVIII

* The protecting benzyl group can be removed from the hydroxyl by a treatment with sodium in liquid ammonia [577]. This debenzylation method has been checked in a synthesis of anomeric vinyl-D-glucopyranosides [578].

Acylation of α-O-alkenyl glycerols (LXVIII) is followed by formation of isomeric heterocyclic compounds of an acetal nature (LXIX and LXX) [580]; this confirms the results indicating the sensitivity of this substance to the action of acidic agents [310–312] and heat [314–315].



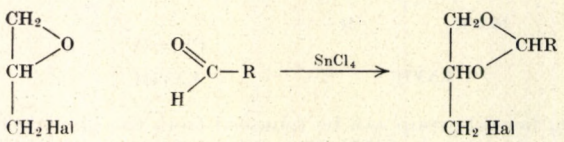
XVIII LXIX LXX

The practicability of Scheme 2 in the synthesis of neutral plasmalogens has been confirmed by obtaining α-O-1-alkenyl glycerols (XVIII) with C_{14} [580], C_{15} [581–582], C_{16} [582–583], C_{17} [584] and C_{18} aldehyde chains and by producing from them (±)-α-O-1-*cis-trans*-alkenyl-β,α-diacylglycerols (I) with palmitic, stearic and (in a single case) linoleic [580] acid residues.

3. The Synthesis of Phosphatide Aldehydogenic Lipids

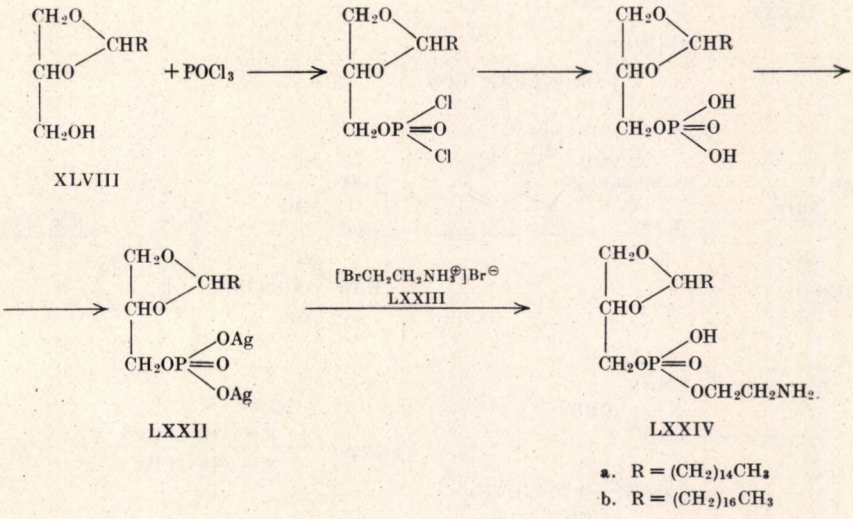
The synthesis of acetalphosphatides (III) does not involve great difficulties. It can be accomplished starting either from α,β-O-alkylidene glycerols (XLVIII) or from the corresponding cyclic acetals of glycerol-halohydrin (LXXI). T. Bersin and his co-workers were the first to study both possibilities.

Bersin *et al.* obtained various α,β-cyclic acetals of glycerol-halohydrins (LXXI) in good yield by reactions between epihalohydrins and aldehydes in the presence of stannic chloride [585–587]. The silver salt of choline phosphoric acid has been reported to interact with chloride (LXXI, Hal = Cl) [588]; nevertheless, attempts failed to give ethanolamine-containing acetal phosphatide (IIIa) by means of inducing neutral silver salts of ethanolamine phosphate to react with the halo compound [587].



LXXI

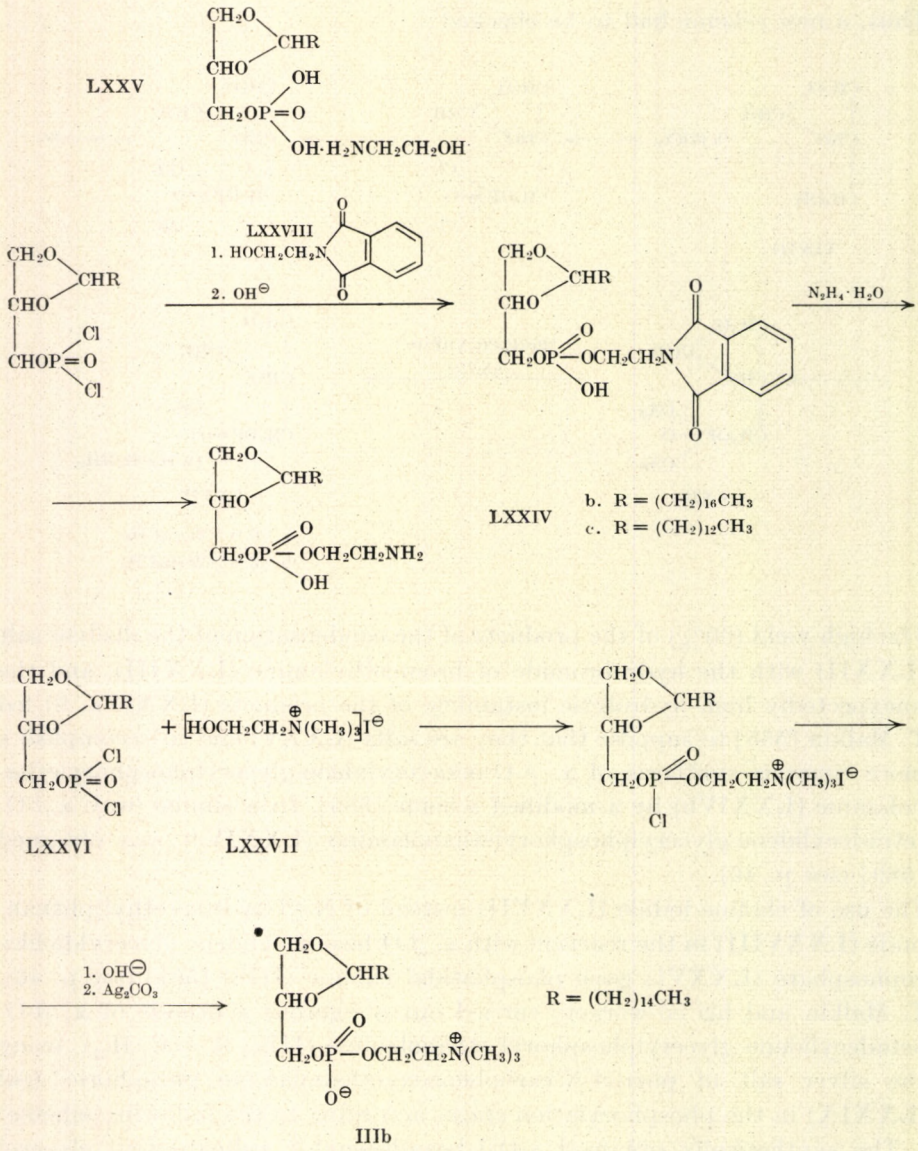
Thus, a new scheme had to be checked:



The high yield (60%) of the products of the condensation of the disilver salt (LXXII) with the hydrobromide of bromoethylamine (LXXIII), and the unexpectedly high hydrolytic instability of the products (LXXIVa, b) led T. Malkin [555] to suppose that they are salts (LXXV) and to accomplish a more rigorous synthesis of α, β-O-octadecylidene-glycerylphosphorylethanolamine (LXXIVb) by a modified scheme [555]. In a similar way α,β-O-tetradecylidene-glycerylphosphorylethanolamine (LXXIVc) was obtained [589] (see p. 46).

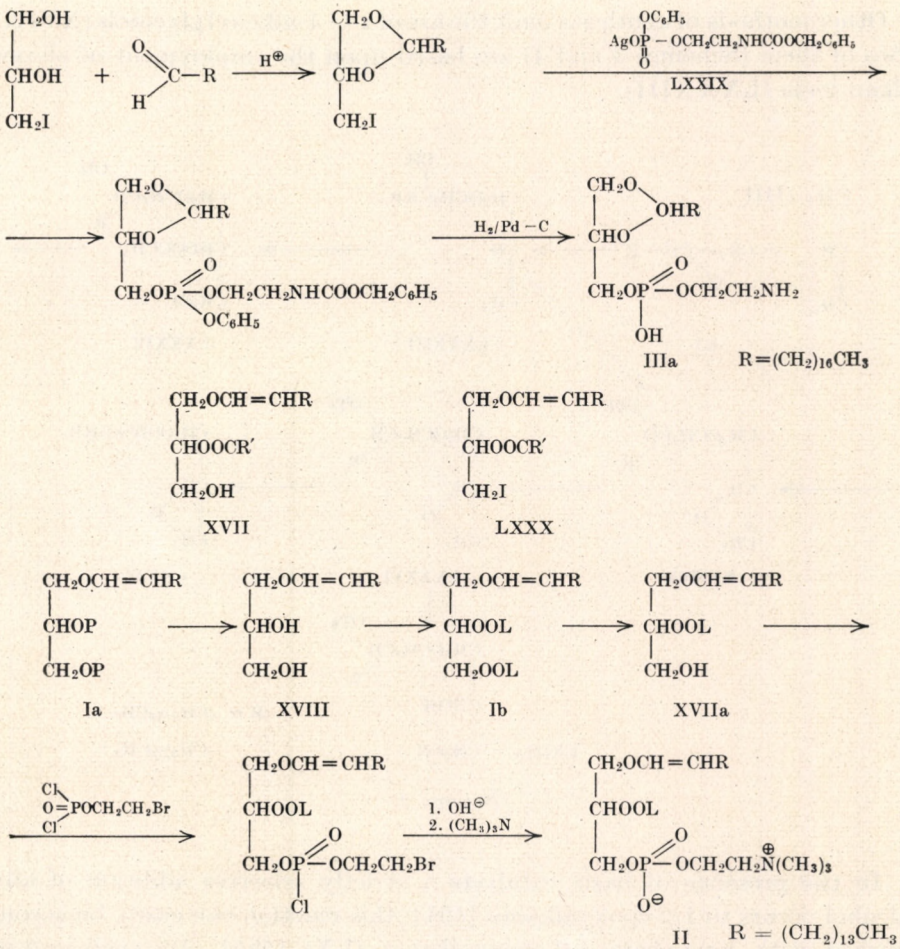
The use of choline iodide (LXXVII) instead of N-(β-hydroxyethyl)phthalimide (LXXVIII) in the reaction with α, β-O-hexadecylidene glyceryldichlorophosphate (LXXVI) gave phosphatidal choline (IIIb) [561] (see p. 46). T. Malkin and his co-workers carried out a rigorous synthesis of α, β-O-octadecylidene glycerylphosphoryl-ethanolamine (IIIa, R $= C_{17}H_{35}$), using the silver salt of phenyl-N-carbobenzoxyethanolamine phosphoric acid (LXXIX) in the phosphorylation stage, according to the following scheme:

The synthetically obtained α-O-1-hexadecenyl-β, α-dipalmitoyl glycerol (Ia, Scheme 3) is saponified by alkali to yield α-O-1-hexadecenyl glycerol (XVIIIa) and subsequently acylated by oleyl chloride. Unlike neutral plasmalogen (Ia) containing saturated fatty acid residues, α-O-1-hexadecenyl-β,α-dioleoyl glycerol (Ib) is easy to deacylate by pancreatic lipase at the α′-position [453]. The resulting α-O-1-hexadecenyl-β-oleoyl glycerol (XVIIa) gives lecithin-plasmalogen (IIa) if reacted successively with the dichloride



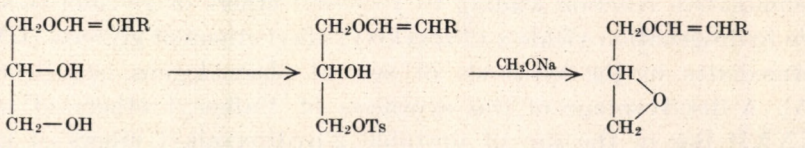
of β-bromoethylphosphate and trimethylamine according to the well-known method [592] (Scheme 3).

Methods for the synthesis of complex plasmalogens using 1-alkenyl ethers of glycidol (LXXXI) are much more practicable. Recently a number of methods for obtaining these compounds have been worked out. According



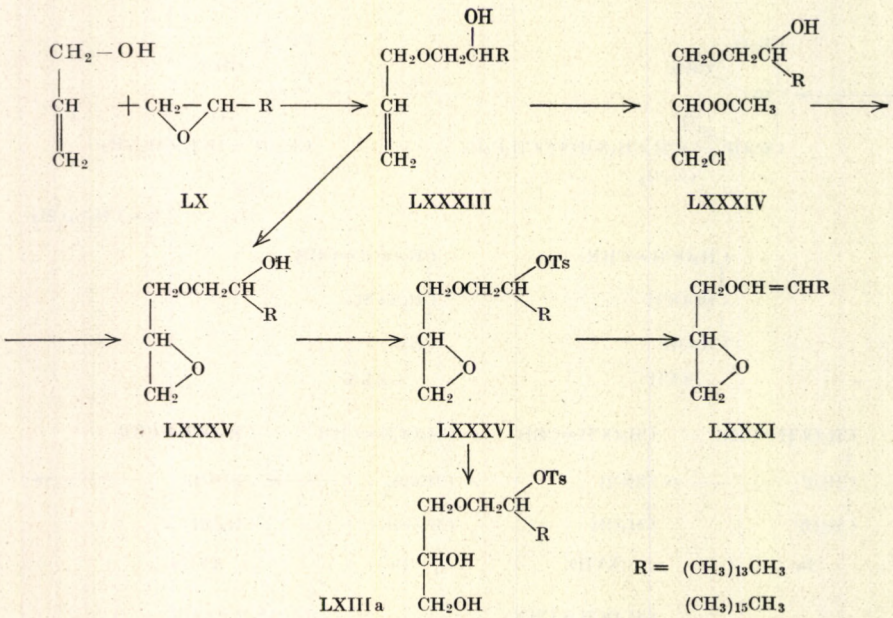
Scheme 3

to the first, α-O-1-alkenyl-α′-O-tosylglycerols (LXXXII) are treated with sodium methoxide, the former compounds being obtained from α-O-1-heptenyl (XVIIIb) and α-O-1-heptadecenyl glycerols (XVIIId) [584]. By this method optically active ethers of glycidol [593] can be produced.



h. R = $(CH_2)_4CH_3$
d. R = $(CH_2)_{14}CH_3$

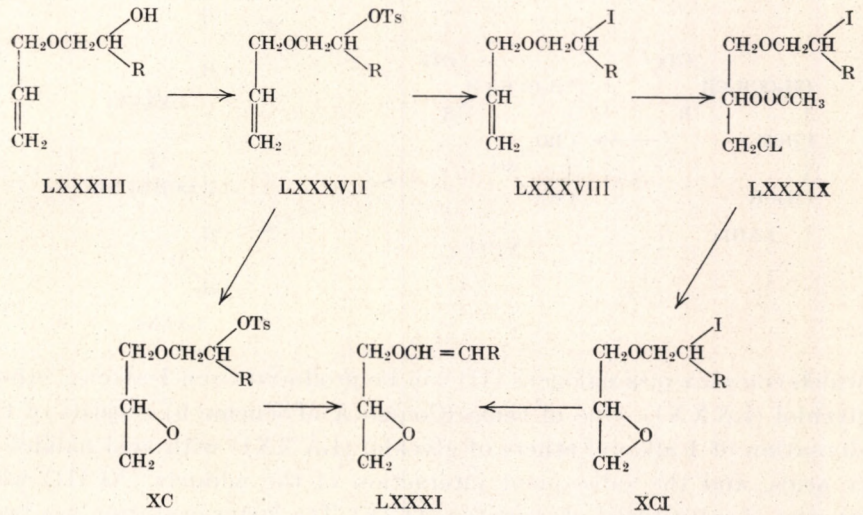
Other methods of synthesis omit the use of α-O-1-alkenyl glycerols (XVIII). Two of them (Schemes 3 and 4) are based upon the employment of alloxyalkan-2-ols (LXXXIII).



Scheme 4

In the presence of basic catalysts a strictly selective addition of allyl alcohol occurs to 1,2-epoxyalkanes [594]; this reaction can easily be accomplished with long-chain 1,2-epoxyalkanes (LX) [595]. Alloxyalkan-2-ols (LXXXIII) can be converted into hydroxyethers of glycidol (LXXXV) either by means of oxidation of the double bond using perbenzoic acid, or through intermediate chloroacetates (LXXXIV). Following the treatment of 2-hydroxyalkyl ethers of glycidol (LXXXV) with *p*-toluene-sulphonyl chloride the resulting 2-tosyloxyalkyl ethers of glycidol (LXXXVI) undergo an elimination reaction leading to 1-alkenyl ethers of glycidol (LXXXI), while hydrogenation yields α-(2-tosyloxy)-alkyl ethers of glycerol (LXIIIa), intermediates in the synthesis of neutral plasmalogens (see Scheme 2) [595]. A disadvantage of this synthesis of 1-alkenyl ethers of glycidol (LXXXI) lies in the use of unstable 2-hydroxyalkyl ethers of glycidol (LXXXV); since these compounds are heat-sensitive, they will isomerize above 30°C, even in neutral media.

Changing the reaction sequence gives a more practicable synthesis scheme [596].

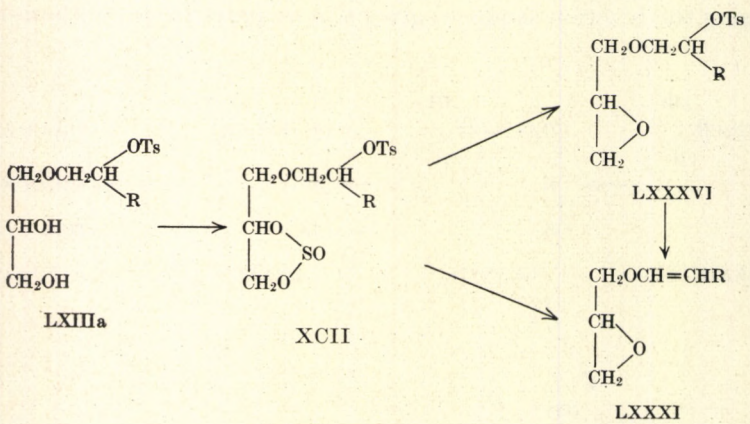


Scheme 5

Within the scope of this scheme, parallel with the reactions of tosylates (LXXXVII → XC → LXXXI), a comparative study of conversion of iodides (LXXXVIII → LXXXIX → XCI → LXXXI) has been carried out. It has been found that the yield of 1-alkenyl ethers of glycidol (LXX) was twice as high when they were produced by dehydroiodation than by dehydrotosylation, the overall yield, however, being lower in the iodide variant.

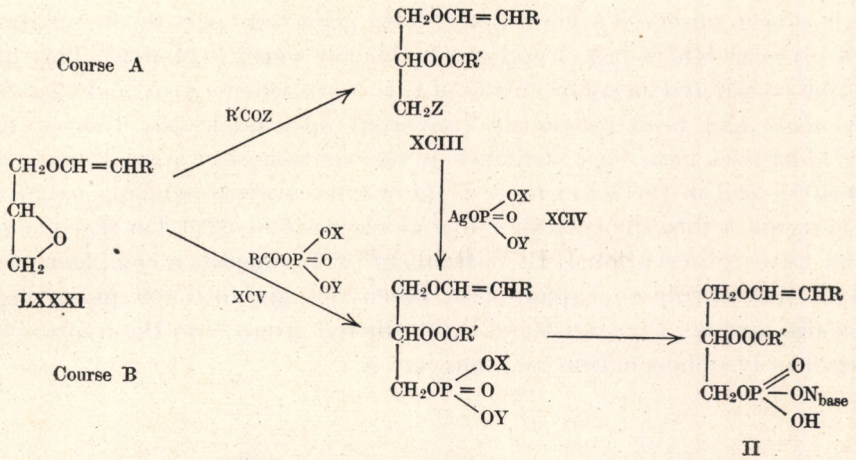
An even more simple synthesis scheme uses α-O-(2-tosyloxy)-alkyl glycerols (LXIIIa) [597] as starting materials. Cyclic sulphites of α-O-(2-tosyloxy)-alkyl glycerols (XCII) are easy to prepare by interaction of glycols (LXIIIa) with thionyl chloride in the presence of pyridine. The closing of the α-oxide ring and the cleavage of *p*-toluenesulphonic acid from the sulphites (XCII) takes place under the action of strong bases and can be carried out in one or two steps (in the second case being accompanied by the formation of intermediate 2-tosyloxyalkyl ethers of glycerol, (LXXXVI)) depending upon the amount of base used.

In view of the reports describing interaction of the water-soluble α-oxides with the phosphate anion [598] a suggestion was made [490] to use this reaction to obtain phosphates of α-O-1-alkenyl glycerols from 1-alkenyl ethers of glycidol (LXXXI). This suggestion has yet not been verified. In the laboratory of N. A. Preobrazhenskii two routes are being investigated

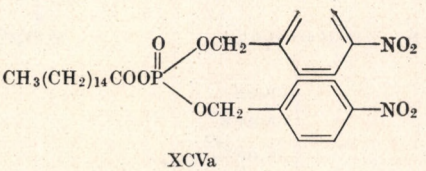


by which complex plasmalogens (II) can be produced from 1-alkenyl ethers of glycidol (LXXXI). One of them (Course A of Scheme 6) consists of the condensation of 1-alkenyl ethers of glycidol (LXXXI) with acid halides of fatty acids, and the subsequent interaction of the adducts (XCIII) with silver salts of substituted phosphates (XCIV). The latter operation has been thoroughly elaborated in the synthesis of phosphatides, among them phosphatides containing polyunsaturated residues of higher fatty acids [599]. Long-chain 1-alkenyl ethers of glycidol (LXXXI) seem to react easily with acid chlorides of carboxylic acids, leading to high yields of α-O-1-alkenyl-β-acylchlorohydrins of glycerol (XCIII, Z = Cl) [600]. Thus, the competing reaction of addition of acid chlorides to the active enol ether bond [601, 602] does not occur. The replacement of the chlorine in the chloro compound (XCIII, Z = Cl) by a more active halogen, e.g. iodine is possible [603, 604]. The use of available [605] acid bromides of higher fatty acids to condense with 1-alkenyl ethers of glycidol (LXXXI) offers an even more practicable route. As bromides are sufficiently reactive and in addition more stable than iodides, they are preferred in the reaction with silver salts of phosphates [606, 607].

When the synthesis is carried out according to Course B of Scheme 6 the residues of both higher fatty acids and substituted phosphate are introduced simultaneously into the glycerol molecule by means of acylphosphates (XCV). Condensation of model α-oxides (1,2-epoxy-octadecane or the 1-hexadecenyl ether of glycidol (with acylphosphate (XCVa)) gives yields of 30–35% in presence of boron trifluoride ester as catalyst [566, 608]. In this reaction palmitoyl-bis-*p*-nitrobenzylphosphate (XCVa) has been used which had been obtained in individual form. The interaction of this acylphosphate with 1-octadecenyl-rethe of glycidol is more complicated [566].



Scheme 6

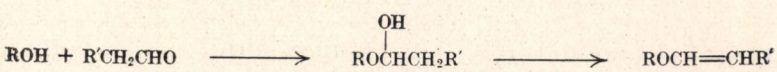


XCVa

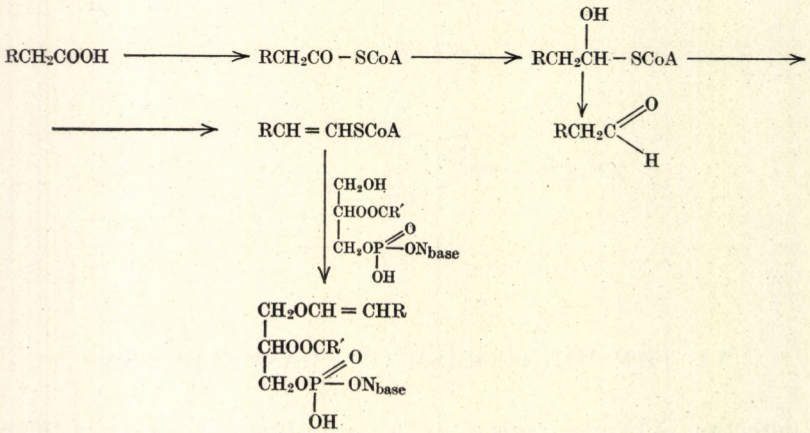
VI. THE METABOLISM OF PLASMALOGENS

The detection of three groups of lipids (according to the type of the ether bond at the α-position of glycerol) in the same natural sources, and their structural similarities, lead to the suggestion that they are closely related in their biogenetic behaviour, too. Nevertheless, experimental data available at present do not allow a definite scheme for the biosynthesis of plasmalogens to be postulated [609–613]. Within this scheme the formation of the 1-alkenyl-ether bond seems to be a most interesting step.

For the time being, the mechanism of formation of this bond has not been discussed, nor has the role of this reaction in the biosynthesis of neutral and complex plasmalogens. J. C. Craig [614] suggests a mechanism by which a direct interaction between aldehyde and glycerol takes place giving rise primarily to hemiacetal formation:

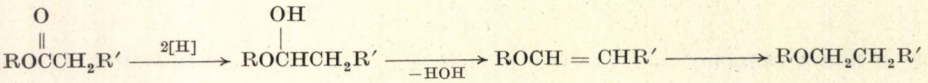


As has been observed several times, fatty acids take part in the building-up of glycerol ethers (alkyl and aldehydogenic ones) [615–619]. This fact could be considered as confirmation of the above scheme provided that free fatty acids had been previously converted into aldehydes. Indeed, free fatty aldehydes have been detected in various tissues of animal organisms [404–405], and in the brain there is an enzyme system reducing palmitate of coenzyme A into the corresponding aldehyde [620–622]. On the strength of the latter observation J. B. Wittenberg [623] suggests a complementary step in the scheme to explain how aldehydes get into the plasmalogen molecules, namely the transfer of the 1-alkenyl group onto the hydroxyl of glycerol in lysophosphatide by coenzyme A.



Nevertheless, attempts to incorporate labelled palmitaldehyde into the vinyl ether section of a plasmalogen molecule by the use of a rat brain homogenisate in the presence of coenzyme A and ATP were unsuccessful [624]. Though other investigators [5, 625] have been able, under different circumstances, to detect radioactivity originating from palmitaldehyde both in alkenyl and acyl chains of phospholipids, they came to the conclusion that direct introduction of aldehydes does not occur.

Another possibility for the formation of the 1-alkenyl ether bond is a biological reduction of the ester bond (again through a hemiacetal phase).



In this case 1-alkenyl ethers precede the formation of alkyl ethers. This conception has been shared by a large number of authors [38, 108, 126, 456, 609, 626–629]. It has been confirmed by various observations, e.g. the increase of the monoenic aldehyde level in plasmalogens of erythrocytes following the introduction of triolein [626]; the close correspondence between the increase of the linoleate level in cell membranes and the increase of the amount of octadienal-containing plasmalogens [626, 630]; and the similarity of fatty acids and aldehydes in lipids obtained from the same source, a similarity of both a qualitative and a quantitative character [92, 294, 628, 631], especially if strict attention is paid to positional characteristics [366, 613].

There exists a third possible mode of formation of the 1-alkenyl ether group under discussion, namely the biological dehydrogenation of alkyl ethers of glycerol [632].

$$ROCH_2CH_2R' \xrightarrow[-2H]{} ROCH = CHR'$$

A number of papers [629–631, 633–636] deal with comparative studies on the metabolism of lipids containing alkyl- and 1-alkenyl ether groups. According to them, biological dehydrogenation of ethers seems to be the most probable way for the formation of the aldehydogenic chain in plasmalogens. There is good reason to suppose that the biosynthesis of aldehydogenic and acyl forms of neutral and complex lipids involves a similar but independent pathway [637]. This conclusion has been confirmed by studies on the introduction of radioactive phosphorylethanolamine into phospholipids [638, 639].

Investigations of the introduction of palmitic acid [631, 633] and long-chain alcohols [619, 631, 640] failed to answer the question of which stage in the series of biological transformations is that in which the vinyl ether bond is formed. There seems to be some reason to suppose that this transformation takes place at the stage of disubstituted glycerol. The chemically inert chimyl and batyl-alcohol, for example, suffer a rapid metabolic change in the intestines [640] and liver [641–643], and hence the enzymatic systems of intestine walls and of liver acting on the ether bond of alkoxyglycerols seem to be different [644].

An enzymatic system detected in microsomae of rat liver [645], and further isolated from them [646], gives rise to oxidative destruction of glycerol ethers in presence of molecular oxygen and NADP. In the first step a hemiacetal is formed which further breaks down giving glycerol and aldehyde. A portion of the aldehyde is reduced to fatty alcohol with the help of NADP-H_2, while the greater part of it is oxidized to give fatty acids [645–646]. There

is no evidence as to the participation of the above-mentioned enzymatic system in the biosynthesis of plasmalogens.

It is worth mentioning that according to M. Hack and F. Helmy [647] a conversion of cardiolipine of cardial muscle into both plasmalogenic and other lipids has been observed, aldehyde not being detectable in cardiolipine itself. The cardiolipine degradation rate increases under the relatively anaerobic conditions of experimental infarctus.

There seems to be no place for lysoplasmalogen (IV) in the scheme of biosynthesis since acyltransferase, which easily acylates α- and β-isolecithins [648], seems to be inactive in the case of lysoplasmalogens or their saturated analogues [337, 649]. On the other hand, the plasmalogenic diglyceride (XVII) can be converted into choline- or ethanolamine-containing plasmalogen (IIb, a) by a fraction isolated from rat liver [457, 650] or by a brain homogenizate [651] in the presence of CDP-choline or CDP-ethanolamine.

As to the biosynthesis of the hydrocarbon section of the aldehyde it is known that incorporation of 1-C^{14}-acetate into the aldehydes takes place at a slower rate than into fatty acids [166]. Monoene and cyclopropane aldehydes may arise from octanoic and decanoic acids [5], while saturated hydrocarbon chains may originate from higher fatty acids [5, 618, 619].

Apparently, mutual transformations can take place between ethanolamine-, choline- and serine-plasmalogens [652]; these belong to the same type as the reactions established in the case of the analogous diacyl phosphatides.

There are enzymes which will act upon the enol ether bond of plasmalogens. The enzymes of microsomatic particles of rat liver will hydrolyze lysoplasmalogen-choline [653] and lysoplasmalogen-ethanolamine [654]. Unlike the enzymatic systems just mentioned, a rat brain enzyme which is maximally active in the presence of magnesium ions acts on plasmalogenic ethanolamine containing a fatty acid residue [346, 655, 656].

VII. THE BIOLOGICAL ROLE OF PLASMALOGENS

There is no doubt that aldehydogenic lipids are necessary for the vital activity of animal organisms. A convincing evidence of this is their widespread occurrence, and the high content in certain tissues. Nevertheless, the relation between the level of a biologically active substance and its significance for the organism should not be oversimplified. There is for example an inverse relation between the stage of evolution of the nervous system and

its lecithin content among different vertebrates and different invertebrates (philogenetic lines) [657–659]. The same inverse relationship can be observed during ontogenesis, too [660, 661]. Similarly, trained rat muscle contains a smaller amount of plasmalogens than does untrained, and myocardium of wild rabbits contains less than that of domestic ones [112]. We know practically nothing about the role of aldehydogenic lipids in the biochemical processes at the molecular level.

Investigations on plasmalogen metabolism of particularly important systems of the organism such as the nervous system have shown that this lipid group displays only a low metabolic activity. [631, 662–669]. In nerve tissue, the biosynthetic activity of plasmalogens can be observed only during the short period of the formation of the myelin membrane [146, 632, 651, 670–673]. Comparative studies [668, 669] on phosphorus metabolism concerning diacyl and plasmalogenic forms of phospholipids in the brain of young and adult rats, as well as both forms of phospholipids in liver, kidneys and heart muscle, suggest that in each case the phosphorus exchange rate is much lower with plasmalogens. The authors of the above mentioned reports, while explaining this difference in the phospholipid metabolism by a certain localization of plasmalogenic forms of phospholipids in discrete microstructures of cells, do not answer the question whether plasmalogens perform identical functions in various tissues or whether their role is determined in each case by the specific features of the tissue itself. Anyway, it would hardly be right to consider the plasmalogens merely as structure-forming substances.

It is known that some vitally-important processes occurring in biological membranes, such as active transport or a number of fermentation reactions [674], may take place only with the aid of lipids. Nevertheless, though it has been suggested [83] that choline-plasmalogens in the gills of the Chinese crab *Eriocheir sinensis* act as ion-transporters in the 'ion-pump' mechanism (plasmalogens showing preference to bind potassium) [675], the role of concrete lipid varieties in the biological membranes has not yet been studied practically.

It may be supposed that the structural divergence of aldehydogenic lipids compared with lipids of the acyl type and with lipids containing a saturated ether bond gives rise to the functional differences too (though only in certain cases). With respect to this, an interesting fact is that though plasmalogenic compounds make up as much as 66% of the sum of the ethanol-containing lipids in thrombocytes [22], plasmalogens, unlike diacyl phospholipids, do not participate directly in the blood coagulation mechanism [197, 676, 677].

Some investigators suggest that the biological activity of plasmalogens is related with the special features of the chemical behaviour of the latter [54, 57], above all with addition reactions of nucleophilic reagents at the active vinyl ether bond. In this case, addition of alcohol leads to formation of the corresponding acetal [546], while addition of water gives rise to release of aldehyde and of lysophosphatide. According to these authors, the decrease of the plasmalogen level in some developing tissues [678, 679] is connected with the participation of plasmalogens in the metabolism of other lipids [680–682], and hence plasmalogens are supposed to be capable of changing into the acetal form [681]. The hypothesis concerning this type of participation of plasmalogens in the biosynthesis of triglycerides, however, has not yet been confirmed [377]. According to E. Titus and his co-workers [683], cholineplasmalogen may serve as an inactive precursor, a transporting form of lysolecithin, a stimulator of cardial muscle. Similarly, plasmalogens seem to represent a latent form of plasmalogenic phosphatide acid, which in turn is a stimulator of muscle contractions [684, 685]. The chemical reactivity of the amino group of aminoethanol-plasmalogens has been noted [686].

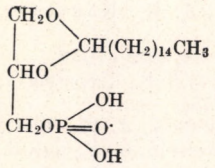
There seems to be strong evidence for assuming that choline plasmalogens should be regarded, similarly to carbohydrates, as substrates for oxidation, thus serving as an energy source of spermatozoids [204, 244, 611, 685, 687]. The assumption that choline plasmalogens might be capable of acting as an energy source for muscle contractions also [112] has not been confirmed by recent experiments [119, 688, 689]. The role of plasmalogens in the mobilization of fats, too, seems to be connected with their participation in the production of energy needed for the transportation of fats through the membrane [690].

J. Kimmig [691] has suggested that cetyl alcohol, the main component of tporous scales, arises from plasmalogens.

Systematic investigations on plasmalogen levels in various organs and tissues concerning characteristics of age as well as of physiological [101, 115, 692–702] and seasonal [86, 112, 178, 703] states of the organism might throw light on the pathways of biosynthesis, and on the function of plasmalogens. It has been stated e.g. that while plasmalogens are not to be found in the eggs and in the first nympha stage of the cockroach *Periplaneta americana*, they appear later and their amount increases during the subsequent metamorphosis stages up to the fully developed state [13]. A similar increase of ethanolamine plasmalogen content has been observed during the development stages of fruit-fly and of the flour-beetle *Tenebrio molitor* [13]. This phenomenon, however, seems not to be common for all insect types. Clini-

cians have long been interested in cases of increase [132, 135, 210, 632, 704–710] and decrease [711–717] of plasmalogen level, bearing in mind the utilization of these phenomena in prognosis and observation of the course of diseases. Some facts, e.g. the presence of neutral plasmalogens and alkyl ethers of diglycerides in reserve fats of cardial muscle and in bone-marrow of adult people, and their absence in the case of new-born infants [46]; the unusually high content of neutral plasmalogens in preputial sebaceous glands of rats and mice and the absence of lipids of the alkyl type [7]; the detection of alkyl ethers of diglycerides in aorta walls and in certain tumours while neutral plasmalogens are not to be found in the same tissues [41]; etc., are of great interest in order to understand the origin of neutral plasmalogens and of their interrelation with alkyl ethers of diglycerides. The increase of plasmalogen level in certain stress states [187, 718] might be considered as a defensive reaction of the organism. It is to be noted e.g. that plasmalogen content increases as a result of X-ray irradiation [187, 719]. On the other hand, a good correspondence in the fatty alcohol and aldehyde composition of erythrocyte lipids [274, 720] has been observed, as has a radiation-protecting effect of batyl- and chimyl alcohol [721–724]. The alkyl ethers of glycerol being metabolically unstable [641, 643, 725], the physiological activity must be related with some form of their metabolites [643] instead of the alkyl ethers of glycerol themselves. Since it is known that alkyl ethers of glycerol are able to stimulate the growth of certain microorganisms and to inhibit the growth of others, to stimulate haemopoesis [726, 727; and references therein] (this may be illustrated by the use of these substances for curing tuberculosis [728, 729] and in some toxicosis cases [730, 731]) it is worth performing comparative studies to examine the effect of 1-alkenyl ethers of glycerol from this point of view.

Though reports are lacking on any clinical use of native aldehydogenic lipids, it is known that fragments of their structures display physiological activity, lysoplasmalogens (IV) [732–734] and the preparate 'Darmstoff' [735] acting as stimulators of non-striated muscles. Considering the method of preparation of this substance (extraction of intestines using hot alkali [736]) as well as its physical and chemical characteristics, it seems indubita-



XCVI

ble that its main component is α, β-O-hexadecylidene-α-phosphate of glycerol (XCVI) [737].

Extensive investigations are making natural and synthetic plasmalogens more and more accessible for comprehensive studies, allowing the possibilities of their practical utilization to be explored.

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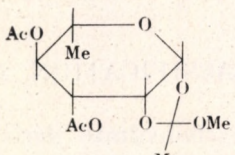
AND

A. F. BOCHKOV

SUGAR ORTHOESTERS AND THEIR SYNTHETIC APPLICATIONS

I. INTRODUCTION

The first representative of sugar orthoesters, 1,2-methyl-orthoacetyl-3,4-di-O-acetyl-β-L-rhamnopyranose (I), was obtained by Emil Fischer and co-workers in 1920 during studies of the condensation of 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide with methanol [1]. The compound, formed along with isomeric methyl glycosides, was obviously quite unexpected and exhibited unusual properties. Thus, of its three constituent acetyl groups only two were susceptible to alkaline hydrolysis. On the other hand, fission of its methyl group occurred under extremely mild acidic conditions, and the compound differed markedly in this respect from the known glycosides. The structure of the compound, named 'γ-rhamnoside', was not established at that time.



I

During the following decade, analogous 'γ-glycosides' of D-mannose [2, 3], maltose [4] and D-lyxose [5] were synthesized. However, it was not until 1930 that the correct orthoester structure of the compounds was proposed simultaneously and independently by Bott, Haworth and Hirst [6] and Freudenberg and Braun [7]. The former group made the proposal on a theoretical basis,* and the latter on the basis of experimental evidence (no absorption in the UV-region characteristic of the carbonyl grouping in the so-called 'γ-methyl-L-rhamnoside monoacetate'). Later on, several tens of sugar orthoesters were described, involving the derivatives of pentoses,

* Curiously enough, less than a year earlier the group of Haworth postulated that the 'γ-glycoside' structure involved a new, hitherto unknown type of stereoisomerism in carbohydrates [8].

hexoses (aldoses and ketoses) and disaccharides (see Table III). It is noteworthy that until 1963–1964 the synthesis of new sugar orthoesters was more or less spasmodic because the workers were usually looking primarily for routes to other compounds and obtained orthoesters as by-products during the synthesis of glycosides according to the Koenids–Knorr method (for reviews, see [9, 10]).*

Only a small number of the papers of this period are specially concerned with the orthoesters (e.g. [12–18]). As a result of this situation, although a great number of publications in this field are known, our knowledge of sugar orthoester synthesis and chemistry is still fragmentary and limited. It was only recently that the synthesis and properties of sugar orthoesters became a subject of more directed studies after it was demonstrated that the compounds are important as agents for stereospecific glycoside synthesis.

The results of the earlier studies on sugar orthoesters are surveyed in a rewiew by Pacsu [19], those of the more modern ones in the monographs by Stanek and co-workers [11, 20] and by Kochetkov and co-workers [21]. The increasing importance of sugar orthoesters in synthetic carbohydrate chemistry, and the publication of a considerable amount of new information, now demands a critical discussion of the earlier papers and some generalization of the results of modern studies. The present review is an attempt at such a discussion and generalization.

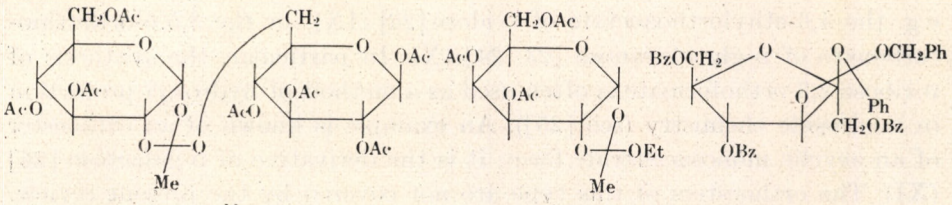
II. STRUCTURE, CLASSIFICATION AND NOMENCLATURE

Sugar orthoesters are carbohydrate derivatives whose hydroxyls are esterified by an orthocarboxylic acid. One, two or three sugar hydroxyls may be involved in the orthoester formation; these may be both hemiacetal and alcoholic hydroxyls. However, in the majority of the known compounds of the class the orthoester is formed by acylation of the hemiacetal hydroxyl of a reducing sugar and one or two alcoholic hydroxyls. Hence, the compounds are cyclic orthoesters. In the first group of this series, which we name bicyclic orthoesters,** the orthoester group is formed by hemiacetal hydroxyl, by one of the neighbouring alcoholic hydroxyls and by an exo-cyclic alkoxy-

* The former attitude to sugar orthoesters as a class not very interesting for synthetic purposes is well characterized by a phrase from the monograph by Stanek and co-workers [11], published in 1963, stating that "Most interesting of all reactions characteristic of orthoesters is the formation of acylated halogenoses from acylated orthoesters . . . ", i.e., reverse reaction of the most important method of orthoester synthesis.

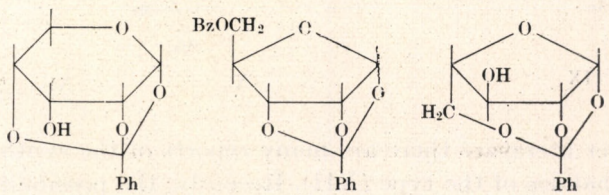
** This name is arbitrary, since the molecule may also involve other rings, as is the case, for example, in orthoesters of oligosaccharides or of alkylidene derivatives.

grouping (the role of the latter may be played, e.g., by another carbohydrate residue as in orthoester II). Examples of orthoesters of this group are compounds I–IV.



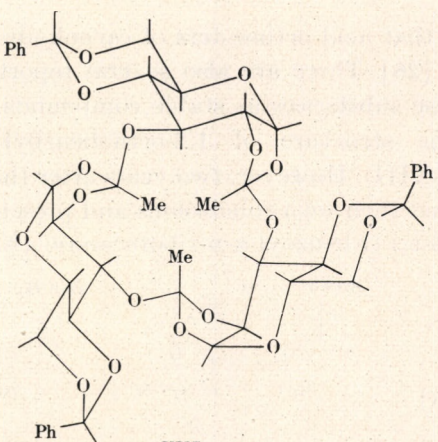
II III IV

The second group, which we arbitrarily name tricyclic orthoesters, contains compounds with orthoester groups formed by acylation of the hemiacetal hydroxyl and two alcoholic hydroxyls of the same carbohydrate residue. Examples are the tricyclic orthobenzoates of D-ribopyranose (V), of D-ribofuranose (VI) and of L-arabinofuranose (VII).



V VI VII

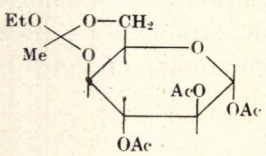
Finally, at present there is a single representative of one more group — an oligomeric macrocyclic D-glucose orthoacetate (VIII).



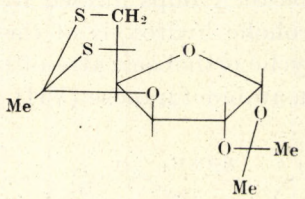
VIII

The known orthoesters of the above groups are usually orthoacetates and orthobenzoates (see Table III).

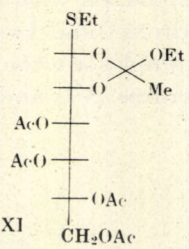
Along with the above types of orthoesters, compounds are known with orthoester groups composed of acylated alcoholic sugar hydroxyls only, e.g. the 4,6-ethylorthoacetate of D-idose [22] (IX), or the 3,5,6-orthothiocarbonate of D-glucofuranose [23, 24] (X). In particular, the synthesis of D-ribose 2,3-orthoformate is often used as a method of hydroxyl protection in nucleoside chemistry (see [25]). An example is known of an orthoester of an acyclic monosaccharide form; it is the derivative of D-galactose [26] (XI). The orthoesters of this type are not covered by the present review. In the literature available we did not find any report on acyclic sugar orthoesters, i.e., compounds with only one sugar hydroxyl esterified by orthoacid.



IX



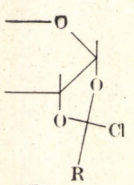
X



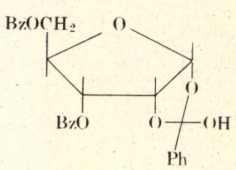
XI

In the earlier literature there are many reports of the synthesis of sugar acyloxonium halides of the type (XII). Recently, the possibility of the formation of these compounds under the conditions described was re-investigated and rejected [27]. It may be expected that if one does obtain compounds of this type, they will be extremely unstable and will tend to have ionic structures.

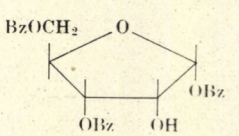
It is postulated that acid orthoesters of carbohydrates are intermediates of acyl migration [28]. There are also several reports concerned with the preparation of these substances as stable compounds. Experimentally best grounded was the structure of 1,2-orthobenzoyl-3,5-di-O-benzoyl-α-D-ribofuranose [29] (XIII). However, two years after the first report the same authors reconsidered their own conclusions and presented evidence that the compound is 1,3,5-tri-O-benzoyl-α-D-ribofuranose (XIV) [30].



XII



XIII

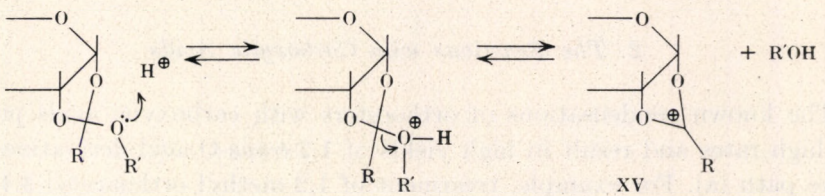


XIV

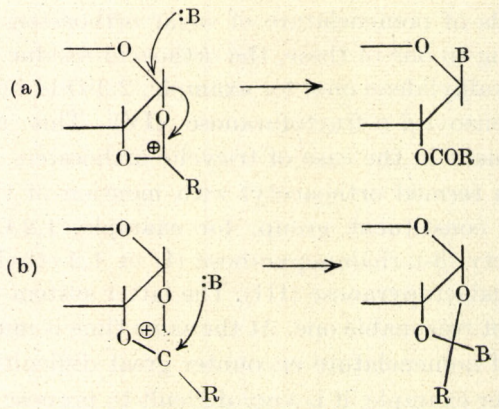
Different methods of nomenclature of sugar orthoesters are used in the literature. According to one of these, the orthoacid residue in orthoesters is termed an α-alkoxyalkylidene one; for example, 2,3-O-(α-benzyloxybenzylidene)-1,4,6-tri-O-benzoyl-β-D-fructofuranose (IV). This nomenclature is obviously inconvenient in the case of tricyclic orthoesters. More often, the orthoester group is termed orthoacetyl with mention of the third (or the fourth) orthoester constituent group, for example, 1,2-O-(methyl-orthoacetyl)-3,4-di-O-acetyl-β-L-rhamnopyranose (I) or 1,2-ethylorthoacetyl-3,4,6-tri-O-acetyl-α-D-galactopyranose (III). The latter system of nomenclature seems to us the most reasonable one. At the same time it must be mentioned that all methods of nomenclature encounter great difficulties with complicated structures. For example, it is very difficult to propose a rational name for the trimeric macrocyclic orthoester (VIII).

III. CHEMICAL PROPERTIES

For the above-mentioned reasons, the properties of sugar orthoesters have been insufficiently studied, and only over a narrow field. The most characteristic property of the compounds is their readiness to take part in numerous acid-catalyzed reactions. The mechanisms of these reactions in the majority of cases have not been studied in detail, but by analogy with the other reactions of nucleophilic substitution at the sugar glycoside centre (see [31]) and with the reactions of similar systems of a non-carbohydrate nature (see [32]) it is commonly accepted that the initial stage is protonation of the alkoxyl oxygen atom followed by dissociation of the resulting oxonium ion into an alcohol and a relatively stable acyloxonium ion (XV):



The existence of these cations has recently been confirmed by their isolation as tetrafluoroborates and hexachloroantimonates [22, 33]. Acyloxonium ions of the type (XV) are typical representatives of ambidentate cations [32] and in accord with this may react with nucleophilic reagents in two major ways:



Reaction (a) occurs by opening of the orthoester ring and by inversion of the configuration at the glycoside centre leading to 1,2-*trans*-carbohydrate derivatives. Reaction (b) is nucleophilic substitution at the electrophilic centre of the cation with retention of the orthoester ring, and leads to other orthoesters or to their analogues.

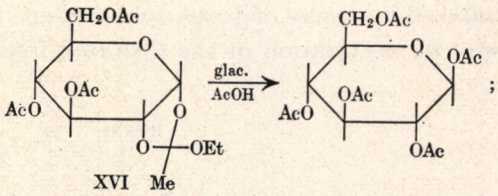
1. Condensation with Alcohols

Of the above reactions the most important is the condensation of sugar orthoesters with alcohols. This condensation when proceeding in accordance with Scheme (b) leads to a new orthoester (re-esterification), but results in 1,2-*trans*-glycosides when proceeding by Scheme (a) (glycosylation). Both the routes, which are the bases of the important synthetic methods, are discussed below (see pages 105, 119).

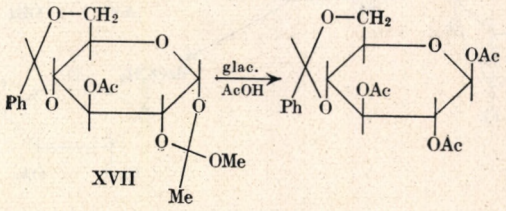
2. The Reactions with Carboxylic Acids

The known condensations of orthoesters with carboxylic acids proceed at high rates and result in high yields of 1,2-*trans*-O-acyl derivatives, i.e., take path (a). For example, treatment of 1,2-methyl orthoacetyl-3,4,6-tri-O-acetyl-α-D-glucopyranose [16] (XVI) or of 1,2-methylorthoacetyl-3-O-acetyl-4,6-O-benzylidene-α-D-glucopyranose [34] (XVII) with glacial acetic acid immediately results in the corresponding β-acetates (see p. 83).

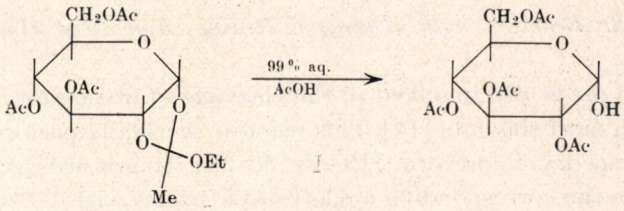
It is interesting that the presence of 1% of water in the acetic acid completely changes the direction of reaction from solvolysis to hydrolysis of the orthoester [16] (hydrolysis is discussed below).



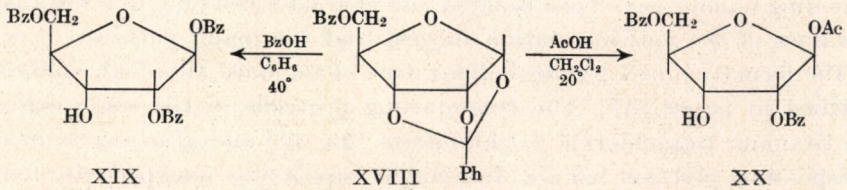
XVI



XVII



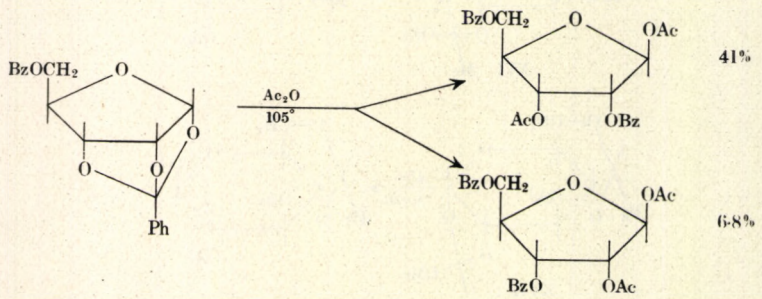
Analogous opening of the ring takes place on treating tricyclic sugar orthoesters with carboxylic acids. Usually, the acyloxy-grouping originating from the ortho-acid residue is situated at the second carbon atom after such treatment. Obviously, the selectivity of the protonation is due to the increased stability of the five-membered cyclic acyloxonium cations. For example, 1,2,3-orthobenzoyl-5-O-benzoyl-α-D-ribofuranose (XVIII) is opened by dry benzoic or acetic acid to form 1,2,5-tri-O-benzoyl-β-D-ribofuranose (XIX) and 1-O-acetyl-2,5-di-O-benzoyl-β-D-ribofuranose (XX), respectively.



XIX XVIII XX

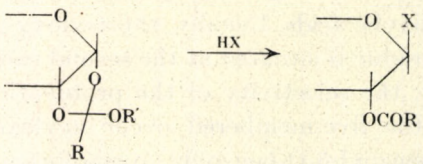
The reaction of this orthoester with acetic anhydride under heating proceeds in an analogous manner, but less selectively [18]. In this case it seems that

the reaction is catalyzed by traces of acetic acid present in the reaction mixture and is followed by acetylation of the liberated hydroxyl:

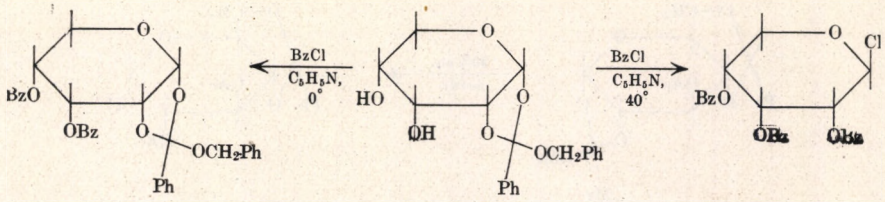


3. The Reaction with Hydrogen Halides and Acid Halides

Mechanism (a) is also involved in the cleavage of orthoesters with hydrogen halides in inert solvents [13]. This reaction is usually performed at room temperature in dry chloroform [13, 18, 35–38]. It proceeds at a high rate and results in the corresponding acylglycosyl halides with 1,2-*trans*-configuration:



In the earlier studies of the thirties-forties this reaction was usually applied to prove the orthoester structure of compounds, and the criterion was the high rate of the exchange evaluated polarimetrically. Although the corresponding halides have been isolated and characterized only in a few cases, the values of the optical rotation suggest that the major path-way was in fact the formation of 1,2-*trans*-halides. One of the most thorough studies is described in paper [37]. The ring-opening proceeds in the same manner with titanium tetrachloride in chloroform [35, 39] and also results in the corresponding glycosyl halides. In a single case it was unexpectedly found that cleavage of the orthoester ring may be performed by benzoyl chloride in pyridine at 40°, whereas at 0° treatment with the same reagents results in simple benzoylation [15].

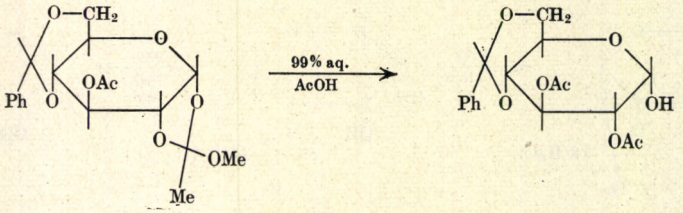


*4. Acidic Hydrolysis**

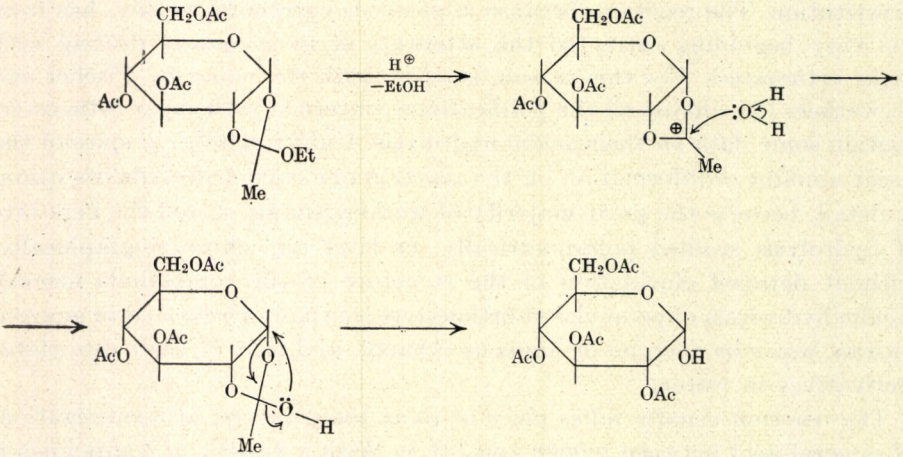
The hydrolysis of orthoesters also belongs to acid-catalyzed nucleophilic substitution. The reaction, because it proceeds extremely readily, has from the very beginning attracted the attention of investigators dealing with sugar orthoesters. For this reason, starting with the paper by Fischer and co-workers [1], almost all the publications concerned with sugar orthoesters contain some data on their acidic hydrolysis. Unfortunately, in spite of the great amount of information on the reaction one cannot describe its course in detail, because the great majority of workers simply stated the high rate of hydrolysis studied polarimetrically, or later on, chromatographically, without detailed elucidation of the structure of the compounds formed. Acidic hydrolysis of peracylated orthoesters is usually performed in aqueous dioxan, aqueous acetone or aqueous ethanol, and that of their deacylated derivatives in water.

The reaction usually takes place at room temperature, at concentrations of mineral acid between 0·0006 and 0·1 N. With a variety of compounds it was demonstrated that the hydrolysis results in an acyloxy-group at C_2 and in the liberation of the sugar hemiacetal hydroxyl, as the hydrolysis products reduce Fehling's reagent and are saponified by alkalies [6, 12, 16, 41, 42]. The same result was found with the orthoacetates of β-D-mannopyranose: NMR-spectroscopic studies and oxidation of the reaction product with lead tetraacetate suggested that the major direction of the reaction was the formation of 2-acetate; chromatographic evidence revealed that at the same time minor amount of free mannose are formed [43]. Treatment of the orthoesters with 99% acetic acid (see also page 82) afforded the derivatives characterized as reductants and having the 1,2-*cis*-configuration [16, 34].

* Cf. [40].



On this basis, the mechanism of hydrolysis has been proposed, postulating that the first stage proceeds according to Scheme (b), and that this is followed by rearrangement of the acid orthoester with retention of configuration at the glycoside centre [16]:



This scheme is in accordance with the majority of the evidence available. At the same time, the structure of the final product may strongly depend on migration of the acyl group and on the mutarotation of the primary reaction product, as well as on the acidic hydrolysis of ester groups. Hence, it is very difficult to make any final conclusion on the detailed mechanism of this reaction. In particular, there is some evidence, although based only on polarimetric studies, that acidic hydrolysis of acetates of β-D-mannopyranose and of β-D-lyxopyranose methylorthoacetates affords free monosaccharides [44]. An analogous result was obtained when 1,2-methylorthoacetyl-3,4,6-tri-O-benzoyl-β-D-mannopyranose was hydrolyzed with acetic acid [45], the major product being 3,4,6-tri-O-benzoyl-D-mannose. On the other hand, hydrolysis of 1,2-ethylorthoacetyl-3,4,6-tri-O-acetyl-α-D-glucopyranose in aqueous dioxan leads to two products: 2,3,4,6-tetra-O-acetyl-D-glucopyranose and 1,3,4,6-tetra-O-acetyl-α-D-glucopyranose [46]. Finally, the studies by Lemieux [47] revealed that hydrolysis of 1,2-alkyl-

orthoacetates of α-D-glucopyranose leads to 1-O-acetyl-D-glucose. Hence, the evidence concerning the hydrolysis of sugar orthoacetates is quite controversial — the different research groups observed the formation of three different types of products under very similar conditions. Thus, it appears that all of the three possible results are realized, i.e. (*i*) formation

Table I

The Conditions for Complete Acidic Hydrolysis of Some Sugar Methylorthoacetates

The parent carbohydrate	Solvent	Temperature °C	Concentration of acid	The time of complete hydrolysis	Ref.
Free orthoesters:					
β-D-mannopyranose*	D_2O	20	1×10^{-2} N HCl	2 min	[43]
β-D-mannopyranose	H_2O	20	1×10^{-2} N HCl	1 h	[6]
α-maltose	H_2O	20	buffer, pH 4·8	20 h**	[48]
β-L-arabinofuranose***	H_2O	20	1×10^{-2} N H_2SO_4	immediately	[49]
β-L-rhamnopyranose	H_2O	20	$6{\cdot}6\times10^{-4}$ N HCl	25 min	[12]
(?) turanose	H_2O	20	2×10^{-3} N HCl	30 min	[41]
	H_2O	20	$3{\cdot}3\times10^{-4}$ N HCl	35 min	[41]
Acetylated orthoesters:					
α-D-glucopyranose+	98% dioxan	20	4×10^{-2} N H_2SO_4	3 min	[46]
β-D-mannopyranose	50% ethanol	98	0·1 N HCl	1·5 h	[44]
β-D-lyxopyranose	50% ethanol	98	1×10^{-2} N HCl	1·5 h	[44]
α-D-ribopyranose	50% ethanol	98	1×10^{-2} N HCl	1·5 h	[50]
α-D-glucopyranose α-D-galactopyranose α-D-galactofuranose β-L-arabinopyranose β-L-arabinofuranose≠	90% Me_2CO	20	1×10^{-2} N H_2SO_4	10–30 min	[51, 52]
β-L-arabinofuranose§	90% Me_2CO	20	1×10^{-2} N H_2SO_4	10–30 min	[53]

* Benzylorthoacetate;
** The extent of hydrolysis is 74%;
*** Methyl orthobenzoate;
+ Ethylorthoacetate;
≠ Methyl- and ethylorthoacetates and orthobenzoates (acetylated or benzoylated respectively);
§ Tricyclic orthobenzoates.

of free hemiacetal hydroxyl and of an acetoxy group at C_2; (*ii*) formation of free C_2-hydroxyl and of an acyloxy group at C_1; and (*iii*) complete removal of the orthoester group with formation of free hydroxyls at both C_1 and C_2.

Up to the present, no systematic study has been published concerned with the rates of sugar orthoester hydrolysis. However, the literature contains many uncoordinated data on the subject. A number of papers report the conditions and the times needed to bring the reactions to completion. These data (surveyed in Table I) make it possible to evaluate approximately the relative rates of the hydrolysis of different orthoesters. In a number of papers, the rate constants of orthoester hydrolyses are reported (Table II).

Table II

The Rate Constants of the Hydrolysis of Some Sugar Methylorthoacetates

The parent carbohydrate	Solvent	Temperature °C	Concentration of acid	K_1 (ln, min^{-1})	Ref.
Free orthoesters					
β-L-rhamnopyranose	H_2O	20	1×10^{-2}N HCl	5·5	[12]
α-maltose	H_2O	23	Buffer, pH 4·8	$5{\cdot}60\times10^{-2}$	[42]
Acetylated orthoesters					
(?)-turanose	95% ethanol	20	$6{\cdot}32\times10^{-2}$N HCl	$1{\cdot}114\times10^{-2}$	[41]
	75% ethanol	20	$9{\cdot}1\times10^{-3}$ N HCl	$8{\cdot}29\times10^{-3}$	[41]
β-D-mannopyranose	95% dioxan	23·5	5×10^{-3} N HCl	$1{\cdot}5\times10^{-2}$	[54]
α-D-glucopyranose	95% dioxan	23·5	5×10^{-3} N HCl	0·29	[54]
methyl ester of α-D-glucopyranoseuronic acid	95% dioxan	20	5×10^{-3} N HCl	$2{\cdot}5\times10^{-2}$	[55]
α-D-glucuronolactone	95% dioxan	20	5×10^{-3} N HCl	$5{\cdot}4\times10^{-2}$	[55]

Comparison of all these data leads to the conclusion that, although the rates of sugar orthoester hydrolysis are always high, the variation of the rates for compounds of different structure is wide. For example, orthoacetates of D-mannose are relatively stable to acidic hydrolysis (see Tables I and II), whereas 1,2-methylorthoacetyl-3,4,6-tri-O-acetyl-β-D-talopyranose is rapidly hydrolyzed even in neutral aqueous and ethanolic media [56], probably due to carbon dioxide of the atmosphere. The effect of the structure of the alkoxyl group on the rate of orthoester hydrolysis has not been investigated. There is only qualitative data that t-butyl- and phenylorthoacetates are

extremely acid-sensitive compared with other orthoesters [57]. Obviously, at present it is difficult to deduce any relationship between the structure of orthoesters and the rates of their acidic hydrolysis.

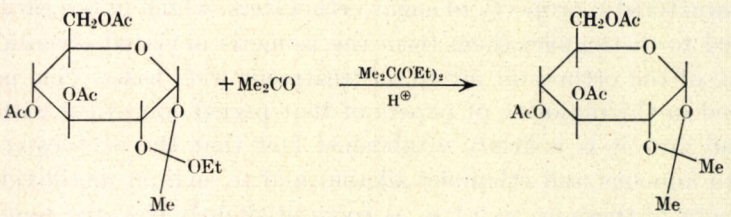
5. Interaction with Alkalies

A characteristic property of sugar orthoesters, which in the earlier studies was used to distinguish them from the isomeric acylated glycosides, is the stability of the orthoester group on treatment with bases. This property is described in the majority of papers of that period concerned with the subject, and now it is a firmly established fact that the orthoester group is stable to aqueous and ethanolic alkalies and to sodium methoxide at concentrations of these up to 0·1 M, at room or slightly elevated temperatures, as well as to methanolic ammonia. Thus, orthoesters survive the treatment usually applied to perform alkaline hydrolysis, alcoholysis or ammonolysis of esters. Orthoesters may be methylated according to Haworth in the presence of concentrated alkali [6, 12, 58] and benzylated in the presence of solid alkali [59] with retention of the orthoester group. The orthoester group also survives acylation with anhydrides and acid halides in pyridine (e.g., [6, 15, 17, 53]). The only exception mentioned above (p. 84) is due to reactivity of the acid chloride, rather than of pyridine. Hence, sugar orthoesters are stable to bases over a wide range of conditions. At the same time it was demonstrated that the orthoester group may be partially cleaved by more prolonged and drastic alkaline treatment: 1,2-methylorthoacetyl-β-L-rhamnopyranose is stable to 0·1 N NaOH at 70° C during 1·5 h, but is cleaved with this reagent to an extent of 64% [12] in 3 h at 80° C; 1,2-methylorthoacetyl-β-D-mannopyranose is stable to 0·75 N NaOH at 15° C during 50 h, but is cleaved to a small extent at 50° C in 2 h [6]; 1,2-methylorthoacetyl-α-D-glycero-D-guloheptopyranose is stable to 0·1 N NaOH in aqueous acetone solution during 2 h, but is cleaved in 6 h [60]. The above-cited three papers are of the earlier period, and obviously there is a need for additional experimental evidence, since the more recent publications contain no information on the matter.*

* Only the review by Pacsu [19] has discussed the possible mechanism of this hydrolysis.

6. Other Reactions

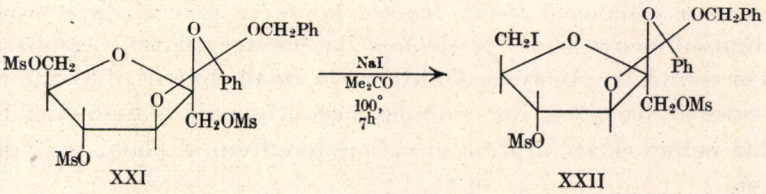
All the other reactions of sugar orthoesters have been studied very briefly. The condensation of 1,2-ethylorthoacetyl-3,4,6-tri-O-acetyl-α-D-glucopyranose with ketones in the presence of catalytic amounts of *p*-toluenesulphonic acid results in substitution of the orthoester group by an alkylidene one [61]:



After more detailed investigation the reaction may appear of preparative importance, providing a new route to alkylidene derivatives of this type which are difficult to obtain by other methods.

A relatively small number of the routine reactions of carbohydrate chemistry have been applied to sugar orthoesters. There are the above-mentioned deacylation, acylation in pyridine, methylation according to Haworth and benzylation, which proceed with retention of the orthoester group. Methylation with sodium amide and methyl iodide in liquid ammonia [16] probably does not affect the orthoester group. At the same time, methylation according to Purdie with methyl iodide in the presence of silver oxide usually proceeds without complication [6, 15, 34], but sometimes results in cleavage of the orthoester group, as e.g., in the case of 1,2-methylorthoacetyl-β-L-rhamnopyranose [8]. In the latter paper it is also claimed that the result of the methylation (cleavage or retention of the orthoester group) depends on the method of silver oxide preparation. Obviously, the cleavage could well be due to the insufficiently effective removal of hydrogen iodide evolved — the latter compound may catalyze the usual acid-induced conversions of the orthoester. Hence, it may be recommended to use for orthoester methylation the conditions that provide guaranteed alkaline media, for example, the procedure of Haworth.

A single case among orthoester reactions is described of the substitution of the primary mesyloxy-group by iodide; the treatment of 1,4,6-tri-O-mesyl-2,3-benzylorthobenzoyl-β-D-fructofuranose (XXI) with sodium iodide in acetone at 100° affords 1,4-di-O-mesyl-2,3-benzylorthobenzoyl-6-iodo-6-deoxy-β-D-fructofuranose (XXII) [14].



XXI XXII

It is noteworthy that in this case the action of a strong nucleophilic agent in the absence of acidic catalyst also leaves the orthoester group intact.

At present, there seem to be no other data on sugar orthoester chemistry. In particular, their reactivity to such important and widely applied reagents in sugar chemistry as periodate and sodium borohydride remain unknown (the only exception is [15]). It may be anticipated, however, that in neutral and weakly alkaline media the orthoester grouping will be stable to the above compounds as well as to other usual reductants and oxidants.

Recently, an extensive study has been started of the reactivity of acyloxonium cations, particularly of cations of a carbohydrate nature (see below, Part VIII). Obviously, sugar orthoesters may serve as convenient starting compounds for the generation of such cations as subjects of either theoretical studies or of synthetic applications.

7. Some Technical Features of the Work with Sugar Orthoesters

As may be seen from the above discussion, sugar orthoesters are extremely acid-labile. For this reason the major technical difficulty is the need to protect these compounds from any acidic action, particularly from atmospheric carbon dioxide. Neglect of this requirement may lead to serious and unexpected failures. The following practical recommendation based on the experience of our laboratory will probably help to avoid some typical mistakes.

Many sugar orthoesters decompose when stored open to the air. Although this is not the case for all the class, it is nevertheless recommended to store the compounds in a desiccator over alkali, preferably in vacuo. Some orthoesters are so acid-labile that even these precautions appear insufficient. In such cases it is reasonable to store them mixed with small amounts of tertiary amines of weak basicity, e.g. pyridine or lutidine. For example, 1,2-methylorthobenzoyl-β-L-arabinofuranose in the absence of bases is immediately cyclized (see p. 106), but is quite stable in the presence of minor amounts of lutidine.

Heating or prolonged (from several hours to several days) storage of neutral unbuffered aqueous or alcoholic orthoester solutions results in their partial or complete solvolysis. Addition of a small amount of pyridine makes such treatment harmless. For example, a small amount of base should always be added before crystallization of orthoesters from alcohols, to avoid their solvolysis.

The solvents which may contain acidic contaminants must be throughly purified. In this respect, the greatest care must be taken with chloroform. In practice, the safest and the most convenient treatment seems to be its distillation over calcium carbonate. We never use conventional 'absolute' chloroform, because as well known its storage after removal of ethanol results in rapid accumulation of acidic impurities.

All our attempts to perform preparative chromatography of sugar orthoesters on silica gel failed.* On the other hand, preparative chromatography of the compounds on neutral alumina never results in any complications.

Acylglycosyl halides are the usual starting material in the synthesis of sugar orthoesters (see below). Traces of these compounds may sometimes be present in prepared orthoesters because they have similar solubilities and chromatographic behavior. Storage of such products results in rapid decomposition of the orthoesters because of the action of hydrogen halide formed from the acylglycosyl halides. For this reason, it is reasonable to make sure of the complete absence of these impurities by purification using, for example, the reaction with silver nitrate in aqueous acetone.

Finally, the following two routine operations must be slightly modified when working with sugar orthoesters. Treatment of the reaction mixture after acylation in pyridine usually involves washing the solution of the reaction product with dilute acid. With sugar orthoesters it is reasonable to substitute for this procedure washing with a solution of bicarbonate for removal of acid, followed by evaporation and removal of excess pyridine as the azeotrope with n-heptane in vacuo.

Deacylation according to Zemplén is usually followed by removal of cations with a cationite. With cationites in the H^+-form, a rapid destruction of orthoesters takes place. Good results are obtained when the pyridinium form of the cationite is used in the presence of an excess of pyridine, or when deacylation is performed with triethylamine in methanol, or, finally, when the reaction mixture after deacylation with methoxide is diluted with chloroform followed by washing with water to remove alkali (the last oper-

* Quite recently, a paper appeared reporting that chromatography of orthoesters on silica gel may be successfully performed in the presence of 2,6-lutidine [61].

ation is applicable, obviously, only to relatively hydrophobic substances, as e.g. 1,2-methylorthoacetyl-4,6-O-benzylidene-α-D-glucopyranose).

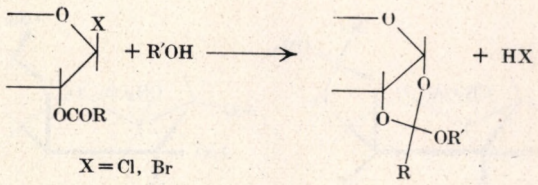
With these simple precautions, the work with sugar orthoesters is not more difficult than with any other class of carbohydrate derivatives.

IV. METHODS OF SUGAR ORTHOESTER SYNTHESIS

The majority of the synthetic routes to orthoesters are based upon the condenzation of acylglycosyl halides* with alcohols. The mechanism and the experimental techniques of this reaction are essentially dependent on the configuration of the glycoside centre relative to that of the neighbouring carbon atom in the starting compounds. For this reason, the methods starting with 1,2-*trans*- and 1,2-*cis*-glycosyl halides are discussed below separately. Along with these conventional routes, a few methods have been elaborated recently on the basis of orthoester interconversions, mainly via re-esterification. No other synthetic routes to orthoesters have been studied as yet.

*1. The Synthesis of Sugar Orthoesters from 1,2-*trans*-Acylglycosyl Halides*

The major direction of the condensation of 1,2-*trans*-acylglycosyl halides with alcohols is the formation of the corresponding orthoesters whose ortho acid residue is formed from the acyl-group adjacent to the glycoside centre.

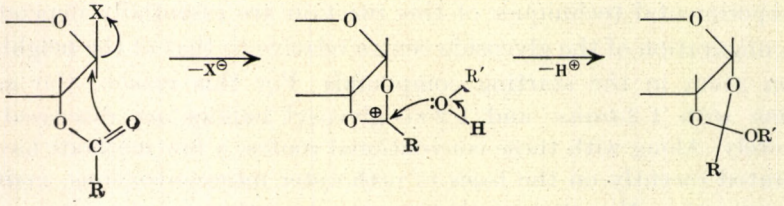


Because of the high sensitivity of orthoesters to acids, the necessary condition of successful synthesis is the rapid and efficient removal of the hydrogen halide formed. For this purpose, hydrogen halide acceptors are added to the reaction mixtures (usually silver oxide or carbonate, or tertiary amines).

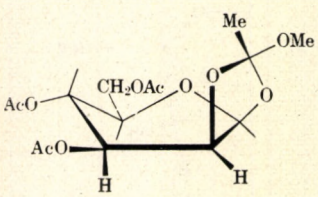
* For acylglycosyl halides see reviews [9, 10, 31] and monographs [11, 20, 21].

(i) Mechanism of the Reaction

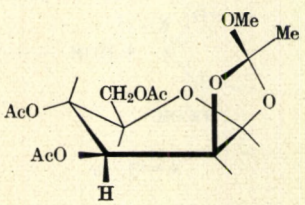
The mechanism of the formation of orthoesters from 1,2-*trans*-acylglycosyl halides was first proposed by Isbell [13, 62]. It was discussed later by Lemieux [31] and remains commonly accepted up to the present. The essence of this mechanism is nucleophilic substitution of the halogen atom at the glycosidic centre with participation of the neighbouring acyloxy-group resulting in a 1,2-*cis*-cyclic acyloxonium ion whose reaction with alcohol gives rise to the orthoester:



Although no strict experimental proof of this mechanism has been ever presented, there is no reason to doubt the general concept. At the same time, according to Perlin (cf. [43, 63]), the ratio of the diastereoisomers at the newly formed asymmetrical centre (C—CH_3-*endo*, XXIII, and *exo*, XXIV) depends on the nature of the hydrogen halide acceptor. For example, in the case of mannose, condensation of the bromide with methanol in the presence of silver carbonate resulted in the ratio XXIII: XXIV = 3 : 2, whereas in the presence of 2,6-lutidine only the isomer XXIII was formed.



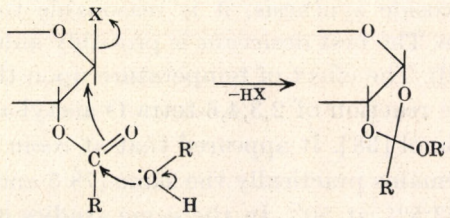
XXIII



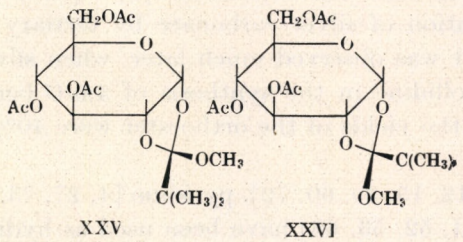
XXIV

Thus, it follows, that the details of the mechanism of orthoester formation may vary with the reaction conditions. For example, along with the above scheme, a synchronous mechanism may also be involved.

It seems improbable that any interconversion of isomers XXIII and XXIV may take place under the conditions of the synthesis of orthoesters from acylglycosyl halides, because all the known cases of re-esterification of



orthoesters take place only in acidic media (see below). Hence, the ratio of the reaction products must be determined by a kinetic factor, i.e. by the relative rates of attack of alcohol at the *endo*- and *exo*-positions of the acyloxonium ion. It is interesting from this viewpoint, that the condensation of the derivatives of glucose* with an acetoxy- or pivaloxy-group at C_2 with methanol in the presence of 2,6-lutidine resulted in both cases in orthoester mixtures with prevailing (about 90%)-OCH_3-*exo*-isomer [57] (XXV and XXVI, respectively). This fact suggests that the reaction product stereochemistry does in fact depend on kinetic factors, since otherwise the equilibrium mixture with orthopivalate would be rich in the $-OCH_3$ *endo*-isomer whose t-butyl grouping occupies the less hindered *exo*-position.



XXV XXVI

(ii) Reaction Conditions

The first, and thereafter widely applied route to orthoesters starting with 1,2-*trans*-acylglycosyl halides was the condensation of the compounds at room temperature in a medium of the corresponding alcohol in the presence of silver oxide or carbonate as hydrogen halide acceptor [1–3, 5, 16, 50, 54–56, 64–68]. The reaction of hydrogen halide with these acceptors produces water which may hydrolyze the starting compounds, and, as in the

* In the studies cited, the starting compound was the 1,2-*cis*-bromide (see below), but the authors believe that the 1,2-*trans*-bromide is formed intermediately in the reaction mixture.

Koenigs–Knorr glycoside synthesis, it is reasonable to add desiccants to the reaction mixture. The best desiccant is probably anhydrous calcium sulphate (see, e.g., [13]). The effect of temperature upon the condensation has been studied for the reaction of 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide with methanol [38]. It appeared that at room temperature and at −15°C the yield remains practically the same (78·5 and 78·1%, respectively), decreasing to 53·5% at 50°. In the same studies it was demonstrated that use of a large excess of alcohol is not necessary to obtain a good yield of orthoester. For example, substitution of the major part of the methanol in the reaction mixture by ether does not considerably decrease the yield. In a number of studies the reaction was performed in inert (aprotic) solvents [36, 37, 43, 49]. Sometimes the reaction conditions were more similar to those of the Koenigs–Knorr glycoside synthesis* (see, e.g., [37]). In the case of D-fructofuranose, a mixture of zinc oxide and anhydrous calcium sulphate in dry benzene was applied to remove hydrogen halide [70, 71]. In other studies, this acceptor of hydrogen halide was not applied. The use of inert solvent and amounts of alcohol close to stoichiometrical requirements permits the application of the reaction to alcohols of complex structure (cf. [37]).

The first paper on orthoester syntheses [1] reported that the yield increases on substitution of silver carbonate by tertiary amine (quinoline). An analogous effect was observed much later, when silver oxide was compared with 2,4,6-collidine in the synthesis of 4,6-O-benzylidene-D-glucose orthoacetate [34]: the yields of the orthoester were 40% and 87%, respectively.

Quinoline [5, 8, 12, 15, 29, 60, 72], pyridine [4, 27, 73, 74], 2,4,6-collidine and 2,6-lutidine [34, 52, 53, 63] have been used as hydrogen halide acceptors. The two latter amines seem to be preferred, because tertiary amines may condense with glycosyl halides to form quaternary glycosylammonium salts (see e.g., [75]); the sterically hindered amines like 2,6-lutidine or 2,4,6-collidine react only with great difficulty. The condensation of 2,3-di-O-acetyl-4,6-O-benzylidene-β-D-glucopyranosyl chloride with methanol affords a 27% yield of orthoester in the presence of quinoline and an 87% yield in the presence of 2,4,6-collidine [34]. As with silver oxide or carbonate, the condenzation in the presence of tertiary amines may be performed in inert

* Since the time of its proposal [69] this method has been subject to considerable modifications (see, e.g., [9, 10, 21]), aimed at increasing the yield and reliability. One of the best modifications is the use of inert solvent as reaction medium with silver carbonate and drierite in the presence of iodine as activator.

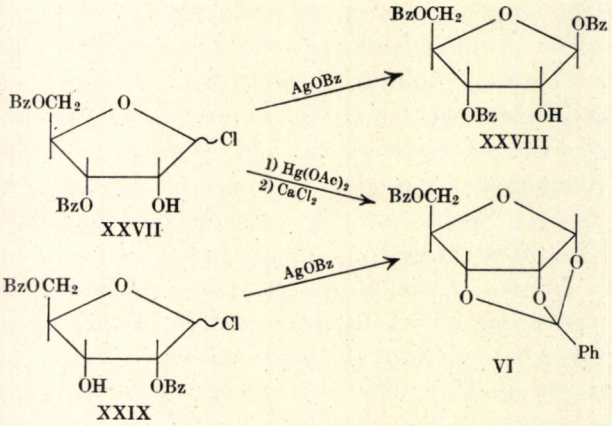
solvents like ether, benzene, chloroform, etc. [29, 63, 72, 74]. It has been found [63] that the optimal conditions for the condensation of 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide with a number of alcohols are room or slightly elevated temperatures, inert solvents and a moderate excess of alcohol in the presence of a 2–3-fold excess of 2,6-lutidine.

When silver compounds are used as hydrogen halide acceptors, the reaction mixture is heterogeneous, and the result depends on such factors (not always easy to control) as the size of particles and character of surface of the reagent, velocity of stirring, etc. Moreover, it was in the presence of these acceptors that the formation of by-products (glycosides, isomeric to the desired ortho esters (see, e.g. [38])) was observed. In a number of cases it was noted that relatively small changes of the conditions during the condensation of 1,2-*trans*-acylglycosyl halides with alcohols may completely alter the results of the reaction. For example, 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl bromide in the presence of silver carbonate in methanol affords the corresponding methylorthobenzoate, but reacts with 1,2,3,4-tetra-O-acetyl-α-D-glucopyranose in methylene chloride in the presence of silver oxide to give a mixture of anomeric disaccharides in which the α-anomer easily predominates [67]. Obviously, the above-mentioned factors are the reason for the difficulties encountered sometimes in attempts to reproduce the syntheses (see, e.g., [63]). Thus syntheses in the presence of 2,6-lutidine or 2,4,6-collidine appear more reliable and convenient, since the reaction mixtures with these acceptors are homogeneous, and no by-product formation has been ever observed.

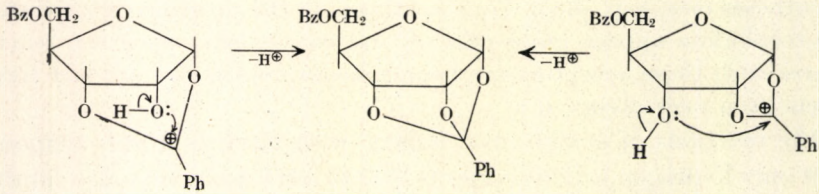
In the reactions of acylglycosyl halides with alcohols silver compounds are not only hydrogen halide acceptors, but to a certain extent also catalysts of the halogen elimination (see [31]). Obviously, for this reason pyridine and silver nitrate have been applied together in studies [68]. This modification has not yet found further applications.

A single example is described of the condensation of a 1,2-*trans*-halide (2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl bromide) with sodium methoxide in toluene [76]; the orthoester was obtained in a very high yield.

Finally, two special cases will be discussed of orthoester synthesis from 1,2-*trans*-acylglycosyl halides. The condensation of 3,5-di-O-benzoyl-D-ribofuranosyl chloride (XXVII, configuration at C_1 unknown) with mercuric acetate in benzene, and subsequent treatment of the reaction product with an aqueous solution of calcium chloride resulted in the tricyclic orthoester (VI). At the same time, condensation of the above chloride with silver benzoate in benzene resulted in the formation of 1,3,5-tri-O-benzoyl-β-D-ribo furanose (XXVIII) [17].



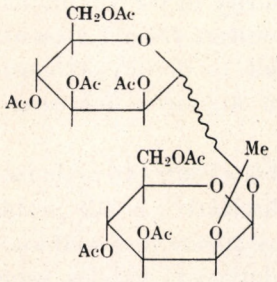
The same tricyclic ester (VI) was obtained by condensation of 2,5-di-O-benzoyl-D-ribofuranosyl chloride (XXIX) with silver benzoate in benzene [18]. It is probable that in both cases the reaction proceeds via a bicyclic acyloxonium ion which is cyclized into the tricyclic orthoester in a way similar to that of re-esterification (see below):



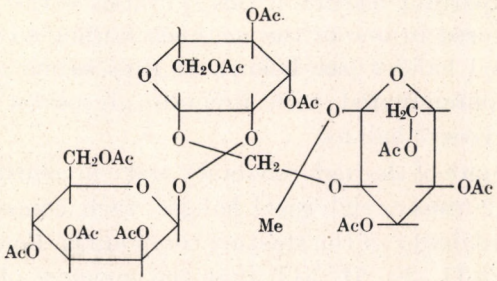
A very interesting feature of these syntheses is the unique participation of the acyloxy-group at C_3 in the nucleophilic substitution at C_1 (if the reaction does not proceed in fact via preliminary migration of the benzoyl residue in compound XXVII from position 3 to position 2).

A much more complicated case of orthoester formation was discovered during the studies of the by-products of the Koenigs–Knorr reaction [77, 78]. Two new orthoesters, one of the dimeric (XXX) the other of the trimeric (XXXI) type, were obtained when 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide was stirred with silver oxide in absolute benzene at room temperature.

The former of these is obviously formed by the usual manner of condenzation of the starting bromide with the major reaction product, 2,3,4,6-tetra-O-acetyl-D-mannopyranose. To explain the formation of the second product

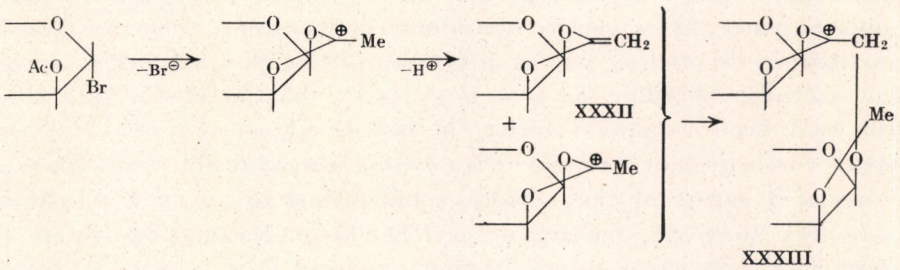


XXX



XXXI

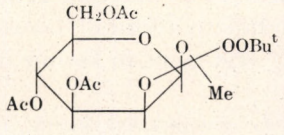
the following scheme was proposed involving the formation of ketene acetal (XXXII) from the intermediate acyloxonium ion and its subsequent dimerization; the subsequent formation of XXXI from cation XXXIII can be readily explained.



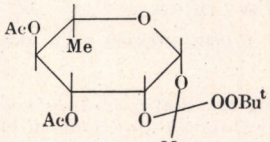
XXXIII

Analogous mechanisms of the dimerization involving an intermediate formation of ketenacetals were proposed earlier for other acyloxonium ions of a non-carbohydrate nature (see [32]).

Finally, one more modification for the synthesis of orthoesters of a somewhat unusual type — the hydroperoxide derivatives — has been proposed by Schulz and Boeden [79, 79a]. The condensation of 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide and of 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide with tert-butyl peroxide in nitromethane in the presence of 2,4,6-collidine afforded the following orthoesters (XXXIV and XXXV):



XXXIV



XXXV

An important feature of this synthesis is the similarity of its conditions to those used in one of the methods leading to orthoesters from 1,2-*cis*-acylglycosyl halides (see below). It thus seems possible to obtain orthoesters not contaminated with isomeric glycosides from anomeric mixtures of acylglycosyl halides.

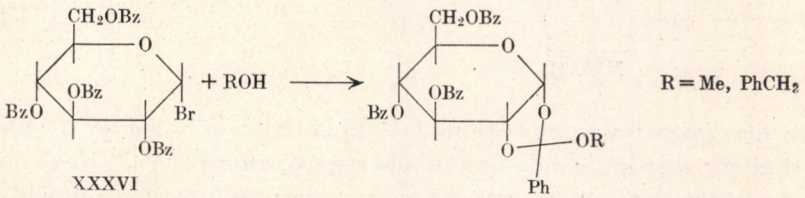
In spite of the high reliability and convenience of the routes to orthoesters via 1,2-*trans*-acylglycosyl halides, their common limitation is the problem of availability of the starting compounds. For many sugars it is well known (see [9–11, 20, 21, 31]) that the anomers of acylglycosyl halides usually have very different stabilities and reactivities; the less stable anomers are referred to as the "acylglycosyl halides of the unstable series". In pyranose forms of sugars, the relative stabilities are determined mainly by the anomeric effect [80]. The synthesis of 1,2-*trans*-acylglycosyl halides of sugars for which these derivatives belong to the 'stable' series (for example, sugars of the manno- and lyxo-configuration) is simple. Quite the contrary is true of 1,2-*trans*-glycosyl halides belonging to the 'unstable' series (e.g., the compounds of gluco-, xylo-, galacto or arabino-configuration); their synthesis is sometimes confronted by serious difficulties. Although a number of convenient methods affording 1,2-*trans*-acylglycosyl halides of the 'unstable' series have been elaborated during the last decade (see [11, 20, 21]), the starting compounds of these syntheses are not always readily available (i.e. per-acylated sugars of the 1,2-*trans*-configuration or acylated alkylthioglycosides). Moreover, the acylglycosyl halides of the unstable series, in accord with their name, usually cannot be stored.* In order to overcome these difficulties, a modification was proposed omitting the isolation stage — it was proposed to use all of the reaction mixture after the synthesis of the halide to obtain the orthoester [73, 81–83].

2. *The Synthesis of Sugar Orthoesters from 1,2-*cis*-Acylglycosyl Halides*

1,2-*cis*-Acylglycosyl halides are the typical starting compounds in the Koenigs–Knorr synthesis and usually afford glycosides on condensation with alcohols. For this reason, it formerly seemed impossible to obtain orthoesters using 1,2-*cis*-acylglycosyl halides as starting compounds. Thus, in 1955 the ability of an acylglycosyl halide to afford orthoesters in reaction with alcohol was considered as a strong argument in favour of its 1,2-*trans*-

* In particular, the compounds are usually decomposed during preparative chromatography, and thus the purification becomes difficult when they are amorphous or do not readily crystallize.

configuration [10]. Later on, it was established that under special conditions the condensation of 1,2-*cis*-acylglycosyl halides with alcohols may lead also to orthoesters. The fact was first discovered in 1956 by Helferich and Weiss [84], who obtained high yields of the corresponding orthoesters by reaction of 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl bromide (XXXVI) with a five-fold excess of methanol or benzyl alcohol in nitromethane in the presence of 2,4,6-collidine:



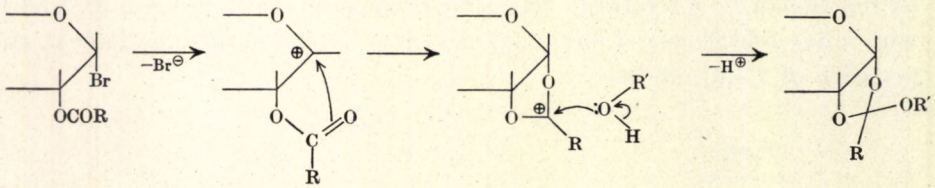
XXXVI

Subsequently, these [79a, 85, 86] and slightly modified [52, 87, 88] conditions were applied to obtain from 1,2-*cis*-acylglycosyl halides a number of orthoesters, including those of pentoses, hexoses and disaccharides. The result of the reaction is to some extent dependent on the ratio of alcohol: halide. For example, studies of the synthesis of β-L-arabinopyranose orthoacetate from the corresponding bromide revealed [52, 88] that the orthoester obtained with a five-fold excess of methanol is contaminated to a considerable extent with isomeric glycoside, whereas the use of a two-fold excess of alcohol removes this difficulty. Lemieux and Morgan [57], after their proposal for the mechanism of orthoester formation (see below), suggested on this basis the addition of soluble ionic halides to the reaction mixture; the best results have been obtained with tetrabutylammonium bromide. As reported in the above paper [57], this modification increases the yield of orthoester to a nearly quantitative one. The formation of orthoester during the condensation of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide with ethanol in acetonitrile in the presence of triethylamine has been also reported [46].

Another method of orthoester synthesis from 1,2-*cis*-acylglycosyl halides is the condensation with alcohols in ethyl acetate in the presence of lead carbonate and drierite under reflux [51, 52]. By this method ethylorthoacetates of glucopyranose and galactopyranose have been obtained.

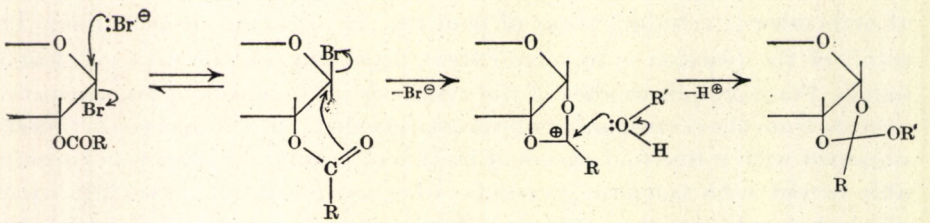
The mechanism of the formation of orthoesters from 1,2-*cis*-acylglycosyl halides has not been studied in detail, and the schemes proposed are rather speculative. For example, to explain the formation of orthoesters under the conditions of the Helferich synthesis, it was proposed that the starting

halide dissociates in the polar solvent to afford the carbonium ion XXXVII and this cyclizes into a more stable acyloxonium ion. The latter reacts with alcohol to afford orthoester:



XXXVII

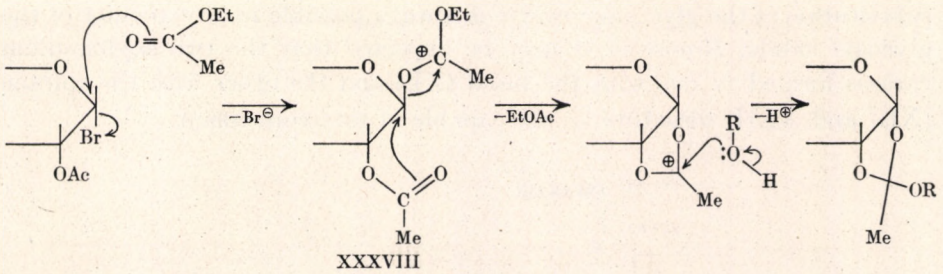
Another, probably more reasonable, explanation is based on the assumption that the starting halide first anomerizes to afford the 1,2-*trans*-anomer, and the latter then reacts with alcohol in the usual manner giving rise to orthoester [57]:



The assumption is confirmed by the fact that addition of tetrabutylammonium bromide — a donor of halogen ion in the anomerization — increases the yield of orthoester. This mechanism seems to contradict the known fact that the 1,2-*cis*-anomer strongly predominates in the equilibrium mixture [89], but the authors overcome the difficulty by making a quite reasonable assumption that 1,2-*trans*-acylglycosyl halide must react with alcohols much faster than does the 1,2-*cis*-anomer.

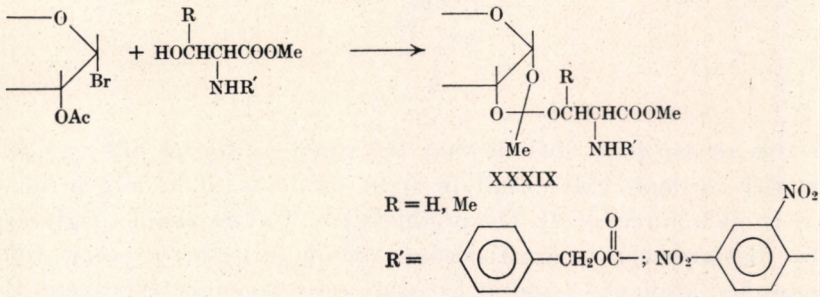
It is assumed by analogy with the participation of neighbouring acyloxy-groups that the synthesis of orthoesters in ethyl acetate involves participation of solvent in substitution at the glycoside centre via intermediate formation of acyloxonium cation XXXVIII and its subsequent transformation to cyclic acyloxonium ion [51, 52].

Finally, a very unexpected and not yet explained fact is the formation of orthoesters (XXXIX) in the attempts to glycosylate the hydroxyl groups of protected serine and threonine with 1,2-*cis*-acylglycosyl halides under



XXXVIII

the conventional conditions of glycoside synthesis by the Koenigs–Knorr reaction, i.e., in nonpolar solvents in the presence of lead or silver carbonates [90–93]:



XXXIX

R = H, Me

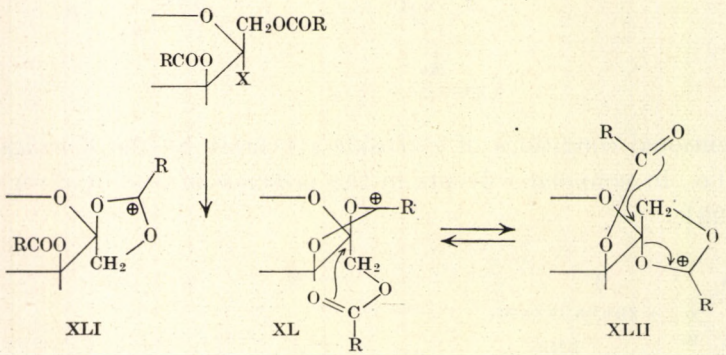
R′=

Development of the methods affording sugar orthoesters from 1,2-*cis*-acylglycosyl halides makes the compounds much more readily available, because it is now possible to take as starting compounds the more available anomers of glycosyl halides for each sugar (either the 1,2-*cis*-, or the 1,2-*trans*-anomer). However, 1,2-*cis*-acylglycosyl halides always tend to form some glycoside in the condensation with alcohols, and this tendency is sometimes difficult to suppress completely (see [51, 52, 88]). For this reason the methods still need further improvement to make them more reliable.

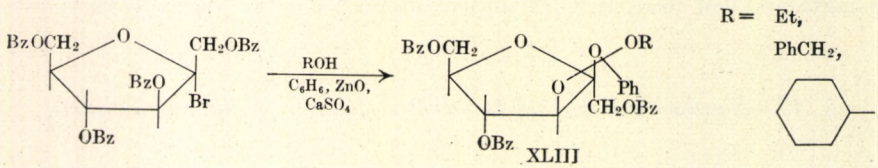
3. The Synthesis of Ketose Orthoesters from Acylglycosyl Halides

In the case of ketoses, the condensation of acylglycosyl halides with alcohols has some peculiar features, making it reasonable to discuss the problem separately. An important fact which was pointed out for the first time by Frush and Isbell [62] (see also [21]), is the presence in acylketosyl halides, unlike in aldoses, of two acyloxy-groups adjacent to the glycoside centre, so that participation of one of these groups in the reactions of nucleophilic

substitution at the glycosidic centre is always possible in any anomer of the glycosyl halide. Moreover, it may be expected that the two acyloxonium cations formed — one with the fused (XL) and the other with the spirane (XLI and XLII) structure — are capable of interconversion:



For this reason it is obvious that the condensation of any acylketosyl halide with alcohols may result in eight products (if hydrogen halide is efficiently enough removed). The products are: (*i*) two anomeric glycosides, (*ii*) two diastereomeric 2,3-orthoesters, (*iii*) four 1,2-orthoesters, differing in the configuration at C_2 and at the orthoester assymmetric centre. Hence it is not surprising that the attempts to perform these reactions always afforded complicated orthoester-containing mixtures [39, 41, 64, 68, 94]. For none of the orthoesters thus obtained has the structure ever been firmly established (it seems that sometimes the authors in fact dealt with mixtures of orthoesters). Exceptions are a number of crystalline β-D-fructofuranose orthobenzoates [14, 70, 71] obtained for the first time by Helferich and co-workers [14, 70] in the following way:

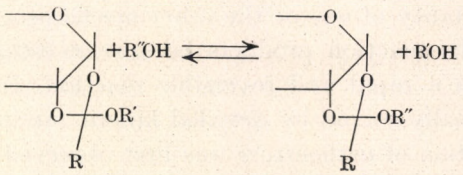


A number of reactions were performed with these orthoesters, and the results were in accord with structure (XLIII). However, in fact the structure remains not finally proved (see below).

Hence, the synthesis, properties and structure of ketose orthoesters form a very complicated field demanding more detailed investigation.

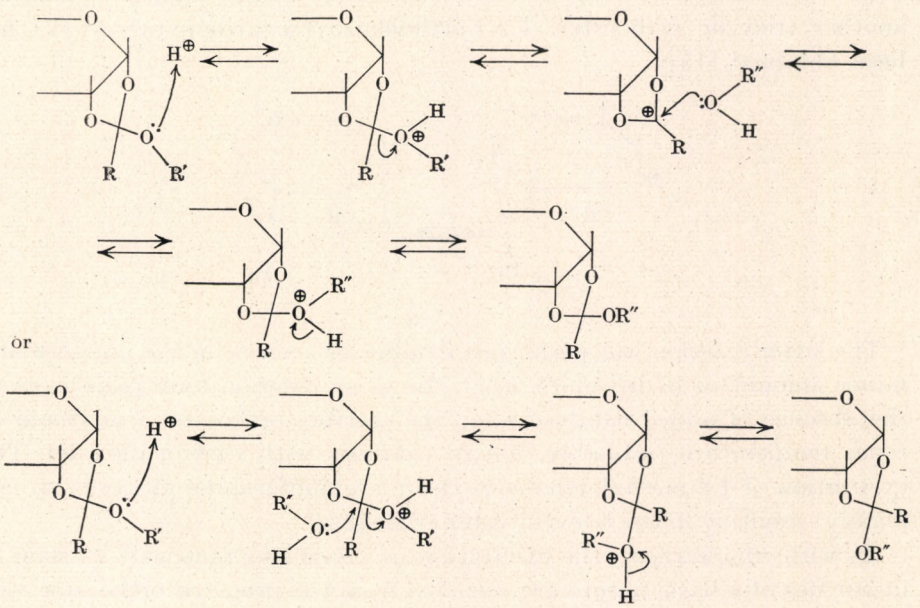
4. The Interconversions of Sugar Orthoesters

With only one exception (see below), all the interconversions of sugar orthoesters known up to date are re-esterifications, i.e. exchange of alcohol radicals in the orthoester group, and proceed according to the following scheme:



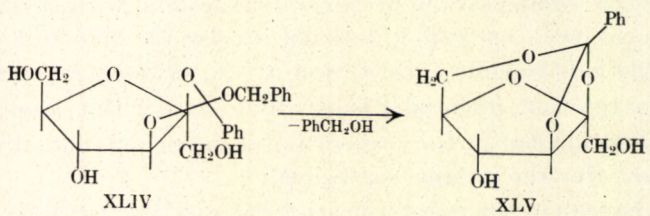
The reaction is the most typical one of the two possible directions of the acid-catalyzed condensation of sugar orthoesters with alcohols. It goes along path (b) (see page 82), it does not involve the glycosidic centre and it is essentially nucleophilic substitution at the orthoester carbon atom. To perform the reaction, orthoester is usually treated with a large excess of the corresponding alcohol in the presence of a minor amount of alcohol in an inert solvent (for the details, see below).

The mechanism of the re-esterification has not been specially studied. It is believed that the reaction may run along one of the alternative pathways presented below, via either mono-, or bimolecular nucleophilic substitution:



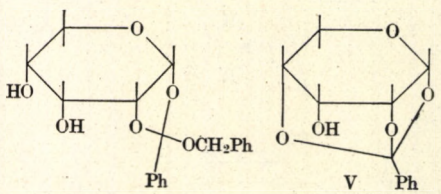
It is also possible that the proton elimination in the last steps of the above paths takes place simultaneously with the nucleophilic attack, but this modification is not very essential. The two alternative mechanisms result in different stereochemistry of the products: in the former case, racemization takes place at the orthoester group carbon atom, whereas in the latter the result is inversion of the configuration. However, one cannot arrive at a decision on the validity of one or the other mechanism on the basis of the configuration of the reaction products because re-esterification under the usual conditions is a rapid and reversible reaction. It is hoped that the problem will be finally solved by detailed kinetic studies.

The re-esterification of orthoesters was first observed in 1953 [70], when 2,3-benzylorthobenzoyl-β-D-fructofuranose (XLIV) was cyclized into 2,3,6-orthobenzoyl-β-D-fructofuranose (XLV):



XLIV XLV

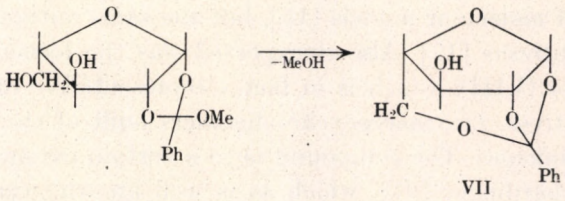
This intramolecular reaction took place when a chloroform solution of XLIV was shaken with aqueous calcium chloride. In an analogous manner, another tricyclic orthoester, 1,2,4-orthobenzoyl-α-D-ribopyranose (V) has been obtained [15]:



V

The latter reaction was performed in aqueous acetone in the presence of a minor amount of hydrochloric acid. The same reaction took place even in the absence of added catalyst when the starting orthoester was stored at room temperature (probably due to catalysis with carbon dioxide). The cyclization of 1,2-methylorthobenzoyl-β-L-arabinofuranose proceeds no less readily, resulting in the tricyclic ester (VII) [53].

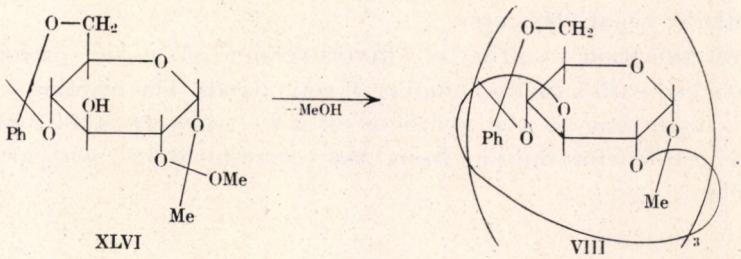
As with ribofuranose, the cyclization proceeds spontaneously as soon as impurities of a basic nature are removed from the prepared orthoester, and



VII

the process is so rapid that the authors failed to characterize the starting orthoester.

Finally, the above-discussed (see p. 97) syntheses of tricyclic ribofuranose orthoesters must obviously also involve re-esterification. A very interesting example of re-esterification which, although intermolecular, also affords a cyclic system, is the re-esterification of 1,2-methylorthoacetyl-4,6-O-benzylidene-α-D-glucopyranose (XLVI) resulting in the macrocyclic trimer (VIII) [95, 96].



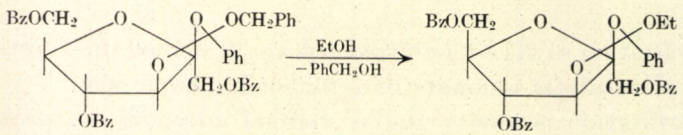
XLVI VIII

The cyclization of XLVI occurred readily in boiling dichlorethane in the presence of catalytic amounts of *p*-toluenesulphonic acid.

Re-esterification is now a unique method affording the tricyclic orthoesters and the related macrocyclic orthoester (VIII). This is the major preparative value of the reaction because orthoesters of this type are applied as starting compounds in the synthesis of polysaccharides (see p. 135). It is interesting that such cyclizations, if they are stereochemically possible, proceed very readily. On the other hand, the mere fact of the existence of orthoesters of this type presents important theoretical problems still waiting investigation. First to be considered must be the conformations of fused cyclic systems in the carbohydrate series, and the stereochemical factors providing the possibility of the formation of the compounds. In particular, at present it is very difficult to predict the possibility or impossibility of obtaining tricyclic orthoesters. For example, in 1955 the possibility of the formation of tricyclic orthobenzoate of ribofuranose (VI) was rejected after

consideration of molecular models [15], but the same authors in two years reported its synthesis [17]. Also unexpected was the formation of macrocyclic orthoester (VIII), which is in fact a representative of the new type of sugar derivatives, i.e., macrocyclic oligomers built of monomers bonded by orthoester linkages. The compound is to a certain extent similar to the dextrines of Schardinger [97], which as is well known, were obtained by microbiological rather than by chemical synthesis. It is noteworthy that the synthesis of (VIII) is strictly directed to the formation of trimer, and no other oligomers have been found in the mixture. The fact points to the role of steric factors as regulators of cyclization. Obviously, it is desirable to study tricyclic orthoesters in accordance with a programme similar to that now accomplished for sugar alkylidene derivatives, i.e., to study the rules of the cyclization, the relative stabilities of the isomers, their conformations, etc. It is believed that the investigation will on the one hand make the compounds more readily available, and on the other will provide a promising new type of trifunctional protection which may open new possibilities in synthetic sugar chemistry.

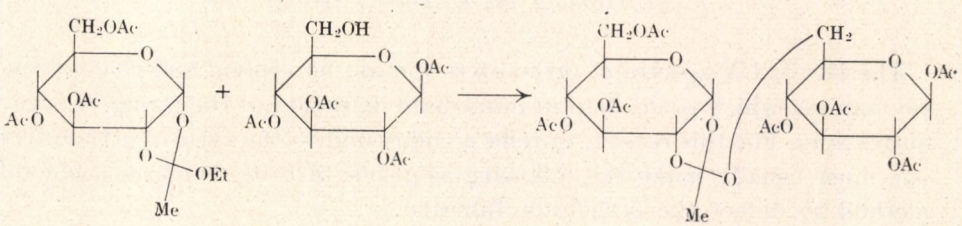
Re-esterification resulting in interconversion of bicyclic orthoesters has been studied with a limited number of compounds. The reaction was applied for the first time to the synthesis of 1,4,6-tri-O-benzoyl-2,3-ethylorthobenzoyl-β-D-fructofuranose from the corresponding benzylorthobenzoate [71]:



The rate of the reaction was high (completion within 20 min. at room temperature) in 0·035 N HCl in a mixture of dioxan with excess of ethanol. Another example of re-esterification has been reported recently. This was a side-reaction during the rearrangement of 3,4,6-tri-O-benzyl-1,2-methylorthoacetyl-β-D-mannopyranose into the isomeric glycoside [59] (see p. 119) in methylene chloride in the presence of *p*-toluenesulphonic acid. Along with the major reaction, the starting orthoester which was practically pure C—CH_3-*endo*-isomer rapidly rearranged into the compound with the *exo*-configuration. Obviously, the reaction involved fission of methanol and its subsequent addition according to S_N1 mechanism (see scheme on p. 105).

Recently, the conditions of the re-esterification were studied in detail for the interaction of acetylated methyl- and ethylorthoacetates of α-D-glucopyranose with cholesterol [52, 98]. Approximately equimolar amounts of orthoester and cholesterol were introduced into an inert solvent. To shift the equilibrium, the lower alcohol was distilled off azeotropically with the solvent. It was found that the re-esterification proceeds readily in a wide variety of solvents (in nitromethane, acetonitrile, ethyl acetate, dichloroethane, etc.) and is catalyzed by many acidic agents (*p*-toluenesulphonic acid, mercury, copper and titanium halides, etc.).

It seems that the optimum conditions of the re-esterification, at least with the relatively complex alcohols, are short boiling of the solution of starting compounds in dichloroethane with a minor amount of *p*-toluenesulphonic acid (0·001–0·01 mole per mole of orthoester). The conditions were successfully applied thereafter to condense 1,2-ethylorthoacetyl-3,4,6-tri-O-acetyl-α-D-glucopyranose with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose, and a high yield was obtained of the re-esterification product, orthoester II [99].

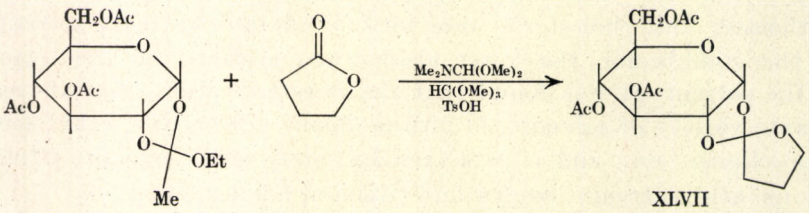


II

It is characteristic, that the orthoester obtained was practically pure C—CH_3-*endo*-isomer, as revealed by its NMR spectrum (see p. 112). In this respect, the re-esterification which is a reversible reaction differs from the other methods affording orthoesters in its considerably higher steric selectivity. In fact, the configuration of the reaction product is controlled by thermodynamic factors resulting in a considerable predominance of one of the two isomers. On the contrary, the synthesis of orthoesters from acylglycosyl halides affords mixtures of isomers because it is controlled by kinetic factors.

Although re-esterification cannot be considered an independent synthetic method, it provides at present the only route to tricyclic orthoesters (see above) and is very convenient to obtain orthoesters with relatively complex alcohol residues (as e.g., in II), which are important for the synthesis of glycosides from orthoesters (see below).

Finally, a single example is described of a new type of orthoester re-esterification, involving complete exchange of the orthoester grouping [61]:



XLVII

If this reaction, reported recently as a short communication, proves general, it will probably find interesting synthetic application.

V. THE METHODS OF THE ELUCIDATION OF STRUCTURE OF SUGAR ORTHOESTERS AND ANALYSIS OF THESE COMPOUNDS

1. Detection of the Orthoester Group

The IR- or UV-spectra of orthoesters contain no general and characteristic maxima which could help in immediate detection of the group in sugar derivatives. For this reason, to refer a compound to the class of orthoesters one must usually make the following sequence of tests which is a general method to detect the orthoester function.

After removal of ester groups, one obtains a compound whose elemental analysis formally corresponds in composition to the monoacyl derivative of a glycoside (for the bicyclic orthoesters) or to that of the monoacyl derivative of a monosaccharide (for the tricyclic ones). In these compounds, the acyl grouping is stable to bases. Mild acidic hydrolysis results in removal of 'aglycone' and the remaining ester group appears susceptible to alkaline hydrolysis or alcoholysis. This sequence has been widely applied in a majority of the studies concerned with orthoesters, from the time of their discovery [1]. Formerly, the studies of the products of these reactions were based only on elemental analysis, and analysis for the ester group. At present, the same investigation may be based on spectral evidence, e.g. on the appearance of a carbonyl band in the IR-spectrum of the orthoester after its mild hydrolysis or alcoholysis. Another method, which was also widely applied in the earlier studies, is based on the demonstration of the rapid solvolysis of the compounds (usually hydrolysis or methanolysis) in the presence of minor amounts of acid. The reactions were usually followed by measuring the

rapid change of the optical rotation. It is easy to understand that for any studies of this kind one must have pure substances.

Recently, a simple and reliable test was proposed for the detection of the orthoester group in sugars in pure substances and in orthoester-containing mixtures [52, 88]. The method is based on the outstanding acidic lability of orthoesters and involves the following procedures. A few milligrams of the substance or mixture studied are hydrolyzed at room temperature by 0·01 N H_2SO_4 in aqueous acetone (10–30 min). The hydrolysate and the starting substance are subjected in parallel to thin-layer chromatography in an appropriate solvent system. A marked decrease of the R_f value serves as an indication that the substance (or a compound of the mixture) is an orthoester. The specificity of the procedure is high enough, because no sugar derivatives other than orthoesters are hydrolyzed so rapidly. It will be mentioned in this connection, that the rates of orthoester hydrolysis are usually 10^{10}–10^{11} times higher compared with those of the isomeric glycosides. Some orthoesters are more stable to acidic hydrolysis; for example, we have found that 1,2-methylorthoacetyl-3,4-di-O-acetyl-β-L-rhamnopyranose and 1,4,6-tri-O-benzoyl-2,3-ethylorthobenzoyl-β-D-fructofuranose are relatively stable. In such cases, the hydrolysis must be prolonged, or the concentration of acid increased (the data on the rates of orthoester hydrolysis are surveyed in Tables I and II).

The above method of analysis, although not very strict, is very convenient in routine practical work because it is general, simple and substance-saving. In particular, it has been applied to express analysis of reaction mixtures [81, 99].

A general method for the detection of the orthoacetate group in sugar orthoesters is NMR-spectroscopy. As demonstrated by the groups of Perlin [43, 63] and Lemieux [57], the C—CH_3-grouping of orthoacetates exhibits in NMR-spectra (taken in $CDCl_3$ or in D_2O) a singlet with chemical shift between 8·2 and 8·5 p.p.m.* The presence of this singlet is a convincing indication, because this region contains no other signals in the spectra of usual sugar orthoesters. The change of the intensity of the singlet has been used to follow the course of a reaction [59]. Unfortunately, the data available have been obtained only with the pyranose form orthoacetates.

To prove the orthoester structure of 1,2-methylorthobenzoyl-β-D-mannopyranose, the following approach has been applied. The UV-spectrum of the compound contains a maximum corresponding to absorption of the benzene ring, whereas the IR-spectrum does not contain the band of car-

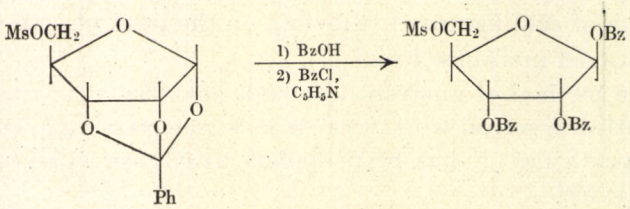
* The τ-scale is used here and below.

bonyl [67]. Obviously, the approach may be applied also to other substances containing no other aromatic substituents.

2. *Elucidation of the Position of the Orthoester Group*

The method of synthesis usually determines the position of the orthoester group in bicyclic orthoesters of aldoses. Nevertheless, the method of methylation [6, 8, 12, 16, 37] has often been applied to confirm the structures. Mentioned above (see p. 90) were some precautions important for successful methylation of orthoesters. Oxidation with lead tetraacetate has been applied for the purpose during the elucidation of the structure of benzylorthobenzoates of mannose [43] and of orthoester XXXI [78].

A much less clear question is the position of the orthoester group in tricyclic orthoesters. In the case of the ribofuranose orthoacetate, the free hydroxyl at C_5 was mesylated, the product subsequently transformed into 1,2,3-tri-O-benzoate and this identified with the compound prepared by an alternative route [17].



The structure of 1,2,5-orthobenzoyl-β-L-arabinofuranose was deduced on the basis of the fact that only one structure is possible for steric reasons [53]; this conclusion has been confirmed by studies of the NMR- and mass-spectra of the orthoester and of its acetate [58].

In the macrocyclic orthoester VIII, the position of hydroxyls involved in the orthoester group is obvious, because a benzylidene group is present at position 4,6. The sequence of the monosaccharide units in the ring follows from the presence of the characteristic singlet in the NMR-spectrum at 8·23 p.p.m. corresponding to the C-CH_3-*endo* grouping of the 1,2-orthoacetate [95].

The proof of the structure of ketose orthoesters must be done most strictly since at least three types of isomeric orthoesters are possible: two anomers of the 1,2-orthoester, and the 2,3-orthoester (the stereoisomerism at the orthoester asymmetric centre is not considered). Unfortunately, no final

proof of the structure of any compound of the series has been ever presented. This conclusion is true also for the series of crystalline fructofuranose orthobenzoates XLIII [14, 70, 71] — the only representatives of the class of ketose orthoesters whose purity is authentic and for the structure of which the most serious data have been presented.*

3. Determination of the Molecular Weight

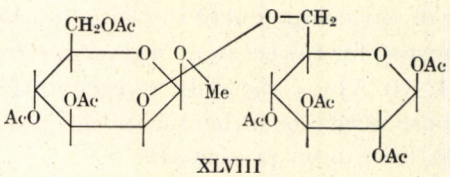
In the earlier studies on the synthesis of tricyclic orthoesters their monomeric character was not doubted. However, discovery of the trimeric macrocyclic orthoester VIII demonstrated the need to determine the molecular weight in all cases of the synthesis of tricyclic orthoesters. The molecular weight of orthoesters VIII and VII has been determined by isothermal distillation [53, 95]. The molecular weight of arabonofuranose orthobenzoate VII has been additionally confirmed by detection of the molecular ion in the mass-spectra of the orthoester and its acetate [58].

4. Elucidation of the Configuration of the Orthoester Asymmetric Centre

The configuration of the orthoester centre in the tricyclic sugar orthoesters is clear from stereochemical considerations. With the bicyclic orthoesters, this configuration must be specially investigated. The separation of the corresponding diastereoisomers has been achieved in a few cases, mostly in the recent studies, and the method of elucidation of the configuration was elaborated only in 1963. For this reason the stereochemistry of the orthoester centre was not discussed (with one exception [37]) in the earlier studies, and we have no information on the configuration of the orthoester centre or the isomer ratio for the majority of the bicyclic sugar orthoesters described.

The first successful separation of orthoester diastereoisomers was made in 1943 with the orthoester (XLVIII) [37], but the configurations of the two isomers obtained was not determined.

* The fact of the formation of a tricyclic orthoester from one of these is not a final proof, since isomerization of the intermediate acyloxonium ion may occur during the re-esterification (see p. 104). In particular it is strange, considering the structure proposed, that the substitution of the mesyloxy-group in the trimesyl ester of this orthobenzoate proceeds only at C_6 and does not touch the mesyloxy-grouping at C_1 (see p. 90).



XLVIII

20 years later, Perlin [43] described the separation of the diastereoisomeric pairs of methyl- and benzylorthoacetates of β-D-mannopyranose. In the same paper it was demonstrated that the C—CH_3- and the alkoxyl groups in the NMR-spectra of diastereoisomeric orthoesters exhibit different chemical shifts. The signals of these groups situated at the *endo*-position of the bicyclic system are shifted 0·25–0·30 p.p.m. to weaker field compared with those of the *exo*-isomers. These investigations have been continued by Canadian and German workers [57, 63, 79a] and generalized for a large number of orthoacetates of mannose, glucose, rhamnose, xylose and lyxose. As a result, the first conclusions of Perlin were confirmed for a considerable number of compounds. It appeared that the most characteristic singlet of the C—CH_3-group in the *endo*-position of sugar orthoacetates exhibits the chemical shift between 8·20 and 8·30 p.p.m., whereas that of the group in the *exo*-position is situated at ca. 8·50 p.p.m. Similarly, the O—CH_3-*endo*-group produces a singlet with chemical shift 6·52–6·55 p.p.m., whereas that of the O—CH_3-*exo*-group is situated at 6·67–6·75 p.p.m. An analogous relationship has been found between the signals of the CH_2-group of benzylorthoacetates [43, 63].

The discovery of the rules discussed above made possible the unequivocal elucidation of the configurations of a number of other sugar orthoesters: of the macrocyclic orthoester (VIII) [95] of orthopyruvate acetal (XXXI) [78], of the orthoacetate II [99] and of a number of mannose orthoacetates [59]. In all cases the ortho-acid radical was found in the *endo*-position.

5. Conformation of Sugar Orthoesters

Before the introduction of NMR-spectroscopy to carbohydrate chemistry, all considerations concerning sugar orthoacetate conformation were speculative. The above-cited studies [43, 57, 63, 79a] revealed that the spin–spin coupling constants $J_{1,2}$ are equal to 2·7–3·0 c.p.s. for the orthoacetates of the pyranose forms of mannose and rhamnose, so that in accordance with the Karplus [100] equation the dihedral angle H—C_1—C_2—H is about 50°. On the other hand, the derivatives of arabinose, lyxose, xylose, glucose and

galactose have coupling constants about 4·5–5·6 c.p.s., corresponding to a dihedral angle of about 35°. Hence, the compounds of the former group have conformation close to C1, whereas in those of the latter this is strongly distorted and more resembles the half-chair conformation. The macrocyclic orthoester VIII has the coupling constant $J_{1,2} = 5{\cdot}5$ c.p.s. [95]. The conformations of a number of acetylated glucopyranose C—H_3-*endo*-orthoacetates, including methyl-, ethyl-, isopropyl-, t-butyl- and phenylorthoacetates, appeared very similar, as suggested by the spin–spin coupling constants [57]. The following dihedral angles have been found: $C_1 - C_2$ 35°; $C_2 - C_3$ 120°; $C_3 - C_4$ 120°; $C_4 - C_5$ 180°. Values close to these have also been obtained for orthoesters XLVII [61]. However, methylorthopyvalate XXV with O—CH_3-*exo*-configuration, unlike orthoacetates has all dihedral angles strongly distorted except the $C_1 - C_2$ one [57]. This fact was regarded by the authors of this paper as convincing evidence in favour of the correct assignment of the orthoester assymmetric centre configuration for the group of compounds studied.

There are no data in the published literature concerning the conformation of furanose from orthoesters or that of the tricyclic orthoesters.

6. Analysis of Sugar Orthoesters

Considered above were the methods of orthoester group detection (p. 110). For the quantitative determination of the content of mannose orthoesters, the specific rotation has been measured of the mixture after treatment with a saturated solution of hydrogen chloride in dry chloroform [38]. The method is based on the ready cleavage of orthoesters by hydrogen halides (see p. 84).

VI. THE SYNTHESIS OF GLYCOSIDES FROM SUGAR ORTHOESTERS

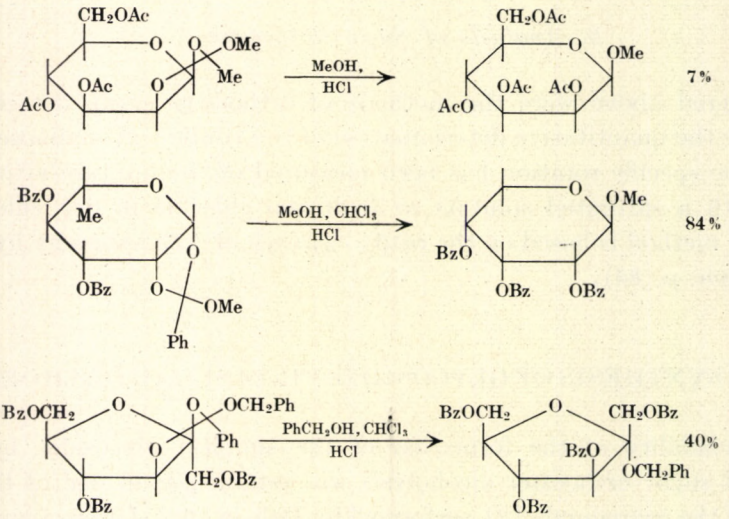
The possibility of the formation of the simplest glycosides by acid-catalyzed sugar orthoester alcoholysis was established in one of the first studies of the compounds [2] performed by Dale in 1924. Later a number of refinements appeared, but the preparative methods based on the reaction have been elaborated only recently, mainly by the authors of the present review and by their collaborators. The syntheses of glycosides from sugar orthoesters fall into two groups which differ considerably in both the results and the conditions. These are (*i*) alcoholysis of orthoesters with a high

excess of alcohol and (*ii*) condensation of orthoesters with stoichiometrical amounts of alcohols in inert solvents. The groups are considered below separately.

1. Alcoholysis of Sugar Orthoesters

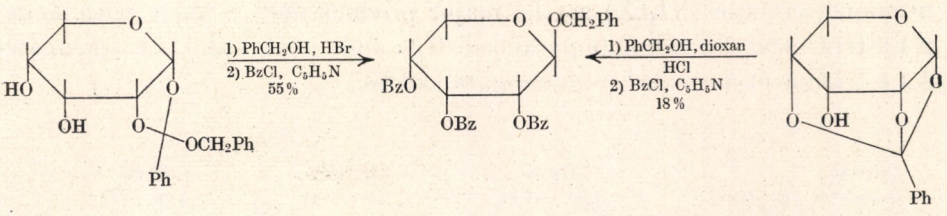
A number of papers have reported that the alcoholysis of sugar orthoesters in excess of alcohol in the presence of minor amounts of acid may result in the formation of glycosides and in recovery of the acyloxy-grouping [2, 14, 15, 18, 72]. The alcoholysis is performed at room temperature in the medium of the alcohol or in its mixture with an inert solvent; minor amounts of hydrogen chloride [2, 14, 72], hydrogen bromide [15], zinc bromide [14] or boron trifluoride etherate [18] are used as catalyst. The same result is obtained when the orthoester is heated to 140°C in benzyl alcohol [18] (obviously, catalysis with acid contaminants or with atmospheric carbon dioxide is the case).

The alcoholysis of acylated bicyclic orthoesters of mannose [2], rhamnose [72] and fructose [14] afforded the per-acylated glycosides:

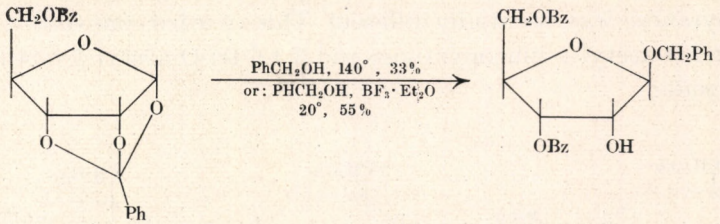


Probably, the alcoholysis of 1,2-benzylorthobenzoyl-α-D-ribopyranose [15] proceeds in the same manner, although the data published are not enough to make a definite conclusion (the authors subjected the product to per-benzoylation prior to identification and the formation of the acyloxy-

group at C_2 was not proved). The same uncertainty was the case with the alcoholysis of tricyclic 1,2,4-orthobenzoyl-α-D-ribopyranose [15]:



A glycoside with a hydroxyl group at C_2 and benzoyloxygroups at C_3 and C_5 has been obtained by alcoholysis of 1,2,3-orthobenzoyl-5-O-benzoyl-α-D-ribofuranose [18], probably due to migration:

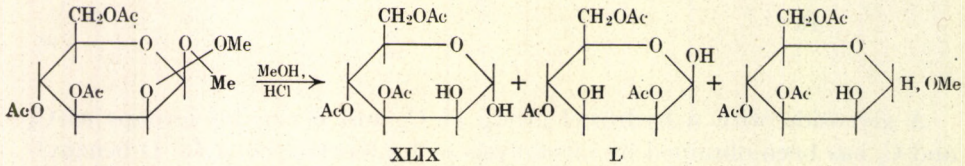


It is amazing in the above-cited studies that the yields of the glycosides vary considerably (from 7 to 84%, see Schemes), and that the stereochemical results are also different: the retention of configuration at the glycoside centre with mannose, and inversion with all the other orthoesters.

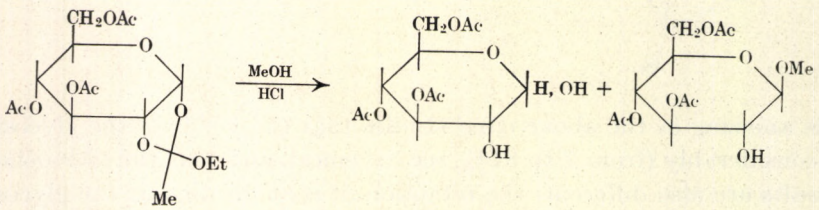
Remarkably different results have been obtained by another group of workers who used very similar conditions of alcoholysis. Treatment of 1,2-metyhlorthoacetyl-3,4-di-O-acetyl-β-D-lyxopyranose with 0·1% HCl in methanol at room temperature resulted in practically complete deacetylation [5]. Methanolysis of 1,2-methylorthoacetyl-3,4-di-O-acetyl-β-L-rhamnopyranose, unlike that of the above benzoylated analogue, results in only a small yield of methyl rhamnoside, whereas the major product of the reaction seems to be 3,4-di-O-acetyl-L-rhamnose [101]. A similar result, i.e. complete removal of orthoester group by alcoholysis, has been obtained by Perlin and coworkers [77, 78] during the mild methanolysis of the orthoesters XXX and XXXI: the major product of reaction in both the cases was 3,4,6-tri-O-acetyl-D-mannose.

The most detailed studies of sugar orthoester alcoholysis were performed by Perlin [102]. First of all, the author failed to reproduce the above-men-

tioned result of Dale [2], who obtained β-methyl-D-mannoside by methanolysis of the mannose derivative. The methanolysis of 1,2-methylortho-acetyl-3,4,6-tri-O-acetyl-β-D-mannopyranose afforded 3,4,6-tri-O-acetyl-α-D-mannopyranose (XLIX) as the major product (80%), along with some 2,4,6-tri-O-acetyl-β-D-mannopyranose (L) and a mixture of anomeric 3,4,6-tri-O-acetyl-methyl-D-mannopyranosides:



The result of methanolysis of 1,2-ethylorthoacetyl-3,4,6-tri-O-acetyl-α-D-glucopyranose was essentially different. This afforded comparable amounts of 3,4,6-tri-O-acetyl-D-glucopyranose and 3,4,6-tri-O-acetyl-β-methyl-D-glucopyranoside:

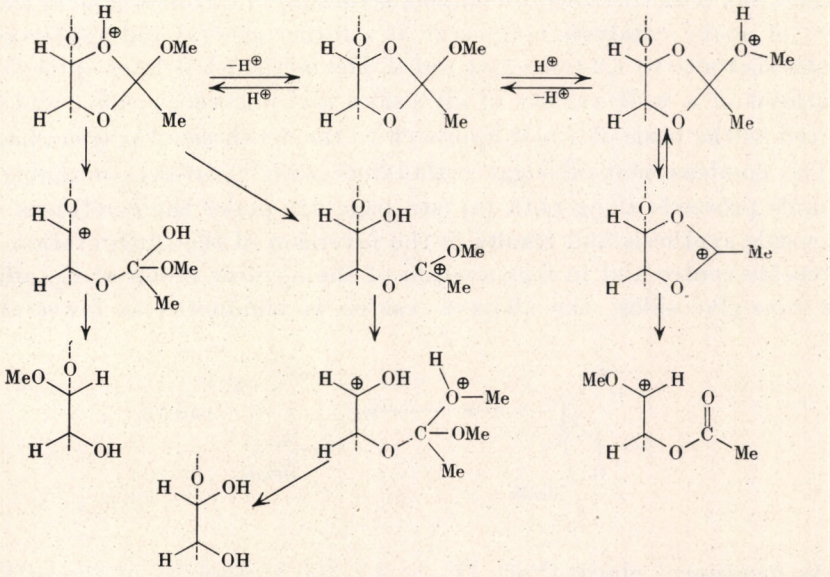


The rate constants of the methanolysis of the above orthoesters of glucose and mannose in 0·00125 M methanolic HCl at 25°C, measured polarimetrically, were 0·056 min^{-1} and 0·10 min^{-1}, respectively.

To explain all the results, the author proposed a mechanism (see p. 119). The formation of (L) is explained by migration from C_3 to C_2 of the acetyl group in (XLIX) with simultaneous anomerization.

It should be noted that the paper [102] contains no mention of the quality of the methanol applied. Hence, it may be guessed that some of the products obtained could be due to hydrolysis of orthoesters by water present in the solvent.

The above data suggest that alcoholysis of sugar orthoesters cannot be considered a convenient preparative method, because different authors sometimes obtain contradictory results, and because the glycoside formation is not stereospecific and is accompanied by a number of side reactions.



Finally, the use of a large excess is possible only with the simplest alcohols and makes it practically impossible to glycosylate the less readily available compounds.

2. *The Orthoester Method of Glycoside Synthesis*

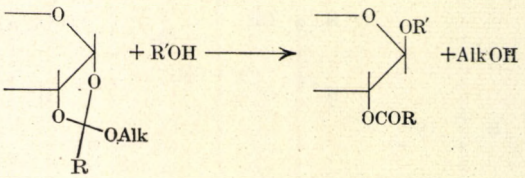
(i) Introduction

The so-called orthoester method of the glycosidic bond formation, permitting the synthesis of the glycosides of alcohols (aglycones) of complex structure, particularly of di- and oligosaccharides, is based on the condensation of sugar orthoesters with alcohol taken in amounts close to stoichiometric requirements, in inert solvents. The experimental conditions, the results and the preparative importance make this method essentially different from the above-considered alcoholysis of orthoesters.

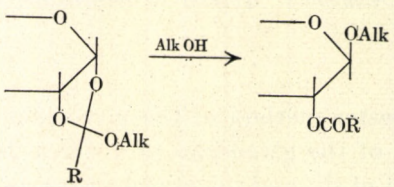
Helferich and Weiss in 1956 described the transformation of 1,2-methylorthobenzoyl-3,4,6-tri-O-benzoyl-α-D-glucopyranose into isomeric 2,3,4,6-tetra-O-benzoyl-β-methyl-D-glucopyranoside, which proceeds at room temperature in nitromethane in the presence of mercuric bromide and hydrogen chloride [84]. In 1963–1964 the authors of the present review demonstrated for the first time [103, 104] with chloresterol and 1,2; 3,4-di-O-isopropylidene-α-D-galactopyranose as aglycones that the condensation of sugar

orthoesters with alcohols of complex structure in nitromethane in the presence of acidic catalysts may serve as a rather general and a stereospecific synthesis route to 1,2-*trans*-glycosides. The method was later applied to the synthesis of a wide variety of glycosides and oligosaccharides, and finally served as the basis of a new approach to the synthesis of polysaccharides.

The condensation of sugar orthoesters with hydroxyl-containing compounds proceeds along path (a) (see page 82) under the conditions of the glycoside synthesis and results in the inversion of the configuration at the glycoside centre and in regeneration of the acyloxy-group at C_2, affording 1,2-*trans*-glycosides; the alkoxyl residue is eliminated as lower alcohol:



As mentioned above (Part IV., — 4.), the alcoholysis of the orthoester group may also lead to a new orthoester via re-esterification. Hence, the formation of orthoesters isomeric with the desired glycosides is a side-reaction of the orthoester method. Another important side-reaction is the glycosylation of the lower alcohol eliminated leading to a glycoside isomeric with the starting orthoester:



The latter reaction sometimes lowers the yields considerably, because the lower alcohols are usually glycosylated more readily than the aglycones treated. The experimental procedures and the methods of the suppression of side-processes will be discussed below.

(ii) The Conditions of the Synthesis

Catalysts and solvents. The direction of the reaction of sugar orthoesters with alcohols depends on the nature of the solvent and on the type and amount of catalyst. These factors may shift the condensation either to re-

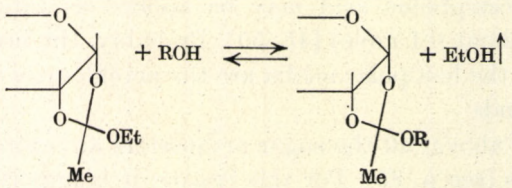
esterification, or to glycosylation [52, 98, 104]. The conditions used in the majority of syntheses were boiling nitromethane in the presence of 0·02–0·05 moles of mercuric bromide [52, 87, 88, 98, 104–112]. Similar results are obtained with mercuric chloride or copper chloride as catalyst, and acetonitrile as solvent [52, 98]. With all the solvents and catalysts, a critical effect was found of the ratio catalyst: orthoester upon the direction of the reaction. Above the critical value the reaction results in glycosylation, below it there takes place re-esterification [52, 98]. It appeared that the critical point is characteristic of the given pair solvent-catalyst; the minimum value of the ratio in the cases studied is characteristic of the reactions in nitromethane catalyzed with mercuric bromide. The other catalysts studied, such as *p*-toluenesulphonic acid, mercuric acetate, cupric acetate and titanium tetrachloride in a polar solvent bring about only re-esterification. In the less polar solvents, such as dichloroethane, all the catalysts mentioned induce only re-esterification [52, 98]. For successful glycosylation in these solvents, catalysts of another type are needed. These are the perchlorates of heteroaromatic amines, such as pyridinium, 4,4′-dipyridinium or 2,6-lutidinium perchlorates [81, 99, 113, 114]. In the two former cases, it was also necessary to add some *p*-toluenesulphonic acid to the reaction mixture. The three perchlorates are efficient catalysts of the glycosylation at low values of the ratio catalyst: orthoester (0·02 moles per mole). A positive result is also obtained with a mixture of silver perchlorate and perchloric acid in the less-polar media, and with boron trifluoride etherate [79, 79a]. In the less-polar media, *p*-toluenesulphonic acid may be applied as catalyst in relatively high amounts (about 0·1 mole) [45, 59]. Probably, the best catalysts of the glycosylation in the less-polar media are the perchlorates of pyridinium and related compounds.

As mentioned above, all the sugar orthoesters are extremely sensitive to acidic hydrolysis (see p. 85). For this reason, it is important for successful glycoside synthesis to protect the reaction mixtures thoroughly from atmospheric moisture. Only absolute solvents are applied. Moreover, it is recommended to distil off the solvents from the reaction mixtures continuously before the addition of catalyst, and during the reaction to remove moisture azeotropically. Without this precaution a considerable part of the starting orthoester is usually hydrolyzed [81]. For the same purpose drierite was formerly added to the reaction mixture [79].

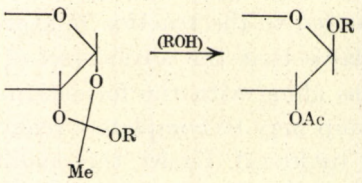
It was mentioned above that the alcoholysis of orthoesters leads to a number of side-reactions along with the formation of glycosides; in particular, the orthoester group may be completely removed to afford glycosides with an unsubstituted hydroxyl. Under the conditions of the orthoester

method, these side-reactions do not usually take place. It was only with *p*-toluenesulphonic acid as catalyst at its relatively high concentrations that 1,2-*trans*-glycosides with hydroxyl at C_2 were detected in the reaction mixture, along with the normal products [45, 59]. The same result was obtained with nitromethane-mercuric bromide [59], but at 46·5° rather than under reflux as recommended in the above-cited papers. This side-reaction has never been observed under other conditions of the glycoside synthesis, and seems to be limited to the conditions used in the studies [45, 49].

Two-stage glycosylation procedure. As mentioned above, a serious complication of the glycoside synthesis by the orthoester method is the glycosylation of the eliminated lower alcohol, leading to a glycoside isomeric with the starting orthoester. To overcome this difficulty, azeotropic distillation was usually applied to remove the lower alcohol continuously from the reaction mixture [52, 87, 88, 98, 99, 104–112, 114]. The technique, however, was of little value with the less reactive compounds, and the yield of the major product was considerably decreased by this side-reaction. To rule out the complication, a two-stage glycosylation procedure has been proposed [99]. In the first stage of the procedure, the condensation of orthoester with the starting aglycone is performed under the conditions resulting in re-esterification (see above) and affording a new orthoester, isomeric with the desired glycoside. Due to the reversibility of the reaction, the equilibrium may be practically completely shifted to the right by continuous azeotropic distillation of the lower alcohol:



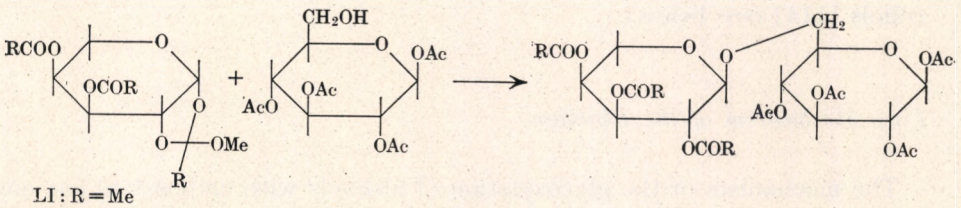
In the second stage, the products obtained are treated under the conditions resulting in glycosylation. The newly-formed orthoester now glycosylates the minor amount remaining of the starting aglycone to afford the desired glycoside, whereas the aglycone is continuously regenerated and enters the reaction:



Obviously, this procedure practically eliminates any formation of the glycosides of lower alcohols, isomeric with the starting orthoesters. About a two-fold increased yield (from 35 to 60·5%) was obtained when this procedure was applied to the synthesis of gentiobiose octa-acetate. The two stages may be performed in the same reaction vessel, without the isolation of the intermediate orthoester [99].

(iii) Structure of the Orthoester Group

In the majority of the studies on the application of the orthoester method, methyl- and ethylorthoacetates of sugars were applied as glycosylating agents. The effect of the nature of the ortho-acid radical on the result of reaction has been studied during the synthesis of vicianose according to the following scheme [52, 88]:

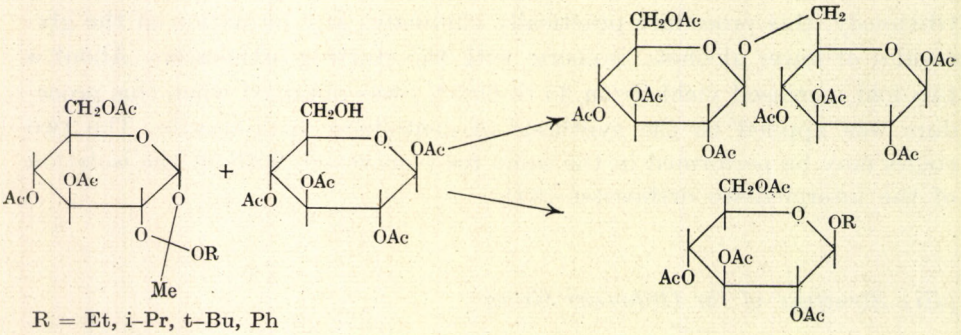


LI : R = Me
LII: R = Ph

It appeared that under standard conditions of the glycosylation (nitromethane, mercuric bromide) the reactivity of orthobenzoate (LII) is markedly higher compared with that of orthoacetate (LI); the yields were 93 and 54·5%, respectively. The orthobenzoates have also been successfully applied to the synthesis of a trisaccharide [87], to the synthesis of fructose glycosides [112] and to the synthesis of polysaccharides (see below).

To investigate the possibility of the inhibition of the side-reaction, i.e., the glycosylation of the lower alcohol, a study has been made of the dependence of the yield of the desired glycoside on the structure of the orthoester alkoxyl residue [81]; the synthesis was that of gentiobiose octa-acetate (see p. 124).

It appeared that the reactivity of the hydroxyl-containing compound eliminated falls along the sequence R = Et, iso-Pr, t-Bu, Ph. In accord with this reactivity, the yields of the by-products (the glycosides isomeric with the starting orthoester) fell, together with an increase of the yield of gentiobiose octa-acetate. On this basis, the use of sugar t-butyl orthoacetates (or orthobenzoates) as glycosylating agents was suggested to reduce the

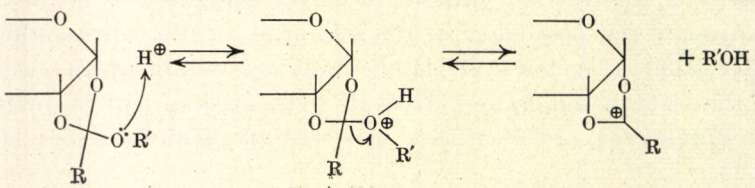


R = Et, i-Pr, t-Bu, Ph

side-reaction to a minimum. The use of t-butyl orthoacetate in combination with the two-stage procedure resulted in fact in an increase of the yield of gentiobiose to 75% (compared with 35% obtained by the single-stage procedure) [114]. The high efficiency of t-butyl orthoacetates has also been demonstrated during the glycosylation of the less reactive secondary alcohols [114] (see below).

(iv) Mechanism of the Reaction

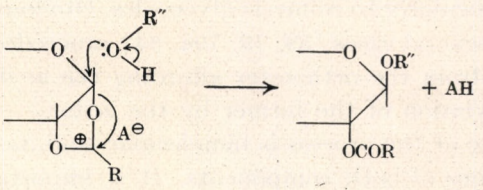
The mechanism of the glycosylation of alcohols with orthoesters has not been specially studied. Nevertheless, the data available concerning the chemistry of the reaction and the effect of the conditions upon its course suggested the following scheme [52, 81, 98, 99], which is in accord with the majority of the evidence available. The primary step of the reaction is protonation of the orthoester alkoxyl residue and the break-down of the oxonium ion affording acyloxonium cation:



The direction of the subsequent reaction of the ambidentate acyloxonium ion with alcohol depends on the nature of the catalyst anion and on the state of this in the solvent. The anion of an acidic catalyst may be capable of forming an intimate ionic pair with the acyloxonium cation, rather than a covalent bond, so that the electrophilic centre of the cation appears screen-

ed by the anion, the direct attack on the electrophilic centre is thus hindered, and the competitive attack at the glycosidic centre prevails.

This direction results in the 1,2-*trans*-glycoside and in regeneration of the acyloxy-group at the carbon atom adjacent to the glycoside centre:



Coordinated mercuric or cupric halide anions may act as such screening anions after dissociation in polar solvents according to the following scheme:

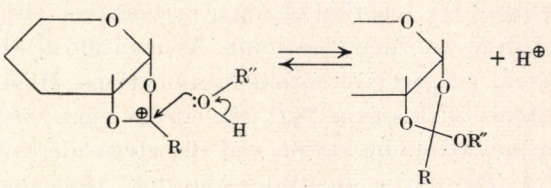
$$2MX_2 \rightleftarrows MX^+ + [MX_3]^-$$

$$M = Hg^{++},\ Cu^{++}$$

$$X = Cl^-,\ Br^-$$

The necessary condition of glycosylation thus appears to be the presence of polar solvent, and this is in fact the case. A similar role may be played by co-ordinated anions formed during the catalysis of the glycosylation with boron trifluoride etherate, or by the perchlorate ion. In the latter case the polarity of the solvent is obviously not critical, so that the glycosylation can be performed in the less polar media.

In the cases when anions of this type are absent, or their concentration is too low, no screening of the electrophilic centre takes place; the attack is directed to the electrophilic centre and results in re-esterification:

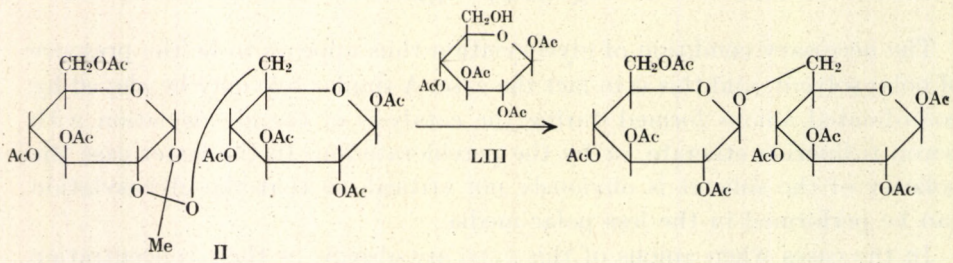


Obviously, screening is not the case when catalysts like mercuric bromide are applied in the less polar media, where no dissociation is possible. Similarly, the same catalyst in the polar media, but taken at low concentrations, cannot efficiently shield the electrophilic centre, due to dissociation of

ionic pairs. The tosylate anion seems also to be incapable of the ionic pair formation with the acyloxonium cation, since a covalent bond may be formed on intimate contact. In fact, re-esterification rather than glycosylation has been the case under all the above-mentioned conditions.

The mechanism proposed leaves no possibility for the orthoesters to rearrange intramolecularly to isomeric glycosides. Obviously, the rearrangement observed in several cases [59, 79, 79a, 84] proceeded in fact via elimination of alcohol from the orthoester affording the acyloxonium ion with subsequent glycosylation of the former by the latter.

The second stage of the process is bimolecular, and its rate must depend on the concentrations of both components. It is important that in all the studies cited the 'rearrangement' has been observed at relatively high concentrations of acid catalyst (which determines the stationary concentrations of alcohol and acyl oxonium ion). Vice versa, it has been demonstrated [99] that at low concentrations of catalyst the conversion of orthoester II to gentiobiose octa-acetate is a slow reaction in the absence of glucose tetra-acetate (LIII), but is considerably accelerated by addition of this alcohol:



II

The fact clearly points to the intermolecular rather than intramolecular character of the 'rearrangement', which thus proceeds as glycosylation.

The two directions of the reaction of sugar orthoesters with alcohols may also be considered from another viewpoint. As mentioned above, the dual reactivity is a general property of ambidentate cations. Hence, by analogy with the other systems of this type [32], the energy curve of the system involving orthoester, acyloxonium cation and the glycoside can be presented as shown in Fig. 1. The curve illustrates the fact, that the formation of orthoester from acyloxonium ion is a rapid and reversible reaction, whereas the glycosylation must overcome a large energy barrier and proceeds slowly and irreversibly. Hence, the re-esterification is the direction of reaction controlled by the kinetic factor, whereas the glycosylation is controlled thermodynamically. A review [32] surveys some data indicating that the

formation of thermodynamically-controlled reaction-product from ambidentate cations proceeds readily at higher temperatures. Similarly, in the cases when glycosylation occurred with difficulty, and the major reaction was re-esterification, the desired glycosides were readily obtained by increasing the temperature [91, 114].

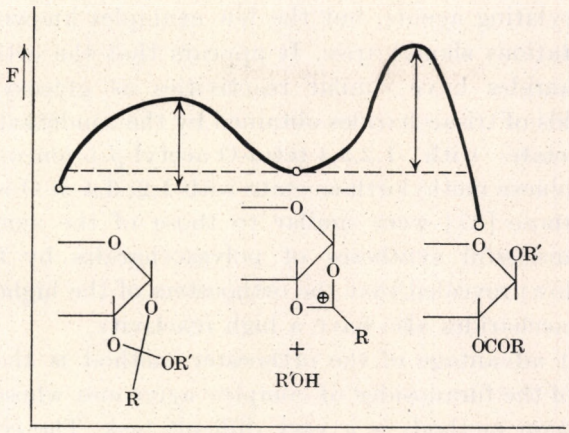


Fig. 1. Schematic energy curve of the system: orthoester–cation–glycoside

(v) Applications of the Orthoester Glycosylation Method

The orthoester method of glycoside synthesis was introduced for practical synthesis only a few years ago, but even now it is obvious that its scope is very extensive. The known cases of its application to different orthoester (glycosylating) and aglycone (glycosylated) components will be briefly considered below. A complete survey of the present data is given in Table IV.

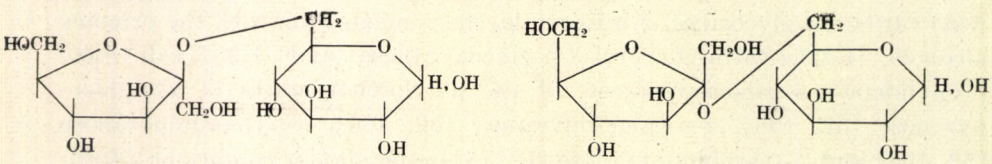
The orthoester components. The present knowledge of the reactivity suggests that orthoesters of a wide variety of carbohydrates of different structure may be used successfully in the glycosylation reactions to afford a wide variety of glycosides. For example, the condensation with the primary alcohols, 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose and 1,2; 3,4-di-O-isopropylidene-α-D-galactopyranose, of the alkylorthoacetates of α-D-glucopyranose [52, 106], α-D-galactopyranose [52, 106], β-L-rhamnopyranose [52, 106] and β-L-arabinopyranose [52, 88] under similar conditions afforded similar yields of the corresponding disaccharides. High yields in the glycosylation of lower alcohols and of t-butyl hydroperoxide have been also obtained with the orthoacetates of β-D-mannopyranose [59, 79, 79a],

β-L-rhamnopyranose [79, 79a], α-D-glucopyranose [79a], α-D-galactopyranose [79a] and the orthobenzoate of β-L-arabinopyranose [79a]. Hence, the configuration (gluco-, galacto-, manno-, arabino-) and the structure (pentose, 6-deoxyhexose, hexose) of the monosaccharide do not considerably affect the glycosylation.

There is not yet enough data on the use of di- and oligosaccharide orthoesters as glycosylating agents, but the few examples known indicate that no serious limitations should arise. It appears that the orthoesters of di- and monosaccharides have similar reactivities as glycosylating agents, because the yields of trisaccharides obtained by the condensation of maltose methyl-orthoacetate with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose [52, 87] and of cellobiose methylorthoacetate with 1,2; 3,4-di-O-isopropylidene-α-D-galactopyranose [87] were similar to those of the syntheses considered above. Successful syntheses of polysaccharides by the orthoester method (see below) revealed that the orthoesters of the higher oligosaccharides and polysaccharides also have a high reactivity.

An important advantage of the orthoester method is the ready availability from it of the furanosides of complex aglycones whose synthesis by other glycosylation methods is a very difficult task. The condensation of 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose with benzoylated methyl orthobenzoates of β-L-arabinopyranose and of β-L-arabinofuranose under similar conditions afforded the corresponding disaccharides; the yields were 93 and 90%, respectively [52, 88]. On this basis it was concluded that the glycosylating activity of sugar orthoesters does not depend upon the size of the sugar oxide ring. The yields of a number of β-D-galactofuranosides synthesized from the corresponding methylorthoacetate [52, 105, 107, 110, 111] were close to those of the pyranosides obtained by the same method from alcohols of similar structure.

The introduction of a ketosyl residue has been studied in only a single case [112], the condensation of 1,4,6-tri-O-benzoyl-2,3-methylorthobenzoyl-β-D-fructofuranose with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose. The reaction afforded two anomeric disaccharides:



In spite of the lack of stereospecificity caused by reasons that are discussed below, the reaction is the first successful synthesis of a ketoside with an aglycone of complex structure.

Hence, the orthoester method is applicable to the synthesis of the glycosides of the major types of natural neutral carbohydrates in the pyranose and in the furanose form. At present the orthoesters of uronic acids are being studied as glycosylating agents in our laboratory.

Aglycones. The orthoester method of glycoside synthesis has been successfully applied to glycosylate a wide variety of hydroxyl-containing compounds, including the major types of the aglycones of the natural O-glycosides, and in particular of the carbohydrate derivatives (oligosaccharide synthesis).

Although the relationship between the nature of the aglycones and their reactivities in the condensations with orthoesters is not yet investigated in detail, it is established that primary alcohols usually react much more readily than secondary ones and afford higher yields of the corresponding glycosides. Glycosylation of acidic hydroxy-groups (e.g., those of phenols) is difficult, and the successful synthesis of the corresponding glycosides needs special techniques.

The lower alcohols. A number of convenient methods have been elaborated for the synthesis of the glycosides of the simplest alcohols. Hence, the glycosylation of the compounds with orthoesters has not been studied in detail, because it is not very interesting as a preparative method. High yields of the tetrabenzoates of β-methyl- and β-benzyl-D-glucopyranosides have been obtained from the isomeric orthoesters [84]. In the same manner, 2-O-acetyl-3,4,6-tri-O-benzyl-α-methyl-, α-isopropyl- and α-cyclohexyl-D-mannopyranosides have been obtained from the corresponding isomeric orthoesters [59]. Comparison of the relative reactivities of ethanol, isopropanol and t-butanol as competing aglycones in the reaction of glucose alkyl-orthoacetates with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose revealed [81] that their activity as acceptors of glycosyl decreases along the above sequence, and t-butanol is glycosylated with great difficulty (see also page 123). The syntheses of the simplest glycosides from sugar orthoesters under the conditions of alcoholysis have been discussed above (see Section VI. 1.).

The polyols. The orthoester method has been applied to the synthesis of the natural β-D-galactofuranosides of polyols: D-mannitol [52, 105], D-arabitol [111] and glycerine [110]. Appropriately protected polyols with the free hydroxyls at the desired positions were introduced into the reactions (the hydroxyls at C_3–C_4 in D-mannitol, at C_2 in D-arabitol and at C_1 in D-glycerol). The yields of the glycosides obtained were high (see Table 4). The possibility of the preparation of polyol glycosides is of special importance because the compounds are the products of the Smith method of polysaccharide degradation.

Polycyclic compounds. Many natural glycosides have a steroid or a triterpenoid polycyclic system as aglycone. The oligosaccharide chain is attached to the C_3 hydroxyl of this system. Glycosylation of these hydroxyls has been studied with cholesterol [52, 98] and oleanolic acid methyl ester [109] as aglycones. Their β-D-glucopyranosides and β-D-galactopyranosides have been obtained by direct glycosylation in nitromethane in a 44–45% yield. The carboxylic group of oleanolic acid appeared practically inert in the condensation with orthoesters, and glycosylation of the C_3-hydroxyl of free oleanolic acid was successfully performed without the protection of this carboxyl [109].

Hydroxyamino acids. The synthesis of the O-glycosides of hydroxyamino acids is important for the studies of the structure and properties of glycoproteins. The specific difficulty of this synthesis is the marked tendency of the hydroxyamino acid derivatives to give orthoesters rather than glycosides when introduced into condensation under the conditions of the Koenigs–Knorr synthesis (see p. 103). By the orthoester method, these orthoesters were successfully transformed into the isomeric glycosides. The derivatives of L-serine β-melibioside and β-maltoside [92] and of L-threonine β-D-glucopyranoside [91] were obtained. In the latter case the glycosylation of the secondary hydroxyl was especially hindered, but at elevated temperature proceeded readily. Direct condensation of 1,2-methylorthoacetyl-3,5,6-tri-O-acetyl-α-D-galactofuranose with the methyl ester of N-carbobenzoxy-L-serine afforded the corresponding β-D-galactofuranoside [107].

The synthesis of oligosaccharides. The synthesis of oligosaccharides is the major problem of glycoside synthesis, and it is the field where the orthoester method gained its major importance. The syntheses were usually performed by condensation of the orthoesters of mono- and disaccharides with suitably protected monosaccharide derivatives containing an unprotected hydroxyl at the desired position. The mild conditions of orthoester synthesis made it possible to use the routinely protected compounds such as acetates, benzoates, methylglycosides, isopropylidene and benzylidene derivatives (see Table IV). Migration of the protective groupings has been observed only once, during the glycosylation of 1,2; 5,6-di-O-isopropylidene-α-D-glucofuranose.*

The highest yields are obtained when primary hydroxyls of the monosaccharide derivatives are glycosylated. For example, the condensation of 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose [52, 81, 87, 88, 99, 104, 106, 114]

* We and T. A. Sokolovskaya have now found that this difficulty can be overcome using t-butylorthoacetate under gentle conditions of glycosylation.

and of 1,2 : 3,4-di-O-isopropylidene-α-D-galactopyranose [52, 87, 104, 106] with a number of mono- and disaccharide orthoesters resulted in yields of oligosaccharides varying between 35 and 93%, but usually these exceeded 40–50%. The lowest yield in this series of syntheses (35%) was obtained during the synthesis of gentiobiose octa-acetate by direct glycosylation in nitromethane. However, the use of the two-stage procedure leads to an increase of the yield to 60·5% [99], and to 75% with the same procedure starting with glucose t-butyl orthoacetate [114]. Abnormally low yields were obtained only in the case of 1,2,3,4,2′,3′,4′-hepta-O-acetyl-α-melibiose [83] (see also p. 139).

Secondary hydroxyls of the carbohydrate derivatives are less reactive in the glycosylation compared with the primary ones. With these compounds, the side reaction of the eliminated lower alcohol glycosylation (see p. 120) considerably decreases the yields of disaccharides. For example, the condensation of 1,2-ethyl-orthoacetyl-3,4,6-tri-O-acetyl-α-D-glucopyranose with 1,2-O-isopropylidene-3,6-di-O-acetyl-α-D-glucofuranose, with 2-O-acetyl-4,6-O-benzylidene-α-methyl-D-glucopyranoside and with 4,6-O-benzylidene-α-methyl-D-glucopyranoside under the direct glycosylation conditions in nitromethane afforded 10, 28 and 20% yields of the disaccharides, respectively. In the latter case, 7% of the product of the C_3-hydroxyl glycosylation (laminaribiose derivative) was obtained along with the major soforose derivative [52, 106, 108]. On this basis, the hydroxyls of the glucose derivatives may be arranged into the following sequence in accordance with their reactivity: $C_6 > C_2 \geq C_3 > C_5$. The difficulties encountered during glycosylation of the secondary sugar hydroxyls may be overcome to a considerable extent by the methods discussed above (see pp. 122, 123). For example, the use of glucose t-butylorthoacetate and glycosylation at elevated temperature (in boiling chlorobenzene) increases yields of the laminaribiose derivative from 28 to 50%, and of the derivative of 5-O-(β-D-glucopyranosyl) glucose from 10 to 25% [114].

Phenols. Attempts at direct glycosylation of phenolic hydroxyl have failed, because in this case the predominant reaction was the side glycosylation of the lower alcohol. Obviously, the difficulty is due to the acidic nature of the hydroxyls of phenols. However, use of the two-stage procedure helped to by-pass the difficulty. Using this method, the tetra-acetates have been synthesized of the β-D-glucopyranoside of β-naphthol, of the monomethyl ether of hydroquinone and of hydroxyacetophenone [113]. The condensation of glucose ethylorthoacetate with phenols was peculiar and in some respects different from the usual glycosylation of alcohols. First of all, with β-naphthol it was demonstrated that the glycosylation takes place

in dichloroethane in the presence of *p*-toluenesulphonic acid alone, i.e., under the conditions resulting in re-esterification with the aliphatic alcohols. Secondly, the glycoside was formed in the absence of any catalyst by *p*-hydroxy-acetophenone, probably, due to the acidity of the phenol itself.

Obviously, some correction of the above mechanisms must be made at least for the glycosylation of phenols.

Other aglycones. A somewhat special case is the synthesis of the t-butyl hydroperoxide glycosides accomplished by 'rearrangement' of the isomeric orthoesters in the less polar media, catalyzed by perchloric acid and silver perchlorate, or with boron trifluoride etherate [79, 79a]. In all the cases studied, high yields have been obtained of the compounds desired.

(vi) The Steric Specificity of Glycosylation with Orthoesters

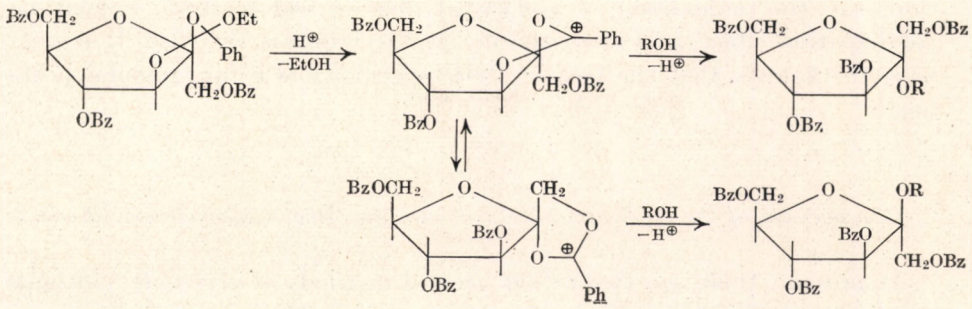
The 1,2-*trans*-configuration of the glycoside bonds in the compounds obtained using the orthoester method has always been confirmed either by identification with the known substances, or by calculation of the molecular rotation. In a number of studies concerned with the development and applications of the orthoester method (see e.g. [52, 81, 87, 88, 98, 99, 105–109, 114]) the composition of the reaction mixture was investigated after the condensation by thin-layer chromatography in solvent systems, resolving the anomeric pairs [115]. With two exceptions (see below), no 1,2-*cis*-glycosides anomeric to the desired compounds have been ever detected.

The most strict proof of the stereospecificity of glycosylation has been obtained during the synthesis of arabinans (see below): polysaccharides were obtained that were completely digested with the *exo*-enzyme α-L-arabinofuranosidase. Unlike the situation with the low-molecular compounds, any possibility was excluded here of the loss of 1,2-*cis*-glycosides during the isolation, since the corresponding bonds (if their formation is possible) must be included in a polysaccharide structure and would be easily detected by enzymatic hydrolysis.

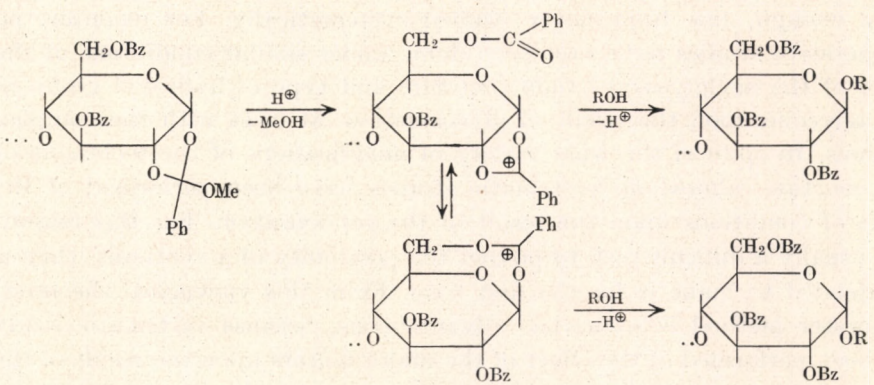
As mentioned above, the preparation of fructosides, unlike the other glycoside syntheses, results in two anomers. The reason of the difference is the possibility of the isomerization of the intermediate acyloxonium cation (see p. 104) according to the scheme shown on p. 133.

On condensation with alcohol, one of the isomeric ions affords the α-, and the other, the β-fructoside.

An analogous explanation has been proposed for the as yet single case of non-stereospecificity of the glycosylation with aldose orthoesters, i.e.



the synthesis of a trisaccharide by condensation of 3,6,2′,3′,4′,6′-hexa-O-benzoyl-1,2-methylorthobenzoyl-α-cellobiose with 1,2 : 3,4-di-O-isopropylidene-α-D-galactopyranose [87]. In this case a minor amount (4%) of the α-anomer was obtained along with the major product, the β-trisaccharide (71%). The abnormal course of the reaction was explained by the presence in position 6 of the glycosyl residue of a strongly nucleophilic benzoyloxy-group, whose participation in the reaction at the glycoside centre results in the 1,6-acyloxonium ion, which subsequently reacts with alcohol to afford the α-glycoside:



This exception to the general rule of stereospecificity obviously does not result in fundamental difficulties. In both cases the formation of the other anomer is due to the high ability of the benzoyloxy-group to participate in the nucleophilic substitution reactions. Hence, it is anticipated that this abnormal behaviour will be characteristic only for the benzoyl protecting group appropriately situated in space relative to the orthoester group. To overcome the difficulty, one must use another protective group, e.g., acetyl, instead of the benzoyl. In fact, the orthoesters of an analogous configura-

tion, e.g. the orthoesters of acetylated glucose and maltose, exhibit no participation effect, and stereospecific glycosylation is the case. It is only with the ketoses that the lack of steric specificity is a fundamental problem.*

3. Comparison of the Orthoester Method with the Other Glycosylation Methods

At present, there are two rather general methods of glycoside synthesis valid with the aglycones of complex structure. These are the Koenigs–Knorr synthesis, and the orthoester method. The two methods must not be opposed since they are to a considerable extent complementary, but it seems reasonable to consider their comparative characteristics.

The Koenigs–Knorr method (see [9, 10, 21, 31]) has been known from the beginning of the 20th century [69], and since that time has been applied to a great number of syntheses. In many cases these were successful and afforded the desired compounds stereospecifically and in high yields. At the same time, a number of failures of its application are known, and the complications have usually been the poor reproducibility of yields and the lack of stereospecificity. The preparative scope of the method, surprisingly enough, has been never studied systematically. For example, no comparative studies have ever been done, under similar conditions, of the effect of the acylglycosyl halide structure and type of hydroxyl group in the aglycone upon the result of glycosylation, at least with the complex alcohols. In spite of the wide variety of modifications of the technique of this reaction, almost no systematic studies have been performed of the effect of conditions upon the result of the condensation. For this reason, it is usually a difficult task to predict the possibility of a synthesis when it is intended to make it for the first time. From this viewpoint, the novel orthoester method is somewhat advantageous, because systematic study has been performed of the effect of the major factors upon the result of the glycosylation, and because the condensations proceeding in homogeneous media are readily reproducible.

* In 1964 Lemieux and Morgan [116, 117] published (without the experimental details) a preliminary report that the lower 3,4,6-tri-O-acetyl-α-alkyl-D-glucopyranosides are formed (along with the β-anomers) during the acid-catalyzed condensation of the corresponding glucose alkylorthoacetates with alcohols. The mechanism suggested by these authors involves the protonation of the oxygen atom at C_1 followed by elimination of all the orthoester group and formation of the glucosyl cation 3,4,6-triacetate, whose interaction with alcohol gives rise to anomeric glycosides. No analogous evidence has been published, since that time, and the possibility of the preparation of 1,2-*cis*-glycosides via sugar orthoacetates still remains a problem.

The scope of the Koenigs–Knorr method is limited to the availability of the 1,2-*cis*-acylglycosyl halides. Although a number of syntheses of this type have also been performed with the 1,2-*trans*-acylglycosyl halides, the compounds still remain inconvenient as starting materials, since the formation of orthoesters rather than of the glycosides is the more typical direction of their reactivity (see above).

The limited availability of the starting compounds severely limits the applicability of the Koenigs–Knorr method to furanoside synthesis. Up to the present, this route has afforded only a few furanosides of aglycones of complex structure. Vice versa, the above-outlined evidence demonstrates that no difficulty arises in this case with the orthoester method, and a number of previously unavailable furanosides have been successfully synthesized. Moreover, the orthoester method has afforded polysaccharides with furanose units for the first time (see below).

As mentioned above, the synthesis of even the simplest glycosides of ketoses by the Koenigs–Knorr method results in many complications. The orthoester method, although resulting in anomer mixtures, has nevertheless now made it possible to obtain the ketosides of complex aglycones by chemical synthesis.

Stereospecificity of the glycosylation has been found to be absent in many cases of the Koenigs–Knorr reaction, and there exists at present no reasonable explanation of the phenomenon. On the contrary, the orthoester method normally results in the strictly stereospecific formation of 1,2-*trans*-glycosides. The most important advantage of the orthoester method is the fact that on its basis the first chemical route to polysaccharides of predetermined structure has been elaborated (see below).

VII. THE SYNTHESIS OF POLYSACCHARIDES BY THE ORTHOESTER METHOD

1. Introduction

It is well known that studies of the model polypeptides and polynucleotides obtained synthetically has been of outstanding importance for the investigation of the physical and chemical properties of these biopolymers as well as of their biological functions. Mention will be made of the role of synthetic polypeptides in studies of the protein secondary structure and of the decisive role of synthetic polynucleotides in elucidation of the code of protein synthesis.

There is no doubt that synthetic polysaccharides of regular and predetermined structure could play a role of similar importance in the chemistry and biochemistry of the natural polysaccharides. However, until recently the synthesis of these models has been a problem too difficult for modern organic chemistry. The difficulty has been primarily the polyfunctionality of the monomer unit, the fact that it may exist in either pyranose or furanose form, and, consequently, the multiplicity of the types of inter-monomer bonding. For this reason, to obtain synthetically polysaccharides of regular structure one must devise monomers with strictly oriented possibilities of polymerization (polycondensation). Moreover, the formation of glycoside bonds results in new asymmetric centres, and the synthesis of polysaccharides, unlike that of polypeptides or polynucleotides, must be strictly stereospecific. Finally, unlike the linear chains of polypeptides or polynucleotides, the polysaccharide chains are usually branched.

The possibility of preparation of model polysaccharides seems to be limited principally to chemical synthesis, because the biochemical methods, like those employed in polynucleotide synthesis, may not be of general importance here. The fact is that one cannot hope to isolate a universal synthesizing enzyme (like polynucleotide phosphorylase), non-specific to the type of monosaccharide residue and to the type of bonding involved.*

All the above-mentioned difficulties lead to the present situation, where the numerous polysaccharide syntheses described (for reviews, see [118–122]) usually afford compounds with random distribution of various types of bonding, of the ring size and of the glycoside bond configuration. Only a few syntheses are known of regular polysaccharides. These are the resynthesis of cellulose [123] and the synthesis of 1,6-α-glucan [124].

It is noteworthy that the Koenigs–Knorr method was also unsuccessful in this field, mainly because of the problem of availability of acylglycosyl halides with free hydroxyl.**

Elaboration of the orthoester method introduced a potent new type of glycosylating agent into carbohydrate synthesis that made it possible for the first time to find approaches to the synthesis of polysaccharides of predetermined structure.

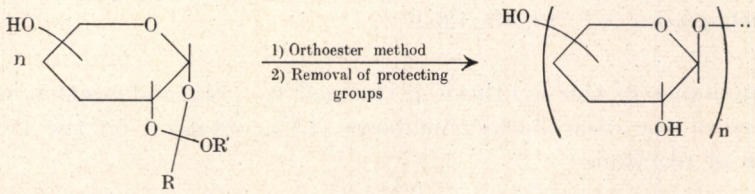
The possibility of the application of sugar orthoesters as monomers for polysaccharide syntheses is due to two circumstances. The first of these is the high efficiency and stereospecificity of the orthoester glycosylation

* Dr. M. A. Grachev (Novosybirsk) first called our attention to this point.

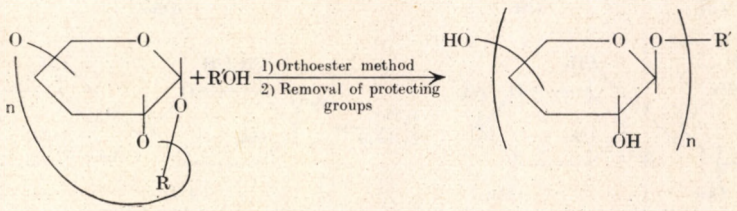
** The best result in this direction has been obtained by Haq and Whelan, who obtained a very small yield of 1,6-glucodextrines by polycondensation of 2,3,4-tri-O-acetyl-α-D-glucopyranosyl bromide. [125]

method. The second is the stability of the orthoester group to a variety of chemical agents; this makes it possible to introduce and to remove various protective groups and thus provides a wide variety of monomers for polysaccharide synthesis.

Two different approaches to polysaccharide synthesis are now studied on the basis of the orthoester method. The first is the polycondensation of monosaccharide or oligosaccharide orthoesters having a free hydroxyl at the desired position, according to the following scheme:



The second approach is the polymerization of tricyclic sugar orthoesters and related compounds according to the following general scheme (pyranose form is shown as an example):



2. Polysaccharide Synthesis

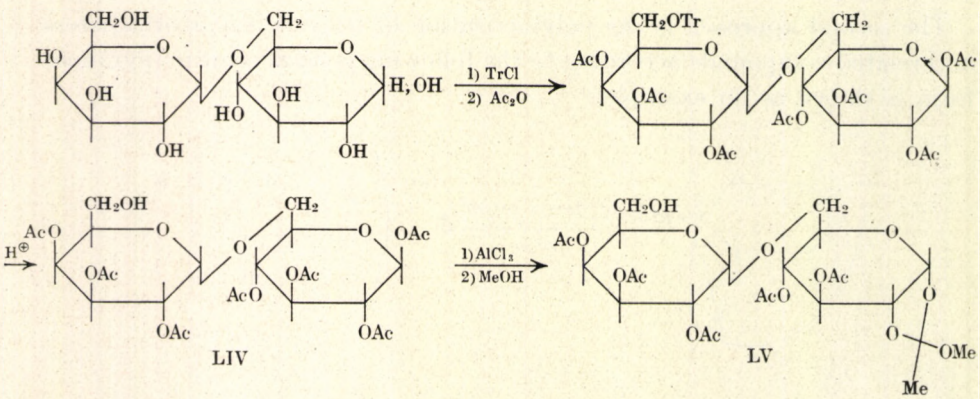
Four syntheses of polysaccharides starting with orthoesters are at present described. All of them have been accomplished using the following experimental techniques. The polymerization or polycondensation of monomer under the conditions of orthoester synthesis resulted in a mixture of derivatives that was first of all subjected to mild acidic hydrolysis in order to destroy the orthoester group that might survive the reaction. This stage was followed by removal of the protective groups by routine methods of carbohydrate chemistry. The mixture of sugars obtained was then analyzed by paper chromatography. In all cases a series of compounds was obtained from mono- to higher oligosaccharides, along with some polysaccharides immobile in paper chromatography. The latter were isolated by fractionation

on Sephadex or by ethanol precipitation. The polysaccharide fraction thus obtained, containing no lower, chromatographically mobile oligosaccharides, was subjected to the analyses needed for proof of the structure.

In the published papers concerned with the synthesis of polysaccharides by the orthoester method, the usual nomenclature of polysaccharides is applied (arabinan, glucan, etc.), but with the index 'S' (synthetic) and the ordinal number.

(i) Galacto-glucan S-1 [82, 83, 126]

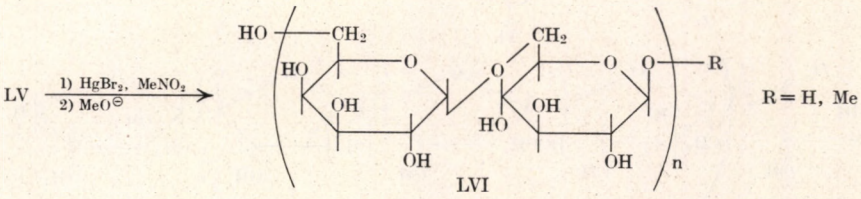
The monomer of this synthesis performed by polycondensation was the derivative of the disaccharide melibiose (LV), obtained by the following sequence of reactions:



The position of the free hydroxyl in the hepta-acetate LIV and in the orthoester LV was proved by methylation and by NMR-studies. The presence of the orthoester group was confirmed by the above-considered methods. The polycondensation was performed in nitromethane in the presence of mercuric bromide. The reaction products were fractionated by gel-filtration. The yield of the polymer fraction was 4·3%.

The structure of the galactoglucan S-1 in formula LVI has been confirmed by hydrolysis, affording glucose and galactose in a ratio 1 : 1, and by periodate oxidation. During the latter, 1·97 moles of periodate was consumed, and 0·99 moles of formic acid was formed per anhydro-hexose unit. The degradation according to Smith afforded glycerol as the major reaction product and traces of hexoses ($< 2\%$); no treitol was found above the sen-

sitivity of the method ($\ll 1\%$). Hence, more than 98% of the polysaccharide constituent glycosidic bonds are linked $1 \rightarrow 6$. The specific rotation of galacto-glucan S-1 was in a good agreement with that calculated for structure LVI.



LVI

Some of the polymer molecules should obviously contain methanol residues (as methylglycosides) at the reducing termini because of the fission of methanol in the course of the reaction, and the others should have free reducing termini formed by hydrolysis of the orthoester group. For this reason, the exact determination of the molecular weight of galacto-glucan S-1 by terminal analysis was difficult. From gel-filtration data authors believe that the compound has a relatively small molecular weight.

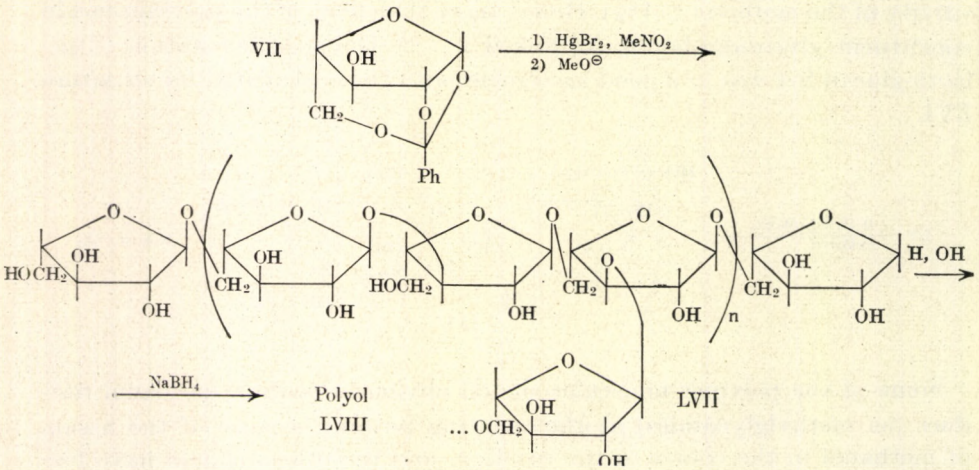
The reason of the small yield and of the low molecular weight of the polycondensation product is the abnormally low reactivity of the hydroxyl group at C_6 in the acylated derivative of melibiose. This low reactivity (compared with the usual sugar primary hydroxyls) has been found for the compound in a number of other reactions, such as methylation, tritylation or the synthesis of tetrasaccharide by condensation of the hepta acetate LIV with per-acetylated melibiose orthoacetate. No explanation has yet been proposed for the phenomenon.

(ii) Arabinan S-1 (Branched) [127–130]

The synthesis was performed by the polymerization of the tricyclic arabinofuranose orthobenzoate (VII) according to the scheme shown on p. 140.

The polymerization was performed in nitromethane in the presence of mercuric bromide during 22 h. The reaction products were fractionated by gel-filtration on Sephadex. The yield of polysaccharide was 50%. Reduction of the arabinan S-1 with sodium borohydride afforded the corresponding polyol that was used to prove the structure of polysaccharide.

The configuration of the glycoside bonds and the size of the oxide rings in arabinan S-1 were confirmed by the complete acidic hydrolysis, and by the enzymatic hydrolysis with α-L-arabinofuranosidase which also proceeds



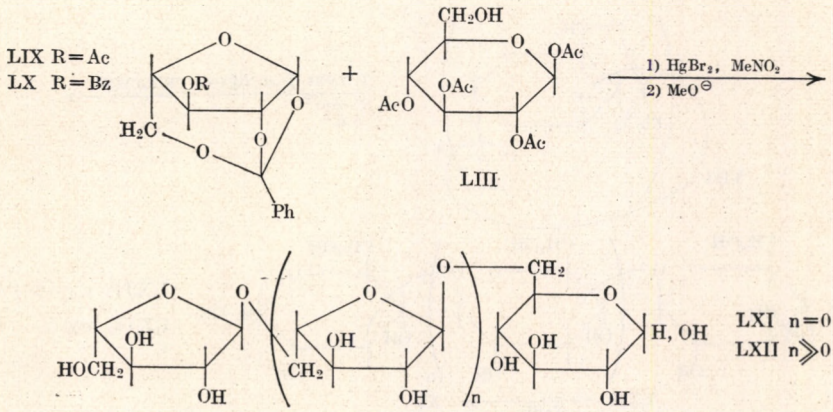
to completion. In both cases the only product of hydrolysis was arabinose. The number-average polymerization degree, as determined by the yield of formaldehyde after the periodate oxidation of polyalcohol LVIII, was 59·5.

As the starting monomer (VII) contained a free hydroxyl at C_3, and one more hydroxyl at C_5 should be formed in the course of polymerization after opening of the orthoester ring, one could expect the formation of an extensively branched polysaccharide with $1 \rightarrow 3$ and $1 \rightarrow 5$ bonds. The structure of arabinan S-1 established by the methods discussed below corresponded in fact to the average formula LVII. The periodate oxidation of arabinan S-1 and of the polyalcohol LVIII revealed 30% of non-oxidizable arabinose units. The methylation of polyalcohol LVIII, followed by hydrolysis and methanolysis, afforded the mixture of partially methylated arabinoses expected for the structure LVII. The ratio of 2,3-di-O-methyl-L-arabinose to 2-O-methyl-L-arabino-pyranose, the data of the periodate oxidation, and the molecular weight revealed that of the 59·5 arabinose units of arabinan S-1 39 contain $1 \rightarrow 5$ bonds, and 15–16 units are bound by branching at C_3. A minor amount of the units (3–4 per molecule) are bonded with $1 \rightarrow 3$ bonds. The number of chains per molecule is 16–17. No $1 \rightarrow 2$ bonds or pyranose units are present. This structure is very similar to that of natural plant arabinan [131].

(iii) Arabinan S-2 (Linear) [126, 130, 132]

Unlike the orthoester VII affording the above branched polysaccharide, the polymerization of its derivative with protected hydroxyl must give rise to linear arabinan. However, an alcohol must now be added to the reaction mixture to initiate the polymerization; this role was formerly played by the hydroxyl at C_3 of the orthoester VII.

The synthesis of the linear arabinan was performed by polymerization of the acetate LIX in a mixture with 0·05 mol of glucose tetra-acetate (LIII) under conditions similar to those of the synthesis of arabinan S-1, according to the following scheme:



For complete polymerization, a prolonged reaction period was needed (130 h). The polysaccharide fractionation was performed by gel-filtration on Sephadex G-25. The yield of arabinan S-2 was 20%.

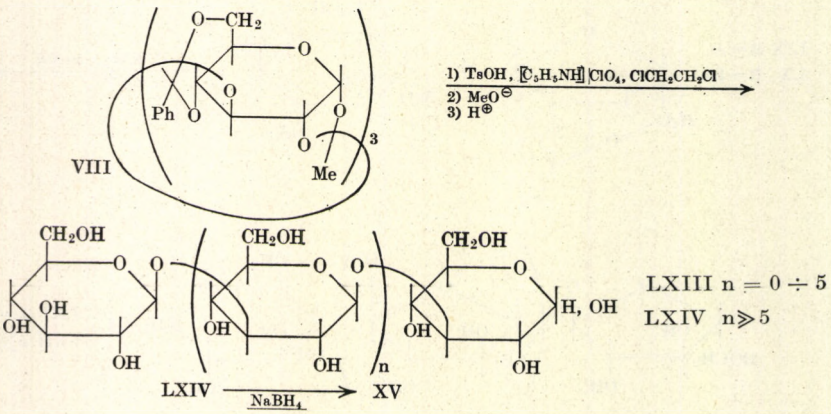
It appeared that polymerization of the acetate LIX or of the benzoate LX practically does not take place in the absence of alcohol-initiator. Vice versa, with the monomer: initiator ratio equal to 1 : 1 the reaction is rapid (4·5 h) and results in disaccharide LXI as the major product, along with minor amounts of its polymer-homologues. Thus, the rate of the reaction and the final extent of polymerization depend critically on the monomer: initiator ratio.

The structure of arabinan S-2 corresponding to formula LXII has been confirmed by the same series of methods as that applied to arabinan S-1. In the same manner it was established that arabinan S-2 is a linear polysaccharide of number-average polymerization degree 23·6, built exclusively of α-L-arabinofuranose residues linked to a D-glucose residue at the reducing

terminus by a 1 → 6 bond. 92% of the arabinose residues are linked 1 → 5, and 8% of the residues are linked 1 → 2. No branching or 1 → 3 bonding is present.

(iv) Glucan S-1 [96]

The starting compound of this synthesis was the macrocyclic orthoester VIII whose behaviour as monomer in the polymerization was much like that of the tricyclic orthoesters. The synthesis of glucan S-1 was performed according to the following scheme:



The polymerization of orthoester VIII was performed in dichlorethane in the presence of catalytic amounts of *p*-toluenesulphonic acid and pyridinium perchlorate. The product was fractionated on Sephadex or by fractional precipitation from water with alcohol. The yield of glucan S-1 was 13%. The structure corresponding to formula LXIV was proved by the following data.

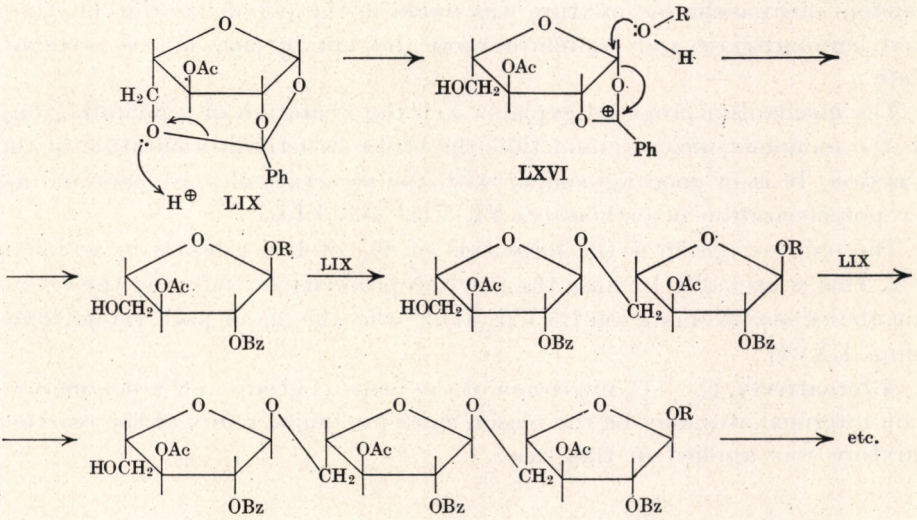
Complete hydrolysis of glucan S-1 afforded glucose as the only reaction product. Partial hydrolysis of the glucan S-1 and of the corresponding polyalcohol LXV gave a series of oligosaccharides that are chromatographically identical to laminaridextrines (from laminaribiose to laminariheptaose). The oligosaccharide fraction (LXIII) of the polymerization product had the same composition. Periodate oxidation of the polyalcohol LXV under controlled conditions resulted in consumption of 0·099 moles of periodate and in the formation of 0·033 moles of formic acid and 0·033 moles of formaldehyde per anhydrohexose unit, in good agreement with the Malaprade

oxidation of polyol LXV obtained from polysaccharide LXIV that has the number-average polymerization degree $(\overline{DP})_n = 30$. Hence, glucan S-1 is regular β-(1 → 3)-glucan. This structure and similar molecular weights ($(\overline{DP})_n \sim 20$–25) are characteristic of the G-chains of the insoluble laminarans [133–135].

3. Mechanism of the Polymerization and Polycondensation of Sugar Orthoesters

Obviously, the formation of polysaccharides from sugar orthoesters proceeds according to the same glycosylation scheme as that leading to low-molecular glycosides. In accordance with the above outlined mechanism (see p. 124), the polycondensation of orthoester LV must involve a nucleophilic attack of the corresponding acyloxonium ion upon the free hydroxyl of another monomer molecule. Thus, the elongation of polymer chain in this case takes place from both the reducing and the non-reducing termini.

In the polymerization of the tricyclic orthoesters (VI, VIII and LIX), the protonation of monomer results in acyloxonium ion and in simultaneous liberation of hydroxyl in the same molecule. Attack of the cation formed upon the other hydroxyl results in an intermediate product devoid of the orthoester group. Its glycosylation by another orthoester molecule leads to liberation of a new hydroxyl, etc. Hence, the elongation of polysaccharide chain occurs from the non-reducing terminus by stepwise glycosylation as shown in the following scheme (the compound LIX is taken as example):



This scheme is confirmed by a number of facts that are observed in polysaccharide synthesis. According to the scheme, the concentration of hydroxyl-containing compounds in the reaction mixture remains constant and equal to the original initiating alcohol concentration during the whole process.

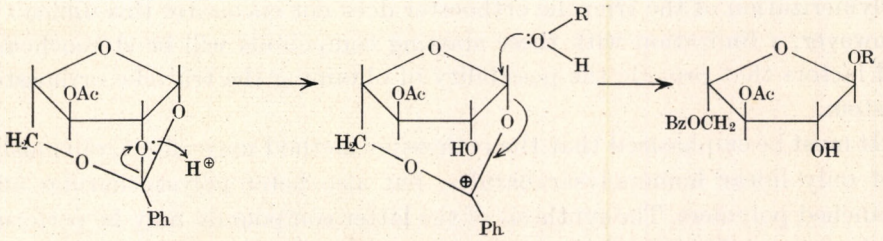
The scheme explains why an alcohol-initiator is necessary for the polymerization of orthoesters LIX and LX, and why the ratio monomer: initiator affects the rate and the degree of polymerization. Practically no polymerization of LIX and LX takes place in fact in the absence of an alcohol-initiator. At the ratio LIX : LIII = 20 : 1, the polymerization is slow and affords high-molecular products. Vice versa, at LIX : LIII = 1 : 1 the reaction is rapid and affords disaccharide as the major product. On the other hand, the polymerization of orthoester VI containing the initiating group in each monomer molecule proceeds relatively rapidly and does not need addition of any initiator. Elongation of the polymer chain from the non-reducing terminus is confirmed by the fact that the initiator residue in the polysaccharide appears at the reducing terminus (it is glucose in arabinan S-2 and the numerous branching in arabinan S-1).

Obviously, the same mechanism is valid in the synthesis of glucan S-1 from the macrocyclic orthoester. Here, *p*-toluenesulfonic acid was applied as catalyst and atmospheric moisture could act as initiator. If the polymerization of the macrocyclic trimeric orthoester proceeded by intramolecular rearrangement rather than by the stepwise scheme, the products of polymerization would be mainly oligosaccharides with degrees of polymerization divisible by 3 (laminaritriose, laminarihexaose, etc.). On the contrary, a random oligosaccharide mixture was found in the polymerization mixture, and laminaritriose and laminarihexaose did not by any means predominate.

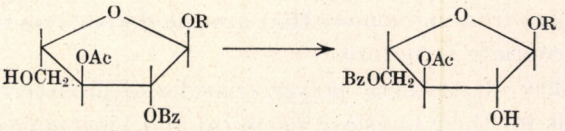
The mechanism proposed explains well the liberation of a definite group in the monomer molecule and thus the strict structural orientation of the reaction. It is in good agreement with the structure of products obtained by polymerization of orthoesters VI, VIII and LIX.

The only exception is the formation of 8% of $1 \rightarrow 2$ bonds in arabinan S-2. This is probably because the reaction proceeds partially via the seven-membered acyloxonium ion LXVII (along with the major path via acyloxonium LXVI).

Alternatively, $C_2 \rightarrow C_5$ migration of the benzoyl group in the polymerization intermediates may be the reason since prolonged reflux of the reaction mixture was applied in this case.



LXVII



4. Conclusion

Although the polysaccharide syntheses via sugar orthoesters are few as yet, it seems reasonable now to evaluate the prospects of the approach. As mentioned above, the glycosylating activity of sugar orthoesters only depends to a small extent on the structure of the parent sugar, on its stereochemistry and on the size of the oxide ring. It is thus anticipated that the synthesis of polysaccharides from orthoesters will have a wide scope. To a certain extent the data available confirm this conclusion, because glucose, galactose and arabinose units in the pyranose and in the furanose form are the constituents of the novel synthetic polysaccharides. More serious limitations will probably arise because of the lowered monomer hydroxyl reactivity, as in the case of galacto-glucan S-1. Whether this will appear a general problem must be revealed by further syntheses.

At present it is difficult to estimate the relative values of the two routes to polysaccharides, the polycondensation and the polymerization. These seem to a certain extent complementary. The first one must become of a more general character, principally because its starting monomers can be obtained for any predetermined polysaccharide structure. However, the limitation of this method at present is the termination of chain growth due to glycosylation of the lower alcohol eliminated, leading to formation of alkylglycosyl termini. Probably, it will be possible to overcome this difficulty by applying the two-stage glycosylation procedure, or by using t-butyl orthoesters. The latter approach seems the most promising, because the t-butylglycosides formed at the termini can be removed by mild acidic hydrolysis, not affecting the glycoside bonds of the polymer chain (cf. [136, 137]). The

polymerization of the tricyclic orthoester does not encounter this difficulty. However, a limitation with these starting compounds will be stereochemical factors that provide the possibility of obtaining the tricyclic orthoester system.

It must be emphasized that the orthoester method may afford, obviously, not only linear homopolysaccharides, but also heteropolysaccharides and branched polymers. The synthesis of the latter compounds may be performed by using bifunctional monomers, as with arabinan S-1.

Alternatively, branching (as short side chains) may be attained by synthesis of polymers from monomers that are the derivatives of oligosaccharides of an appropriate structure.

The availability of synthetic polysaccharides of predetermined structure provides a basis for their physical, chemical and biochemical studies. More detailed discussion of these problems is beyond the scope of the present review. It will only be mentioned that these model compounds may be used to elucidate the structure of natural polysaccharides by direct comparison of the physical-chemical properties; to study the relationship between the physical-chemical properties of polysaccharides, or their behaviour in biological systems, with their structure; to investigate the reactivity of different functional groups of polysaccharides and the chemistry of their derivatives; to study the secondary structure of polysaccharides, etc.

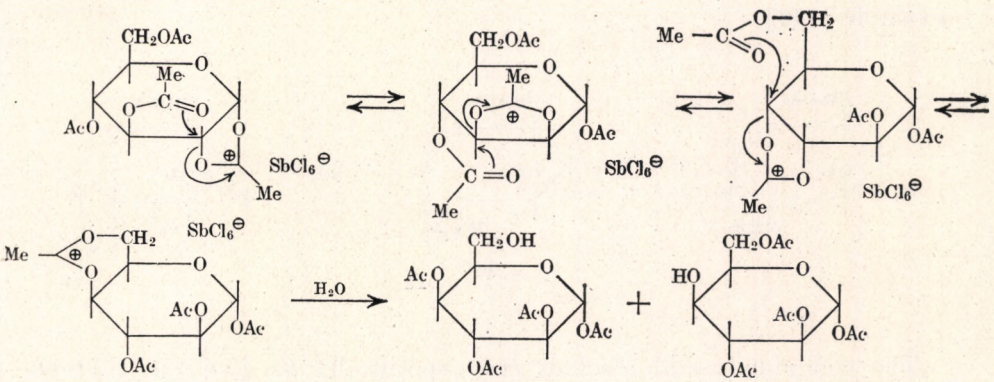
VIII. REACTIONS OF THE COMPOUNDS RELATED TO SUGAR ORTHOESTERS

Some sugar derivatives have structure and reactivity closely related to those of the sugar orthoesters. In the present section selected examples of the compounds of this type will be discussed.

1. Acyloxonium Ions

1,2-Acyloxonium ions have been many times mentioned above as the hypothetical intermediates in some nucleophilic substitution reactions at the glycoside centre of sugars, and in the reactions of orthoesters. In many instances (see, e.g. [32]), the compounds of a non-carbohydrate nature of this type have been isolated and characterized as stable salts. Recently, Paulsen, Heyns *et al.* have for the first time described the isolation of such cations in the carbohydrate series [22, 138–141]. These have been prepared

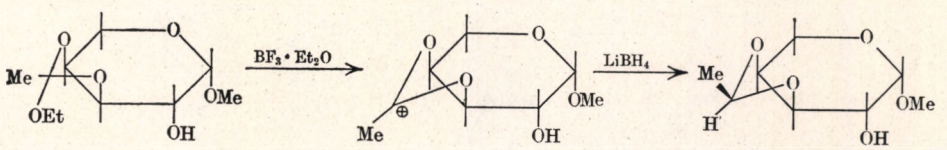
from glucose, galactose and arabinose as hexachloroantimonates or fluoroborates of the corresponding peracetylated acyloxonium cations by reacting peracetylated sugars or acetylated glucosyl halides with antimony pentachloride or boron trifluoride etherate. The reactivity of the compounds is quite unusual. For example, in the case of glucose the hydrolysis of the hexachloroantimonate with aqueous sodium acetate involves consecutive participation of the acetyls at C_3, C_4 and C_6, so that inversion of the configuration at C_2, C_3 and C_4 takes place to give the derivatives of D-idose:



It is interesting that the same acyloxonium ion but stabilized with fluoroborate affords (under the same hydrolytic conditions) only the derivatives of D-glucose. Evidently, the development of these studies will open new important synthetic possibilities. In particular, the above synthesis of the difficultly available D-idose is outstandingly simple and convenient.

Although the present review does not cover sugar orthoesters whose orthoester group is formed only by alcoholic sugar hydroxyls, the following reaction seems interesting as it could also be applied in principle to usual sugar orthoesters.

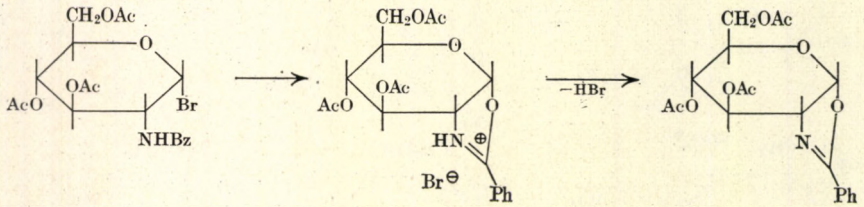
The treatment of 3,4-ethylorthoacetyl-β-methyl-L-arabinopyranoside with boron trifluoride etherate affords amorphous acyloxonium fluoroborate, whose reduction with lithium borohydride proceeds smoothly and stereospecifically to give the corresponding 3,4-O-ethylidene derivative [42]:



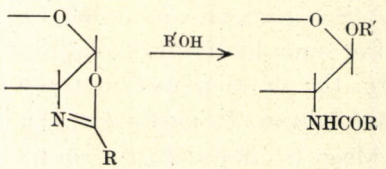
It is anticipated that the application of this reaction to 1,2-orthoesters will be of preparative importance.

2. *Oxazoline Derivatives*

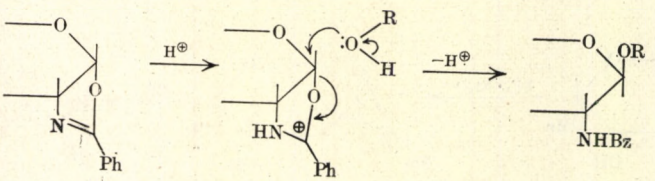
The closest analogues of orthoesters in reactivity are the carbohydrate derivatives obtained by rearrangement of peracetylated 2-amino-2-deoxy-sugar glycosylhalides, followed by elimination of hydrogen halide, for example [143]:



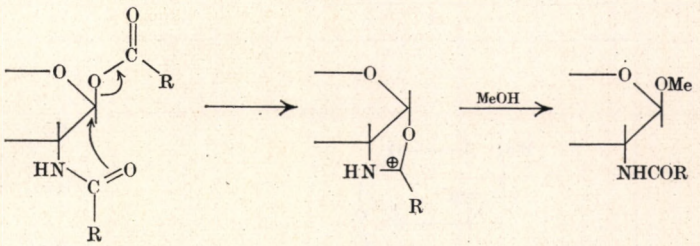
The compounds add alcohols stereospecifically to give the 1,2-*trans*-glycosides:



The reaction was observed for the first time by Micheel [143–145], who synthesized by this method serine glucosaminide [143], and later was used by other authors [146, 147]. Obviously, this glycosylation is analogous to the reactions of sugar orthoesters, as it must proceed via the cation of the protonated form of oxazoline:



It is interesting that oxazoline derivatives can also be obtained directly from the peracetylated aminosugar derivatives by their solvolysis and subsequent ring opening to afford the glycosides [148]:



3. Conclusion

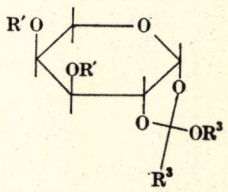
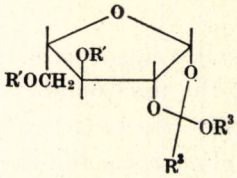
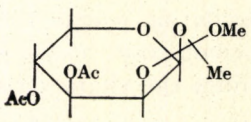
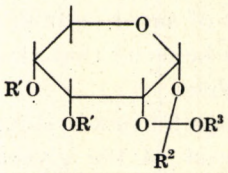
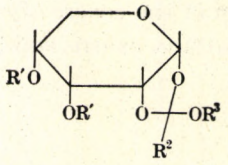
The fundamental difference between sugar orthoesters and the other glycosylating agents is the fact that the nucleophilic substitution at the glycosidic centre of these compounds proceeds via charged intermediates (acyloxonium ions), whose centre of charge is not the glycoside centre, while the configuration of the latter is fixed by the cyclic system. Hence, unlike acylhalogenoses and other glycosylating agents, they are practically incapable of S_N1 reaction, and no 'racemization' at the glycoside centre occurs. It is this difference that determines the characteristic stereospecificity of substitution of orthoesters.

In this respect, close analogues of sugar orthoesters are the above-mentioned oxazoline derivatives. It is believed that this type of reagent is not limited to orthoesters and oxazolines.

Hence, it seems interesting to look for new systems of optimum structure for stereospecific reactions, at least at the glycoside centre. Obviously, during this research one must remember the characteristic features of the reaction desired, i.e. the separation of the centre of charge from the reaction centre, and the fixation of the reaction centre configuration until the final reaction step.

Table

Physical Properties

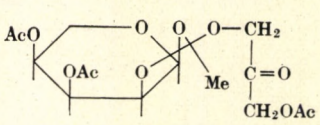
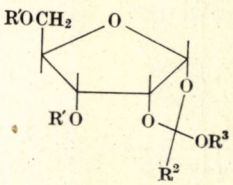
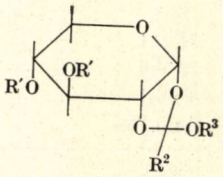
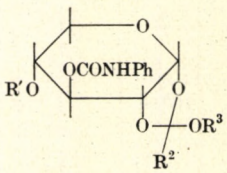
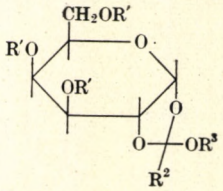
	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
			1. Bicyc (a) Al
1.		Bz	Ph
2.		Ac	Me
3.		Bz	Ph
4.		H	Ph
5.		Bz	Ph
6.		H	Ph
7.		Bz	Me
8.		Bz	n-NO_2Ph
9.		Bz	Ph
10.		H	Ph
11.			
12.		H	Ph
13.		Bz	Ph
14.		Bz	Ph
15.		Ac	Me

* Homogeneous C—Me-*endo*-isomer.

III

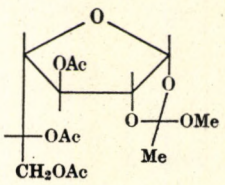
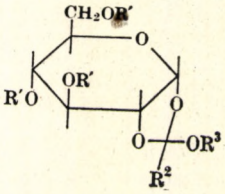
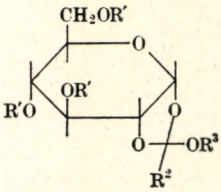
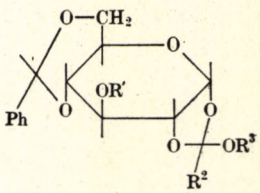
of Sugar Orthoesters

R^3	M. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
lic orthoesters dopentoses			
Me	—	—	[52, 88]
Me	1·4596 (17)	+ 52 ($CHCl_3$)	[52, 88]
t-BuO	128–9	+101·1 ($CHCl_3$)	[79a]
t-BuO	115–7	+ 27·8 ($CHCl_3$)	[79a]
Me	1·5610 (37)	+ 18 ($CHCl_3$)	[53]
Me	—	− 5 ($CHCl_3$)	[49, 53]
Me	120–1	+ 15 ($CHCl_3$)	[49]
Me	155–6	+ 36 ($CHCl_3$)	[49]
t-BuO	130–1	+ 1·5 ($CHCl_3$)	[79a]
t-BuO	148–151	+ 36·8 ($CHCl_3$)	[79a]
	89·5	−103·1 ($CHCl_3$)	[5]
	90–3*	−107·3 ($CHCl_3$)	[63]
$PhCH_2$	103–4	+ 6·6 ($CHCl_3$) + 21 (dioxane)	[15]
$PhCH_2$	—	—	[15]
Me	—	—	[65, 66]
CH_2— | CO | CH_2OAc	97–8	− 11·6 ($CHCl_3$)	[36]

	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
16.			
17.	15+16 (racemate)		
18.		Bz	Me
19.		Bz	Ph
20.		Bz	*o*-NO_2Ph
21.		Bz	Ph
22.		Ac	Me
23.		H	Ph
24.		Ac	Me*
25.		H	Me*
26.		H	Me
27.		Ac	Me
28.		H	Me
			(b) Aldo
29.		Ac	Me
30.		Ac	Me*
31.		H	Me*

* Homogeneous C—Me-*endo*-isomer.

R^3	M. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
	97–8	+ 11·8 ($CHCl_3$)	[36]
	124·5–5·0	0·0	[36]
Me	—	+ 72 ($CHCl_3$)	[49]
Me	—	+ 90 ($CHCl_3$)	[49]
Me	—	+112 ($CHCl_3$)	[49]
$PhCH_2$	—	—	[29]
Me		+ 2·4 ($CHCl_3$)	[149]
Me		+ 31·8 ($CHCl_3$)	[149]
t-BuO	78–9	− 15·3 ($CHCl_3$)	[79a]
t-BuO	89–90	+ 13·1 ($CHCl_3$)	[79a]
Me		+ 14 ($CHCl_3$)	[149]
Me			[149]
Me			[149]
hexoses			
Et	1·4590 (20)	+ 78 ($CHCl_3$)	[51, 52]
t-BuO	96–8	+ 73·2 ($CHCl_3$)	[79a]
t-BuO	82–3	+ 78·4 (H_2O)	[79a]

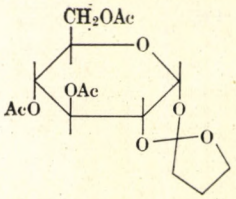
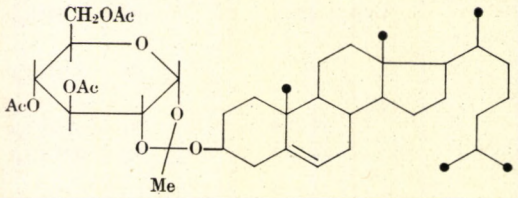
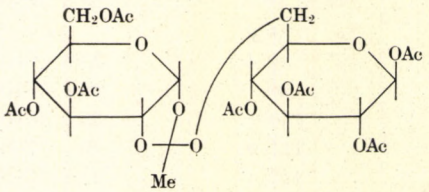
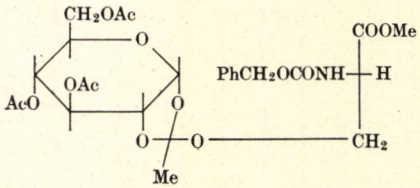
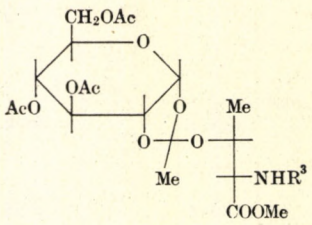
	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
32.			
33.		Ac	Me
34.		Me	Me
35.		Ac	Me
		Ac	Me*
36.		Ac	Me
37.		Ac	Me*
		Ac	Me
38.		Ac	Me
		Ac	Me*
39.		Ac	Me
40.		Ac	Me*
41.		H	Me*
42.		Ac	Ph
43.		Bz	Ph
44.		Bz	Ph
45.		Ac	Me_3C
46.		Ac	MeO
47.		Ac	Me
48.		H	Me
49.		Me	Me

* Homogeneous C—Me-*endo*-isomer.

** Possible mistake in structure—it may really be the corresponding methyl-β.

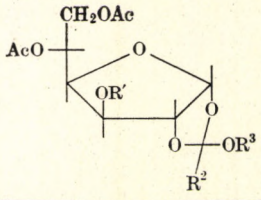
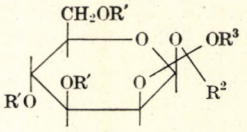
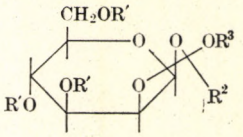
R^3	M. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
	1·4599	+ 24 ($CHCl_3$)	[52, 105]
Me	1·4554 (20)	+ 34 ($CHCl_3$)	[51, 52]
	—	+ 65 ($CHCl_3$)	[54]
Et	—	—	[16]
Et	97–7·5	+ 31 ($CHCl_3$)	[16]
	94–6	+ 32 ($CHCl_3$)	[51, 52]
	94–5; 96–7	+ 30·5 ($CHCl_3$)	[74]
Et	94–6	—	[57]
n-Pr	92–4·5	+ 39·5 ($CHCl_3$)	[16]
	69	+ 30·5 ($CHCl_3$)	[85]
iso-Pr	120–1	+ 30 ($CHCl_3$)	[57]
iso-Pr	117–9	+ 29 ($CHCl_3$)	[81]
t-Bu	152–4	+ 33·8 ($CHCl_3$)	[81]
	150	+ 37·5 ($CHCl_3$)	[85]
t-Bu	152·5–4·5	+ 34·5 ($CHCl_3$)	[57]
Ph	—	—	[57]
	—	+ 39 ($CHCl_3$)	[81]
t-BuO	86–7	+ 10 ($CHCl_3$)	[79a]
t-BuO	120–1	+ 22·8 (H_2O)	[79a]
iso-Pr	—	—	[57]
Me	—	+ 11·25 ($CHCl_3$)	[84]
$PhCH_2$	—	+ 3·2 ($CHCl_3$)	[84]
Me	—	—	[57]
Me	—	—	[57]
Me	148–9	+ 36·0 ($CHCl_3$)	[34]
Me	—	—	[34, 96]
Me**	130–1	— 63·8	[34]

glucoside.

	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
50. 51.			
52.			
53.			
54.			
55. 56.			

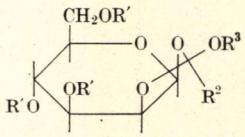
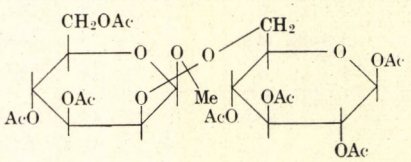
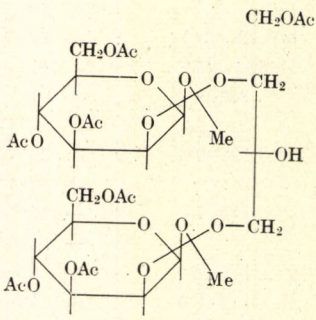
* Homogeneous C—Me-*endo*-isomer.

R^3	M. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
C—O—CH_2-*exo*	—	+ 42 ($CHCl_3$)	[61]
C—O—CH_2-*endo*	116–7	+ 85 ($CHCl_3$)	[61]
	98–100 (dec)	+ 2 ($CHCl_3$)	[51, 52] [98]
	137–8*	+ 39 ($CHCl_3$)	[99]
	—	—	[90, 93]
2,4-di-NO_2Ph	—	+ 28·2 ($CHCl_3$)	[91]
$PhCH_2OCO$	—	—	[91]

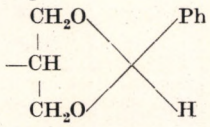
	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
57.		Ac	Me
58.		Ms	Me
59.		Ac	Me
		Ac	Me*
		Ac	Me**
60.		H	Me
61.		Ac	Me
62.		Me	Me
63.		Ac	Me*
64.		H	Me*
		H	Me**
65.		Ac	Me*
66.		Ac	Me*
67.		Ac	Me*

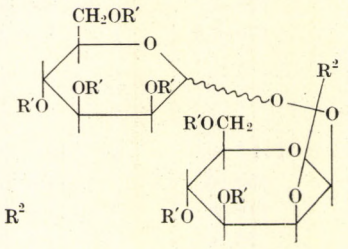
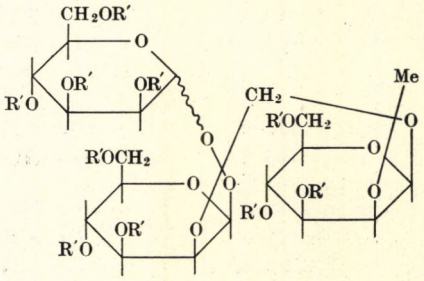
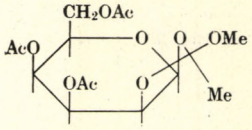
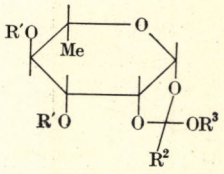
* Homogeneous C—Me-*endo*-isomer.
** Homogeneous C—Me-*exo*-isomer.

R^3	M. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
Et	90	+ 27 ($CHCl_3$)	[74]
Et	65–6	+ 14·5 ($CHCl_3$)	[27]
Me	105	— 26·6 ($CHCl_3$)	[2]
	105	— 31·0 ($CHCl_3$)	[3]
		— 33·4 (MeOH)	
		— 74·0 (C_6H_6)	
	104	— 27 ($CHCl_3$)	[6]
	104–5	— 22·6 ($CHCl_3$)	[76]
Me	106	— 22 ($CHCl_3$)	[43]
	111–3	— 23·5 ($CHCl_3$)	[63]
Me	—	+ 12 ($CHCl_3$)	[43]
Me	—	— 2 (Me_2CO)	[3]
	1·4582 (18)	— 6 (H_2O)	[6]
		— 23° (H_2O)	
Et	81–2	— 27·6 ($CHCl_3$)	[3]
		— 46·5 (EtOH)	
		— 80·2 (C_6H_5)	
		— 38·1 (Et_2O)	
Me	b.p.120°/0·1	— 20 (H_2O)	[6]
	1·4594 (15)	— 11 ($CHCl_3$)	
$PhCH_2$	146–7	— 1 ($CHCl_3$)	[43, 63]
$PhCH_2$	128–30	+ 26·6 (H_2O)	[43]
		+ 42·2 (C_5H_5N)	
$PhCH_2$	115–7	— 14·8 (H_2O)	[43]
		— 17·8 (C_5H_5N)	
n-$IPhCH_2$	178–9	+ 0·9 ($CHCl_3$)	[63]
n-$BrPhCH_2$	182–2·5	+ 1·5 ($CHCl_3$)	[63]
o-$BrPhCH_2$	119–21	+ 3·9 ($CHCl_3$)	[63]

	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
68.		Ac	Me*
69.		Ac	Me*
70.		Ac	Me*
71.		Ac	Me*
71a.		Ac	Me
72.		$PhCH_2$	Me*
73.		$PhCH_2$	Me*
74.		$PhCH_2$	Me*
75.		Bz	Ph
76.		H	Ph
77.		Ac	Me*
78.		H	Me*
79.			
80.			

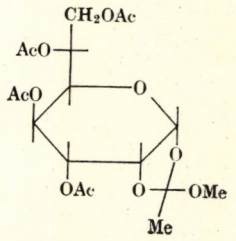
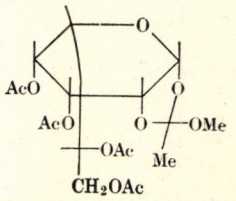
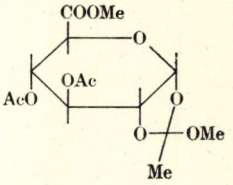
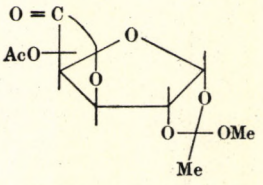
* Homogeneous C—Me-*endo*-isomer.

R^3	M. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
Ph_2CH	150–1	+ 10·4 ($CHCl_3$)	[63]
	176–6·5	— 8·4 ($CHCl_3$)	[63]
iso-Pr	104·5–6	— 13 ($CHCl_3$)	[59]
cyclohexyl	139–41	— 11·4 ($CHCl_3$)	[59]
t-Bu	111–2	— 13·4 ($CHCl_3$)	[79a]
Me	76–8	+ 12·1 ($CHCl_3$)	[59]
	78–81		[45]
iso-Pr	97–100	+ 13 ($CHCl_3$)	[59]
cyclohexyl	87·5–90	+ 13·6 ($CHCl_3$)	[59]
Me	—	—124 (MeOH)	[67]
Me	—	—	[67]
t-BuO	128–9	— 28·9 ($CHCl_3$)	[79a]
t-BuO	133–4	— 12·9 (H_2O)	[79a]
C—Me-*endo*-isomer	172–3	+ 18·3 ($CHCl_3$)	[63]
	168–9	+ 17·1 ($CHCl_3$)	[37]
C—Me-*exo*-isomer	174·0–4·5	— 27·6 ($CHCl_3$)	[37]
C—Me-*endo*-isomer	164–5	— 8·5 ($CHCl_3$)	[63]

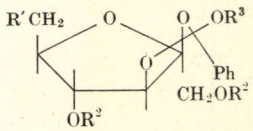
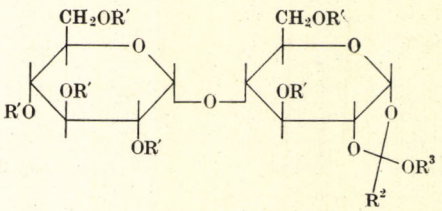
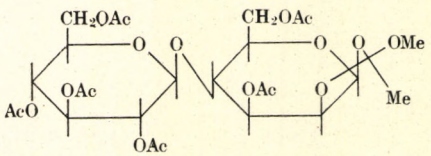
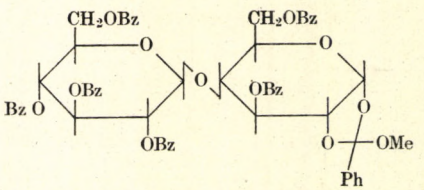
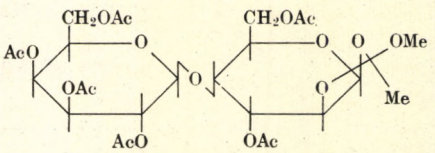
	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
81.		Ac	Me*
82.		H	Me*
83.		Me	Me*
84.	 (C—CH_2-*endo*-isomers)	Ac	—
85.		H	—
86.		Me	
87.			
88.		Ac	Me
		Ac	Me*
89.		H	Me
90.		Me	Me
91.		Ac	Me*
92.		Bz	Ph

* Homogeneous C—Me-*endo*-isomer.

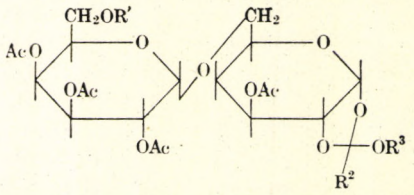
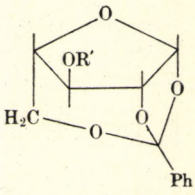
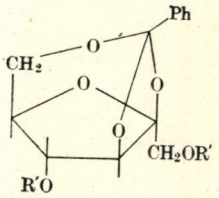
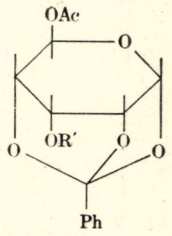
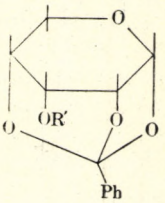
R^3	m. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
—	152–4	— 23·6 ($CHCl_3$)	[77]
—	120	—	[77]
—	142–4	— 36·5 ($CHCl_3$)	[77]
—	216–9	— 26·5 ($CHCl_3$)	[77]
	213–5	— 29 ($CHCl_3$)	[78]
—	156–7	— 5·4 (H_2O)	[77, 78]
	165–6	—	[78]
	91·5–2·5	+ 3·7 ($CHCl_3$)	[56]
Me	83–5	+ 28·05 ($Cl_2CHCHCl_2$)	[1]
	83	+ 35 ($CHCl_3$)	[8]
		+ 35 (EtOH)	
	83–4	+ 35 ($CHCl_3$)	[12]
Me	84–6	+ 34·7 ($CHCl_3$)	[63]
Me	143–4	+ 16·3 (H_2O)	[1]
	—	+ 10 (EtOH)	[8]
	140–1	+ 16·4 (H_2O)	[12]
Me	67 b.p.90°/0·1 $1·4510$ (17)	+ 36 (H_2O)	[8,12]
t-BuO	121–2	+ 35·5 ($CHCl_3$)	[79a]
Me	174–5	+ 37·5 ($CHCl_3$)	[72]

	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
			(c) Aldo
93.			
94.			
			(d) Uron
95.			
96.			
			(e) Keto
97.	D-Fructopyranose methyl orthoacetate		
98.	—„— triacetate		

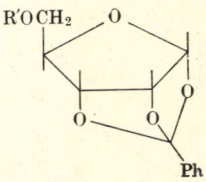
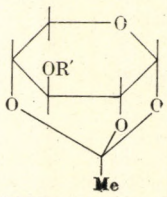
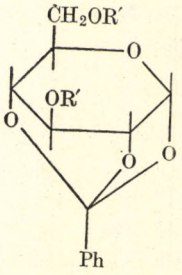
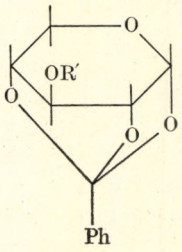
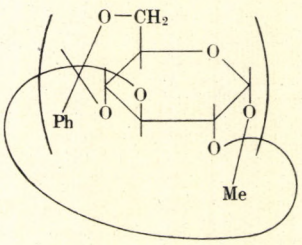
R^3	m. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
heptoses			
	112	+ 43 ($CHCl_3$)	[60]
	106	+ 3·2 ($CHCl_3$)	[13]
ic acids			
	118	+ 54·0 ($CHCl_3$)	[55]
	110–1	+112·5 ($CHCl_3$)	[55]
ses			
	—	— 12·4 ($CHCl_3$)	[68]
	—	— 13·6 ($CHCl_3$)	[68]

	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
99.		BzO	Bz
100.		BzO	Bz
101.		BzO	Bz
102.		BzO	Bz
103.		BzO	Bz
104.		HO	H
105.		MsO	Ms
106.		TsO	Ts
107.		J	Ms
108.	Ethyl orthoacetyl-L--sorbopyranose triacetate		
			(f) Disac
109.		Ac	Me
110.		H	Me
111.		Ac	Me
112.		Ac	Me
113.			
114.			
115.			

R^3	M. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
Me	91–2	0·99 ($CHCl_3$)	[70]
Et	113–5/126–7	— 1·5 ($CHCl_3$)	[71]
n-Pr	140–1	— 4·17 ($CHCl_3$)	[70]
cyclohexyl	111–2	— 9·4 ($CHCl_3$)	[70]
$PhCH_2$	145–7	+ 5·8 ($CHCl_3$)	[70]
$PhCH_2$	—	+ 17·3 ($CHCl_3$)	[70]
$PhCH_2$	96–7	+ 17·4 ($CHCl_3$)	[70]
$PhCH_2$	95–6	+ 21·0 ($CHCl_3$)	[70]
$PhCH_2$	100–2	— 7·4 ($CHCl_3$)	[14]
	—	—	[64]
charides			
Me	163–4	+101·6 ($CHCl_3$)	[4]
	164	+ 98·8 ($CHCl_3$)	[73]
Me	—	+117·12 (H_2O)	[4]
Et	142–3	—	[4]
$PhCH_2OCONH$—CH(COOMe)—CH_2	—	+ 69 ($CHCl_3$)	[92]
	167	— 12·7 ($CHCl_3$)	[150]
	172–3	+ 32·4 ($CHCl_3$)	[87]
	121–2	+ 25·3 ($CHCl_3$)	[13]

	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
116.		Ac	Me
117.		H	Me
118.	Turanose methylorthoacetate		
119.	Turanose methylhexaacetate		
			2. Tricyc
120.		H	
121.		Ac	
122.		Bz	
123.		H	
124.		Ac	
125.		Bz	
126.		Ms	
127.		Ts	
128.		H	
129.		Ac	
130.		Bz	
131.		Me	

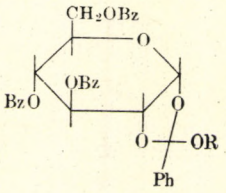
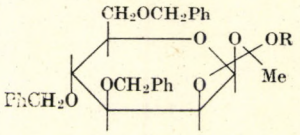
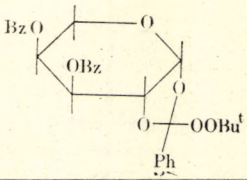
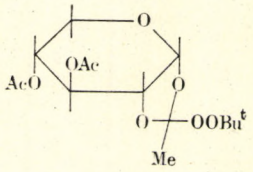
R^3	m. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
Me	—	+104 ($CHCl_3$)	[83]
Me	—	+118 ($CHCl_3$)	[82, 83]
	137	+114·6 (H_2O)	[41, 94]
	162–4	+ 80 ($CHCl_3$)	[41, 94]
lic and macrocyclic orthoesters			
	148–9	+ 28 ($CHCl_3$)	[53]
	128–30	+ 62 ($CHCl_3$)	[53]
	143–5	+ 75 ($CHCl_3$)	[53]
	113 4	— 33·7 (H_2O)	[70]
	73–6/84	— 38·2 ($CHCl_3$)	[70]
	136–7	— 52·6 ($CHCl_3$)	[70]
	96–7	— 12·95 ($CHCl_3$)	[14]
	101–3	— 43·9 ($CHCl_3$)	[14]
	150–1	+ 68·4 ($CHCl_3$) + 75·2 (H_2O-dioxane)	[15]
	96–7 t. subl. 150/0·1	+ 83·5 ($CHCl_3$)	[15]
	170–1	+ 78·1 ($CHCl_3$)	[15]
	103–5	+ 84·3 ($CHCl_3$)	[15]

	Structure (or name for compounds with unknown structure)	R^1	R^2
1	2	3	4
132.		H	
133.		Ac	
134.		Bz	
135.		Ms	
136.		H	
137.		Ac	
138.		Bz	
139.		PhNHCO	
140.		H	
141.		Ac	
142.		Bz	
143.		PhNHCO	
144.		$PhCH_2$	
145.		H	
146.		Bz	
147.		Me	
148.			Homogeneous

R^3	m. p. (°C) or n_D (°C)	$[\alpha]_D$ (solvent)	Ref.
5	6	7	8
	106–8	+ 41·0 ($CHCl_3$)	[17]
	117–8	+ 42 ($CHCl_3$)	[17]
	182–3	+ 43·4 ($CHCl_3$)	[17, 18]
	119–24	+ 42 (CH_2Cl_2)	[17]
		+ 58·4 ($CHCl_3$)	[149]
	67–8	+ 67·3 ($CHCl_3$)	[149]
	83–84	+ 64 ($CHCl_3$)	[149]
	93–94	+ 55 ($CHCl_3$)	[149]
	144–145	+ 67 ($CHCl_3$)	[149a]
	159–161	+ 55 ($CHCl_3$)	[149a]
	136–137	+ 52 ($CHCl_3$)	[149a]
	128–129	+ 53 ($CHCl_3$)	[149a]
	51– 53	+ 44·4 ($CHCl_3$)	[149a]
	146–150	+ 25·5 ($CHCl_3$)	[149b]
	135	0·0 ($CHCl_3$)	[149b]
	100		[149b]
	281–3	+120 ($CHCl_3$)	[95, 96]
C—Me-*endo*-isomer			

Table

Syntheses of Compounds of Low

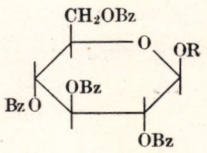
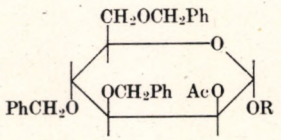
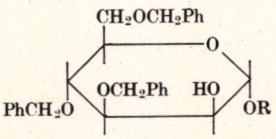
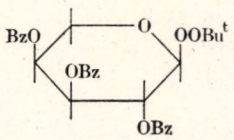
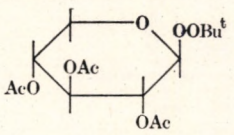
	Starting compounds		Conditions**
1	2		3
			1. Lower
1. 2.		R = Me	A
		R = $PhCH_2$	A
3. 4. 5.		R = Me	B
		R = iso-Pr	B
		R = cyclohexyl	B
			2. Tert-Butyl
6.			C
7.			C

* From the works concerning studies of the effect of reaction conditions on the results,

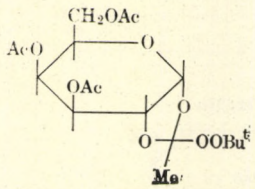
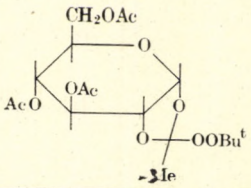
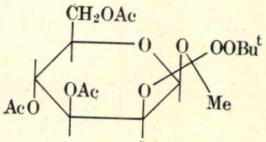
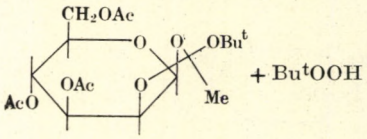
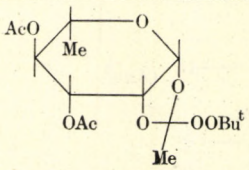
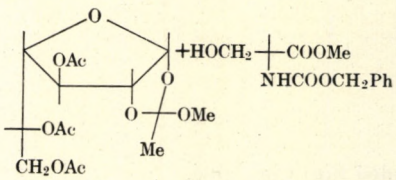
** A — solvent $MeNO_2$, catalyst $HgBr_2$ + HCl, 20°C; B — solvent CH_2Cl_2, catalyst TsOH, $AgClO_4$ + $HClO_4$, 20°C; E — solvent $MeNO_2$, catalyst $HgBr_2$, boiling; F — solvent $ClCH_2CH_2Cl$, double-stage scheme; G — solvent: chlorobenzene, catalyst: 2,6-lutidinium perchlorate,

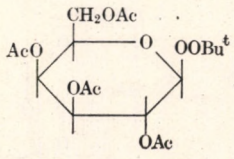
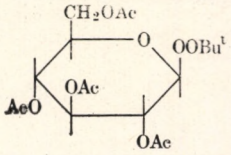
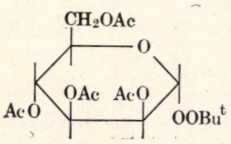
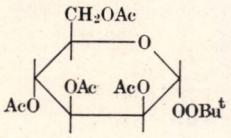
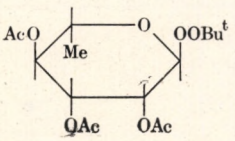
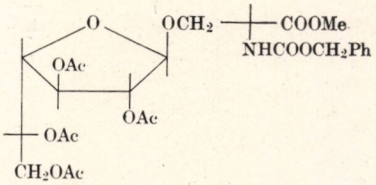
IV

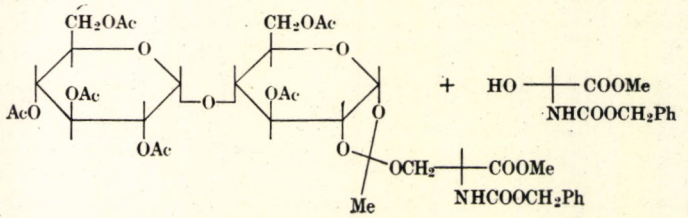
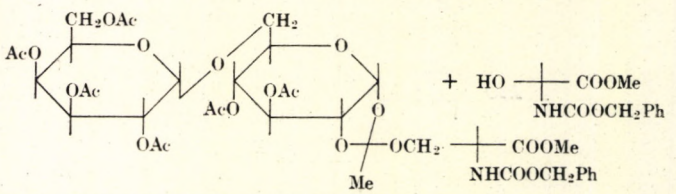
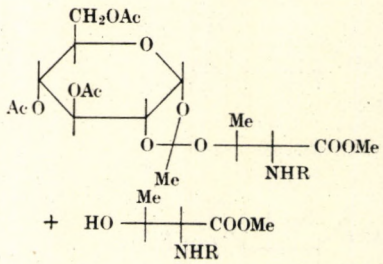
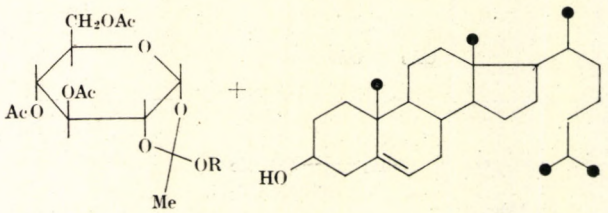
*Molecularity by Orthoester Method**

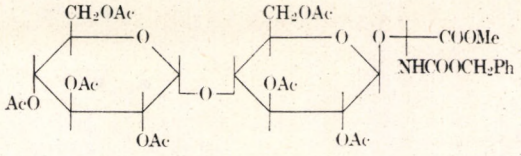
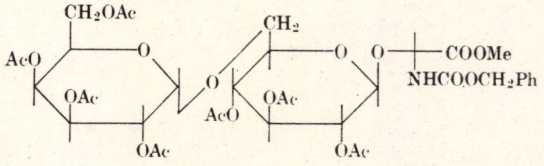
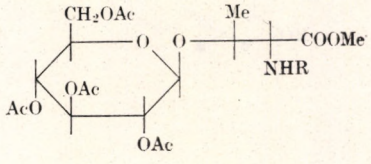
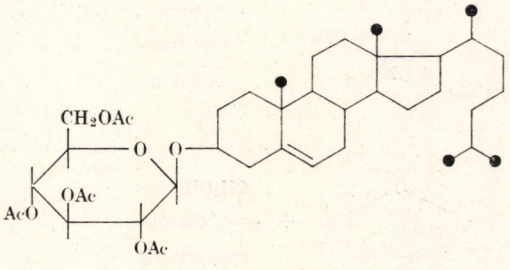
Reaction product for which the yield was determined			Yield %	Ref.
4			5	6
glycosides 	R = Me		57	[84]
	R = $PhCH_2$		48	[84]
 I +  II	R = Me	I	83	[45]
		II	7	[59]
	R = iso-Pr	I	74	[59]
		II	II	
	R = cyclohexyl	I	79	[59]
		II	8	
peroxyglycosides 			71	[79a]
			78	[79a]

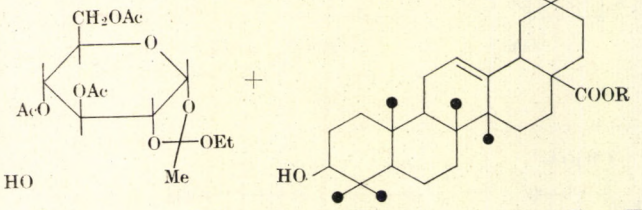
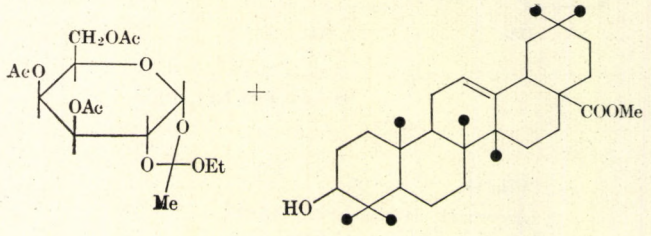
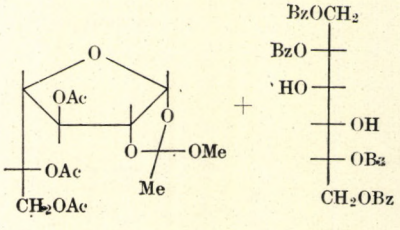
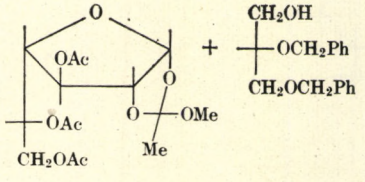
only the best yields are included into the Table.
46·5°C; C — solvent CH_2Cl_2, catalyst $BF_3 \cdot Et_2O$, 20°C; D — solvent benzene, catalyst catalyst $C_5H_5NHClO_4$ (or 4,4′-dipyridyl perchlorate + *p*-toluene sulfonic acid), boiling, boiling.

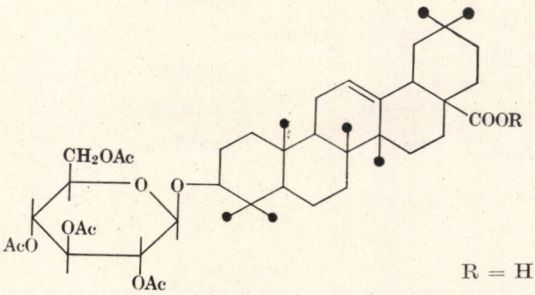
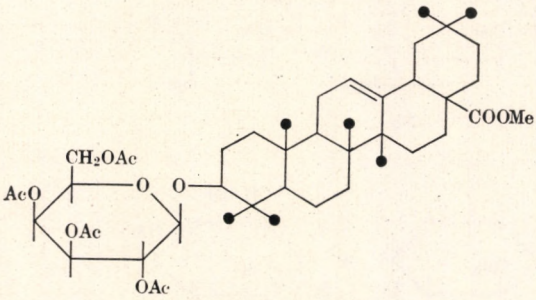
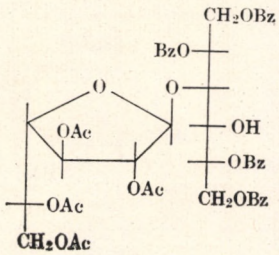
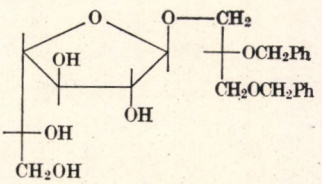
	Starting compounds	Conditions**
1	2	3
8.		C
9.		C D
10.		C D
11.		D
12.		C D
		3. O-glycosides of
13.		E

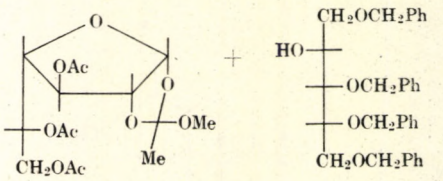
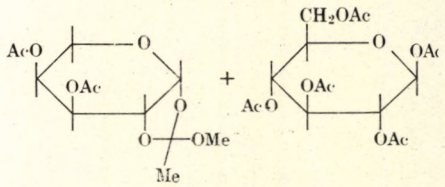
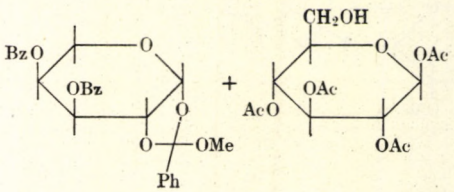
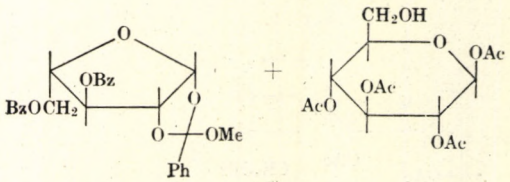
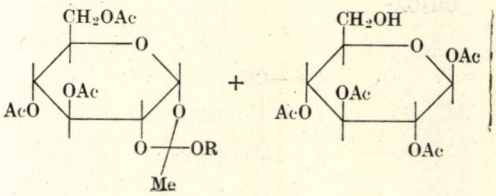
Reaction product for which the yield was determined	Yield %	Ref.
4	5	6
	54	[79a]
	48 52	[79a] [79a]
	75 89	[79,79a]
	30	[79a]
	72 86	[79,79a] [79,79a]
hydroxyamino acids		
	17·3	[107]

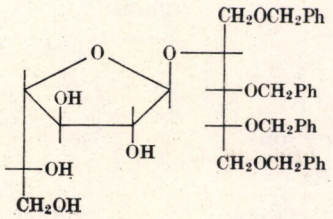
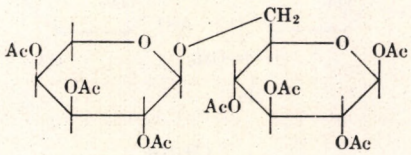
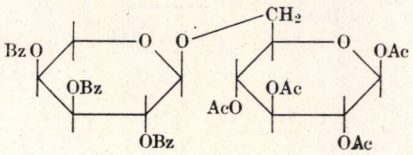
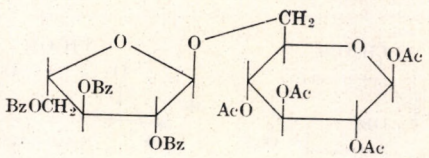
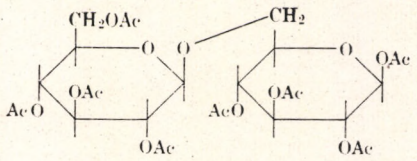
1	Starting compounds 2	Conditions** 3
14.		F
15.		F
16.	R = 2,4-di-NO_2-Ph 	F F
17.	R = $PhCH_2OCO$	(150°)
	4. Glycosides of polycyclic	
18.	 R = Me, Et	E

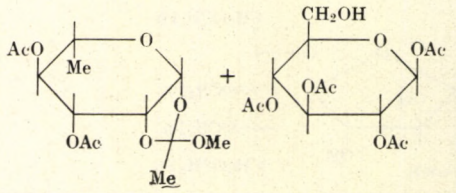
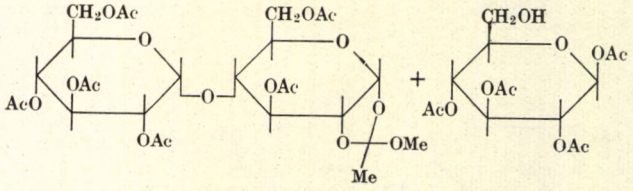
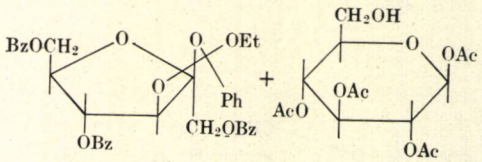
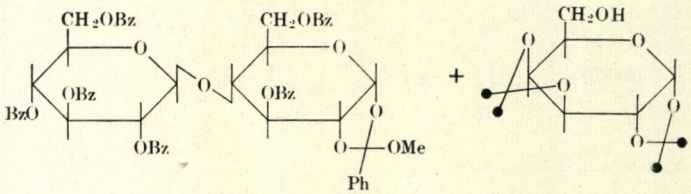
Reaction product for which the yield was determined	Yield %	Ref.
4	5	6
	23	[92]
	32·5	[92]
R = 2,4-di-NO_2-Ph	41	[91]
		
R = $PhCH_2OCO$	37	[91]
compounds		
	45	[52,98] [104]

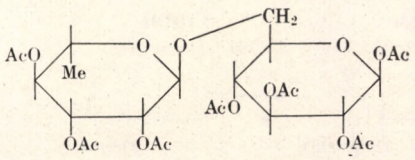
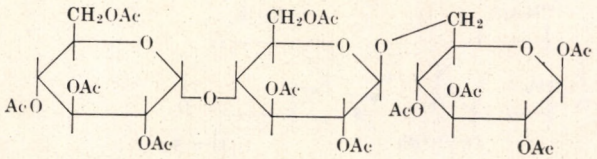
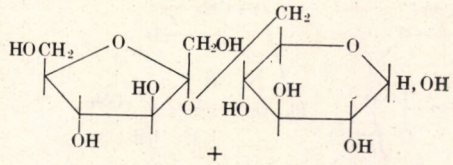
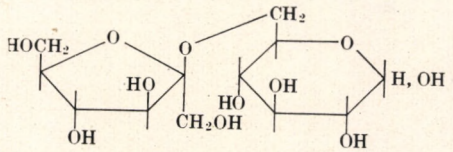
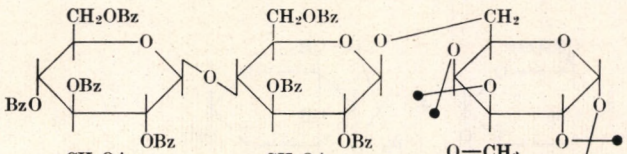
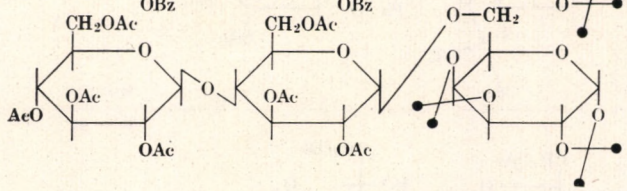
N°	Starting compounds	Conditions**
1	2	3
19.	R = Me 	E
20.	R = H	E
21.		E
	5. Glycosides	
22.		E
23.		E

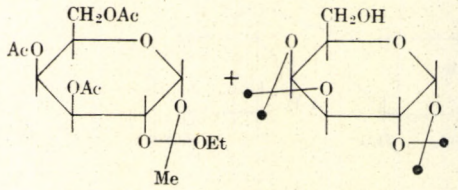
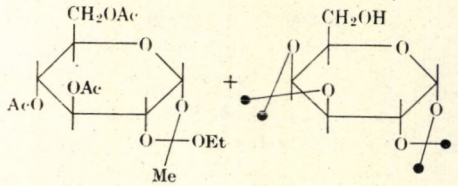
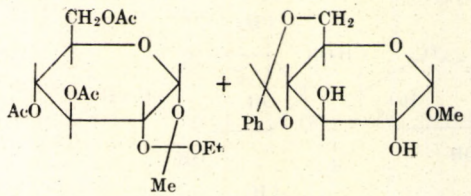
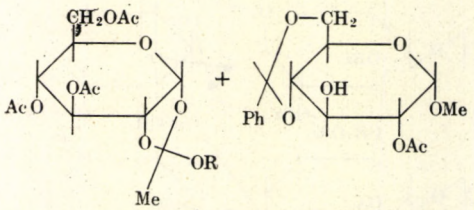
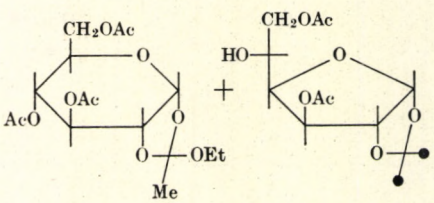
Reaction product for which the yield was determined	Yield %	Ref.
4	5	6
 R = Me R = H	45 28	[109] [109]
	44	[109]
of polyols 	28·3	[52, 105]
	54	[110]

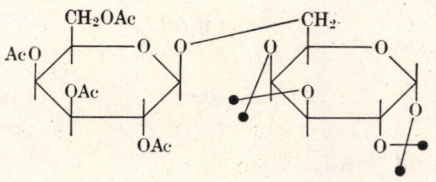
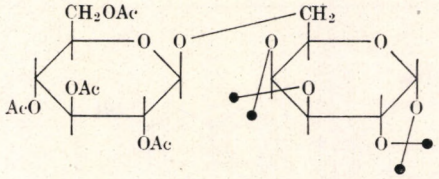
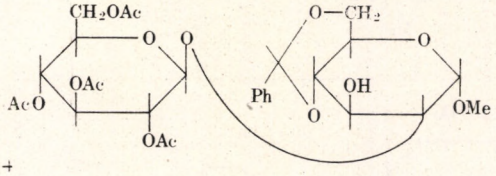
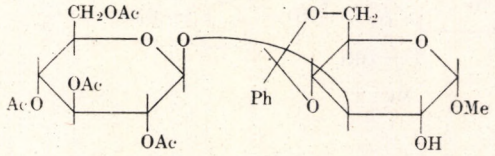
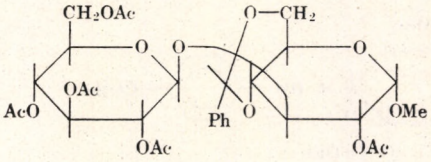
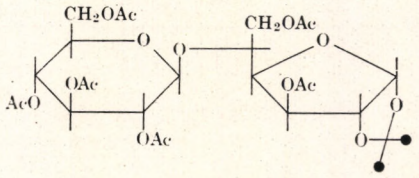
N°	Starting compounds	Conditions**
1	2	3
24.		E
		6. Oligosa
25.		E
26.		E
27.		E
28.	 R = Et	E
29.	R = Et	F
	R = Bu^t	F

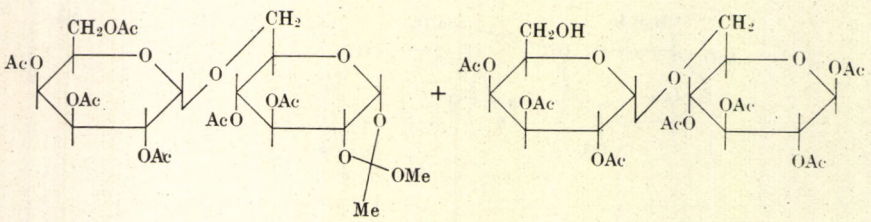
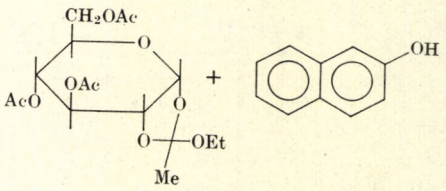
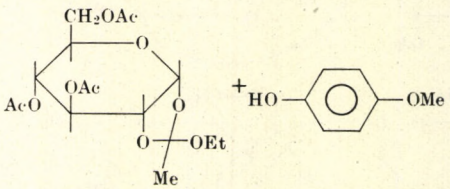
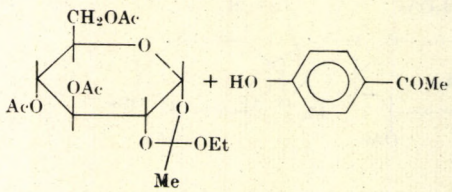
Reaction product for which the yield was determined	Yield %	Ref.
4	5	6
	26·8	[111]
ccharides		
	54·5	[52, 88]
	93	[52, 88]
	90	[52, 88]
	35 60·5 75	[52, 106] [99] [114]

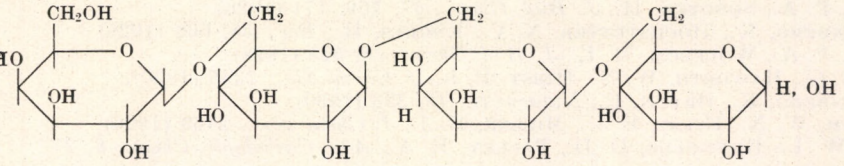
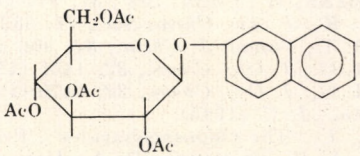
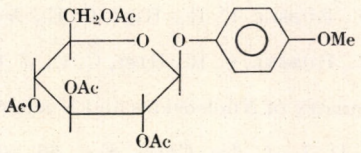
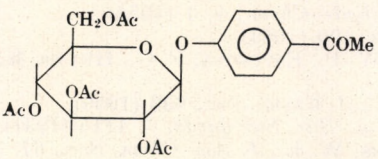
N°	Starting compounds	Conditions**
1	2	3
30.		E
31.		E
32.		E
33.		E

Reaction product for which the yield was determined	Yield %	Ref.
4	5	6
	45	[52, 106]
	55	[52, 87]
	16	[112]
	6	
	71	[87]
	4	

N°	Starting compounds	Conditions**
1	2	3
34.		E
35.		E
36.		E
37.	 R = Et	E
38.	R = Bu^t	G
39.	 R = Et	E
40.	R = Bu^t	G

Reaction product for which the yield was determined	Yield %	Ref.
4	5	6
	64	[52, 104, 106]
	51·5	[52, 104, 106]
 + 	21 7	[52, 106, 108]
	28 50	[52, 108] [114]
	10 25	[52, 106] [114]

N°	Starting compounds	Conditions**
1	2	3
41.		E F
		7. Aryl-
42.		F
43.		F
44.		F

Reaction product for which the yield was determined	Yield %	Ref.
4	5	6
	—	[82]
glycosides 	55	[113]
	21	[113]
	7·5	[113]

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N. M. MOLLOV
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THALICTRUM ALKALOIDS

I. INTRODUCTION

(N. M. Mollov and V. St. Georgiev)

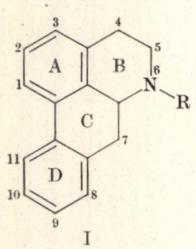
The genus Thalictrum combines different species of perennial herbaceous plants distributed in the temperate geographical regions. They are found at various heights, from 200 to 2000 and even up to 3000 metres above sea level, and under various ecological conditions.

It is well known that the plants belonging to this genus are rich in different isoquinoline alkaloids. It should also be noted that one species of Thalictrum found in different regions of the world, i.e. Asia, Europe, exhibits qualitatively different alkaloid compositions. Inversely, the same alkaloids are often present in different Thalictrum species, for example, thalicarpine — in *Thalictrum dasycarpum*, *Thalictrum minus*, *Thalictrum fendleri*; thalidezine — in *Thalictrum fendleri*, *Thalictrum simplex*; hernandezine — in *Thalictrum hernandezii*, *Thalictrum simplex*.

The first reports concerning phytochemical studies of the Thalictrum plants date from the end of the 19th century. From the roots of *Thalictrum macrocarpum* Gren. was isolated thalicrine, a substance with basic properties and a toxicity similar to that of aconitine [1]. The alkaloid berberine was isolated from *Thalictrum flavum* [1]. In the early 1930's in the USSR began the study of the alkaloid composition of various species of Thalictrum. The school of Orekhov showed the presence of the alkaloids in *Thalictrum minus*, *Thalictrum petaloideum*, *Thalictrum angustifolium*, *Thalictrum flavum* and *Thalictrum simplex*, distributed on the territory of the Soviet Union.

The last 10–15 years is a period typical for the detailed investigation of the alkaloid composition of these plants. The most intensive work is carried out in Japan (Tomita, Furukawa, Tomimatsu), the USA (Shamma, Kupchan, Doskotch, Beal), the USSR (Yunusov,) and Bulgaria (Mollov).

The alkaloids found in the various plants of the genus Thalictrum belong to the isoquinoline type. Monomolecular isoquinoline type alkaloids belonging to the aporphine (I), benzylisoquinoline (II), protoberberine (III) and protopine series have been isolated.



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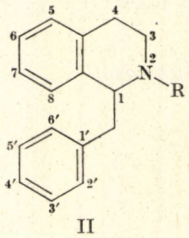

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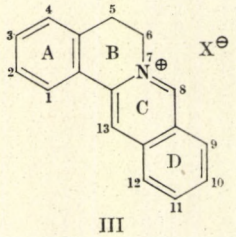
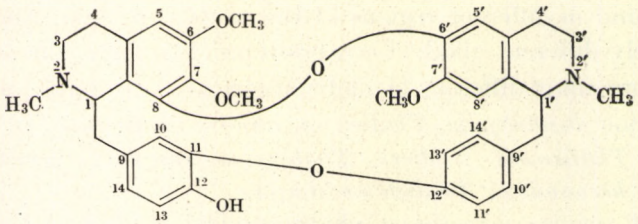


III

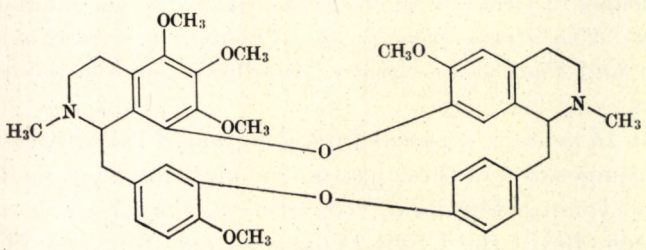
The majority of the compounds isolated are bismolecular isoquinoline alkaloids. Their classification into three groups can be based on the nature of the isoquinoline components:

1. *Biscoclaurine alkaloids.* Their molecules contain two coclaurine moieties (or their derivatives), bridged by two ether oxygens;* for example thalicberine (IV).



IV

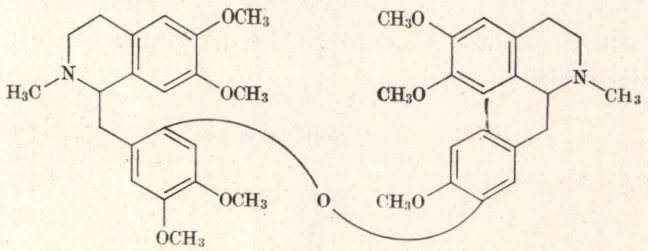
2. *Coclaurine-thalifendlerine alkaloids.* In this case the two ether oxygens join together coclaurine and thalifendlerine moieties (or their derivatives)* for example hernandezine (V).



V

* It is possible to introduce additional classification, based on the position of the bridging oxygen in each of the mentioned groups.

3. *Aporphine-benzylisoquinoline alkaloids.* These molecules contain a benzylisoquinoline part bonded by means of an ether oxygen to an aporphine one; for example, thalicarpine (VI).

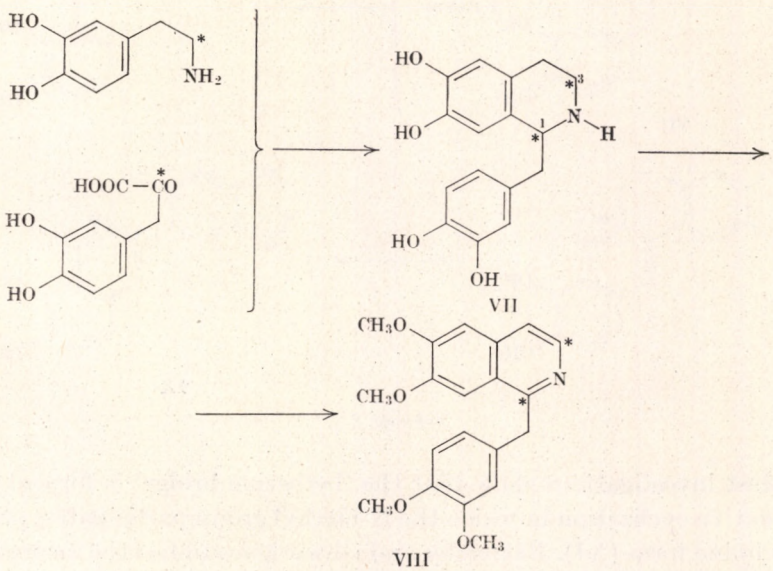


VI

1. Biogenesis

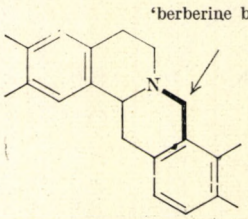
Considerable interest has also been focussed lately on the study of the biosynthesis of various isoquinoline alkaloids [2, 3, 4].

At present it is known that the formation of the benzylisoquinoline alkaloids takes place by way of bonding of two C_6-C_2 units, derivatives of tyrosine (Scheme 1). Thus feeding (±)-tyrosine-(2-^{14}C) to *Papaver somniferum* leads to the synthesis of papaverine (VIII) in which C-1 and C-3 are equally radioactive. A nearer precursor of papaverine is norlaudanosoline (VII).

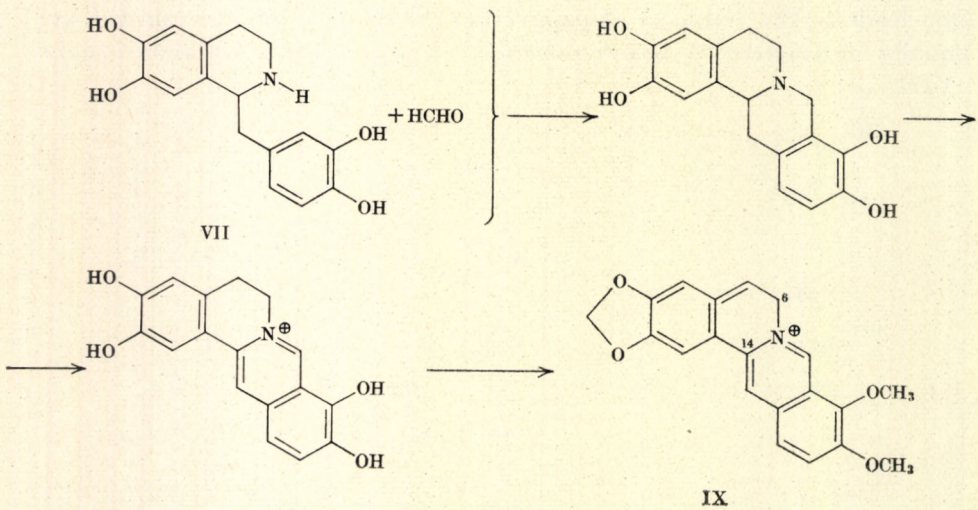


Scheme 1

The biosynthesis of protoberberine alkaloids is closely related to that of the benzylisoquinoline ones. Administration of (±)-tyrosine-(2-^{14}C) to *Hydrastis canadensis* plants leads to the synthesis of berberine (IX) equally labelled at C-6 and C-14 [5]. Of considerable importance in the elucidation of the biosynthesis of these alkaloids is the determination of the origin of the 'berberine bridge'.



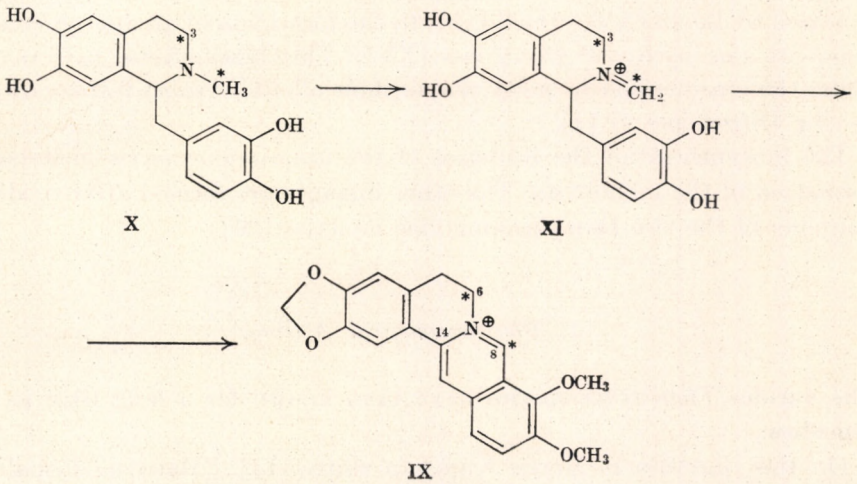
It was considered until quite recently, that the formation of this 'berberine bridge' is due to a Mannich type reaction between the benzylisoquinoline precursor, for instance norlaudanosoline (VII), and a biological equivalent of formaldehyde [6, 7, 8] (see Scheme 2).



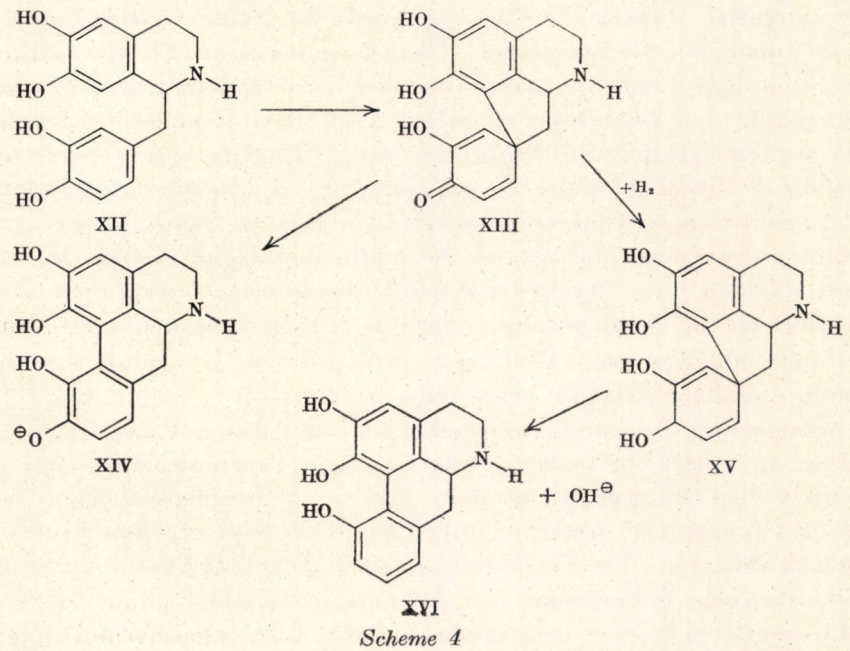
Scheme 2

The latest investigations show that the 'berberine bridge' is formed during an oxidative cyclization in which the N-methyl group participates, probably via its imine form (XI). Battersby and co-workers studied the incorporation of (±)-laudanosoline (X) labelled at C-3 (64% of the total activity) and at

the N-methyl group (36% of the total activity). The isolated berberine (IX) gives 34% of its total activity at C-8 [9]. This result, as well as other studies, confirm unequivocally the conception of the N-methyl origin of the 'berberine bridge' [10, 11, 12] (Scheme 3).



Scheme 3



Scheme 4

The aporphine alkaloids are formed by the joining of the two aromatic nuclei in one benzylisoquinoline type precursor [2, 13]. This process takes place by phenolic oxidation and formation of an intermediate containing a six-membered ring dienone (XIII). This intermediate isomerizes into an aporphine alkaloid (XIV). The isomerization may be preceded by hydrogenation to dienol (XV), which leads to the formation of an aporphine alkaloid with one hydroxyl group less (XVI). This biosynthetic pathway explains the considerable diversity of aporphine alkaloids substituted variously in ring D (Scheme 4) [4].

The biosynthesis of the bis-bases of the isoquinoline series includes the formation of the monobases. The ether linkages are formed after oxidative coupling of the two benzylisoquinoline moieties [29].

2. *Pharmacological Activity*

The various *Thalictrum* species have been known for a long time as folk remedies.

On this question S. Morris Kupchan writes [14]: "Many medicinal uses of Thalictrum species in folk remedies have been recorded. *Thalictrum foliosum* DC. is found throughout the Himalayas and taken as a tonic, aperient, purgative, diuretic, febrifuge, a remedy for atonic dyspepsia, and used as an application for ophthalmia. *Thalictrum thunbergii* DC. is used in Japan as a home remedy against stomach ache and diarrhea. *Thalictrum collinum* L. and *Thalictrum silvaticum* Koch. have been used in Ukrainian folk medicine as diuretics. *Thalictrum fendleri* Engelm. was prepared by the Indians of Nevada as a tea to cure gonorrhea; a decoction of the root was used against colds. *Thalictrum minus* L. is used in South Africa to treat fevers. Intravenous injection of the hydrochlorides of the extract of the total alkaloids from *Thalictrum minus* L. has recently been shown to exert an effect on the blood pressure and pulse of frogs, cats, and dogs". Bulgarian folk medicine uses *Thalictrum aquilegifolium* L. against diphtheria, jaundice and for reducing nerve tension [15].

Alkaloid mixtures or individual alkaloids isolated from various Thalictrum species have been the object of pharmacological screening since only quite recently. In 1956 appeared a report dealing with the pharmacological activity of a preparation obtained from *Thalictrum minus* L. found in the Armenian SSR. [16]. The *Thalictrum minus* L. distributed in the Byelorussian SSR. contains phytoncides and exhibits bactericidal properties against gram-positive and gram-negative bacteria [17]. The pharmacological exam-

ination of the alkaloids of *Thalictrum minus* L., carried out in Bulgaria, shows that they cause a significant, but transient hypotensive effect connected, to a certain extent, with the excitation of the M-cholinoreactive systems. A more detailed investigation of their hypotensive action indicates that it is associated with the noradrenaline metabolism. These alkaloids also possess spasmolytic and diuretic properties [18, 19, 20, 21].

The alkaloid thalsimine isolated from *Thalictrum simplex* L. has hypotensive, tranquillizing, cholinomimetic and adrenolytic properties [22, 23, 24]. Another alkaloid from the same plant, thalsine, also shows hypotensive and adrenolytic properties [25]; the alkaloid hernandezine exhibits a two-phase effect on the blood pressure; the latter increases or decreases depending on the administered dose [16].

A thorough pharmacological evaluation of *Thalictrum foetidum* L. has been carried out in the Soviet Union. It is reported that its alkaloid mixture lowers the blood pressure, produces slight spasmolytic effects in the intestinal muscle and also, depending on the dose and time of its administration, initially depresses the conditioned reflexes and subsequently heightens the excitation of the central nervous system [17, 22, 23].

Clear spasmolytic action caused by the thalisopine alkaloid isolated from *Thalictrum isopyroides* is reported for the first time for Thalictrum alkaloids by a group of Soviet chemists [24].

The alkaloid thalicarpine isolated from *Thalictrum dasycarpum*, *Thalictrum revolutum*, *Thalictrum minus* and *Hernandia ovigera* L. has been evaluated pharmacologically in the United States. It is reported to lower, but not significantly, the blood pressure and not to exhibit anti-inflammatory, anticoagulant, hypoglycemic and diuretic properties [14, 26, 27].

Pharmacological studies of the alkaloid mixture obtained from *Thalictrum rochebrunianum* have indicated that doses of 2 mg/kg introduced intravenously lower the blood pressure [28].

It can be said in conclusion that the alkaloids obtained from Thalictrum plants possess a variety of clearly exhibited pharmacological properties which make them of considerable promise for medicinal use.

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II. STRUCTURE AND PROPERTIES

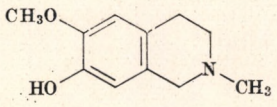
(N. M. Mollov and H. B. Dutschewska)

1. Isoquinoline Alkaloids

It was in 1969 when for the first time some simple isoquinoline alkaloids were found in Thalictrum species. From the four alkaloids isolated so far only corypalline was known; the structures of the other three have been elucidated recently.

(i) Corypalline

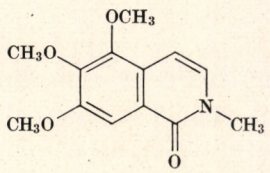
Corypalline, $C_{11}H_{15}O_2N$, m.p. 168°C, $[\alpha]_D$ 0°, had been isolated earlier from *Corydalis* species [1], now it was found in *Thalictrum dasycarpum* Fisch et Lall. [2]. Its structutre is represented by I.



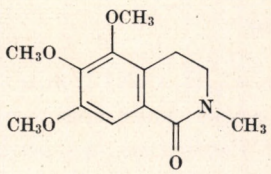
I

(ii) Thalactamine

Thalactamine, $C_{13}H_{15}O_4N$, m.p. 112–4°C, has been isolated from the above-ground parts of *Thalictrum minus* L. [3]. The NMR, IR and mass spectral data are in good agreement with structure II. Hydrogenation of the double bond at C_3-C_4 under high pressure and high temperature gave compound III, which was identical with a synthetic sample.



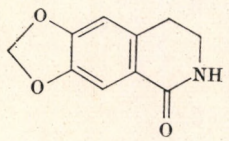
II



III

(iii) Noroxyhydrastinine

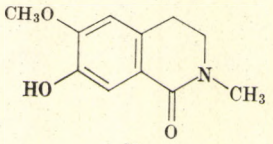
Noroxyhydrastinine, $C_{10}H_9O_3N$, pale yellow crystals, m.p. 182–3°C (methanol), has been isolated from *Thalictrum minus var. adiantifolium* Hort. [4]. The structure was established by physical methods and direct comparison with the compound (IV) prepared by oxidation with alkaline $KMnO_4$ of berberine chloride.



IV

(iv) Thalifoline

Thalifoline, $C_{11}H_{13}O_3N$, colourless crystals, m.p. 210–11°C (methanol), is obtainable from *Thalictrum minus var. adiantifolium* Hort. [4]. On the basis of spectral data structure V of the alkaloid was proposed and proved by synthesis.



V

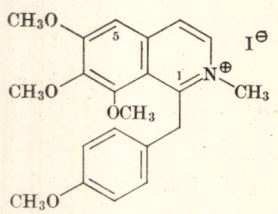
2. Benzyltetrahydroisoquinoline Alkaloids

The benzyltetrahydroisoquinoline alkaloids are not widely distributed in plants of the genus *Thalictrum*. So far only three alkaloids have been discovered: takatonine, thalifendlerine and laudanine.

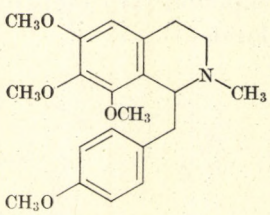
(i) Takatonine

Takatonine, ($C_{21}H_{24}O_4N$) I, m.p. 192–193°C (methanol); $[\alpha]_D$ 0°, isolated from the Japanese crude commercial drug 'Takato-gusa', the dried leaves and stems of *Thalictrum thunbergii* DC (*T. minus, var. hypoleucum*) [5].

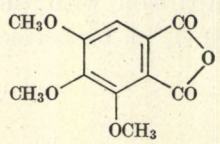
The structure of the alkaloid was first postulated to be 1-(4′-methoxybenzyl)-2-methyl-6,7,8-trimethoxyisoquinoline iodide (VI). The reduction of the quaternary base with zinc and 50% acetic acid gave tetrahydrotakatonine, m.p. 186–187°C (ethanol: petr. ether) (VII). The picrate of VII was identical with *dl*-O-methylcorpaverine picrate. Oxidation of the hydrochloride with potassium permanganate in aqueous solution yielded anisic acid. The isoquinoline part gave, on further degradation, 3,4,5-trimethoxyphthalic anhydride (VIII).



VI

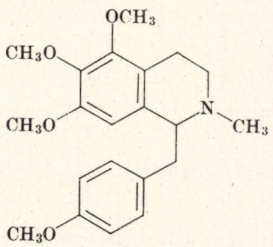


VII

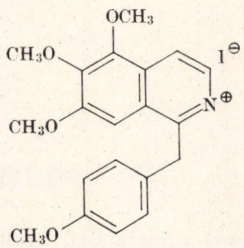


VIII

Recently it has been shown by NMR and IR spectra as well as by TLC that tetrahydrotakatonine, obtained after reduction of the native alkaloid, differs from O-methylcorpaverine, synthesized by the method of Tomita and Okui [6]. The NMR spectrum of the synthetic compound (VII) showed an aromatic proton singlet at 3·63 τ, while tetrahydrotakatonine absorbs at 4·12 τ. The aromatic proton at C-8 in benzylisoquinolines exhibits a different NMR spectral behaviour as compared to the remaining aromatic protons of the benzylisoquinoline. As a result of the shielding by the benzyl aromatic ring, the absorption of the proton at C-8 will be shifted upfield (about 4 τ), while the other aromatic protons absorb at 3–3·62 τ [7, 8]. The analysis of these spectra and the chemical results led to the postulation that the structural formula VII should be replaced by IX, and that of natural takatonine by X. The new structure has been verified by total synthesis [9, 10]. Takatonine was the first benzyltetrahydroisoquinoline alkaloid recognized to contain a substituent at C-5.



IX

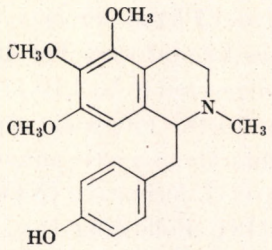


X

(ii) Thalifendlerine

Thalifendlerine, $C_{20}H_{25}O_4N$, m.p. 177–178°C; $[\alpha]_D$ — 108° (methanol); isolated from *Thalictrum fendleri*.

The position of the O-methyl group at C-5 (XI) was proved by interpretation of the NMR spectrum and from a mass spectrometric investigation.

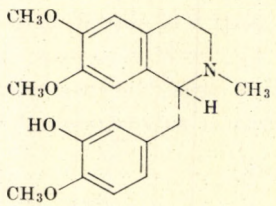


XI

On the other hand, treatment of thalifendlerine with diazomethane gave an O-methyl derivative, m.p. 195–197°C which was found to be identical in terms of NMR spectra and TLC values with tetrahydrotakatonine (IX) [11, 12]. Recently *dl*-thalifendlerine has been synthesized [13].

(iii) Laudanidine

Laudanidine, $C_{20}H_{25}O_4N$ (XII), m.p. 181–2°C, L-(+)-isomer, was isolated from the roots of *Thalictrum dasycarpum* Fisch et Lall. [2].



XII

3. Aporphine Alkaloids

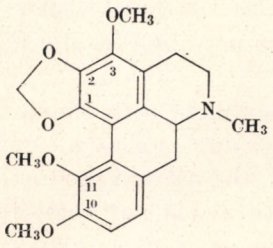
Among the great number of aporphine alkaloids wide-spread in nature, only a small number are so far known from the various representatives of the genus *Thalictrum*: thalicmine, thalicmidine, thaliporphine, glaucine, preocoteine, magnoflorine, thalissimidine, thalicminine and isocorydine.

(i) Thalicmine (ocoteine)

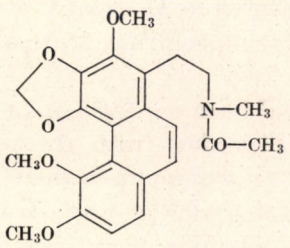
Thalicmine, $C_{21}H_{25}O_5N$ [14] or $C_{21}H_{23}O_5N$ [15], m.p. 137–138°C (methanol); $[\alpha]_D + 255{\cdot}3°$ or $+ 37{\cdot}5°$ (ethanol), isolated from *Thalictrum minus* L., *Thalictrum fendleri* [12] and *Thalictrum isopyroides* [16]. This alkaloid has been investigated in various laboratories. The first proposed structure came from Yunusov *et al.* It was suggested to be 3,10,11-trimethoxy-1,2-methylenedioxyaporphine (XIII) due to the following experiments [17].

Treatment with acetyl chloride or acetic anhydride broke the heterocyclic ring and the resulting N-acetyl derivative (XIV) had neither basic properties nor optical activity [18]. Following this, oxidation with conc. nitric acid changed it into mellophanic acid (XV). Hofmann degradation of tha-

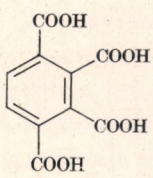
licmine-methiodide yielded 2,5,6-trimethoxy-3,4-methylenedioxy-1-vinylphenanthrene (XVI). These reactions related the alkaloid to the aporphines.



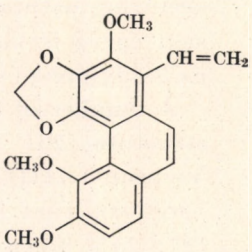
XIII



XIV

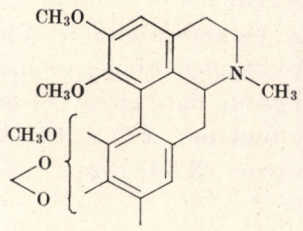


XV

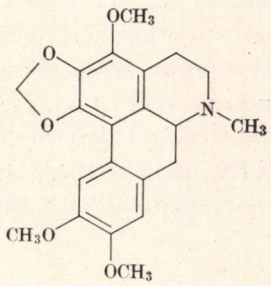


XVI

Later Jacobucci found ocoteine in the roots of *Ocotea puberula* (Rich.) Nees [19]. On the basis of the agreement between the melting points, Vernengo *et al.* assumed that thalicmine and ocoteine must be identical in spite of the difference in the optical rotation [20]. Further investigations [21] changed the position of the substituents (XVII). On the basis of spectral data and optical rotation Vernengo proposed that thalicmine is 1,2-methylenedioxy-3,9,10-trimethoxyaporphine (XVIII): the NMR spectrum shows a signal at 2·43 τ, indicating that one of the positions C-1 or C-11 of the biphenyl system is unsubstituted [22]. In the structure proposed by Yunusov both positions are occupied.



XVII



XVIII

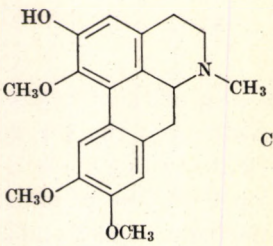
On the other hand, when an aporphine contains a substituent at C-11 (corydine type) the angle of rotation has a higher value and the UV spectrum (see on p. 282) shows two maxima — at 268–272 mμ ($\log \varepsilon \sim 4{\cdot}2$) and at 303–310 m$\mu$ ($\log \varepsilon \sim 3{\cdot}8$). Alkaloids with position C-11 free of substituents (boldine type) show two maxima in the UV spectrum at 282 and 303–310 mμ of about equal intensity ($\log \varepsilon = 4{\cdot}2$) [23, 24]. Ocoteine has a low speci-

fic rotation, + 37·5°. Further, the maxima in the UV spectrum at 283 and 302 mμ (for both $\log \varepsilon = 4{\cdot}25$) indicate that either C-1 or C-11 must be unsubstituted [25]. The substituent must be at C-1 because all aporphines occurring naturally are substituted at C-1 and C-2. The rotational dispersion curve is identical with (+)-dicentrine, so that it may be considered as 3-methoxydicentrine.

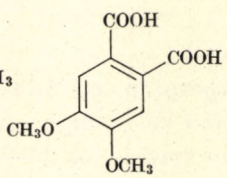
A number of syntheses have been carried out of products with similar structure [26]. The synthetic product with the structure suggested by Yunusov (XIII) showed only one maximum in the UV; the natural product had two. That is further proof that the first structure was not correct [27, 28]. Some years later Shamma compared the synthetic product (XVIII) with the natural one and established their identity. In this way the problem of the structure of thalicmine (ocoteine) was solved [29]. NMR and mass spectral studies of thalicmine confirmed this structure [30, 31].

(ii) Thalicmidine

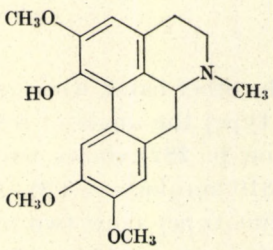
Thalicmidine, $C_{20}H_{23}O_4N$, m.p. 192–193°C (petr. ether); $[\alpha]_D - 84{\cdot}5°$ (ethanol); isolated from the mother liquor of thalicmine of *Thalictrum minus* [14]. On the basis of the following chemical reactions Yunusov proposed structure XIX: methylation of the free phenolic hydroxy group with methyl iodide in alkali yielded O-methylthalicmidine methiodide, which was identical with the methiodide of glaucine or O-methylboldine. Oxidation of thalicmidine with potassium permanganate in aqueous solution gave m-hemipinic acid (XX). It showed that methoxy groups are at both positions 9 and 10 and that the third group may be at C-1 or C-2. The first structure (XIX) showed the phenolic function at C-2 [15], because the alkaloid was not identical with glaucentrine (XXI) [32].



XIX

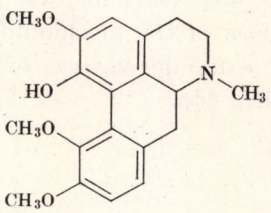


XX

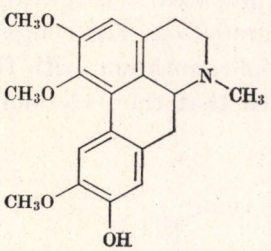


XXI

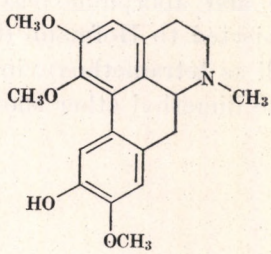
Later Shamma *et al.* corrected the structure of glaucentrine and showed that it is not a 1,2,9,10-tetrasubstituted aporphine as had been believed so far. He proved [33] that it corresponded to corydine (XXII). The comparison on the basis of which Yunusov *et al.* assigned structure XIX to thalicmidine cannot therefore be correct. In fact aporphine alkaloids have lately been synthesized, or isolated from plants, having three methoxy groups and one hydroxy group in the four possible combinations. Thus, Shamma synthesized (±)-1-hydroxy-2,9,10-trimethoxy-aporphine (XXI) and showed that it differs from thalicmidine and glaucentrine [33]. A natural product, the base from *Fagara tinguassoiba* Hoehne [34, 35], was found to have structure XI (in the N-methyl quaternary form), this structure replacing the old one XIX [29, 36]. There has been a suggestion that thalicmidine is a (−)-1-hydroxy-2,9,10-trimethoxyaporphine [35] but the Fagara base can be methylated with diazomethane [36], while thaliomidine remained unreactive under those conditions [15]. The alkaloid with the structure XIX is O,N-dimethyllaurelliptine (O-methylisoboldine) [37] which physical constants corresponds to those of thalicmidine. N-methyllaurotetanine (XXIII) has also been compared but its physical constants did not agree with those of thalicmidine [35]. Shamma proposed the only possible structure XXIV [29, 38]. In 1963 Tomita found in the roots of *Cocculus sarmentosus* the alkaloid cocsarmine and gave the structure XXIV — (+)-N-methyl-1,2,9-trimethoxy-10-hydroxyaporphine [39]. The same structure has been suggested for rogersine [40] but it is uncertain [41, 42]. The physical properties of cocsarmine iodide did not correspond to those of thalicmidine methiodide.



XXII



XXIII

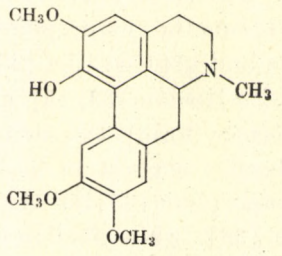


XXIV

Yunusov *et al.* supported structure XIX, because oxidation gives *m*-hemipinic acid; this excluded positions 9 and 10 for the hydroxy group [43]. Now the same authors accept structure (XXI) on the basis of NMR and MS data [30, 31].

(iii) Thaliporphine

Thaliporphine, $C_{20}H_{23}O_4N$, m.p. 170–172°C (methanol), isolated from *Thalictrum fendleri* [44]. The structure (XXV) has been proved by comparison with the compound synthesized by Shamma [33].

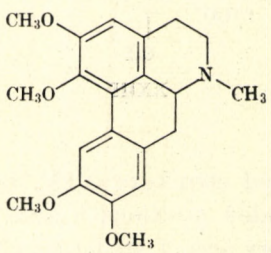


XXV

(iv) Glaucine

Glaucine, $C_{21}H_{25}O_4N$, m. p. 120°C (methanol); $[\alpha]_D$ — 114° (ethanol) was the first aporphine to be found in plants and the only tetramethoxyaporphine occurring naturally. It has been isolated from different species of *Glaucium*, *Corydalis*, *Dicentra* and also from *Thalictrum minus* L. [15] and *Thalictrum fendleri* [12].

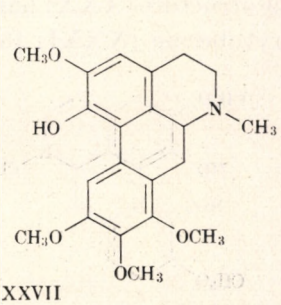
The structure of glaucine (XXVI) was elucidated by Gadamer. It was the first aporphine base prepared synthetically [45]. Glaucine was also subjected to Hofmann degradation. The comparison of the methiodide as well as tetramethoxyvinylphenanthrene with the same derivatives of boldine dimethyl ether showed that they are identical [46].



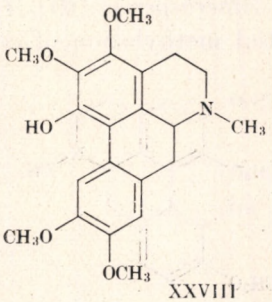
XXVI

(v) Preocoteine

Preocoteine, $C_{21}H_{25}O_5N$, an oil, isolated from *Thalictrum fendleri* [44]. It is the second pentasubstituted aporphine with four methoxy and one hydroxy groups. The NMR spectrum showed that no methoxy is present either at C-1 or C-11. Since all aporphines occurring naturally are substituted at C-1 and C-2, the phenolic group has to be at C-1. The comparison of the O-methylated alkaloid with the synthetic (±)-1,2,8,9,10-pentamethoxyaporphine (XXVII) showed that they are not identical, which proved structure XXVIII for preocoteine.



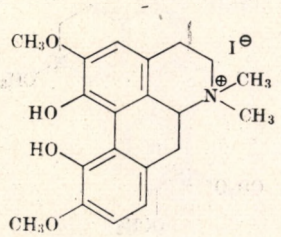
XXVII



XXVIII

(vi) Magnoflorine

Magnoflorine, ($C_{20}H_{24}O_4N$) I, m.p. 248–249°C (methanol), isolated from different species of *Magnoliaceae* [47], *Berberidaceae*, *Menispermaceae* and from the following *Thalictrum* species: *thunbergii* [48, 49, 50], *dasycarpum* [51, 52], *rochebrunianum* [53], *rugosum* [54, 55, 56], *foliolosum* [57], *fendleri* [11], *minus var. adiantifolium* [56], *minus* [58], *flavum* [59], *foetidum* [58], *isopyroides* [58] and *simplex* [60]. According to information of 1963 magnoflorine accumulates in greater quantities in the roots than in stems and leaves [39]. It has been found that the alkaloid thalictrine from *Thalictrum*

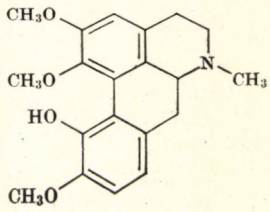


XXIX

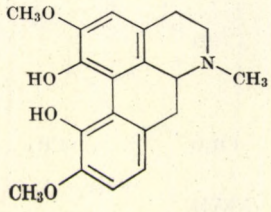
foliolosum [61] is identical with magnoflorine [57] and magnoflorine iodide with corytuberine methiodide [47]. The structure of magnoflorine XXIX has been elucidated by synthesis [62].

(vii) Isocorydine

Isocorydine, $C_{20}H_{23}O_4N$, m.p. 185–186°C; $[\alpha]_D$ + 195° (chloroform), isolated for the first time from *Thalictrum aquilegifolium* [63]. So far it has been found in various species of *Corydalis*, *Dicentra*, *Glaucium*, *Peumus*, *Artabotrys* and *Atherosperma* [64]. The structure (XXX) has been established by the partial methylation of corytuberine (XXXI) [65].



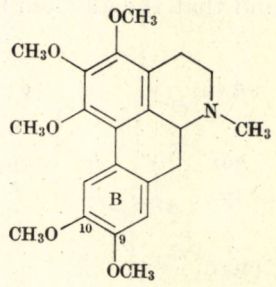
XXX



XXXI

(viii) Thalicsimidine

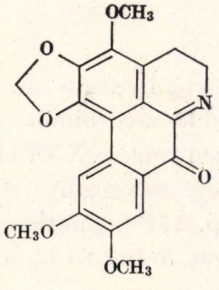
Thalicsimidine, $C_{18}H_{21}O_3N$, m.p. 131–2°C (ethanol), $[\alpha]_D$ + 20·26° (chloroform), and 57·85° (ethanol), can be isolated from *T. simplex* L. [60]. The IR and UV spectra show that it is an aporphine having no substituent at C-11 [66]. The optical rotation is below 100°, therefore the substituents in ring 'B' must be at C-9 and C-10 (XXXII). The mass spectral and NMR data are consistent with this structre [30, 31].



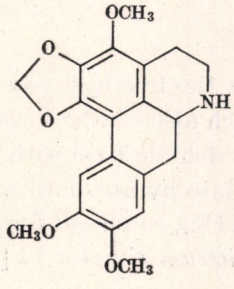
XXXII

(ix) Thalicminine

Thalicminine, $C_{20}H_{15}O_6N$, m.p. 263–265°C (chloroform), isolated from *Thalictrum minus* L., so far known as 'Base No. 5' [14, 15]. In the IR spectrum there is a band for a conjugated carbonyl group, in the UV four maxima similar to the alkaloids of the liriodenine type. Therefore thalicminine is related to 10-ketoaporphines with the structure XXXIII [67]. By reduction of the base with zinc in sulphuric acid a base with a secondary amino group is obtained (XXXIV). The methylation of the nitrogen atom led to the compound XVIII, which is found to be identical with the alkaloid thalicmine. Oxidation of the latter with potassium permanganate in acetone, or chromic acid in pyridine, afforded thalicminine.



XXXIII



XXXIV

4. Protoberberine Alkaloids

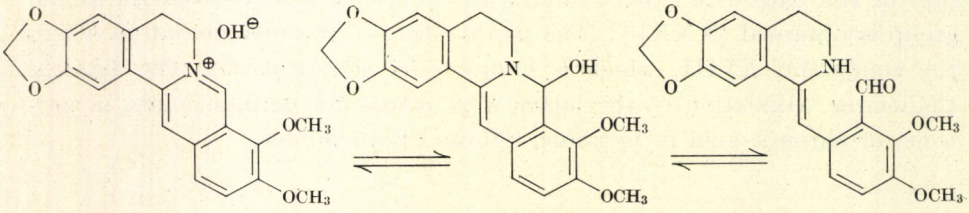
The protoberberine or berberine alkaloids are widely distributed in *Berberidaceae, Ranunculaceae, Anonaceae, Menispermaceae, Papaveraceae* and *Rutaceae.*

(i) Berberine

Berberine, $C_{20}H_{19}O_5N$, m.p. 144°C or 205°C (hydr.) is one of the alkaloids most widely spread in various representatives of the genus *Thalictrum.* It has been isolated from *T. foliolosum* L. [61], *T. flavum* L. [59, 68, 69], *T. thunbergii* DC. [47], *T. fendleri* [11], *T. rochebrunianum* [53, 70], *T. rugosum* Ait. [54, 55, 56, 71, 72], *T. tubiferum* [73], *T. pedunculatum* [74],

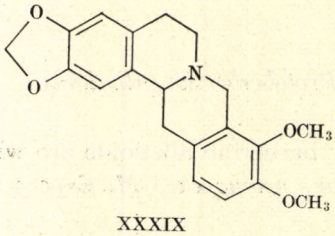
T. minus L. [58, 75, 76], *T. dasycarpum* [51], *T. minus var. adiantifolium* [56], *T. foetidum* [58], *T. longipedunculatum* [58], *T. isopyroides* [58] and *T. simplex* [77].

Fundamental research on the structure of berberine was made by Perkin [78, 79] by studying the degradation products of the oxidation with permanganate. Gadamer proposed that berberine is a quaternary salt (XXXVI).



XXXVI XXXVII XXXVIII

He assumed that the free base existed in the hydrate form, $C_{20}H_{18}O_4$ N.OH [80, 81, 82], which also can be formulated as α-carbinolamine (XXXVII) or as a secondary aldehyde base with on open ring (XXXVIII). The reduction with zinc and dilute hydrochloric acid (or catalytically) yielded tetrahydroberberine (XXXIX), in the *dl*-form, m.p. 171°C (methanol). Thalsine, isolated from *Thalictrum simplex* L. [77] was found to be identical with berberine [68].



XXXIX

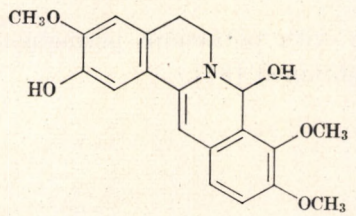
(ii) Canadine

L-Canadine, $C_{20}H_{21}O_4N$, m.p. 135°C (methanol); $[\alpha]_D$ −299°, was isolated from *Thalictrum actaefolium* [83]. The synthesis of tetrahydroberberine (XXXIX) was carried out in 1911 [85].

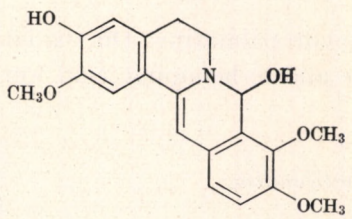
L-Canadine was also isolated from *Thalictrum minus* L. as the chloromethylate, $C_{21}H_{24}O_4Cl$, m.p. 191–3°C (decomp.); $[\alpha]_D$ −158° [84].

(iii) Jatrorrhizine and (iv) Columbamine

Jatrorrhizine, ($C_{20}H_{20}O_4N$) I, m. p. 210°C $[\alpha]_D$ 0° has been found in *Thalictrum fendleri* [11], *T. rochebrunianum* [53], *T. foliolosum* DC. [86]. Columbamine, $(C_{20}H_{20}O_4N)^+X^-$, isolated from *Thalictrum rugosum* Ait. [71] is an isomer (XL) of jatrorrhizine (XLI). The phenolic hydroxyl

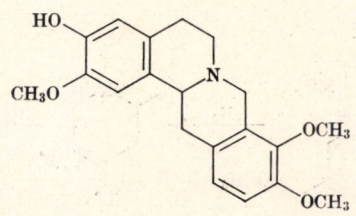


XL

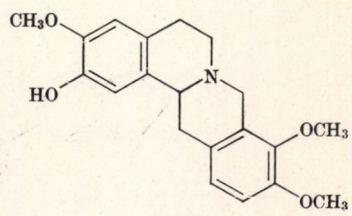


XLI

group in both alkaloids can be methylated with diazomethane. Tetrahydrojatrorrhizine is identical with *dl*-corypalmine (XLII), and tetrahydrocolumbamine with *dl*-isocorypalmine (XLIII) [87].



XLII

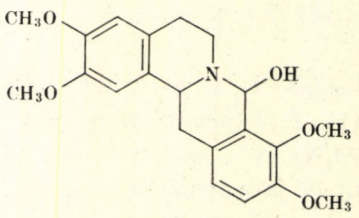


XLIII

Recently Cava *et al.* carried out the synthesis of columbamine from berberine [88].

(v) Palmatine

Palmatine, $C_{21}H_{23}O_5N$; in quaternary salt form was isolated from *Thalictrum foliolosum* DC. [86]. The structure (XLIV) was elucidated by Gadamer [89]. Reduction of the alkaloid yielded

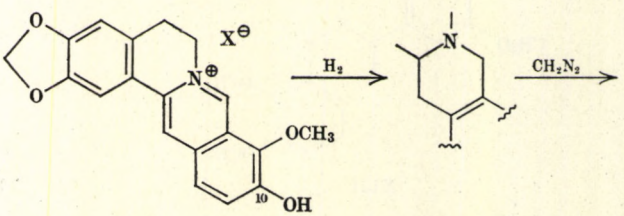


XLIV

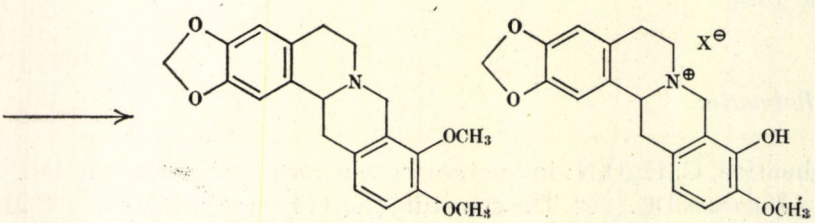
dl-tetrahydropalmatine. On oxidation with potassium permanganate corydaline and *m*-hemipinic acid were obtained [87].

(vi) Thalifendine

Thalifendine, $C_{19}H_{16}O_4N^+X^-$ $[\alpha]_D 0°$ (the chloride sinters $> 230°C$), has been obtained from *Thalictrum fendleri* L. [11, 12]. When reduced with Pt/H_2 the alkaloid (XLV) is converted into tetrahydrothalifendine (XLVI); O-methylation with diazomethane gives tetrahydroberberine (XXXIX). The position of the hydroxyl group at C-10 was proved by heating berberine chloride in an inert atmosphere; the resulting berberrubine chloride (XLVII) differs from thalifendine chloride.



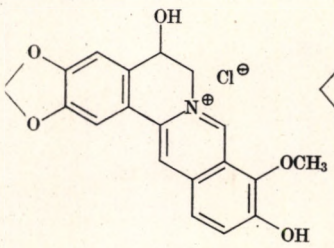
XLV XLVI



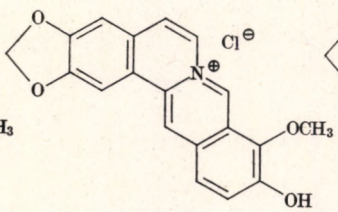
XXXIX XLVII

(vii) Thalidastine

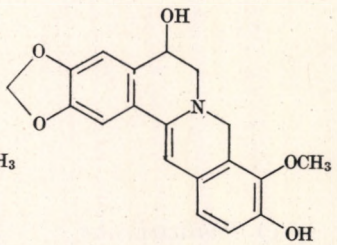
Thalidastine, $C_{19}H_{16}O_5N^+X^-$, the chloride darkened above 230°C (decomp.); $[\alpha]_D 138°$ (methanol), isolated from *Thalictrum fendleri* L. [12, 90]. After dehydration of thalidastine (XLVIII) deoxythalidastine chloride (XLIX) is formed. Reduction of thalidastine chloride with Pt/H_2 in ethanol led to tetrahydrothalidastine (L). Mass spectroscopic investigation of the alkaloid proved the structure; this was confirmed by synthesis [91].



XLVIII

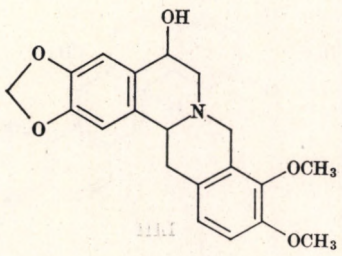


XLIX



L

After berberastine (LI), isolated from *Hydrastis canadensis*, thalidastine is the second alkaloid which belongs to the protoberberine alkaloids, hydroxylated at C-5.



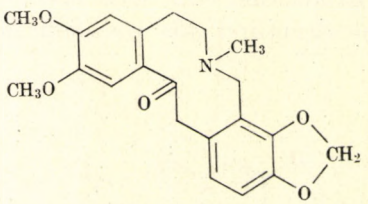
LI

5. Protopine Alkaloids

In recent years it has been found that *Thalictrum* species contained some protopine alkaloids. Such compounds have been isolated from *Thalictrum isopyroides* C. A. M., *Thalictrum minus* L., *Thalictrum simplex* L. and *Thalictrum flavum*.

(i) Thalisopyrine

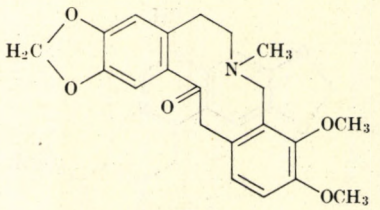
Thalisopyrine, $C_{23}H_{25}O_5N$, m.p. 216–7°C (acetone), had been described as a new alkaloid from *Thalictrum isopyroides* [92] and *T. flavum* [59]. Later it was shown that it was identical with cryptopine (LII) [59].



LII

(ii) Thalictrimine

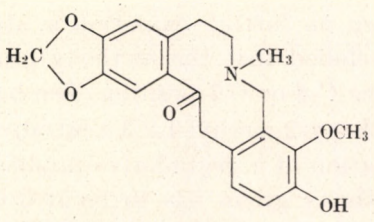
Thalictrimine (LIII), $C_{20}H_{23}O_4$, m.p. 169–170°C (ethanol–chloroform), $[\alpha]_D 0°$, identical with β-allocryptopine [84], is the main alkaloid of *Thalictrum minus* L., growing in the Moldau SSR [93, 94] and of *Thalictrum simplex* L. [60].



LIII

(iii) Thalictrisine

Thalictrisine, $C_{20}H_{21}O_5N$, m.p. 261–3°C (methanol), has been isolated from the roots of *Thalictrum simplex* L. [95]. IR, UV and MS analyses have shown that the alkaloid belongs to the protopine alkaloids. Methylation with diazomethane gives β-allocryptopine; on this basis, structure LIV has been proposed [95].



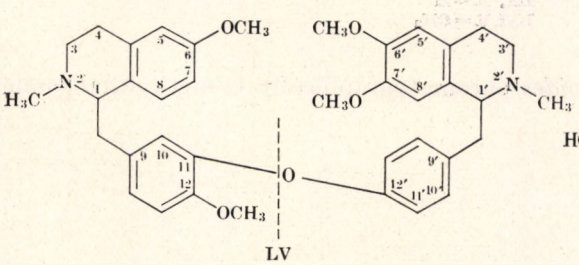
LIV

6. *Bisbenzylisoquinoline Alkaloids*

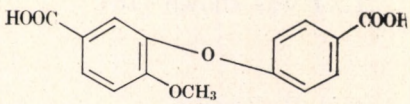
This group of alkaloids is the most widely distributed in *Thalictrum* species. In these alkaloids two benzylisoquinoline units are joined by ether bridges in different ways.

(i) Thalisopine

Thalisopine, $C_{38}H_{44}O_5N_2 \cdot 2\,H_2O$, m.p. 151–153°C (methanol : water, 3 : 1); $[\alpha]_D$ — 104·9° (acetone) or $[\alpha]_D$ — 71·02°C (chloroform), occurs in the roots of *Thalictrum isopyroides* C.A.M. [16, 95a, 96]. The structure (LV) was elucidated by oxidation of the alkaloid to 4′,5-dicarboxy-2-methoxydiphenylether (LVI), a reaction which relates the base to the bisbenzylisoquinolines [97]. Exhaustive Hofmann degradation proceeds in two steps, the second of which yields trimethylamine and a compound containing no nitrogen. Cleavage of thalisopine with metallic sodium in liquid ammonia bisects the molecule into phenolic and non-phenolic parts. The phenolic moiety is converted by diazomethane into O-methylarmepavine (LVII).



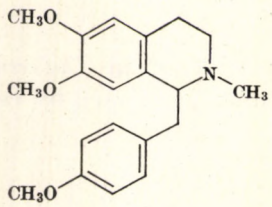
LV



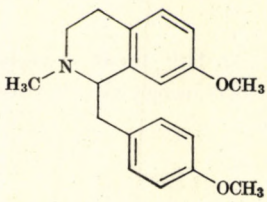
LVI

The non-phenolic part has two methoxyl and one N-methyl group. Oxidation of this moiety with potassium permanganate in acetone gives anisic acid

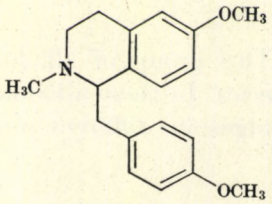
and a weak base which on further oxidation is also converted to anisic acid. The authors concluded that the methoxy group in the isoquinoline moiety is present at the C-6 or C-7 position. The base differs from *d*-1-(4′-methoxybenzyl)-7-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (LVIII) obtained after methylation of a degradative product of cepharantine [98], and therefore the methoxy group has to be at C-6. Thus, it follows that the non-phenolic moiety of thalisopine is *d*-1-(4′-methoxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (LIX).



LVII

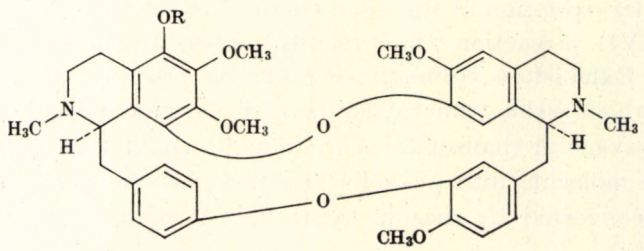


LVIII



LIX

Now, as a more probable structure for thalisopine, $C_{38}H_{42}O_7N_2$ (LX) is accepted on the basis of NMR and MS investigations of the alkaloid [16].



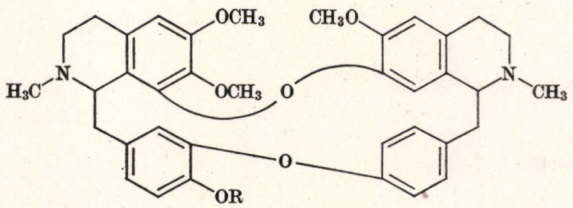
LX, R = H
LXI, R = CH_3

In the roots of *T. isopyroides* chromatographically O-methylthalisopine LXI was shown [16].

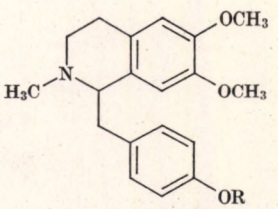
(ii) Berbamine

Berbamine, $C_{37}H_{40}O_6N_2$, m.p. 172°C (methanol); $[\alpha]_D$ + 109° (chloroform), isolated from *Thalictrum foetidum* L. [99]. The structure (LXII) has been established by cleavage of O-ethylberbamine (LXIII) with metal-

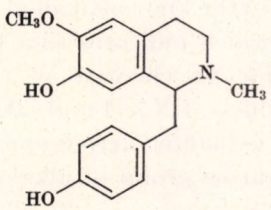
lic sodium in liquid ammonia, resulting in *l*-O-ethylarmepavine (LXIV) and *d*-N-methylcoclaurine (LXV) [100].



LXII, R = H
LXVII, R = CH_3
LXIII, R = C_2H_5



LXIV, R = C_2H_5
LXVI, R = CH_3



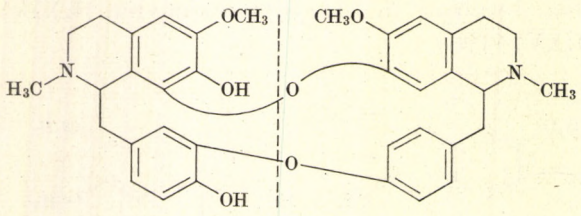
LXV

(iii) Isotetrandrine

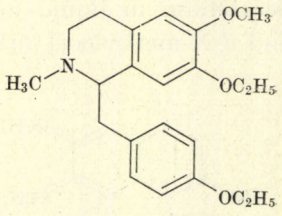
Isotetrandrine, $C_{38}H_{42}O_6N_2$, m.p. 182°C (methanol); $[\alpha]_D$ + 146° (chloroform). The alkaloid has been found in *Thalictrum foetidum* [99]. Cleavage with sodium in liquid ammonia gives *l*-O-methylarmepavine (LXVI) and *d*-N-methylcoclaurine (LXV), leading to the structure LXVII [100].

(iv) Obamegine

Obamegine, $C_{36}H_{38}O_6N_2$, m.p. 171–172°C (methanol); $[\alpha]_D$99° (methanol), first isolated from *Berberis Tschonoskyana* Regel [101], later from *Thalictrum rugosum* Ait. [102]. The strucutre was elucidated by converting the alkaloid into isotetrandrine after O-methylation with diazomethane, and by reductive cleavage of the diethylether of obamegine. The latter reaction yielded *l*-N-methyl-O,O-diethylcoclaurine (LXVIII) and *d*-N-methylcoclaurine (LXV). Later it has been found that obamegine (LXIX) and stepholine, isolated by Tomita [103] from *Stephania japonica* Miers. are identical [104].



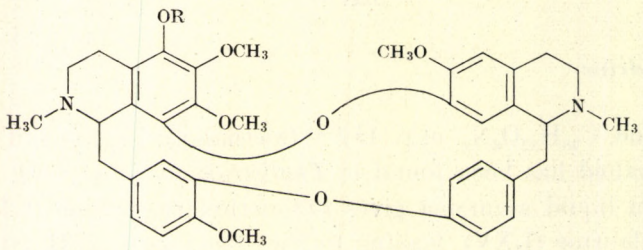
LXIX



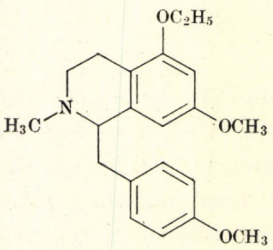
LXVIII

(v) Thalidezine

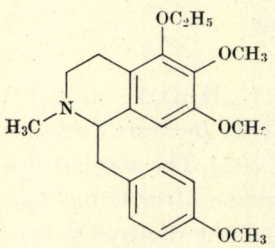
Thalidezine, $C_{38}H_{42}O_7N_2$, m.p. 158–159°C (acetone); $[\alpha]_D$ 235° (chloroform), isolated from *Thalictrum fendleri* [44] and *Thalictrum simplex* [105]. The alkaloid is converted into the known alkaloid hernandezine by O-methylation with diazomethane, which indicates that thalidezine is O-demethylhernandezine (LXX). Reductive cleavage of O-ethylthalidezine (LXXI) gives two non-phenolic products, LXXII and LXXIII. Thalidezine is a new bisbenzylisoquinoline with a thalifendlerine moiety, and therefore belongs to the thalifendlerine-coclaurine group of alkaloids.



LXX, R = H
LXXI, R = C_2H_5



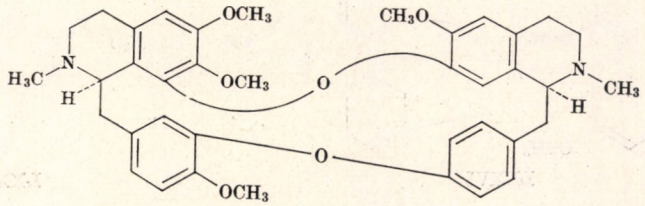
LXXII



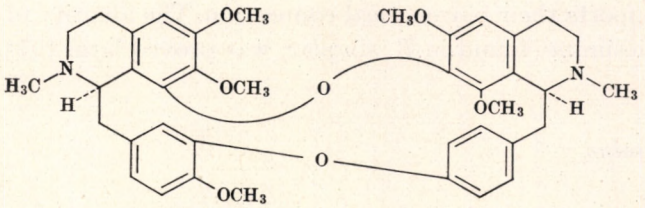
LXXIII

(vi) Hernandezine

Hernandezine, $C_{39}H_{44}O_7N_2$, m.p. 192–193°C; $[\alpha]_D$ – 220°. It was first isolated from *Thalictrum hernandezii* Tausch [106], later from *Thalictrum fendleri* [12], *T. rochebrunianum* [53, 70], *Thalictrum simplex* L. [77, 97, 107]. *Thalictrum flavum* [68] and *Thalictrum alpinum* [108]. Velasquez was the first to describe the alkaloid [106]. Later, Padilla and Herran [109] investigated it chemically, subjecting it to oxidation with permanganate to yield 5,4′-dicarboxy-2-methoxy-diphenyl ether (LVI); its Hofmann degradation was also studied. The dimethiodide of the methine base was further oxidized with chromic acid and the same dibasic acid was obtained. The presence of one methoxy group and one ether linkage in this acid suggested that the remaining five oxygen atoms are in the isoquinoline part of the alkaloid. Four of the oxygens are incorporated in the methoxy groups, the fifth in the second linkage. A comparison of the NMR spectrum [110] with that of the alkaloid tetrandrine (LXXIV) enabled Padilla and Herrán to assign the positions of four methoxy groups. As a result of steric hindrance, they suggested that the fifth is at C-8′ (LXXV).



LXXIV

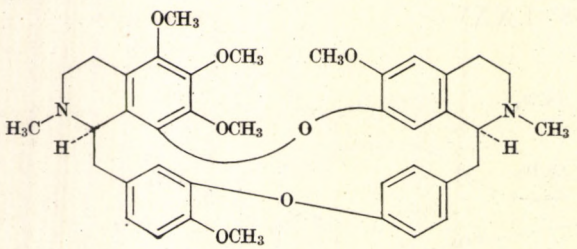


LXXV

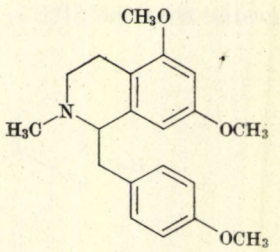
After cleavage of hernandezine with sodium in liquid ammonia the above-mentioned authors isolated only one phenolic base, which they did not characterize further. Maekh and Yunusov [97, 111] carried out the same reaction and obtained a mixture of phenol bases, which after O-methylation were converted into O-methylarmepavine (LXVI). When this mixture is

separated at different pH values, N-methylcoclaurine (LXV) is obtained, a proof that one methoxy group is cleaved at C-8′.

After re-examination of the NMR spectrum of hernandezine, Shamma *et al.* [112] confirmed that one of the aromatic protons absorbs at high field — 3·98 τ. It is known that this signal is characteristic of a C-8 proton in the benzylisoquinolines, due to the high shielding of the aromatic ring of the benzyl group [7, 13]. The same authors proposed structure LXXVI, supported by mass spectrometric data. Reductive cleavage yields a phenolic moiety — N-methylcoclaurine and a non-phenolic base — 5,7,4′-trimethoxybenzyltetrahydroisoquinoline (LXXVII). The absence of a methoxy group at C-6 in the latter was explained by the cleavage during the reduction reaction because of steric hindrance. The authors proved that hydrogenolysis occurs at this C-atom by carrying out the reaction of metallic sodium in liquid ammonia with O-methylthalifendlerine (tetrahydrotakatonine, IX). They obtained the same non-phenolic base. The *S,S* configuration of hernandezine was confirmed as a result of optical rotatory data [114].



LXXVI



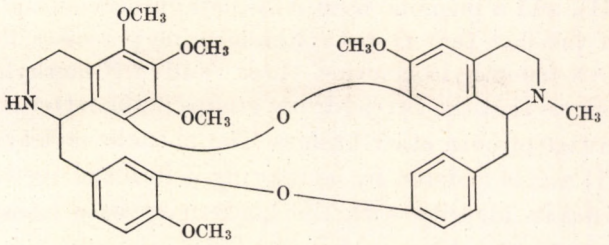
LXXVII

Hernandezine occurs along with thalifendlerine (XI) in *Thalictrum fendleri*, which supports their biogenetical connection. The identity of hernandezine and thalicsimine, found in *T. simplex*, was proved later [97].

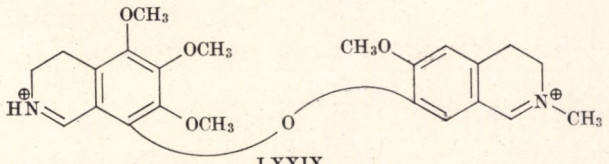
(vii) Thalisamine

Thalisamine, $C_{38}H_{42}O_7N_2$, m.p. 191–194°C (methanol); $[\alpha]_D$ — 138° (chloroform), has been found in *Thalictrum simplex* L. [105]. N-methylation of the alkaloid (LXXVIII) with formaldehyde-formic acid yields hernandezine (LXXVI). In the NMR spectrum there is a signal of only one N-methyl group, which signifies that one of the two nitrogen atoms is not methylated. A comparison of thalisamine with dihydrothalsimine (see under thalsimine), obtained by catalytic hydrogenation of thalsimine, shows that the com-

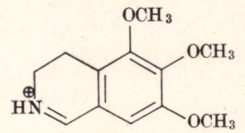
pounds are not identical. It follows that in thalisamine the N-methyl and NH-groups are reversed. The structure of thalisamine is confirmed by the mass spectrum which shows a peak for the doubly charged ion (LXXIX) and peaks due to ions from the fragmentation of the molecule (LXXX, LXXXI)



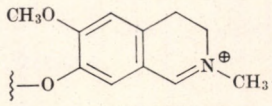
LXXVIII



LXXIX



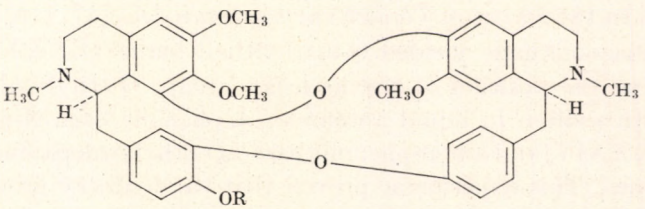
LXXX



LXXXI

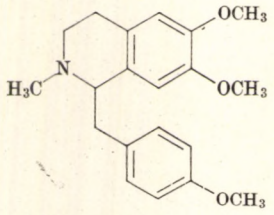
(viii) O-Methylthalicberine

O-Methylthalicberine, $C_{38}H_{42}O_6N_2$, m.p. 186–187°C; $[\alpha]_D$ + 265·9° (chloroform), isolated from the leaves of *Thalictrum thunbergii* DC. as well as from the commercial drug 'Takato-gusa' and from *T. isopyroides* [96]. The alkaloid (LXXXII) was submitted to Hofmann degradation [116, 117].

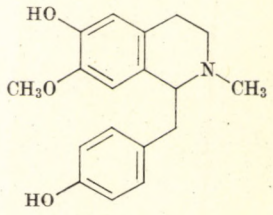


LXXXII, R = CH_3
LXXXVII, R = H

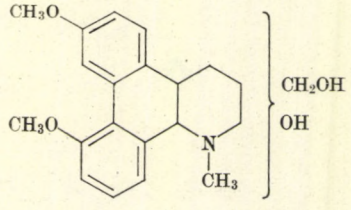
Methylmethine, obtained at the first stage was further oxidized with potassium permanganate to give 5,4′-dicarboxy-2-methoxydiphenyl ether (LVI). In order to elucidate both moieties of the molecule, O-methylthalicberine was cleaved with metallic sodium in liquid ammonia [118]. Two basic products were thereby isolated: a non-phenolic base, *d*-O,O,N-trimethylcoclaurine (LXXXIII), and a phenolic base, *d*-N-methylisococlaurine (LXXXIV). On account of the fact that O-methylthalicberine possesses four methoxy groups, *d*-O,O,N-trimethylcoclaurine three, and *d*-N-methylisococlaurine only one, it follows that the two hydroxy groups in the latter compound are formed by cleavage of both ether bridges. The phenolic moiety of the molecule (LXXXII) was elucidated by converting it into the methiodide [119, 120] and proving its identity with the quaternary base lotusine, isolated from *Nelumbo nucifera*.



LXXXIII



LXXXIV



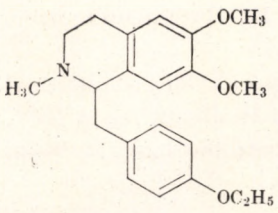
LXXXV

The alkaloid thalmidine, found in the overground parts of *Thalictrum minus* L. was first considered as a triphenylidine alkaloid (LXXXV). Later it was compared with O-methylthalicberine and the identity was established [121, 122].

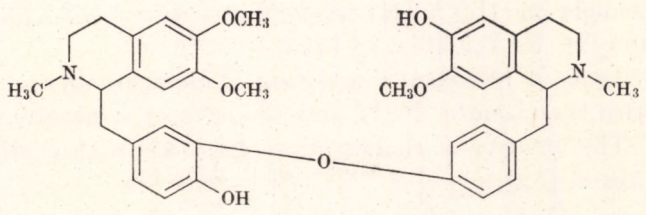
(ix) Thalicberine

Thalicberine, $C_{37}H_{40}O_6N_2$, m.p. 161°C; $[\alpha]_D$ 231·2° (chloroform), has also been found in the leaves of *Thalictrum thunbergii* DC. [87, 115]. O-Methylation with diazomethane yielded O-methylthalicberine (LXXXII). In order to determine the position of the hydroxy group, O-ethylthalicberine was cleaved with sodium in liquid ammonia. A phenolic base N-methylisococlaurine (LXXXIV) and a non-phenolic base *d*,O-ethylarmepavine (LXXXVI) were obtained. This experiment proved that the hydroxy group is in position C-12 (LXXXVII). Thalicberine itself was also submitted to a reductive cleavage, which afforded a phenolic alkaloid (LXXXVIII) of the dauricine

type [123]. After O-methylation with diazomethane it is converted into dauricine [124].



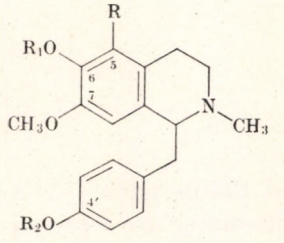
LXXXVI



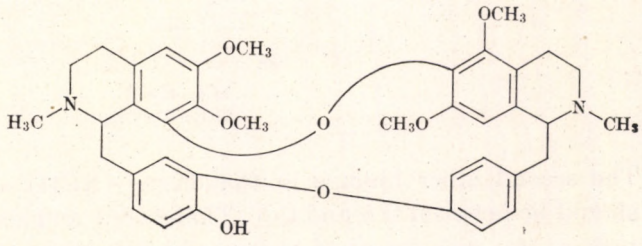
LXXXVIII

(x) Thalfoetidine

Thalfoetidine, $C_{38}H_{42}O_7N_2 \cdot 0{\cdot}5\ (C_2H_5)_2O$, m.p. 168–170°C (ether); $[\alpha]_D$ — 88·6° (chloroform), isolated from *Thalictrum foetidum* [125], *T. longipe-dunculatum* [58] and *T. simplex* [127]. The alkaloid possesses one phenolic hydroxyl group which can be methylated with diazomethane (XC). Reductive cleavage of this methyl-derivative afforded a non-phenolic base *l*-O,O,N-trimethylcoclaurine (LXXXIII); cleavage (under the same conditions) of the O-ethylderivative of thalfoetidine yielded *d*-O-ethylarmepavine (LXXXVI). In this way the nature of one moiety of the molecule and the position of the hydroxyl group were established. By the cleavage of O-methyl-, or of O-ethylthalfoetidine, one and the same compound, with the formula $C_{19}H_{23}O_4N$, was isolated, possessing two phenolic hydroxy groups (LXXXIX). By O-methylation with diazomethane one hydroxy group was easily methylated, the other after a long reaction time. The phenolic base was converted into O-methylthalifendlerine (IX). One of the hydroxyl groups is like the one in thalifendlerine (XI), because in the IR spectrum it absorbs at 3600 cm^{-1} as does the hydroxy group of the latter. It has been suggested, on the basis of



LXXXIX, R = OCH_3; R_1=R_2=H
LXXXIXa, R = OH; R_1=CH_3; R_2=H



XC

a comparison of the NMR spectrum of this base with the spectra of thalifendlerine and tetrahydrotakatonine (IX), that the second hydroxy group is at C-6. The similarity of the IR spectra of thalfoetidine and the alkaloid thalicberine (LXXVII) supports structure LXXXIX for the phenolic base, and XC for the alkaloid [125].

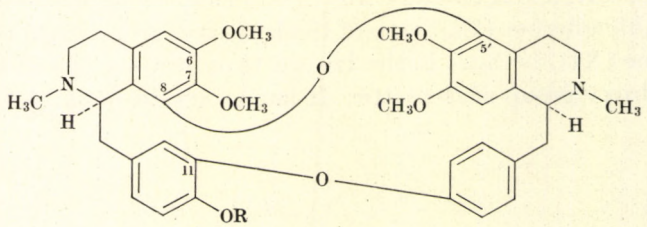
Later it has been found that O-methylthalfoetidine (XCII) is identical with thalidasine (XCII); thus the correct structure of thalfoetidine is XCI.

The identity of thalictrinine [126, 127] with thalfoetidine has also been proved [58].

(xi) Thalidasine

Thalidasine, $C_{39}H_{44}O_7N_2$, an amorphous solid, m.p. 105–7 °C, $[\alpha]_D$ — 70° (methanol), is found in *Thalictrum dasycarpum* [128, 129, 130]. The structure (XCI) has been elucidated by different chemical reactions and spectral investigations. Permanganate oxydation yields 4′,5-dicarboxy-2-methoxydiphenyl ether (LVI), a proof of a bisbenzylisoquinoline skeleton. Treatment with sodium in liquid ammonia affords *l*-O-methylarmepavine (LXVI) and a dihydroxy-dimethoxy-benzylisoquinoline which gives IX on O-methylation.

Spectral data and the reaction with Gibbs reagent show that C-4′, C-6 or C-4′, C-5 could be the positions of the two hydroxy groups in the phenolic product. The compound with C-4′, C-6 hydroxy groups was synthesized and was not identical with the phenolic base. Therefore, C-4′, C-5 have been proposed as the true positions of the hydroxy groups in this base (LXXXIX a).

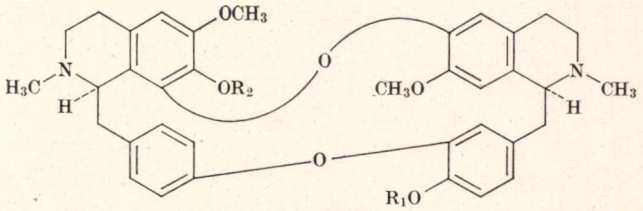


XCI, R = H
XCII, R = CH_3

The second ether linkage in thalidasine (XCII) and thalfoetidine (XCI) should be between C-8 and C-5′. Thalidasine and thalfoetidine are representatives of a new group of bisbenzylisoquinoline alkaloids having 20-membered central heterocyclic ring.

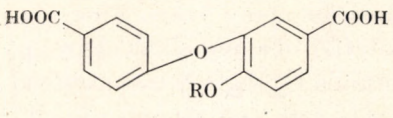
(xii) Thalicrine and (xiii) Homothalicrine

Thalicrine, $C_{36}H_{38}O_6N_2 \cdot H_2O$, m.p. 221–222°C (methanol); $[\alpha]_D + 341{\cdot}2°$; homothalicrine, $C_{37}H_{40}O_6N_2$, m.p. 235–236°C (decomp.); $[\alpha]_D + 425{\cdot}3°$, both isolated from the roots of *Thalictrum thunbergii* [131]. O-Methylation of thalicrine (XCIII) with diazomethane within three days methylated the phenolic hydroxy group in the benzyl part of the molecule and the alkaloid was converted into homothalicrine (XCIV). The second hydroxy group could be methylated very slowly, in one or two weeks to give O,O-imethyl-

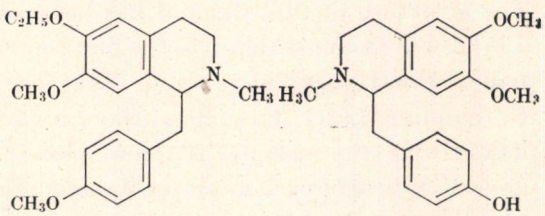


XCV, $R_1 = R_2 = CH_3$
XCIII, $R_1 = R_2 = H$
XCIV, $R_1 = CH_3$, $R_2 = H$

thalicrine (XCV). Hofmann degradation of homothalicrine yielded a methylmethine, which on further oxidation with potassium permanganate in acetone gave 3′,-4-dicarboxy-6′-methoxydiphenyl ether (XCVI). Hofmann degradation of O-ethylthalicrine gave its analogue, 4,3′-dicarboxy-6′-ethoxydiphenyl ether (XCVII). In this way the form of the ether linkage in the benzyl moiety of the molecule of thalicrine and homothalicrine was established. Treatment with sodium in liquid ammonia was carried out to establish the nature of the bonding between the isoquinoline parts of both



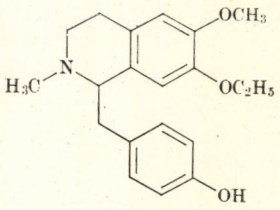
XCVI, $R = CH_3$
XCVII, $R = C_2H_5$



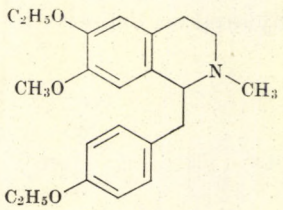
XCIX XCVIII

alkaloids. O-Methylhomothalicrine yielded the phenolic base armepavine (XCVIII) whose hydroxy group was obtained after cleavage of the ether bridge in the benzyl part of the molecule. From the mother liquor of arme-

pavine, after ethylation with diazoethane, *l*-1-(4′-methoxybenzyl)-2-methyl-6-ethoxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (XCIX) was isolated. The presence of one ethoxy group is proof that the hydroxy group is derived from the cleavage of the ether bridge in the isoquinoline part [132]. O,O-Diethylthalicrine obtained after O-ethylation with diazoethane was cleaved with sodium in liquid ammonia and a phenolic base, *d*-1-(4′-hydroxybenzyl)-2-methyl-6-methoxy-7-ethoxy-1,2,3,4-tetrahydroisoquinoline (C) was ob-



C



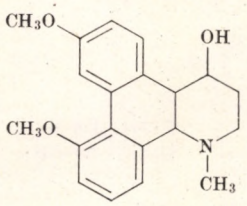
CI

tained. The second phenolic base of this cleavage was also ethylated with diazoethane to give *l*-O,O-diethyl-N-methyl-isococlaurine (CI). The identification of this part was carried out by comparison with a product obtained from O-methylthalicberine by cleavage and subsequent ethylation [133]. It has been reported that thalicrine and aromoline are identical as are homothalicrine and O-methylaromoline. The name aromoline has been proposed for the first base and homoaromoline for the second [134].

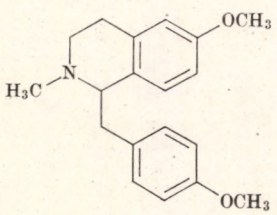
(xiv) Thalmine

Thalmine, $C_{36}H_{40}O_5N_2$, m.p. 252–253°C (decomp.; methanol : chloroform); $[\alpha]_D$ — 64·5°, isolated from the overground parts of *Thalictrum minus* L. The structure of this alkaloid has been studied for many years. First [14, 135] it was assumed that it is the first representative of a new alkaloid group, named the triphenylidine group (CII). The formula $C_{20}H_{23}O_3N$ was ascribed to thalmine. Later the same authors revised this data and classified the alkaloid with the bisbenzylisoquinolines. Oxidation with $KMnO_4$ in acetone gave 4′,5-dicarboxy-2-methoxydiphenylether, showing the position of one of the ether bridges, as well as the position of the phenolic hydroxy group, which must be in the isoquinoline part of the molecule due to the lack of such a group in the acid [97]. The alkaloid has been cleaved with sodium in liquid ammonia resulting in a phenolic and a non-phenolic basic product [121]. The non-phenolic base (CIII) was represented as *d*-1-(4′-methoxybenzyl)-

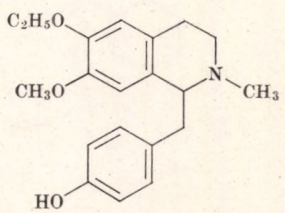
2-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline. This cleavage did not give a crystalline phenolic product and it has not been studied further. For the purpose of characterizing the phenolic moiety of the molecule, O-methyl-



CII

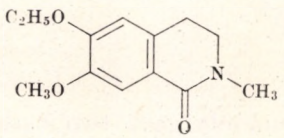


CIII

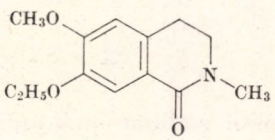


CIV

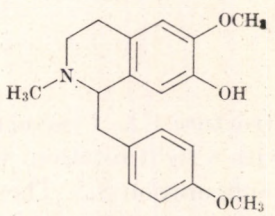
thalmine was submitted to reductive cleavage. An oxalate was obtained from the phenolic fraction, identified as armepavine oxalate. The isolation of the latter proved that the hydroxy groups of the phenolic product was formed after the cleavage of the ether bridge in the benzyl part of thalmine. Further, O-ethylthalmine was treated in the same way and two phenolic bases were obtained: the first was identical with *d*-1-(4′-oxybenzyl)-6-ethoxy 7-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (CIV). When oxidized with potassium permanganate a weakly basic compound 1-oxo-6-ethoxy-7-methoxy-2-methyltetrahydroisoquinoline (CV) was obtained. Thus the position of the hydroxy group at C-6 of the tetrahydroisoquinoline part of the molecule was fixed.



CV



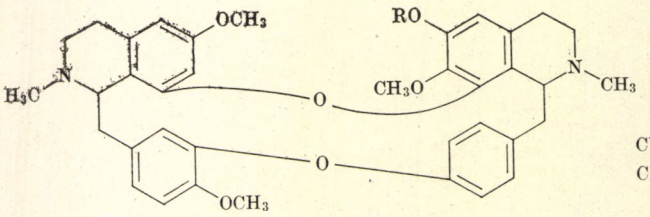
CVI



CVII

The second phenolic base, a product of the degradation of O-ethylthalmine, was converted into O-methylarmepavine with methyl iodide. It has been assumed that in this way the hydroxy group, obtained after cleavage of the ether linkage has been methylated. This base was oxidized with potassium permanganate in acetone after O-ethylation and 1-oxo-6-methoxy-7-ethoxy-2-methyl-tetrahydroisoquinoline (CVI) resulted. Thus it has been

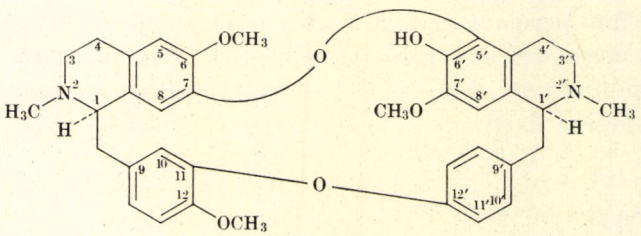
proved that the second product is a 1-(4′-methoxybenzyl)-6-methoxy-7-hydroxy-2-methyltetrahydroisoquinoline (CVII). Structure CVIII was proposed for thalmine. In a later communication about the structure of this



CVIII, R = H
CIX, R = CH_3

alkaloid, there is a new correction as to the manner of bonding of the ether bridge in the isoquinoline part [136], on the basis of the following experiments:

O-Methylmethine should be a diastereoisomer of O-methyloxyacanthine (CIX). The products of the Hofmann degradation of both compounds, containing no nitrogen and lacking an asymmetrical carbon atom, have been compared. It was found that they are not identical, a proof that thalmine cannot be related to the oxyacanthine series. Therefore the authors suggested



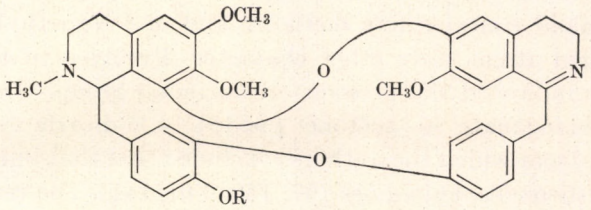
CX

structure CX. Cleavage with sodium in liquid ammonia gave two bases with a right rotation, therefore both asymmetrical carbon atoms have the configuration *S*,*S*. The structure of thalmine was confirmed by NMR and MS investigations [137].

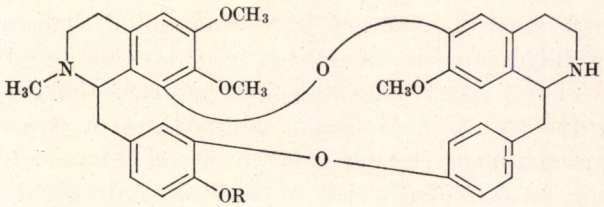
(xv) O-Methylthalmethine

O-Methylthalmethine, $C_{37}H_{38}O_6N_2$, m.p. 245–6°C (benzene), $[\alpha]_D$ 237° (chloroform), is found in *Thalictrum minus* L. [76]. By catalytic hydrogenation (Adams catalyst) one mole of hydrogen is consumed and the alkaloid

(CXI) is converted into the dihydro derivative (CXII), m.p. 278–280°C (benzene). The same product is obtained by $NaBH_4$ reduction. This compound can be methylated on the nitrogen with formaldehyde–formic acid. The base,



CXI, R = CH_3
CXIII, R = H



CXII, R = CH_3
CXIV, R = H

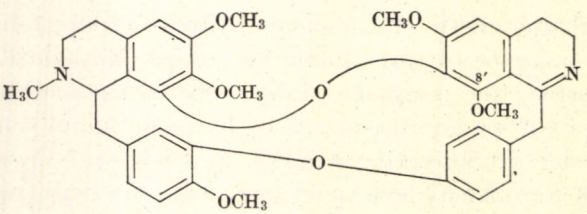
m.p. 184–7°C is identical in its spectral and chromatographic behaviour with O-methylthalicberine (LXXXII). The position of the >C=N group in O-methylthalmethine has been elucidated by cleavage with metallic sodium in liquid ammonia. A non-phenolic base was isolated which was identical with *dl*-O,O,N-trimethylcoclaurine (LXXXIII). Therefore the imino group has to be in the right-hand part of the molecule [138].

(xvi) Thalmethine

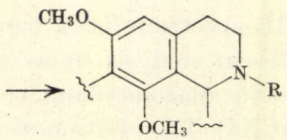
Thalmethine (CXIII), $C_{36}H_{36}O_6N_2$, m.p. 275–7°C (methanol), $[\alpha]_D$ + 200° (in chloroform), has also been isolated from *Thalictrum minus* L. [76]. The IR spectrum has a band characteristic of a phenolic hydroxy group; functional analysis shows the presence of three methoxy groups. Methylation with diazomethane converted the compound into O-methylthalmethine (CXI). The position of the hydroxy group was proved by reduction of thalmethine with $NaBH_4$. The dihydroderivative (CXIV), m.p. 196–197°C was further N-methylated with formaldehyde–formic acid. The new base was identical with thalicberine (LXXXVII), isolated from *Thalictrum thunbergii*.

(xvii) Thalsimine

Thalsimine $C_{38}H_{40}O_7N_2$, m.p. 137–142°C (acetone); $[\alpha]_D$ + 27·45 (ethanol), can be isolated from the leaves, stems and seeds of *Thalictrum simplex* L. [126]. The alkaloid contains five methoxy and one N-methyl group. The remaining oxygen atoms have ether character. Pyrolysis of thalsimine in molten sulphur indicated the presence of an imino group. Oxidation with potassium permanganate in acetone yielded 4,′5-dicarboxy-2-methoxydiphenyl ether, from which the authors concluded that thalsimine belongs to the group of bisbenzylisoquinolines [97, 139, 140, 141]. The remaining four methoxy groups and the second ether linkage must be in the isoquinoline part of the molecule. On reduction with $NaBH_4$, zinc and sulphuric acid, or catalytically, one mole of hydrogen is consumed and thalsimine (CXV) is converted into dihydrothalsimine with a secondary amino group (CXVI). N-Methylation of the latter yielded N-methyldihydrothalsimine, identical with hernandezine (LXXV). Hofmann degradation of N-methyldihydrothalsimine afforded trimethylamine after the second step, as well as a compound containing no nitrogen, which on catalytic hydrogenation consumed eight atoms of hydrogen. This compound is identical with the similar product of the Hofmann degradation of hernandezine. Therefore, thalsimine differs from the latter in the presence of an imino group in place of a N-methyl one. To determine the position of the double bond, thalsimine was hydrogenated and subsequently cleaved with metallic sodium in liquid ammonia. From the reaction mixture a crystalline phenolic base was isolated as the hydrobromide. Further, thalsimine was directly cleaved by reduction; the same hydrobromide with negative reaction of the tertiary nitrogen was obtained. Therefore, by the cleavage of both ether linkages the double bond of thalsimine was reduced. The phenolic base obtained was identical with coclaurine (CXVII). This explained that simultaneously with the reduction the methoxy at C-8′ in the first-proposed structure (CXV) is cleaved. In this way it was proved that the double bond at the nitrogen atom is placed in

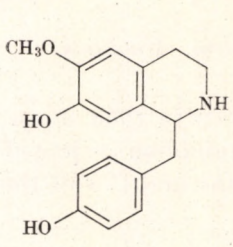


CXV

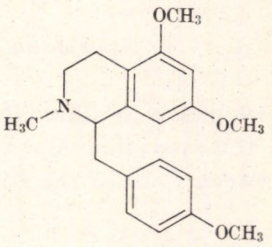


CXVI, R = H
LXXV, R = CH_3

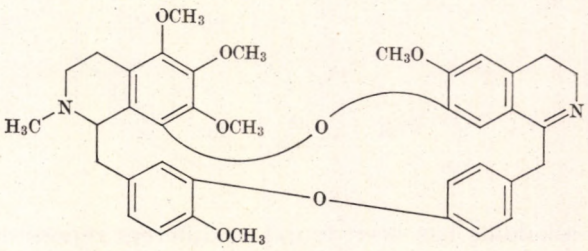
the right-hand benzylisoquinoline part of the alkaloid. A non-phenolic base has not been isolated. The newly-established structure of hernandezine (LXXVI) changed the structure of thalsimine as well. A direct proof of the new structure (CXVIII) was the reductive cleavage undertaken by the authors; a non-phenolic product (CXIX) was obtained as well as the base coclaurine (CXVII). The non-phenolic compound had the same IR and NMR spectrum as the non-phenolic base obtained after the cleavage reaction of hernandezine [142].



CXVII



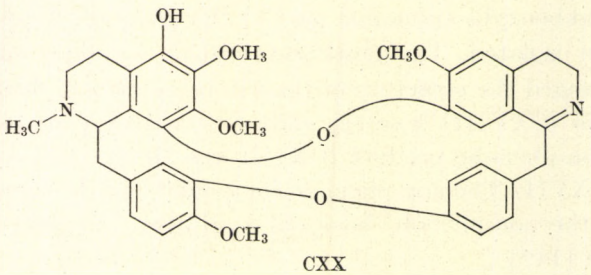
CXIX



CXVIII

(xviii) Thalsimidine

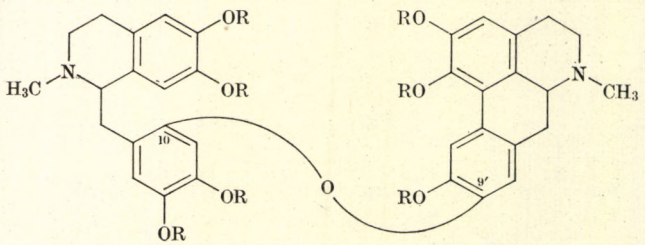
Thalsimidine, $C_{37}H_{38}O_7N_2$, m.p. 195°C (ethanol); $[\alpha]_D + 48°$ (in chloroform), can be isolated from the above-ground parts of *Thalictrum simplex* L. Reduction with Pt/H_2 gives a dihydro compound. O,N-dimethyldihydrothalsimidine yields on Hofmann degradation the same N-desmethine as hernandezine (LXXVI). Methylation with diazomethane affords thalsimine (CXVIII). The new alkaloid is therefore a demethylthalsimine. MS data prove that the hydroxy group is located in the isoquinoline part. The authors suggest position C-5 for the hydroxy group so that the final structure of thalsimidine should be CXX [143, 144].



CXX

7. *Aporphine-benzylisoquinoline Alkaloids*

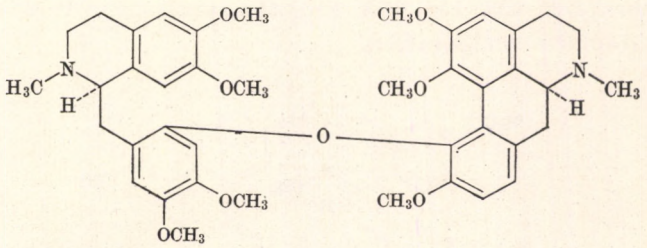
This new group of alkaloids was discovered in 1963. Both parts of the molecule, the aporphine and the benzylisoquinoline are joined by an ether bridge between C-10 of the benzylisoquinoline and C-9 of the aporphine.



This group of alkaloids has been found in different representatives of the genus *Thalictrum* and in *Hernandia ovigera*. The following alkaloids are known up to now: thalicarpine, thalmelatine, dehydrothalicarpine, dehydrothalmelatine, adiantifoline and foetidine.

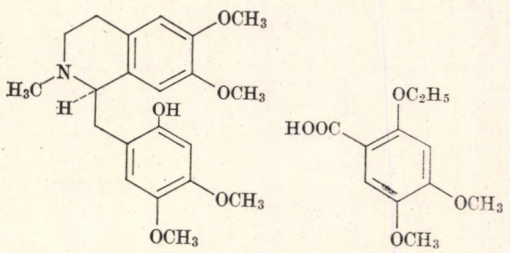
(i) Thalicarpine

Thalicarpine, $C_{41}H_{48}O_8N_2$, m.p. 160–161°C (ether); 108–110°C (methanol); $[\alpha]_D$ + 133° (methanol); +115° (ethanol) and +89° (chloroform), isolated first from roots of *Thalictrum dasycarpum* Fisch et Lall. [52], later from *Thalictrum minus* [145], *Thalictrum revolutum* [146], *Thalictrum fendleri* [44] and from *Hernandia ovigera* L. [147]. Kupchan *et al.* proposed structure CXXI [148] as the first representative of the so-called dimeric aporphine-benzylisoquinoline alkaloids on the basis of the following experiments:



CXXI

By the cleavage of the base with metallic sodium in liquid ammonia, a phenolic and two non-phenolic bases were isolated [149]. The phenolic base was characterized as (—)-6′-hydroxylaudanosine (CXXII). The location of the hydroxy group was determined after converting the phenolic base into the O-ethyl iodide with a further Hofmann degradation. Oxidation of the O-ethyl-des-N-methine with potassium permanganate yielded 2-ethoxy-4,5-dimethoxybenzoic acid (CXXIII).

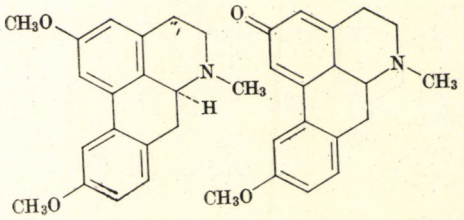


CXXII CXXIII

The one non-phenolic base of the reductive cleavage was characterized as (+)-2,10-dimethoxyaporphine-methiodide (CXXIV) after Hofmann degradation and comparison of the hydriodide with an authentic sample. Simultaneously with the cleavage of thalicarpine into two moieties a hydrogenolysis of the methoxy group at C-1 in the aporphine part was observed. Similar cleavage has been observed in other biphenyl systems and aporphine alkaloids, such as O-methyldomesticine and O,O-dimethylcorytuberine [150, 151].

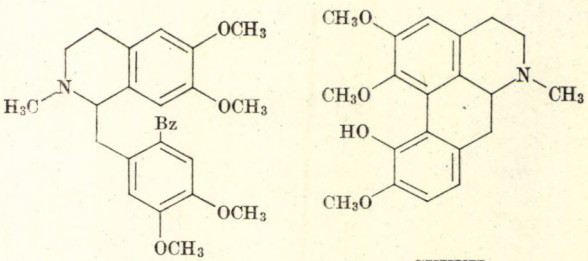
The second non-phenolic compound was identified as a ketone (CXXV). Such a ketone has been isolated by cleavage of aporphine alkaloids. The synthesis of thalicarpine was accomplished earlier by a modified Ullmann condensation of *R*-(—)-6′-bromolaudanosine (CXXVI) with *S*-(+)-isocorydine (CXXVII). Since their configuration has been determined previously by

other authors [152, 153, 154, 155, 156] the synthesis of thalicarpine established its absolute configuration.



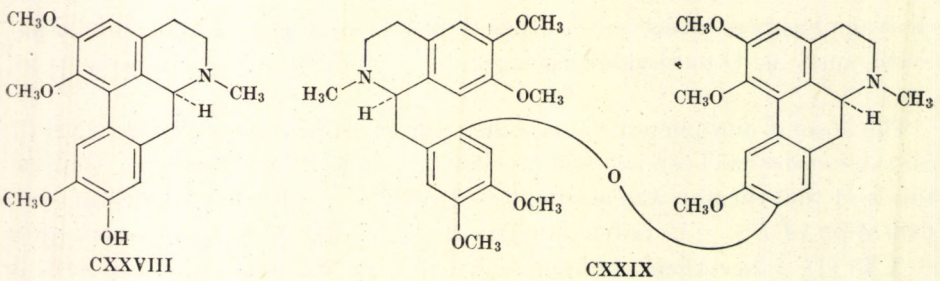
CXXIV CXXV

From a more recent study of the same alkaloid it has been found that in some respects the spectral properties did not satisfy structure CXXI [157, 158]. Thus the UV spectrum, with two maxima at 282 and 302 mμ is typical



CXXVI CXXVII

of aporphines without substituents at C-11 [24]. The NMR spectrum shows a signal for C-11 aromatic proton at 1·77 τ, shifted downfield in comparison with the remaining aromatic protons [157, 159]. These facts raise doubts about the location of the diphenyl ether bridge between the two moieties of the molecule. The re-examination of the synthesis of thalicarpine gave a compound which differed in its spectral properties from those of the natural

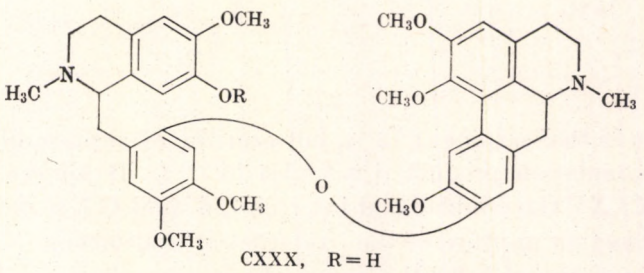


CXXVIII CXXIX

alkaloid. The condensation of S-(−)-6′-bromlaudanosine (CXXVI) with S-N-methyllaurotetanine (CXXVIII) led to a base, identical with the natural one. With the determination of the configuration of N-methyllaurotetanine the new configuration of thalicarpine (CXXIX) was established.

(ii) Thalmelatine

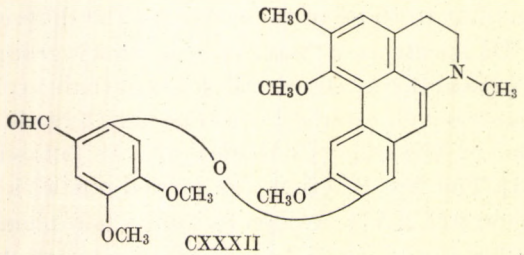
Thalmelatine, $C_{40}H_{46}O_8N_2$, m.p. 131–135°C (ether); 120–123°C (abs. ethanol); $[\alpha]_D + 110°$ (ethanol), isolated from *Thalictrum minus* [145]. Functional analysis shows the presence of six methoxy groups (CXXX). UV and IR spectra are very similar to those of thalicarpine. The difference between them is that in the IR spectrum a band of a hydroxy group is found. O-Methylation with diazomethane converted it into thalicarpine. The structure of thalmelatine has been elucidated on the one hand by sodium–liquid ammonia treatment, and on the other by oxidation with potassium permanganate in acetone [160]. The first reaction was carried out with the O-ethyl derivative of the alkaloid (CXXXI). A phenolic and a non-phenolic fraction have been obtained. The non-phenolic base is identical with 2,10-dimethoxyaporphine, isolated from the same reaction of thalicarpine (CXXIV). The chromatographic behaviour and IR spectrum of the phenolic base, however, did not agree with 6′-hydroxylaudanosine (CXXII). These data show that the hydroxy group is located in the benzylisoquinoline moiety. The reduction with sodium in liquid ammonia gives rise to mixtures which are difficult to separate, and the pure compounds are obtained only in small quantities. Therefore oxidation of O-ethylthalmelatine (CXXXI) was examined in addition to thalicarpine itself. From the reaction mixture of either alkaloid compound CXXXII was isolated as main product. It did not possess an ethoxy group. Therefore the hydroxy group of thalmelatine can only be in the isoquinoline part of the benzylisoquinoline



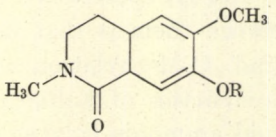
CXXX, R = H
CXXXI, R = C_2H_5

moiety at C-6 or C-7. 1-Oxo-6,7-dimethoxy-2-methyltetrahydroisoquinoline was isolated from the oxidation of thalicarpine (CXXXIII) [161], and 1-oxo-6-methoxy-7-ethoxy-benzyltetrahydroisoquinoline (CXXXIV) from O-ethylthalmelatine (CXXXI) [162].

The isolation of the latter compound located the hydroxy group of thalme-l atine at C-7. The structure of the product CXXXII $C_{29}H_{29}O_7N$ was proved by interpretation of the UV, IR and NMR spectra, by treatment with sodium in liquid ammonia and by hydrogenation [163]. On reductive cleavage, 2,10-dimethoxyaporphine (CXXIV) is produced; the presence of the aldehyde group was proved by the formation of a 2,4-dinitrophenylhydrazone. Cata-

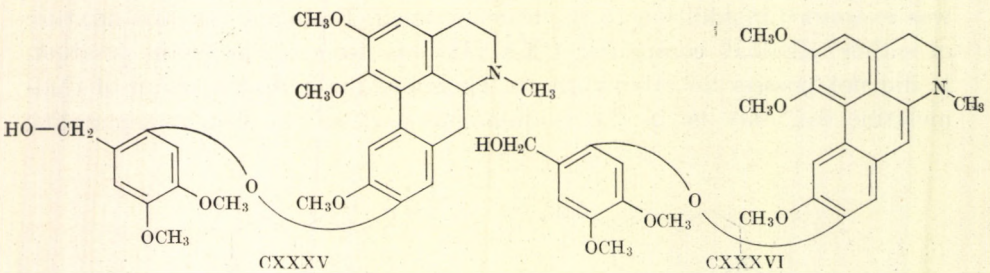


CXXXII



CXXXIII, R = CH_3
CXXXIV, R = C_2H_5

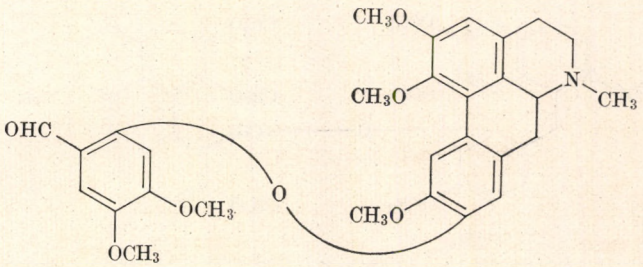
lytic hydrogenation consumed two moles of hydrogen, and a compound (CXXXV) was obtained, characterized by spectral analysis. Hydrogenation with $LiAlH_4$ gave the product CXXXVI, in the IR spectrum of which the aldehyde group band at 1680 cm^{-1} was lacking. In the NMR spectrum one of the aromatic protons absorbs downfield (at 1·03 τ) in comparison with the



CXXXV CXXXVI

same proton in thalicarpine (1·72 τ), but near the absorption of this proton in the main oxidation product (CXXXII) (0·77 τ). It follows that in the product CXXXV the double bond between C-6′ and C-7 is retained [164]. From the reaction mixture of the oxidation of thalicarpine (CXXIX) and O-ethylthalmelatine (CXXXI) a second basic compound, $C_{29}H_{31}O_7N$, m.p.

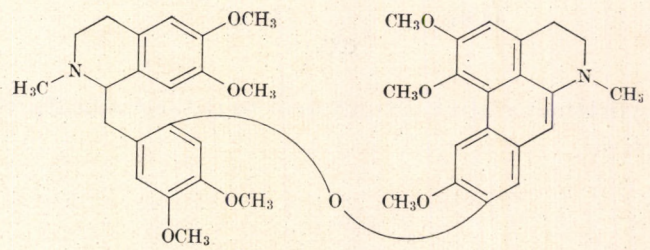
166–8°C (methanol) was isolated [165]. Its IR spectrum shows a band due to the carbonyl group at 1680 cm^{-1} (CXXXVII). The NMR spectrum, like that of thalicarpine, has a signal at $\delta = 1{\cdot}70$ ppm.



These data have led to the assumption that oxidation produces no change in the aporphine moiety of this compound. No double bond is created as in the case of compound CXX. The isolated product is identical with the natural alkaloid hernandaline, isolated from *Hernandia ovigera* [166, 167].

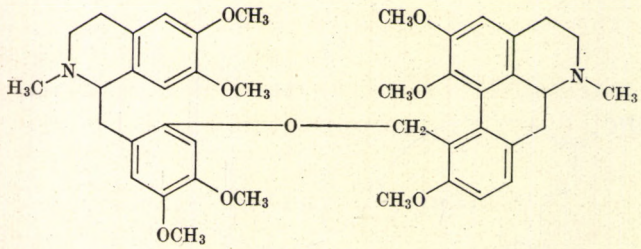
(iii) Dehydrothalicarpine

Dehydrothalicarpine, $C_{41}H_{46}O_8N_2$, m.p. 180–2°C, $[\alpha]_D + 54°$ (in chloroform), has been isolated from *Thalictrum minus* L. [168] and *Thalictrum dasycarpum* [128, 129]. If the alkaloid mixture is allowed to stand, the quantity of this alkaloid is increased. In catalytic hydrogenation dehydrothalicarpine absorbs one mole of hydrogen to give a compound which is identical with thalicarpine (CXXIX) in its chromatographic behaviour as well as according to the NMR, IR and UV spectra. The melting point of the hydrogenated product is lower by 60°C (106–108°C) and the optical rotation is +77° instead of +100° (in thalicarpine), measured at the same concentration. The structure of dehydrothalicarpine was established as CXXXVIII.



CXXXVIII

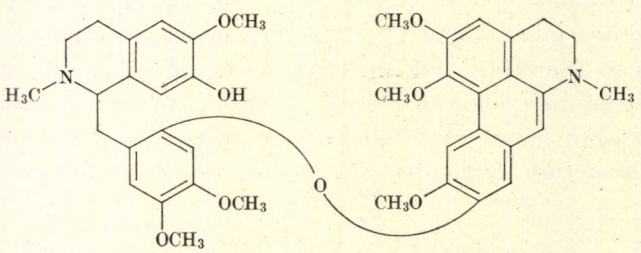
It was found that thalictrucarpine (earlier structure CXXXIX) is identical with dehydrothalicarpine [169]. It has been isolated from *Thalictrum dasycarpum* [128].



CXXXIX

(iv) Dehydrothalmelatine

Dehydrothalmelatine, $C_{40}H_{44}O_8N_2 \cdot H_2O$, m.p. 126–128°C (ether: methanol); $[\alpha]_D + 31{\cdot}9°$ (chloroform), has been isolated from the alkaloid mixture, left as free bases, of *Thalictrum minus* [165]. The UV and IR spectra are very similar to those of dehydrothalicarpine, differing only in the presence of a hydroxy group band in the latter. Catalytic reduction consumed one mole of hydrogen and the chromatographic behaviour and IR spectrum of the compound obtained are in complete agreement with those of the alkaloid thalmelatine (CXL). Therefore on analogy with dehydrothali-

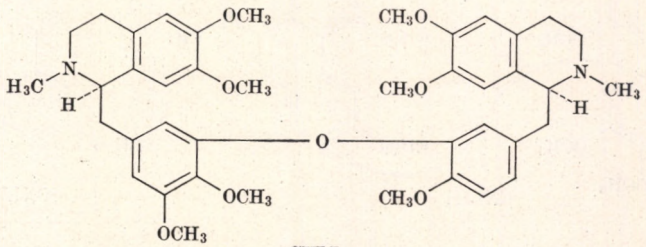


CXL

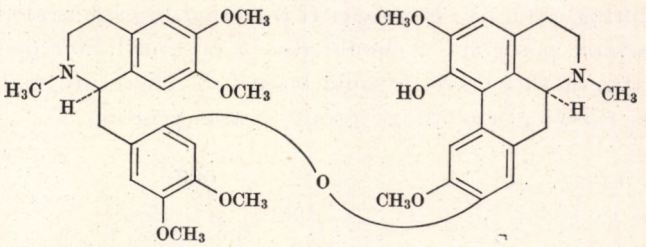
carpine, dehydrothalmelatine is equivalent to thalmelatine dehydrogenated at C-6′ and C-7

(v) Foetidine

Foetidine, $C_{41}H_{50}O_8N_2 \cdot H_2O$, m.p. 132–135°C (ethyl acetate); $[\alpha]_D$ + 121·4° (methanol), was found in *Thalictrum foetidum* L. [170]. Sodium–liquid ammonia treatment afforded a non-phenolic moiety, identical with *d*-laudanosine, and a phenolic part, characterized as laudanine. At first, structure CXLI was proposed. Later, the same authors revised this: the UV spectrum contains not one maximum as had been shown earlier, but two, like the aporphine alkaloids [24, 171]. Moreover, it was found that the alkaloid possesses not seven methoxy groups, but six together with one hydroxy group. Reductive cleavage gave the non-phenolic *d*-laudanosine



CXLI



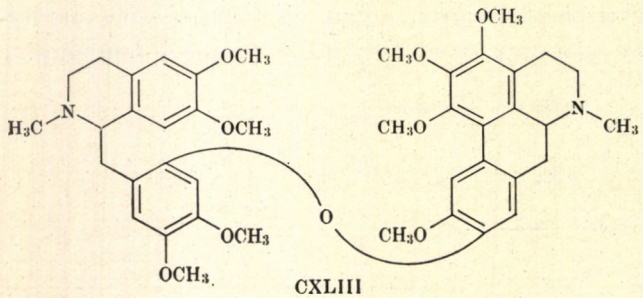
CXLII

and two phenolic bases. One of them is identical with (∓)-laudanine, the other with isoboldine. On the basis of these data structure CXLII has been suggested and a new formula $C_{40}H_{46}O_8N_2 \cdot H_2O$ proposed. As the absolute configurations of *d*-laudanosine and *d*-glaucine have been determined earlier, the absolute configuration of foetidine must be *S,S* (CXLII).

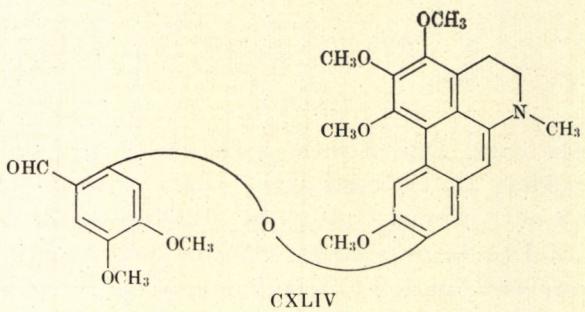
(vi) Adiantifoline

Adiantifoline, $C_{42}H_{50}O_9N_2$, m.p. 143·5–144°C (abs. ethanol), $[\alpha]_D$ + 90° (methanol), has been isolated from *Thalictrum minus* L. *var. adiantifolium* Hort. [172]. The UV and IR spectra are similar to that of thalicarpine, but

the NMR spectrum shows an extra methoxy group (CXLIII). Compound CXLIV, obtained by the oxidation of adiantifoline with $KMnO_4$ in acetone, has very similar spectral data to those of the oxidation product (CXXXII) of thalicarpine and thalmelatine. Cleavage with sodium in liquid ammonia yielded 6′-hydroxylaudanosine as a phenolic part, so that the extra methoxy group must be in the aporphine part of the molecule. As a non-phenolic product the ketone CXLV was isolated; it follows from this that the positions of two oxygens are C-2 and C-10.



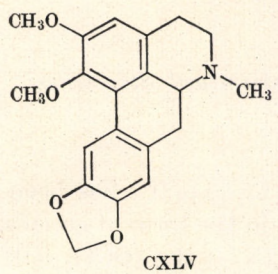
The position C-1 may also be substituted with a methoxy group which is decomposed during reductive cleavage. If the ether linkage is identical with that in thalicarpine, position C-9 should also be occupied. So a possible position for the extra methoxy group could be at C-3. There are many Thalictrum alkaloids carrying a methoxy group at this position.



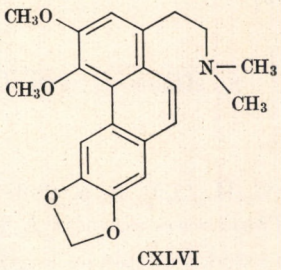
(vii) Thalictuberine

Thalictuberine, $C_{21}H_{23}O_4N$, m.p. 126–7°C, $[\alpha]_D$ 0°, can be isolated from the roots of *Thalictrum thunbergii* DC. [169]. Hofmann degradation of O-methyldomesticine (CXLV) (from *Nandia domestica*) gave a methylmethine,

identical with thalictuberine. The proposed structure is 1-(2-dimethylaminoethyl)-3,4-dimethoxy-6,7-methylendioxyphenanthrene (CXLVI).



CXLV

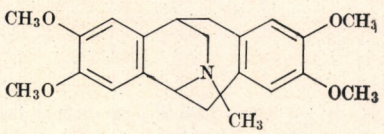


CXLVI

8. Pavine, Isopavine and Other Related Alkaloids

(i) Thalisopavine

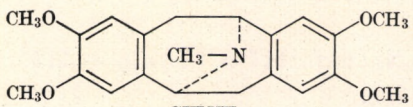
Thalisopavine, $C_{20}H_{23}O_4N$, m.p. 211–2°C, $[\alpha]_D$ — 210° (chloroform), was isolated from *Thalictrum dasycarpum* [2]. Chemical investigations and spectral data have led to structure CXLVI.



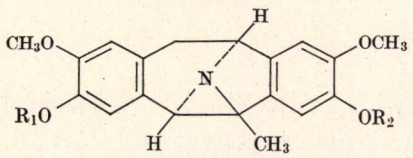
CXLVI

(ii) Argemonine, Norargemonine and Bisnorargemonine

Argemonine, $C_{21}H_{25}O_4N$ (CXLVII), m.p. 156–7°C, $[\alpha]_D$ — 200° (methanol) — 214° (ethanol) [172]; norargemonine, $C_{20}H_{23}O_4N$, m.p. 239–242°C,



CXLVII



CXLVIII, R_1=H; R_2=CH_3 CXLIX, R_1=R_2=H

$[\alpha]_D$ — 154° (methanol) (CXLVIII) and bisnorargemonine, $C_{19}H_{21}O_4N$, m.p. 181–2°C, $[\alpha]_D$ + 87° (CXLIX) have been isolated from *Thalictrum dasycarpum* [2].

9. Alkaloids with Undetermined Structures

(i) Thalicsine

Thalicsine, $C_{21}H_{19}O_6N$, m.p. 263–267°C, $[\alpha]_D$ 0°, has been isolated from the roots of *Thalictrum simplex L.* [77] in the form of greenish yellow crystals. The proposed formula is $C_{19}H_{12}O_4(N—CH_3)$ $(OCH_3)(OH)$.

(ii) Elatrine

Elatrine, $C_{40}H_{56}O_6N_3$, m.p. 180–3°C (decomp.), $[\alpha]_D$ + 288·94° (in ethanol), isolated from *Thalictrum minus var. elatum* [173].

(iii) Thalibrunine

Thalibrunine, $C_{39}H_{46}O_8N_2$, m.p. 172–3°C, $[\alpha]_D$ 160° (in methanol), found in *Thalictrum rochebrunianum* [53].

(iv) Rugosine

Rugosine, $C_{20}H_{27}O_5N$, m.p. 110–115°C, isolated from the roots of *Thalictrum rugosum* Ait. [174, 175].

(v) Thalfine

Thalifine, $C_{38}H_{36}O_8N_2$, m.p. 141–2°C (decomp.), $[\alpha]_D$ + 69° (ethano) isolated from *Thalictrum foetidum* [176].

(vi) Thalfinine

Thalfinine, $C_{39}H_{44}O_8N_2$, m.p. 117–8°C, $[\alpha]_D$ + 115° (ethanol), found in *Thalictrum foetidum* [176].

(vii) Thalisopidine

Thalisopidine, $C_{37}H_{40}O_7N_2$, m.p. 215–6°C (acetone), $[\alpha]_D$ — 9° (ethanol), has been isolated from *Thalictrum isopyroides* C. A. M. [16]. The first proposed formula is $C_{32}H_{23}(N—CH_3)_2(OCH_3)_3(OH)_2(—O—)_2$.

10. Thalictrum Alkaloids

Alkaloid	M. p., °C	$[\alpha]_D$	Origin
1. Isoquinoline alkaloids			
Thalactamine $C_{13}H_{15}O_2N$	112–4°	0°	*T. minus* [3]
Corypalline $C_{11}H_{15}O_2N$	168°	0°	*T. dasycarpum* [1, 2]
Noroxyhydrastinine $C_{10}H_9O_3N$	182–3°	—	*T. minus var. adiant.* [4]
Thalifoline $C_{11}H_{13}O_3N$	210–1°	—	*T. minus var. adiant.* [4]
2. Benzylisoquinoline alkaloids			
Takatonine $C_{21}H_{24}O_4N^+$	iodide 192–3°	0°	*T. thunbergii* [5]
Thalifendlerine $C_{20}H_{25}O_4N$	177–8°	—108° MeOH	*T. fendleri* [11]
Laudanidine $C_{20}H_{25}O_4N$	184°	—94° $CHCl_3$	*T. dasycarpum* [2]
3. Aporphine alkaloids			
Thalicmine (ocoteine) $C_{21}H_{23}O_5N$	137–8°	225° EtOH	*T. minus* [14], *T. isopyroides* [16], *T. fendleri* [12].
Thalicmidine $C_{20}H_{23}O_4N$	192–3°	—84·5° EtOH	*T. minus* [12]
Glaucine $C_{21}H_{25}O_4N$	120°	114°	*T. minus* [15] *T. fendleri* [12]
Magnoflorine $C_{20}H_{24}O_4N^+$	iodide 248–9°	—214°	*T. dasycarpum* [51, 52], *T. minus* [58], *T. minus var. adiant.* Hort. [56], *T. flavum* [59], *T. foliolsum* [57], *T. foetidum* [58], *T. fendleri* [11], *T. isopyroides* [58], *T. thunbergii* DC. [48], *T. simplex* L. [60], *T. rugosum* [54, 55], *T. rochebrunianum* [53].

Alkaloid	M.p., °C	$[\alpha]_D$	Origin
Isocorydine $C_{20}H_{23}O_4N$	185–6°	195° (C)	*T. aquilegifolium* [63]
Thalicsimidine $C_{22}H_{26}O_5N$	131–2°	20·26	*T. simplex* [60]
Thalicminine (Base No. 5) $C_{20}H_{15}O_6N$	263–5°	0°	*T. minus* [14, 67], *T. simplex* L. [95], *T. isopyroides* [16]
Thaliporphine $C_{20}H_{23}O_4N$	170–2°	—	*T. fendleri* [44]
Preocoteine $C_{21}H_{23}O_5N$	oil	—	*T. fendleri* [44]
4. Protoberberine alkaloids			
Berberine $C_{20}H_{18}O_4N$	144° or 205° (hydr.)	0°	*T. dasycarpum* [51], *T. flavum* [59, 68, 69], *T. fendleri* [11], *T. foliolosum* [61], *T. foetidum* [58], *T. minus* L. [58], *T. minus ssp. elatum* [75], *T. minus var. adiant.* [56], *T. longipedunculatum* [58], *T. isopyroides* [58], *T. pedunculatum* [74], *T. rugosum* [54, 55], *T. rochebrunianum* [53, 70], *T. tubiferum* [73], *T. thunbergii* [47], *T. simplex* L. [77]
Tetrahydroberberine $C_{20}H_{21}O_4N$	169–70°	0°	*T. actaefolium* [83]
Chlormethylate of L-Canadine $C_{21}H_{24}O_4N$. HCl	191–3°	—158°	*T. minus* L. [84]
Jatrorrhizine $C_{20}H_{20}O_4N$	—	0°	*T. fendleri* [11] *T. foliolosum* [86] *T. rugosum* [54, 55] *T. rochebrunianum* [53]
Columbamine $C_{20}H_{20}O_4N$	—	0°	*T. rugosum* [71]

Alkaloid	M.p., °C	$[\alpha]_D$	Origin
Palmatine $C_{21}H_{22}O_4N$	—	0°	*T. foliolosum* [86]
Thalifendine $C_{19}H_{16}O_4N$	chloride 230°	0°	*T. fendleri* [11, 12]
Thalidastine $C_{19}H_{16}O_5N$	230°	138° (M)	*T. fendleri* [12, 90]
5. Protopine alkaloids			
Thalisopyrine (Cryptopine) $C_{23}H_{25}O_5N$	216–7°	0°	*T. isopyroides* [92] *T. flavum* [59]
Thalictrimine (β-Allocryptopine) $C_{20}H_{23}O_4N$	169·5–70°	0°	*T. minus* L. [93, 94] *T. simplex* L. [60]
Thalictrisine $C_{20}H_{21}O_5N$	261–3°	—	*T. simplex* [95]
6. Bisbenzylisoquinoline alkaloids			
Thalisopine $C_{28}H_{44}O_5N_2 \cdot 2\,H_2O$	151–3°	—104·9° (A)	*T. isopyroides* [16, 95a, 96]
O-Methyltalisopine $C_{39}H_{46}O_5N_2$	—	—	*T. isopyroides* [96]
Berbamine $C_{37}H_{40}O_6N_2$	172°	109° (C)	*T. foetidum* [99]
Obamegine (Stepholine) $C_{36}H_{38}O_6N_2$	162–6°	99° (M)	*T. rugosum* [102]
Isotetrandine $C_{38}H_{42}O_6N_2$	182°	146° (C)	*T. foetidum* [99]
Thalidezine $C_{38}H_{42}O_7N_2$	158–9°	235° (C)	*T. simplex* [105], *T. rugosum* [130], *T. fendleri* [44]

Alkaloid	M.p., °C	$[\alpha]_D$	Origin
Hernandezine (Thalicsimine) $C_{39}H_{44}O_7N_2$	192–3°	250° (C)	*T. hernandezii* [106] *T. rochebrunianum* [53] *T. flavum* [68], *T. alpinum* [108] *T. simplex* [77, 97, 107] *T. fendleri* [12]
Thalisamine $C_{38}H_{42}O_7N_2$	191–4°	—138° (C)	*T. simplex* [105]
Thalidasine $C_{39}H_{42}O_7N_2$	105–7° amorphous	—70° (M)	*T. dasycarpum* [128, 129]
Thalicberine $C_{37}H_{40}O_6N_2$	161°	231·2° (C)	*T. thunbergii* [115]
O-Methylthalicberine (Thalmidine) $C_{38}H_{42}O_6N_2$	186–7° 192–3°	266° (C)	*T. thunbergii* [115] *T. minus* [121, 122] *T. isopyroides* [96]
Thalfoetidine (Thalictrinine) $C_{38}H_{42}O_7N_2 . 0{\cdot}5(C_2H_5)_2O$	168–70°	—88·6° (C)	*T. foetidum* [125] *T. longipedunculatum* [58]
Thalmine $C_{36}H_{40}O_5N_2$	252–3°	—64·5°	*T. simplex* L. [127] *T. minus* [14, 135]
Thalicrine (Aromoline) $C_{36}H_{38}O_6N_2 . H_2O$	221–2°	341·2°	*T. thunbergii* [131]
Homothalicrine (Homoaromoline) $C_{37}H_{40}O_6N_2$	235–6°	425·3°	*T. thunbergii* [131]
Thalmetine $C_{36}H_{36}O_6N_2 . 1/2\ H_2O$	275–7°	200° (C)	*T. minus* [76]
O-Methylthalmetine $C_{37}H_{38}O_6N_2$	245–6°	237° (C)	*T. minus* L. [76]

Alkaloid	M.p.. °C	$[\alpha]_D$	Origin
Thalsimine $C_{38}H_{40}O_7N_2$	137–142°	27·45° (C)	*T. simplex* [126]
Thalsimidine $C_{37}H_{38}O_7N_2$	195°	48° (C)	*T. simplex* [143, 144]
7. Aporphine-benzylisoquinoline alkaloids			
Thalicarpine $C_{41}H_{48}O_8N_2$	160–1° 103–110° (M)	115° (A) 83° (C) 133° (M)	*T. dasycarpum* [52] *T. minus* [145] *T. revolutum* [146] *T. fendleri* [44] *Hernandia ovigera* [147]
Dehydrothalicarpine (Thalictrucarpine) $C_{42}H_{50}O_8N_2$	180–2°	54° (C)	*T. minus* [168] *T. dasycarpum* [128, 129]
Thalmelatine $C_{40}H_{46}O_8N_2$	131–5°	110° (A)	*T. minus* [145]
Dehydrothalmelatine $C_{40}H_{44}O_6N_2 . H_2O$	126–3°	31·9° (C)	*T. minus* [165]
Adiantifoline $C_{42}H_{50}O_9N$	143·5–144°	90° (M)	*T. minus var. adiantifolium* [172]
Foetidine $C_{41}H_{50}O_8N_2 . H_2O$	132–5°	121·4° (M)	*T. foetidum* [170]
8. Pavine, isopavine and other alkaloids			
Thalisopavine $C_{20}H_{23}O_4N$	211–2°	—210° (C)	*T. dasycarpum* [2]
Argemonine $C_{21}H_{25}O_4N$	156–7°	—200° (M)	*T. dasycarpum* [169]

Alkaloid	M.p., °C	$[\alpha]_D$	Origin
Norargemonine $C_{20}H_{23}O_4N$	239–42°	—154° (C)	*T. dasycarpum* [2]
Bisnorargemonine $C_{19}H_{21}O_4N$	181–2°	87° (C)	*T. dasycarpum* [2]
Thalictuberine $C_{21}H_{23}O_4N$	126–7°	0°	*T. thunbergii* [169]
9. Alkaloids with undetermined structure Thalicsine $C_{21}H_{19}O_6N$	263–7°	0°	*T. simplex* [77]
Elatrine $C_{40}H_{56}O_6N_3$		288·9° (A)	*T. minus ssp. elatum* [173]
Thalibrunine $C_{39}H_{46}O_8N_2$	172–3°	160° (M)	*T. rochebrunianum* [53]
Rugosine $C_{20}H_{27}O_5N$	110–5°	0°	*T. rugosum* [174, 175]
Thalfine $C_{38}H_{36}O_8N_2$	161–2°	69° (A)	*T. foetidum* [176]
Thalfinine $C_{39}H_{44}O_8N_2$	234–5°	135° (H_2O)	*T. foetidum* [176]
Thalisopidine $C_{37}H_{40}O_7N_2$	215–6°	—9° (A)	*T. isopyroides* [16]

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III. SPECTRAL PROPERTIES

(N. M. Mollov and V. St. Georgiev)

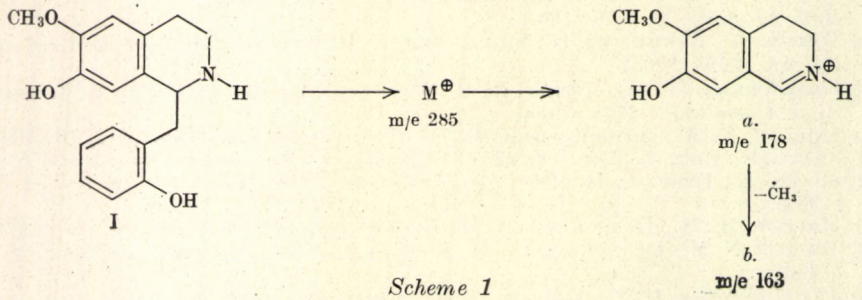
1. Mass Spectrometry

Mass spectrometry only recently found application as a research method in the study of isoquinoline alkaloids to which belong the Thalictrum alkaloids. But the valuable information which the method gives about the structure of these substances makes it a useful tool of the research chemist.

(i) Benzyltetrahydroisoquinoline Alkaloids

It has been found that the benzyltetrahydroisoquinoline alkaloids exhibit typical behaviour under the conditions of mass spectrometrical fragmentation [1]. Thus, for example, the spectrum of 1-(2′-hydroxybenzyl)-6-methoxy--7-hydroxy-1,2,3,4-tetrahydroisoquinoline (I), has the basic peak due to *a*. at m/e 178, as a result of the cleavage of the benzyl group from the molec-

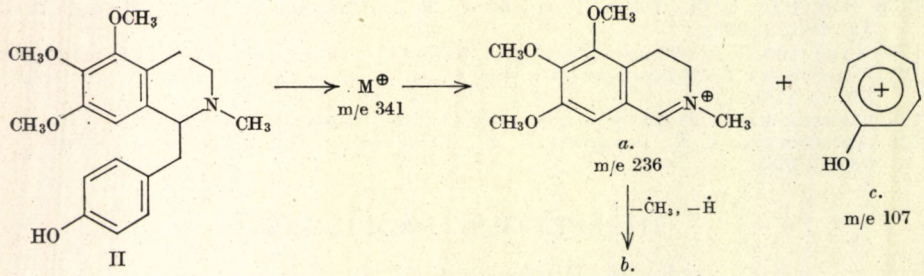
ular ion. A methyl group from *a.* may split off to form the ion *b.*; this corresponds to the intensive peak at m/e 163 (see Scheme 1).



Scheme 1

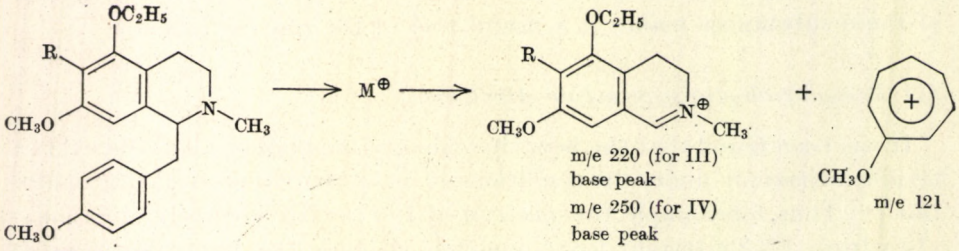
In the literature up to the present there is information only about mass spectral investigations of thalifendlerine (II) [2], of the benzyltetrahydroisoquinoline alkaloids found in Thalictrum species.

The mass spectrum of this alkaloid shows a peak for the molecular ion at m/e 341, a basic peak at m/e 236, corresponding to the ion *a.* and two intensive peaks at m/e 220 and 107 for the fragments *b.* and *c.* (Scheme 2).



Scheme 2

The mass spectral fragmentation of two non-phenolic compounds (III, IV) obtained after sodium–liquid ammonia treatment of O-ethylthalidezine is similar (Scheme 3) [3].

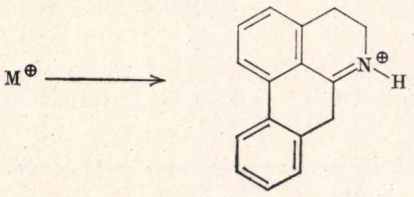


Scheme 3

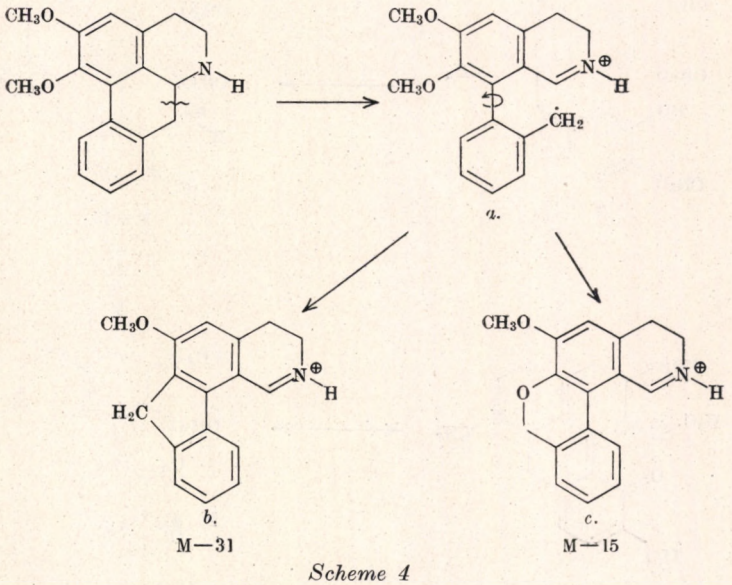
(ii) Aporphine Alkaloids

The aporphine alkaloids are more stable under the conditions of mass spectral fragmentation due to the more difficult cleavage of the biphenyl system.

The mass spectra of these alkaloids [1] show a basic peak, formed after the splitting off of one hydrogen atom, adjacent to the nitrogen atom, from the molecular ion:

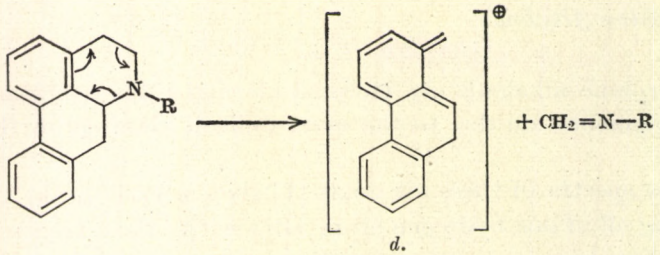


In addition, there are other peaks corresponding to the fragments M-15 and M-31, formed by the loss of one methyl and one methoxy radical, and stabilized after that by the formation of a new ring (Scheme 4).



Scheme 4

The peak M-29 corresponds to a fragment formed after the loss of methyleneimine, as a result of a cyclic transfer of electrons (the peak for alkaloids possessing an N-methyl group will be M-43):

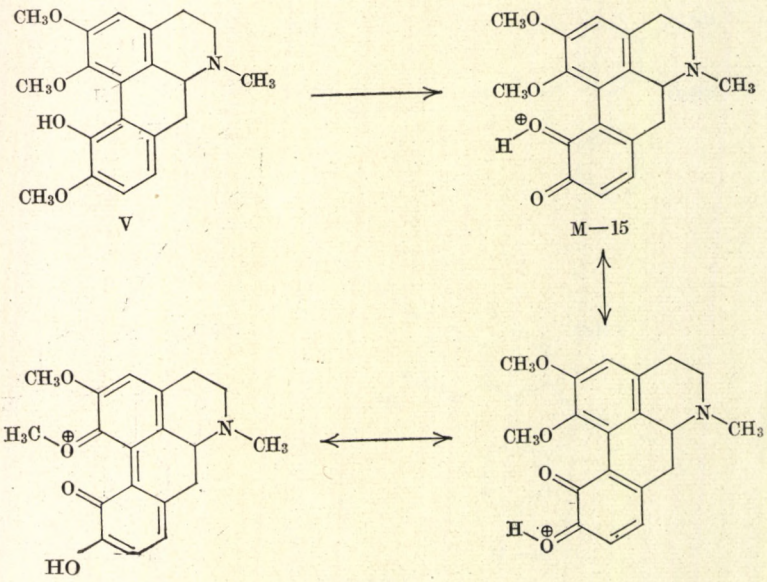


d.

M — 29 (R = H);
M — 43 (R = CH_3);

The further loss of $-OCH_3$ and $-CH_3$ groups from the fragment *d.* is possible.

All peaks described above are observed in the mass spectrum of isocorydine (V) [1]. It is an outstanding fact here that the peak M-15 is basic. This can be explained by the presence of four oxygen functions on the aromatic rings of isocorydine which gives rise to the possibility of stabilization of the positive charge by delocalizing it among several oxygen atoms:

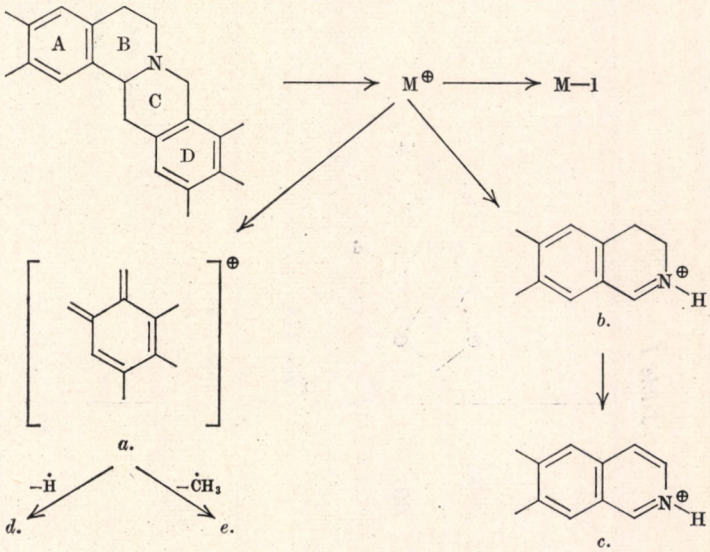


As a conclusion the presence of peaks at m/e 152 and 165 must be noted in the mass spectra of all aporphine alkaloids; these peaks may serve for indication of these bases.

The mass spectral behaviour of the aporphine bases thalicmine, thalicmidine and thalicsimidine were also investigated [37, 38]. The fragmentations of these compounds are in good agreement with the behaviour of the other aporphine alkaloids.

(iii) Protoberberine Alkaloids

The fragmentation scheme of the alkaloids of this group indicates that the cleavage of the ring system C is dominant [1] (see Scheme 5).



Scheme 5

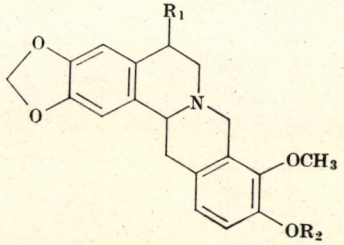
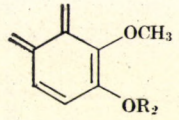
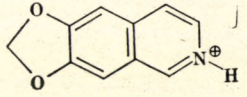
The peaks corresponding to the fragments *a.* and *b.* (or *c.*) are usually the basic peaks. They are obtained by the cleavage of the ring C bonds in the molecular ion. Further, a methyl group (ion *e.*) might split from some methoxy substituent of the ion *a.*

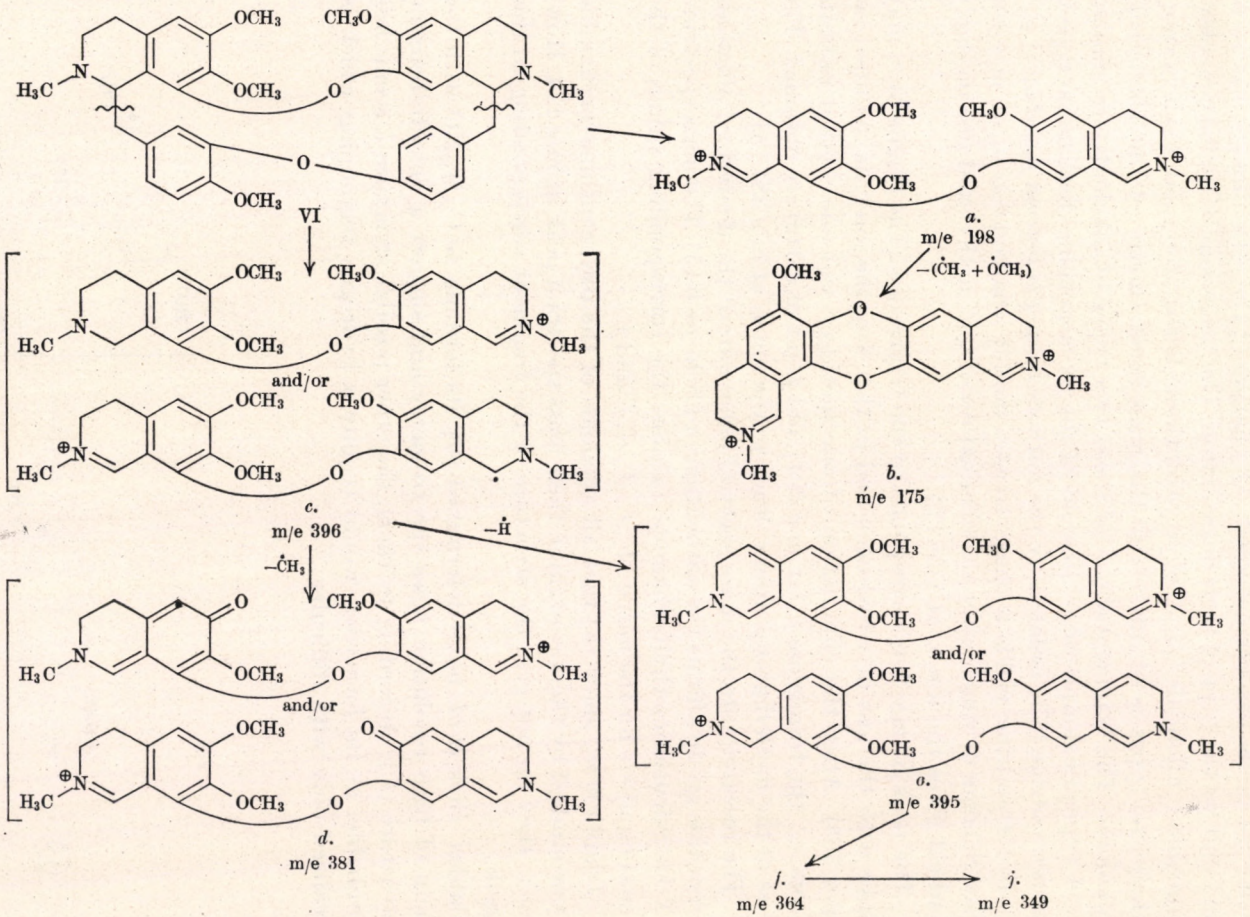
The mass spectral behaviour of the tetrahydro derivatives of berberine [1], thalidastine [4] and thalifendine [2] has been examined from the protoberberine alkaloids found in Thalictrum species. The data of this research are shown in Table I.

(iv) Bisbenzylisoquinoline Alkaloids

Several publications have appeared in the last few years dealing with the behaviour of the bisbenzylisoquinoline alkaloids under mass spectrometry conditions. The mass spectral behaviour of various bisbenzylisoquin-

Table I

	 *a.*	 *c.*	*b.*	*e.*	*d.*
Tetrahydroberberine $R_1 = H$; $R_2 = CH_3$;	m/e 164	m/e 174	—	m/e 149	—
Tetrahydrothalidastine $R_1 = OH$; $R_2 = H$;	m/e 150	m/e 174	—	m/e 135	—
Tetrahydrothalifendine $R_1 = R_2 = H$;	m/e 150	—	m/e 176 basic peak	—	m/e 149


VI
a.
m/e 198
−(ĊH₃ + ȮCH₃)
b.
m/e 175
c.
m/e 396
−Ḣ
−ĊH₃
and/or
d.
m/e 381
and/or
o.
m/e 395
f.
m/e 364
j.
m/e 349

Scheme 6

oline alkaloids has been studied. The nature of the ether linkage has to be in mind for the type of their degradation. The behaviour of the bisbenzylisoquinoline alkaloids of the oxyacanthine–berbamine and thalicberine–thalicrine types is of interest in the mass spectrometry of the Thalictrum alkaloids. The fragmentations of these two types of alkaloids are similar.

A typical example of an alkaloid of the oxyacanthine–berbamine type is isotetrandrine (VI) isolated by the authors from *Thalictrum foetidum*.

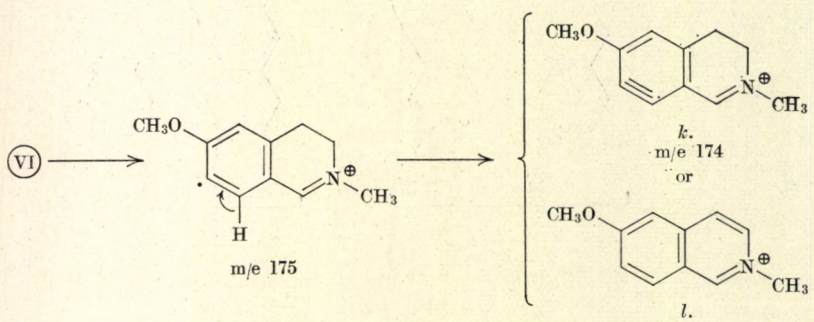
Isotetrandrine yields a characteristic doubly charged ion *a*. at m/e 198 (see Scheme 6) which after the elimination of one methyl and one methoxy radical gives the ion *b*. at m/e 175.

The peak at m/e 396 corresponds to the fragment *c*. The fragment *c*. may split with the loss of a hydrogen and in this way the ion *e*. is obtained (at m/e 395). After the elimination of one methoxy and one methyl radicals from *e*., the framgents *f*. (at m/e 364) and *j*. (at m/e 349) are obtained. The loss of the methyl group from *c*. leads to the ion *d*. at m/e 381 [5].

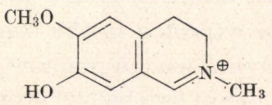
The research into the mass spectral behaviour of the deuterized alkaloids from this group affirms the authenticity of Scheme 6 [5]. The mass spectrum of O-trideuteromethylberbamine exhibits the corresponding values of the mass numbers for the ions *a*., *b*., *c*., *d*., *e*., *f*. and *j*.

Tomita *et al.* show [5] that all alkaloids of the oxyacanthine–berbamine type studied by them contain in their mass spectra a peak at m/e 174. However, they do not give the structure of the fragment corresponding to this peak.

Baldas, Bick *et al.* have determined [6], its formula as $C_{11}H_{12}NO$ with the help of high resolution mass spectrometry and suggest a dehydrobenzene structure (*k*.). According to them, the other possible structure *l*. seems less probable, as its formation would require a hydrogen regrouping which is realized only with difficulty:

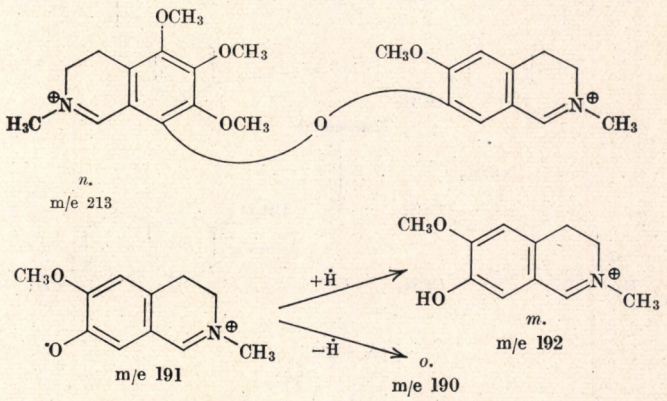


A peak for a singly charged ion, of variable intensity, is also observed at m/e 192. It may have a structure *m.*, in which the origin of the hydroxy hydrogen is unclear. It may be a result of migration [6].



m.
m/e 192

The mass spectrum of hernandezine [7] shows a basic peak for a doubly charged ion *n.* at m/e 213 (isotope 213·5), a peak at m/e 191, corresponding to M-461 and two peaks at m/e 190 and 192 for the fragments *o.* and *m.*:



n.
m/e 213

m/e 191

m/e 192

m/e 190

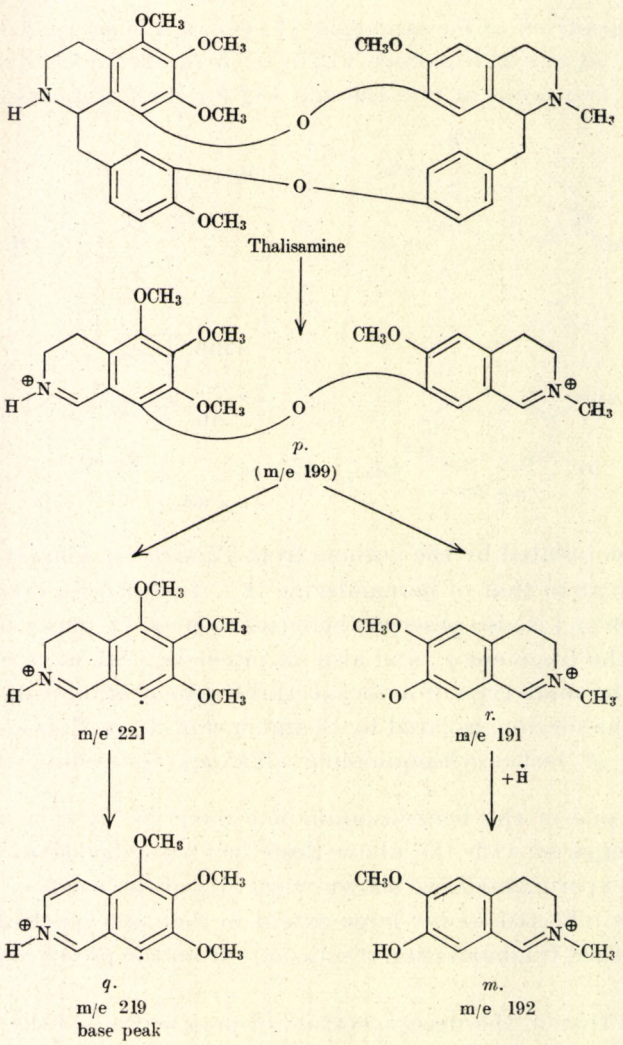
Thalisamine, isolated by the authors from *Thalictrum simplex*, has a mass spectrum similar to that of hernandezine [8]. A peak for a doubly charged ion at m/e 199 (*p.*) is also observed here (see Scheme 7), plus a basic peak at m/e 219, for the fragment *q.*, and also an intensive peak at m/e 191 for the fragment *r*. The same type of mass spectral fragmentation is also shown by the alkaloid thalidezine, isolated by Shamma *et al.* from *T. fendleri* [3].

The group of bisbenzylisoquinoline alkaloids containing the function $-C=N-$ in one of the benzylisoquinoline moieties, have a more special behaviour compared with the above-described bisbenzylisoquinoline alkaloids. As our experiments have shown, variation of the location of the ether linkages is not reflected to any large extent in the mass spectral behaviour. Their more special fragmentation results only from the presence of the imino group.

It is known that in the mass spectrum of papaverine [1] the most intensive peaks are those for the molecular ion, M-1, M-15 (a basic peak) and

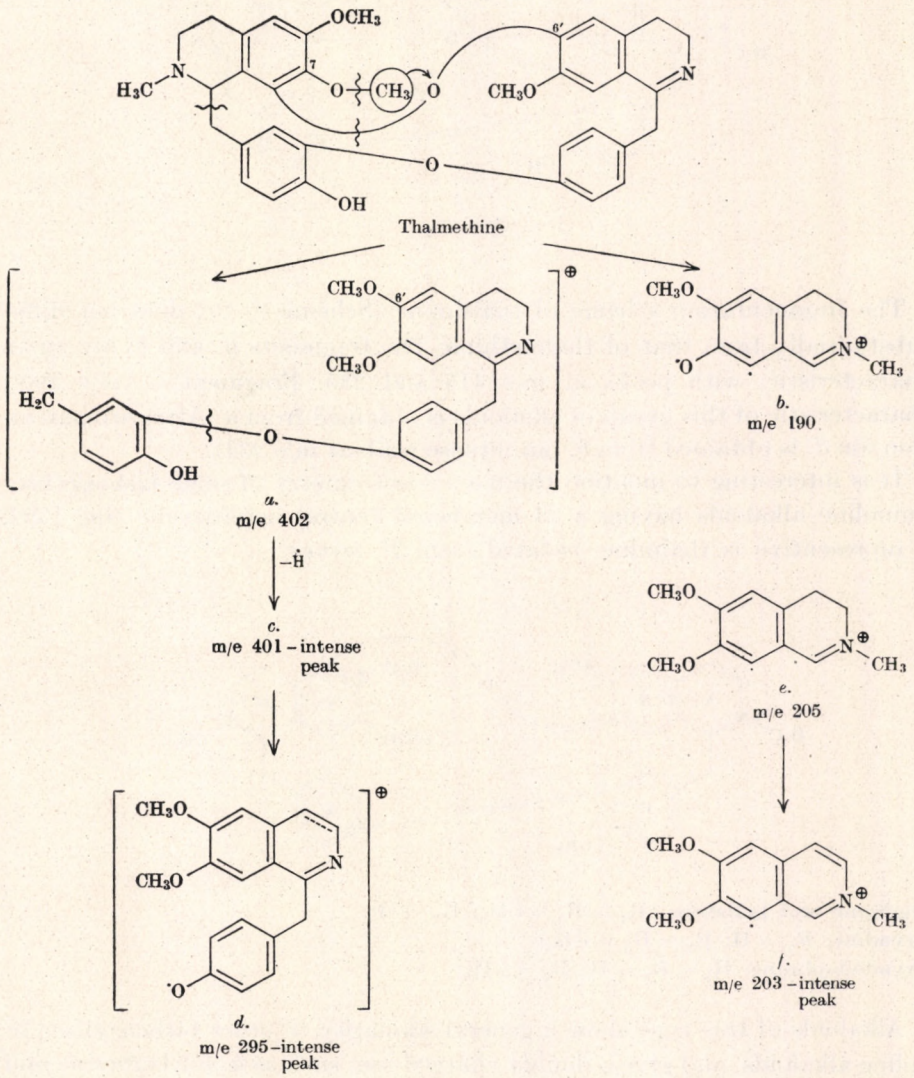
M-31. The rest of the peaks have insignificant intensity. This shows that the predominant cleavage is of the CH_3 and OCH_3 groups without affecting the full carbon skeleton of the alkaloid. In this case a stabilization of the benzyl bond by the adjacent aromatic ring is possible.

A similar effect should be expected in the behaviour of the bisbenzylisoquinoline alkaloids of this group, too. In practice, the typical doubly charged ion, obtained after the cleavage of two benzyl groups from the bisbenzyltetrahydroisoquinoline alkaloids, cannot be observed in their mass spectra.



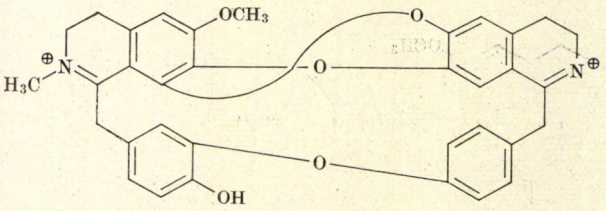
Scheme 7

The fragmentation of thalmethine [9] proceeds according to Scheme 8. First, cleavage of the upper ether linkage and the benzyl group of the left isoquinoline moiety occurs; simultaneously, the methoxy group at C-7 loses a methyl radical to the oxygen of the ether linkage to form a new methoxy group at C-6′. Hence, two ions *a*. and *b*. are produced leading to peaks at m/e 402 and 190, respectively.



Scheme 8

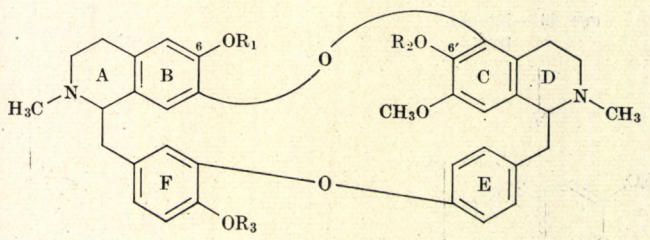
Further, *a.* loses a hydrogen radical to give *c.* (m/e 401 — an intense peak) and this decomposes to the ion *d.* to which corresponds an intense peak at m/e 295. The mass spectrum of thalmethine also shows peaks at m/e 205 and 203 for the fragments *e.* and *f.*, and a peak at m/e 273 for a doubly charged ion *g.* obtained by the loss of one methyl and one methoxy radical.



g.
(m/e 273)

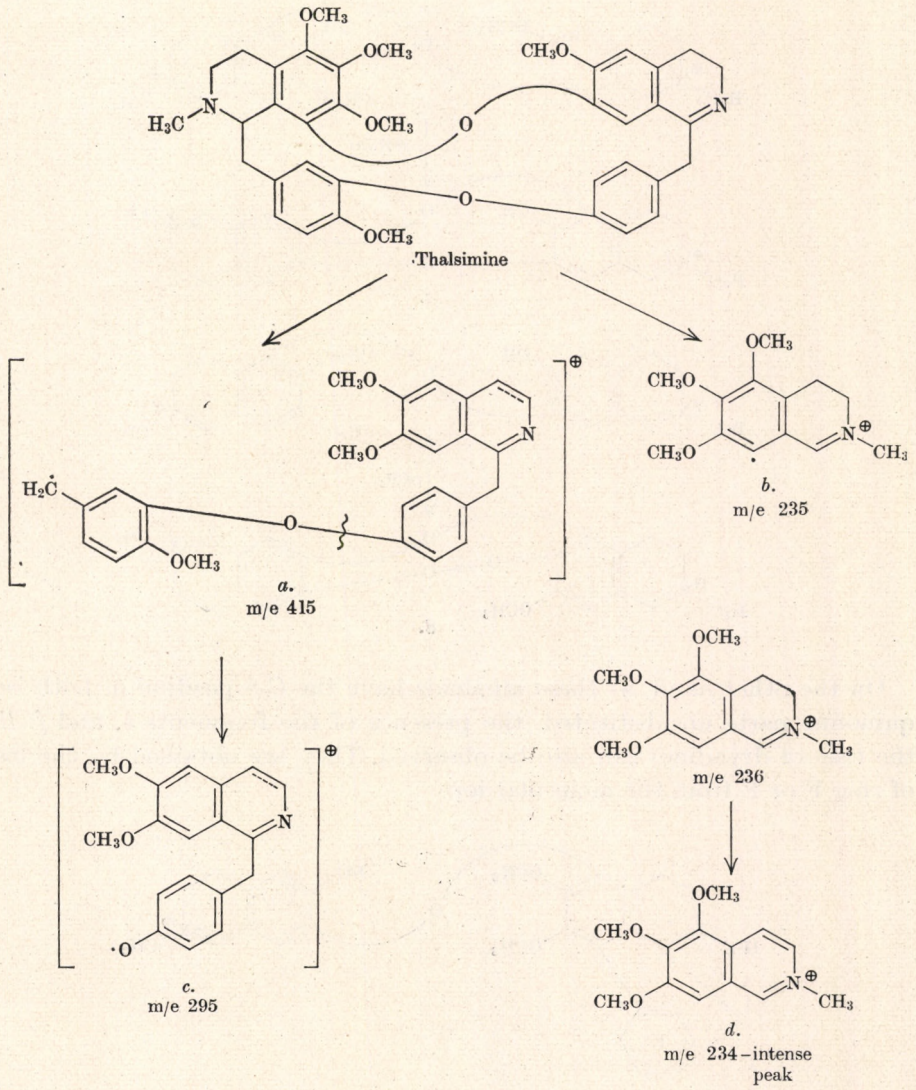
The fragmentation scheme of thalsimine (Scheme 9) [9] does not differ substantially from that of thalmethine. The fragments *a.* and *b.* are again characteristic, with peaks at m/e 415 and 235. Fragment *c.* (m/e 295), characteristic of this group of alkaloids is obtained from *a.* After aromatization ion *d.* is obtained from *b.* (an intense peak at m/e 234).

It is interesting to mention the mass spectrometry of some bisbenzylisoquinoline alkaloids having a 21-membered central heterocyclic ring [39]; a representive is thalmine, isolated from *T. minus.*



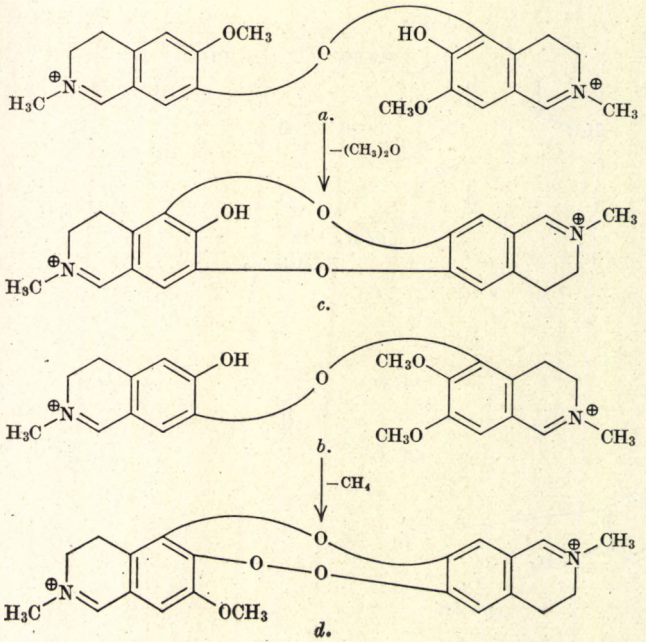
Thalmine and lauberine: $R_1 = R_3 = CH_3$; $R_2 = H$;
Dryadine: $R_1 = H$; $R_2 = R_3 = CH_3$;
Dryadodaphnine: $R_1 = R_3 = H$; $R_2 = CH_3$;

Alkaloids of this type show a general similarity to other bisbenzylisoquinoline alkaloids, and give a doubly charged ion, such as *a.* for lauberine and *b.* for dryadine.

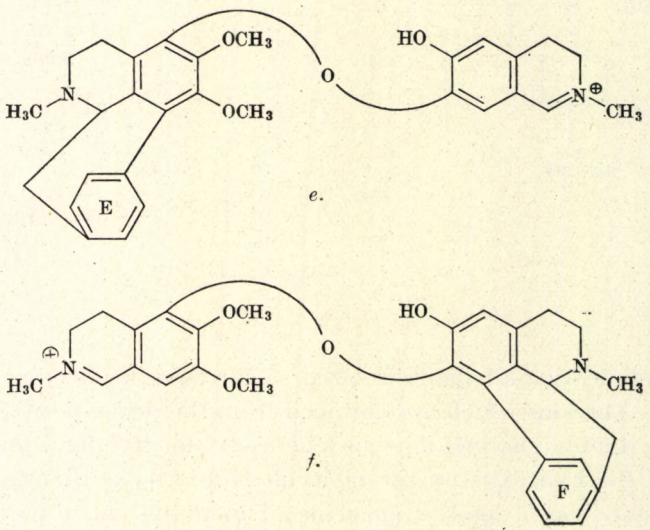


Scheme 9

All thalmine type alkaloids isolated so far have a phenolic hydroxyl at C-6 or C-6′. The ions which are obtained from the decomposition of the corresponding doubly charged ions may be useful for the determination of the position of the hydroxyls in the molecule. Ion *a.* loses dimethyl ether and gives *c.*, while ion *b.* loses a molecule of methane and gives fragment *d.*



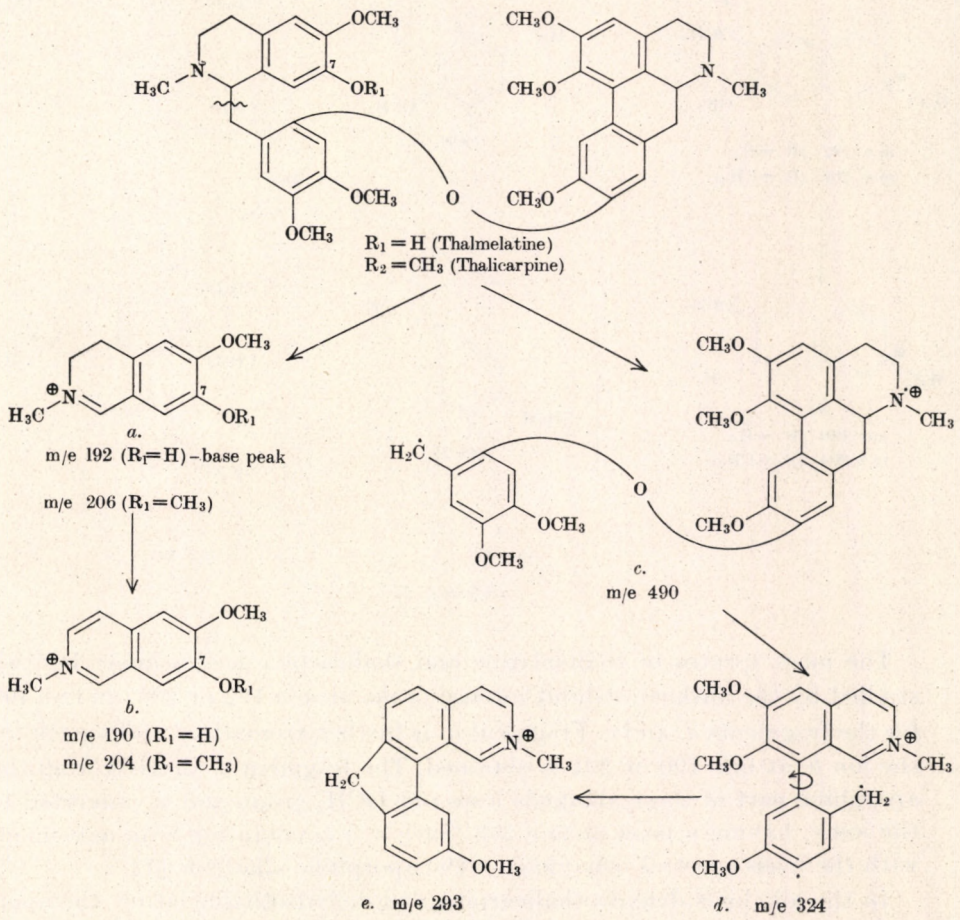
On the other hand, as these alkaloids have the C-8 position of both isoquinoline parts unsubstituted, the presence of the fragments *e.* and *f.* (in the case of dryadine) can also be observed. They are obtained by the loss of ring F or E from the molecular ion.



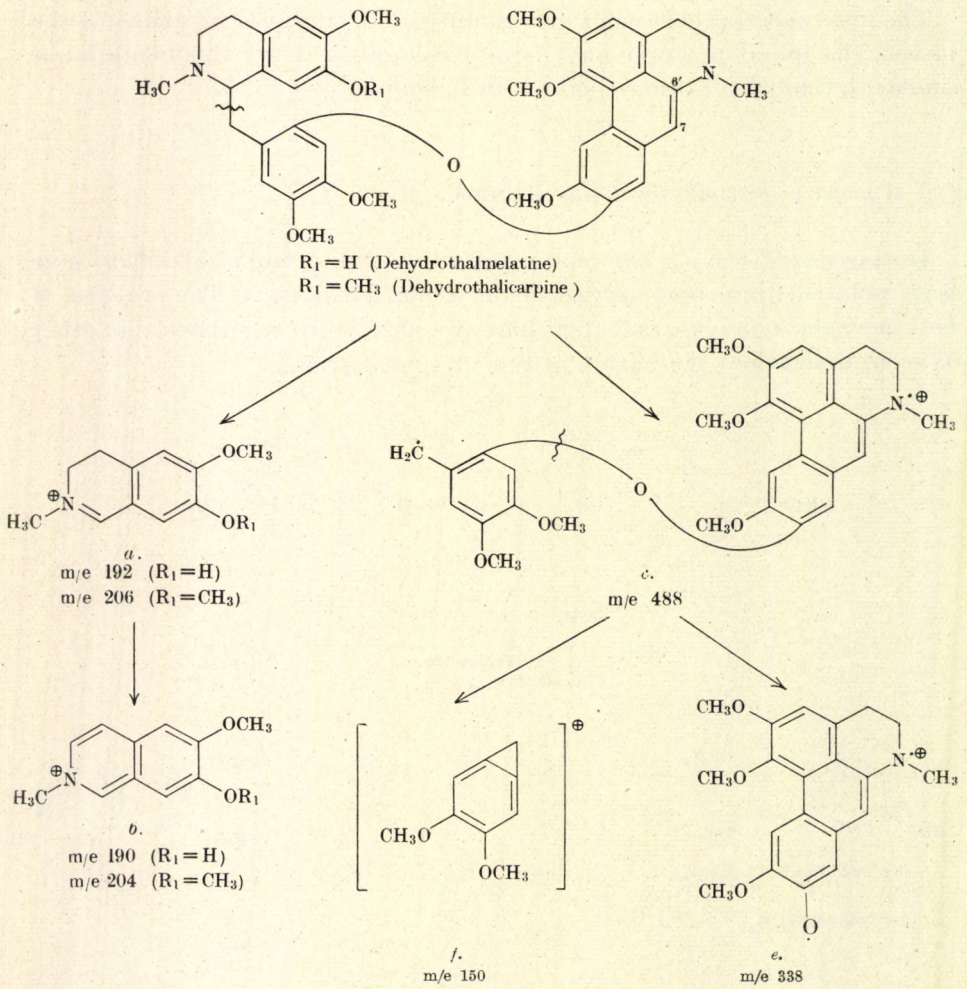
The mass spectral behaviour of a number of Thalictrum alkaloids of the bisbenzylisoquinoline group have been investigated. Their fragmentation is similar to that of the other alkaloids of this group [40].

(v) Aporphine-benzylisoquinoline Alkaloids

During recent years a few representatives of this group of alkaloids have been isolated from some species of the genus Thalictrum. The presence of both benzylisoquinoline and aporphine systems, linked together by an ether oxygen, determines the nature of their fragmentation.



Scheme 10



Scheme 11

The mass spectra of thalmelatine and thalicarpine (see Scheme 10) [9], studied by the authors, exhibit intense peaks at m/e 192 or 206, and at 490 for the fragments *a.* and *c.* Fragment *a.* is further aromatized and a peak for the ion *b.* at m/e 190 or 204 is obtained. The fragment *d.* derived from the aporphine part of these alkaloids loses one OCH_3 group and is converted to the ion *e.* having a peak at m/e 293. Such a fragmentation is in agreement with the mass spectral behaviour of the aporphine alkaloids [1].

In the alkaloids dehydrothalicarpine and dehydrothalmelatine, the aporphine system is additionally aromatized due to the double bond between

C-6′ and C-7. As a result of this fact the aporphine part is rather stable. The basis peaks are *a.* and *c.* (Scheme 11). An intense peak corresponds to the fragment *e.* with m/e 338. The latter should be stable owing to the possibility for stabilization into the fragment (see the case of the alkaloid isocorydine on p. 248).

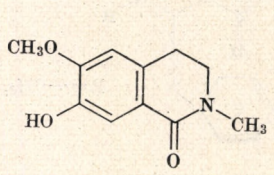
2. Nuclear Magnetic Resonance

(i) Simple Isoquinoline and Benzyltetrahydroisoquinoline Alkaloids

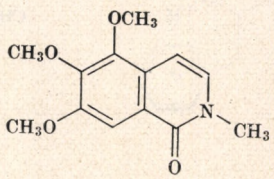
Nuclear magnetic resonance techniques have been widely applied to solve many structural and stereochemical problems.

The information which can be obtained about the nature and the number of functional groups and single hydrogen atoms, as well as about the location of some of them, has made NMR a popular and effective tool in the studies of natural organic substances; it is particularly useful with the Thalictrum alkaloids. Thus in the benzyltetrahydroisoquinoline alkaloids, positive information can be obtained about the N-methyl groups, which usually absorb between 7·2 and 8·0 τ. The lack of signals in that field is sufficient indication that the nitrogen atom is not substituted.

In some cases the absorption of the N-methyl group undergoes down-field shift under the influence of some neighbouring groups. Thus in the alkaloid thalifoline its absorption is at 6·73 τ due to the deshielding effect of the carbonyl group [46]. In thalactamine the N-methyl is deshielded by both the carbonyl group and a neighbouring double bond, so its absorption is at 6·46 τ [47].



Thalifoline



Thalactamine

The carbonyl group in thalifoline and thalactamine shifts the absorption of the aromatic protons at C-8 to lower τ-values. They appear at 2·44 τ, and 2·44 τ, respectively [46, 47].

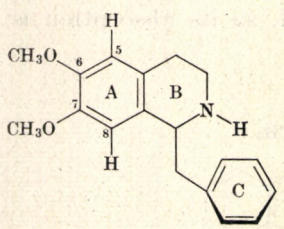
The methoxy groups absorb in the region $\sim$ 6·0–7·0 τ. The information which can be obtained about the number of these substituents is also quite reliable.

The methylenedioxy groups give signals at $\sim$ 5 τ; the form of these signals depends to a great extent on the rest of the substituents of the molecule, and on the conditions of measurement. They absorb as an AB system (singlet, two doublets, or multiplet).

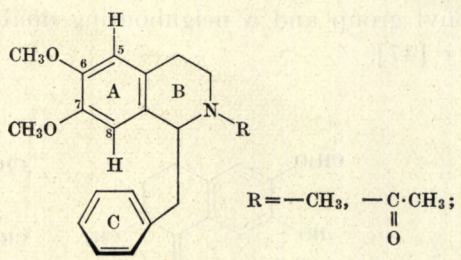
The hydroxy groups absorb over a rather wide range, presenting difficulties in their identification. They can be more easily found by acetylation, when a peak is observed corresponding to the methyl group from the acetyl radical.

The question of the location of the various functional groups is more difficult to solve, but it is possible in some instances.

For example, Dalton, Cava and Buck [10], from a study of the NMR spectra of 1-benzyl-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinolines, found that the absorptions of the methoxy group at C-7 and the aromatic proton at C-8 depend to a great extent on the nature of the substituent on the nitrogen atom. Depending on whether this is hydrogen (VII) or another larger substituent (VIII), in the preferred conformation of the compound, the aromatic ring C will be located just under the nitrogen atom or, conversely, near ring A. In the latter case the aromatic proton at C-8 and the methoxy group at C-7 will be shielded, which will cause a shift of their absorption to higher values of τ, at 4·15 $\pm$ 0·03 and around 6·40, respectively (see Table II).

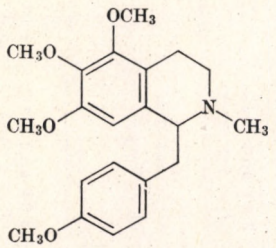


VII



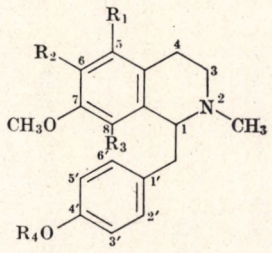
VIII

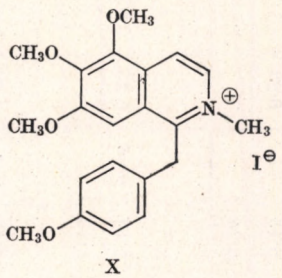
The characteristic absorption of the proton at C-8 was used recently to correct the structure of takatonine. In the NMR spectrum of tetrahydrotakatonine (IX) a signal for a proton at 4·12 τ is observed.



IX

The presence of a peak with such a τ value is in contradiction with the postulation that one of the methoxy groups is at C-8 and Kubota *et al.* proved that in the true structure the methoxy group is not at C-8 but at C-5 [11].





X

IX $R_1 = R_2 = OCH_3$; $R_3 = H$; $R_4 = CH_3$;
XI $R_1 = H$; $R_2 = R_3 = OCH_3$; $R_4 = CH_3$;
XII $R_1 = R_2 = OCH_3$; $R_3 = R_4 = H$;
XIII $R_1 = OCH_3$; $R_2 = R_3 = H$; $R_4 = CH_3$;
XIV $R_1 = OH$; $R_2 = OCH_3$; $R_3 = R_4 = H$;
XV $R_1 = OC_2H_5$; $R_2 = R_3 = H$; $R_4 = CH_3$;
XVI $R_1 = OC_2H_5$; $R_2 = OCH_3$; $R_3 = H$; $R_4 = CH_3$.

The methoxy groups at C-5 and C-6 have a normal absorption at about $6{\cdot}20 \pm 0{\cdot}07$ τ (Table II).

The methoxy group at C-4′ gives a signal at higher τ values probably on account of steric hindrance of the OCH_3 group at C-7 (Table II).

The presence of spin–spin coupling between the aromatic protons (with a definite value of the constant J) makes their identification more reliable.

The coupling of the *o*-located protons in the benzyl group is especially characteristic, leading to two doublets of the type A_2B_2 with a constant $J = 8{\cdot}5 \pm 0{\cdot}5$ cps. When some substituent is present in the benzyl group (e.g. the OCH_3 group), the presence of a quadruplet of the type A_2B_2 unambiguously points out that its position is C-4′ (XIII).

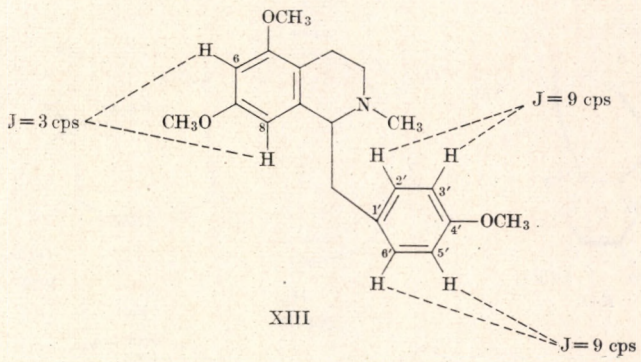
Table II

No.	Name	Chemical shift (in τ) —N.CH$_3$	—OCH$_3$* C-5	C-6	C-7	C-8	C-4′	Aromatic protons C-5	C-6	C-8	Two doublets of the type A_2B_2 for C-2′, C-3′, C-5 and C-6′	—OH
1.	Tetrahydrotakatonine (IX)	7·48	6·15	6·15	6·43	—	6·22	—	—	4·12		—
2.	Takatonine iodide (X)	5·38			6·24			—	—	2·56		—
3.	1-(4′-Methoxybenzyl)-2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline (XI)	7·65	—	6·15	6·18	6·05	6·23	3·63	—	—		—
4.	Thalifendlerine (XII)	7·51	6·15	6·15	6·43	—	—	—	—	4·17	3·40 and 3·15 (J = 8·4 cps)	
5.	Non-phenolic base of hernandezine (XIII)**	7·56	6·28	—	6·47	—	6·28	—	Two doublets at 4·15 and 3·73 (J = 3 cps)		3·15 and 2·95 (J = 9 cps)	—
6.	Phenolic base of thalfoetidine (XIV)**	7·47	—	6·13	6·41	—	—	—	—	4·18	3·34 and 3·03 (J = 8 cps)	4·18 and 4·27
7.	Minor non-phenolic compound of O-ethoxythalidezine (XV)**	7·55	triplet at 8·62 and quadruplet at 6·05 (J = 7 cps)	—	6·46	—	6·24	—	Two doublets at 3·77 and 4·24 ($J_{6,8}$ = 2·5 cps)			
8.	Major non-phenolic compound of O-ethoxythalidezine (XVI)**			6·17	6·42	—	6·22	—	—	4·10		

* The data at C-5 of the two non-phenolic bases of O-ethoxythalidezine are for ethoxy groups. The NMR spectra are recorded at 100 Mc.

** The base is obtained after sodium–liquid ammonia cleavage of the alkaloid.

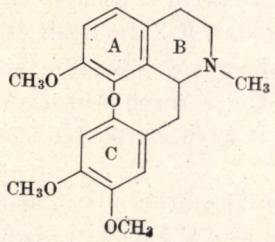
Another characteristic spin–spin coupling is m-interaction between the aromatic protons at C-6 and C-8. In the NMR spectrum of the non-phenolic base (XIII), obtained after sodium–liquid ammonia cleavage of hernandezine, this coupling gives rise to two doublets of the type AB with a constant J = 3 cps (Table II).



XIII

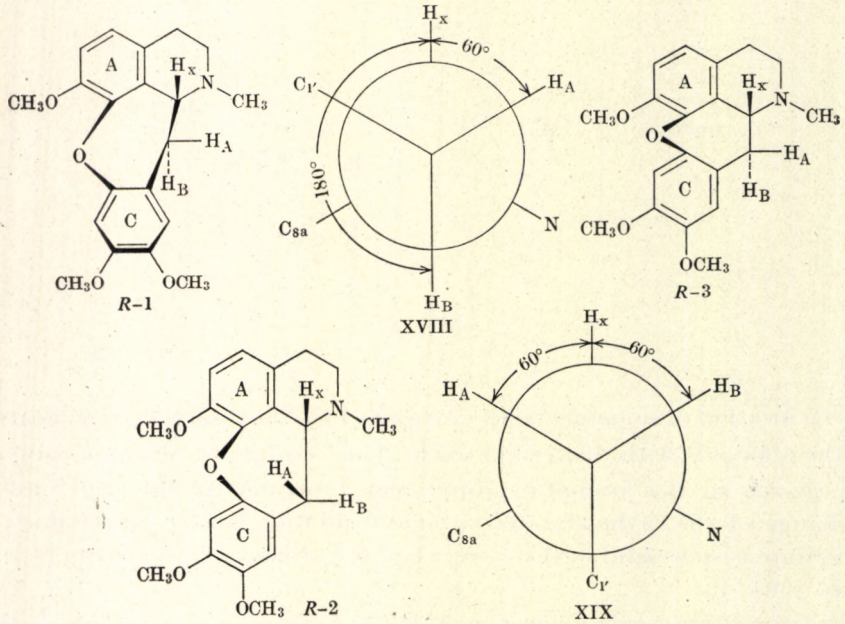
The location of some phenolic hydroxyls may be observed after treatment of the alkaloids with deuterium oxide. This results in the disappearance of the signals in the aromatic proton region because of the acid-catalyzed exchange of the ortho-situated aromatic protons against deuterium. This procedure is successful in the cases when the aromatic protons can be recognized [39].

In the last few years nuclear magnetic resonance has also been used to solve some stereochemical problems. Thus, the difficult problem of the conformation of cularine (XVII) was solved successfully using high resolution NMR spectrometry [12]. Cularine is a representative of the benzyltetrahydroisoquinoline alkaloids which have an oxygen linkage between the rings A and C. The configuration of the asymmetric carbon atom of cularine had been shown to be *D* (or *R*).



XVII

The presence of an oxygen linkage in the molecule is, however, the reason for it to be disymmetric as a whole. So three conformations are possible for cularine (corresponding to *R*-1, *R*-2 and *R*-3) whose Newman's projections are XVIII (the projections of *R*-1 and *R*-3 are the same) and XIX (for *R*-2).



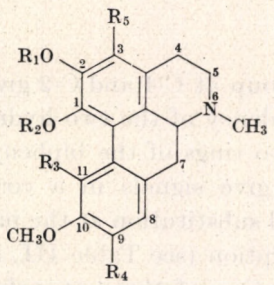
The constants, J, of the spin–spin coupling between the protons H_X, H_A and H_B are J_{XA} = 3–4 cps and J_{XB} = 8–14 cps for the projection XVIII and $J_{XA} = J_{XB}$ = 3–4 cps for the projection XIX. J_{XA} = 4 cps and J_{XB} = = 12 cps were the values actually obtained from the NMR spectrum of cularine. Therefore, one of the conformations *R*-1 or *R*-3 is possible. Comparison of molecular models of *R*-1 and *R*-3 shows that in *R*-1 the oxygen atom is moved away from H_X, while in *R*-3 it shields H_X and the latter will absorb up-field. In practice, H_X absorbs at 5·56 τ in the NMR spectrum of cularine. Moreover, the rotation of the N-methyl group is more free in conformation *R*-3, than in *R*-1, where it is hindered by the equatorial hydrogen atom (H_A).

So the most probable conformation for cularine is *R*-3. The solving of this problem is a fine illustration of the great possibilities of NMR spectroscopy in the research into natural compounds.

(ii) Aporphine Alkaloids

A special characteristic in the NMR spectra of the alkaloids of this group is the absorption of the aromatic protons at C-3 and C-11 [13] (see Table III). When methoxy and methylenedioxy substituents are present at C-1 and C-2 (as is the case with the majority of aporphine alkaloids) the proton at C-11 absorbs downfield, between 1·2 and 2·3 τ [14]. These low values can be explained by the deshielding influence of the neighbouring aromatic nucleus and the probable formation of a hydrogen bond between the aromatic proton at C-11 and the oxygen atom of the substituent at C-1.

The presence of a proton at C-3 may be determined too; it absorbs normally at high field (3·2–3·5 τ) [15], and spin–spin coupling is not observed. The absorption of a proton in this characteristic region is lacking in the spectrum of ocoteine (formerly postulated as XXI). Instead, a peak is observed for the methoxy group with low τ-values (see Table III), in agreement with the revised structure (XXIa) of this aporphine alkaloid, with the OCH_3 group at position 3 [16].



XX $R_1 = R_2 = CH_3$; $R_3 = R_5 = H$; $R_4 = OCH_3$;
XXI $R_1 = R_2 = -CH_2-$; $R_3 = R_5 = H$; $R_4 = OCH_3$;
XXIa $R_1 = R_2 = -CH_2-$; $R_3 = H$; $R_4 = R_5 = OCH_3$;
XXII $R_1 = CH_3$; $R_2 = R_3 = R_5 = H$; $R_4 = OCH_3$;
XXIII $R_1 = CH_3$; $R_2 = R_3 = H$; $R_4 = R_5 = OCH_3$;
XXIV $R_1 = R_2 = CH_3$; $R_3 = OH$; $R_4 = R_5 = H$;
XXIVa $R_1 = R_2 = -CH_2-$; $R_3 = H$; $R_4 = R_5 = OCH_3$;
XXIVb $R_1 = R_2 = CH_3$; $R_3 = H$; $R_4 = R_5 = OCH_3$;
XXIVc $R_1 = CH_3$; $R_2 = R_3 = R_5 = H$; $R_4 = OCH_3$

The other aromatic protons absorb normally.

The methoxy groups appear in a comparatively narrow region (6·01–6·16 τ) with the exception of that at C-1, which gives a signal shifted toward higher field (6·28–6·46 τ) as a result of the influence of the neighbouring aromatic nucleus.

Table III

No.	Name	Chemical shift (in τ)							
			Substituents at the carbon atoms						
		$-N.CH_3$	C-1	C-2	C-3	C-8	C-9	C-10	C-11
1.	Glaucine (XX)	7·41	6·28	6·07	3·32	3·11	6·03	6·03	1·79
2.	Ocoteine (Thalicmine) (XXIa)	7·48	AB-quadruplet at 4·03 (J = 8·5 cps)		6·01	3·22	6·09	6·09	2·39
3.	Thaliporphine (XXII)	7·47		6·17	3·49	3·23	6·11	6·11	1·93
4.	Preocoteine (XXIII)			6·12	6·12	3·26	6·08	6·08	2·01
5.	Isocorydine (XXIV)	7·46	6·28	6·09	3·27	3·12	3·12	6·07	
6.	Isocorydine methochloride	7·50	6·46	6·16	3·30	3·50	3·30	6·11	1·40

The methylenedioxy group at C-1 and C-2 gives a quadruplet of the type AB due to the non-equivalency of the two hydrogen atoms, a result of the non-coplanarity of the two rings of the biphenyl system.

The N-methyl groups give signals in a comparatively narrow region (7·40–7·50 τ) as additional substitution at the nitrogen atom does not significantly affect their absorption (see Table III, isocorydine methochloride).

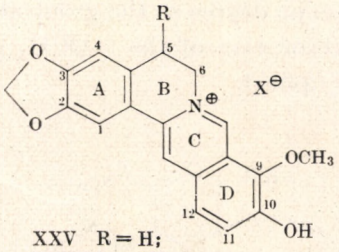
NMR spectral investigation of three aporphine alkaloids isolated from Thalictrum species, thalicmine (XXIa), thalicsimidine (XXIVa) and thalicmidine (XXIVb), has also been carried out. The results support the accepted structures for these alkaloids [41].

(iii) Protoberberine Alkaloids

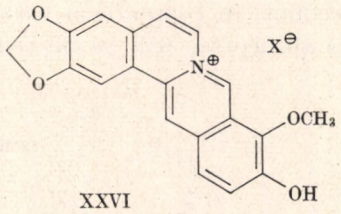
The NMR data [2, 4] for two protoberberine bases, thalifendine (XXV) and deoxythalidastine (XXVI) are given in Table IV.

The methylenedioxy group absorbs at 3·85 τ in the NMR spectrum of thalifendine chloride (XXV). The methoxy group has an absorption shifted downfield (5·72 τ), while the aromatic protons give signals in a wide region between 0·45 and 3·05 τ.

In deoxythalidastine chloride (XXVI), obtained by the dehydration of thalidastine chloride (XXVII), the planarity of the protoberberine system increases overall, as a result of the additional aromaticity of the molecule.



XXV R = H;
XXVII R = OH;



XXVI

This leads to a shift of the absorption of the functional groups and the aromatic protons to lower field (see Table IV).

*Table IV**

No.	Name	Chemical shift (in τ)								
		$-O.CH_2.O-$ C-2 and C-3	$-OCH_3$ C-9	Aromatic protons						
1.	Thalifendine chloride (XXV)	3·85	5·72	3·05	2·48	2·05	2·05	1·48	0·45	—
2.	Deoxythalidastine chloride (XXVI)	3·66	5·68	2·57	1·97	1·91	1·78	0·63	0·12	AB quart. at 2·08 and 1·31 τ (J = 7·2 cps)

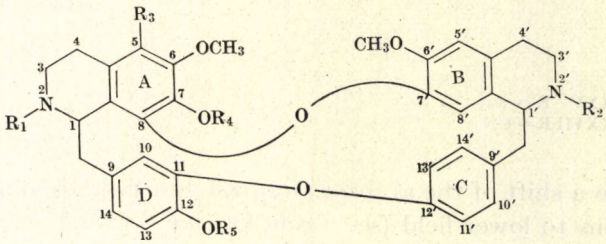
* Spectra recorded in CF_3COOH with TMS as internal standard.

(iv) Bisbenzylisoquinoline Alkaloids

The majority of the bisbenzylisoquinoline alkaloids isolated so far from Thalictrum species have ether linkages of the berbamine or thalicberine type.

The NMR absorption of the C-7 methoxy groups (signals in the region around 6·50–6·80 τ (Tables V and VI) is especially characteristic. Examination

of molecular models shows that these groups lie very near to the aromatic ring B, which shields them to a considerable degree. The methoxy groups at C-5 and C-6′ have chemical shift values of ~ 6·15–6·25 and 6·35–6·65 τ, respectively. One can see from the models that the methoxy group at C-6′ is shielded by ring A to almost the same degree as the group at C-7. On this account, in comparison with the absorption of the $-OCH_3$ group at C-6, its absorption will be shifted more upfield.

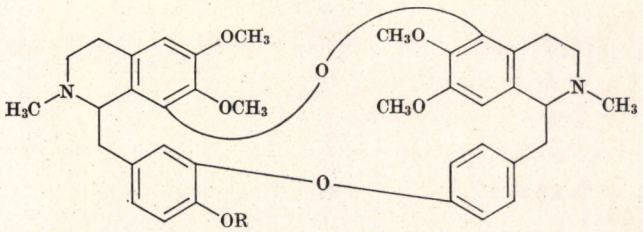


XXVIII $R_1 = R_2 = R_4 = R_5 = CH_3$; $R_3 = OH$;
XXIX $R_1 = R_2 = R_4 = R_5 = CH_3$; $R_3 = OCH_3$;
XXX $R_1 = H$; $R_2 = R_4 = R_5 = CH_3$; $R_3 = OCH_3$;
XXXI $R_1 = R_2 = R_4 = R_5 = CH_3$; $R_3 = H$;
XXXII $R_1 = R_2 = R_4 = CH_3$; $R_3 = R_5 = H$;
XXXIII $R_1 = R_4 = R_5 = CH_3$; $R_2 = H$; $R_3 = OCH_3$

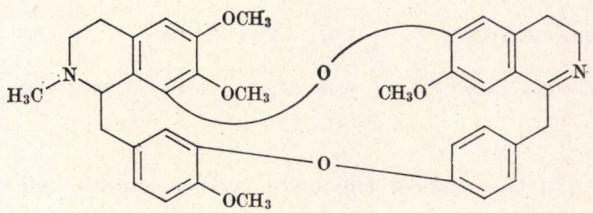
It is observed that when the configuration of the two asymmetric carbon atoms in the molecule is *R,R* or *S,S*, the values of the chemical shift of the

Table V

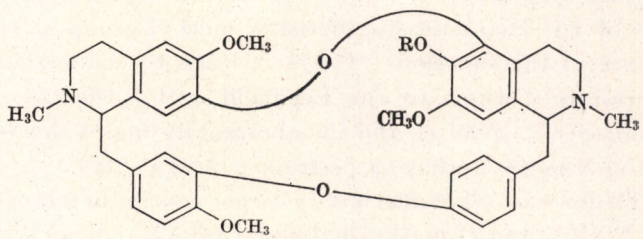
No.	Name	Chemical shift (in τ)						
		$-N.CH_3$		$-O.CH_3$				
		N-2	N-2′	C-5	C-6	C-7	C-12	C-6′
1.	Thalidezine (XXVIII)	7·67	7·36	—	6·22	6·73	6·08	6·63
2.	Hernandezine (XXIX)	7·70	7·37	6·17	6·21	6·76	6·09	6·66
3.	Thalisamine (XXX)	—	7·69	6·16	6·16	6·56	6·06	6·56
4.	Isotetrandrine (XXXI)	7·75	7·35	—	6·18	6·85	6·05	6·35
5.	Berbamine (XXXII)	7·72	7·40	—	6·22	6·82	—	6·37
6.	Dihydrothalsimine (XXXIII)	7·72	—	6·16	6·20	6·70	6·05	6·60



XXXIV R = H;
XXXIVa R = CH_3;



XXXV



XXXVa R = H;
XXXVb R = CH_3;

methoxy group at C-6′ are ~ 6·40 τ. With configuration *R,S* or *S,R*, the values of the chemical shift are near 6·60 τ [17]. In the latter case the shielding influence of ring A will be stronger as it is nearer to the methoxy group at C-6′.

The methoxy groups at C-5 give signals in almost the same region as those at C-6. Nevertheless, the weak shift toward lower field is an indication that the shielding by the neighbouring aromatic rings is minimal.

The behaviour of the methoxy group at C-12 is also similar. The presence of a signal in the region around 6·05–6·10 τ is a sure indication that a methoxy group is present at C-12.

The N-methyl groups of the alkaloids of the berbamine series absorb around 7·40 and 7·70 τ (Table V) [17]. The normal absorption of one N-methyl group in the benzyltetrahydroisoquinoline alkaloids is between

Table VI

No.	Name	Chemical shift (in τ)						
		$-N.CH_3$		$-O.CH_3$				
		N-2	N-2′	C-6	C-7	C-12	C-6′	C-7′
1.	Thalfoetidine (XXXIV)	7·68	7·30	6·23	6·68	—	6·11	6·49
2.	Thalidasine (XXXIVa)	7·75	7·38	6·25	6·73	6·09	6·13	6·50
3.	Thalmine (XXXVa)	7·36	7·78	6·07	—	6·07	—	6·07
4.	O-Methylthalmine (XXXVb)	7·35	7·84	6·10	—	6·09	6·31	6·13
5.	O-Methylthalmethine (XXXV)	8·08	—	6·11	6·30	6·09	—	6·16

7·40 and 7·50 τ. In the case of the bisbenzylisoquinoline alkaloids of the berbamine series, the aromatic ring D, on account of its proximity to the N-methyl group at position 2, will shield it to a higher degree. Thus, the higher value of τ is characteristic of the N-methyl group at this position.

The presence of the function $>C=N-$, leads to changes in its conformation, the results of which are the downfield shift of the absorption of the methoxy group at C-7 (6·30 τ), and the abnormally high value of the chemical shift of the N-methyl group at position 2 (8·08 τ) [42].

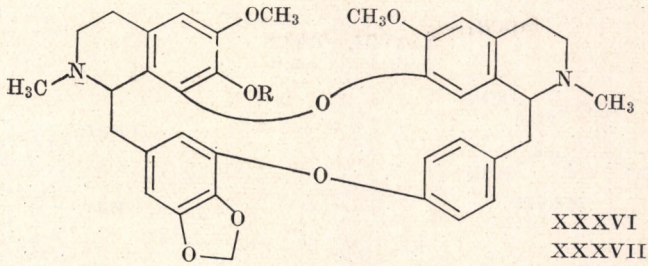
A few Thalictrum alkaloids have a larger central heterocyclic ring. In thalmine (XXXVa) and O-methylthalmine (XXXVb) this ring is 21-membered, because the two ether bridges are between C-7 — C-5′ and C-11 — C-12′, respectively. Thalfoetidine (XXXIV) and thalidasine (XXXIVa) have a 20-membered ring with the ether bridges connecting C-8 — C-5′ and C-11 — C-12′. As a result of this, the O-Me and N-Me groups of thalmine are, in general, less crowded and their absorption is close to the normal with the exception of the case when the substituents are at 6′ and 2′ positions (Table VI) [39].

The ring system of thalfoetidine (XXXIV) and thalidasine (XXXIVa) is not so roomy as in thalmine (XXXVa) because of the positions C-8 and C-5′ of one of the ether linkages. Some aromatic rings of these alkaloids influence the absorption of certain substituents. Their signals appear close to those of the berbamine and thalicberine types of alkaloids (Table VI) [31, 36].

The absorption of the aromatic protons in bisbenzylisoquinoline alkaloids is analogous to that of the monobases. The C-8 proton in the bisalkaloids absorbs also in the highfield (about 4 τ), and can be easily recognized [7,

10]. Thalmine-type alkaloids which have two C-8 aromatic protons show two such singlets in the highfield aromatic region (3·9–4·10 τ) [39].

So far, bisbenzylisoquinoline alkaloids containing the methylenedioxy group have not been discovered in plants of Thalictrum species. But such alkaloids are known in nature.



XXXVI R = CH_3;
XXXVII R = H;

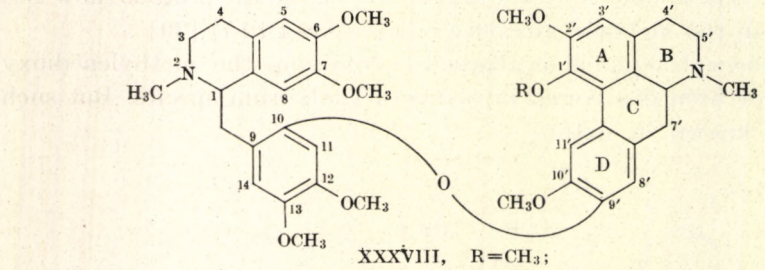
For example, the alkaloids tenuipine (XXXVI) and nortenuipine (XXXVII) belong to the berbamine series. The absorption of the methylenedioxy group of these alkaloids is in the region about 4·02 τ [17].

(v) Aporphine-benzylisoquinoline Alkaloids

The alkaloids of this group contain benzylisoquinoline and aporphine systems linked together by an ether oxygen.

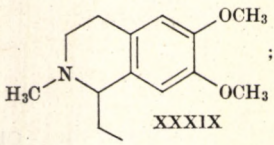
The most characteristic feature in the NMR spectra of these alkaloids is the absorption of the aromatic proton at C-11′ in the aporphine part of the molecule. This proton produces a peak at low field as is the case with the simple aporphine molecule (see p. 267). For example in the spectrum of thalicarpine (XXXVIII) (Table VII) it absorbs at 1·72 τ and in foetidine (XLVIII) 1·93 τ [48].

Recently, the alkaloids dehydrothalicarpine (XXXIX) and dehydrothalmelatine (XLI) were isolated by the authors. They belong to the same group and contain a new double bond in ring C. Hence the biphenyl system of rings A and D is replaced by a phenanthrene system formed by rings A, C and D. As a result of this additional aromaticity, the dihedral angle between the planes of the two aromatic nuclei A and C of the initial aporphine system decreases and the absorption of the aromatic proton at C-11′ s displaced to still lower field. The signal of this proton, at 1·72 τ in thali-



XXXVIII, R=CH_3;
XLVIII, R=H;

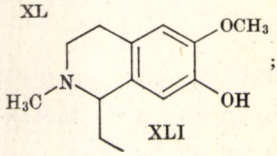
R_1=



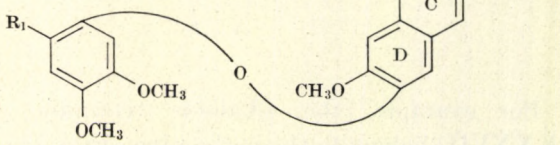
XXXIX

R_1= CHO;
XL

R_1=



XLI



carpine, moves to 0·62 τ in dehydrothalicarpine, and 0·77 τ in the main oxidation product of thalicarpine (XL) [18], which also contains such a phenanthrene system.

The N-methyl group in the benzylisoquinoline part of the molecule absorbs around 7·55 τ, in the aporphine part between 6·98 and 7·50 τ (Table VII). The value around 7·50 τ is normal for the absorption of such groups in an aporphine system (see Table III). Its shifting toward lower field (6·98–7·01 τ) may also be explained by the appearance of the phenanthrene system in this part of the molecule.

The absorption of the methoxyl groups are, on the whole, shifted downfield a little in comparison with their absorption in the benzylisoquinoline alkaloids (see Tables V and VI). The predominant peaks are chiefly in the region between 6·00 and 6·20 τ (Table VII). These lower values of the chemical shift are due to the diminished influence of the neighbouring aromatic nuclei.

The probable reason for this is the presence of only one ether linkage, giving the possibility of freer rotation around C_{arom}-oxygen bonds. The methoxy groups at C-10′ can be interpreted as the most shielded ones. The presence of an ether linkage in their immediate vicinity favours higher values of the chemical shift (6·40–6·50 τ).

Table VII

No.	Name	Chemical shift (in τ)									
		$-N.CH_3$		$-O.CH_3$							Aromatic protons
		N-2	N-6′								
1.	Thalicarpine (XXXVIII)	7·55	7·50	6·03	6·07	6·13	6·17	6·19	6·27	6·39	3·75, 3·43, 3·39, 3·34, 3·32, 2·65 and 1·72 (C-11′)
2.	Dehydrothalicarpine (XXXIX)	7·51	6·98	6·01	6·04	6·12	6·28	6·26	6·26	6·51	3·73, 3·69, 3·48, 3·23, 3·23, 3·12, 2·92 and 0·62 (C-11′)
3.	Main oxidation product of thalicarpine (XL*)	—	7·01	6·00	6·00	6·06	6·06	6·25	—	—	3·58, 3·47 2·96, 2·56 and 0·77 (C-11′)

* In addition to the chemical shift values given in this Table, a peak at 0·36 τ (1 H) due to the —CHO group, and another at 6·73 τ (4 H) due to two methylene groups (corresponding to C-4′ and C-5′) are present in the NMR spectrum of this compound.

The absence of one isoquinoline part of the molecule in compound XL leads to a more sensitive displacement of the absorption of the methoxyl groups towards lower field (Table VII).

3. Infrared Spectroscopy

Of all physical methods of research into Thalictrum alkaloids, IR spectroscopy is the most important. This is a result of the fact that many problems which arise in this research can be solved with its help. Its efficiency increases further when it is combined with other physical methods (UV spectroscopy, NMR, mass spectrometry).

The question of the identity or non-identity of two alkaloids, or of some synthetic product and a sample from an optically active alkaloid, may be solved to a large extent with the help of IR spectra. However, in practice one must be very cautious in the comparison, because the spectra of some alkaloids are very similar to one another owing to their structural similarities. For example, the IR spectra of the bisbenzylisoquinoline alkaloids thalfoetidine, thalicberine and berbamine show only small differences and this might lead to difficulties in their identification.

Table VIII

No.	Name	Frequency (cm^{-1}) =C=N— group
1.	Thalmethine (XLII)	1575, 1610 and 1630
2.	O-Methylthalmethine (XXXV)	1580, 1610 and 1630
3.	Thalsimine (XLIII)	1570, 1590 and 1620

Secondly, on the basis of data derived from IR spectra, conclusions may be made about the presence or absence of many functional groups in the molecules of Thalictrum alkaloids or their synthetic derivatives. The group

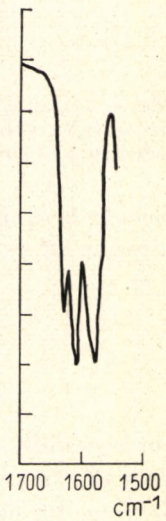


Fig. 1. IR spectrum of thalmethine

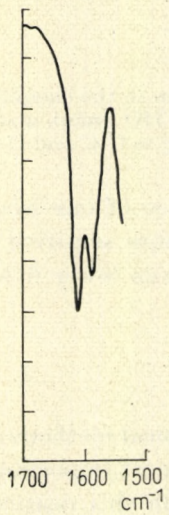


Fig. 2. IR spectrum of 1,2-dihydrothalmethine

$>C=N-$ is very characteristic; it is to be found in some bisbenzylisoquinoline alkaloids. Three bands are observed (Fig. 1) in their IR spectra in the region around 1600 cm^{-1} (see Table VIII). On hydrogenation it is reduced to $>CH-NH-$ and in the spectrum the triplet is no longer to be observed (Fig. 2).

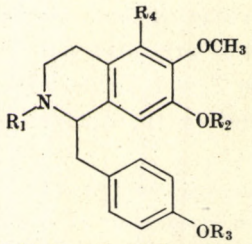
The phenolic hydroxyl groups absorb in the region between 3500 and 3600 cm^{-1} (Table IX). The disappearance of the band for the —OH group

Table IX

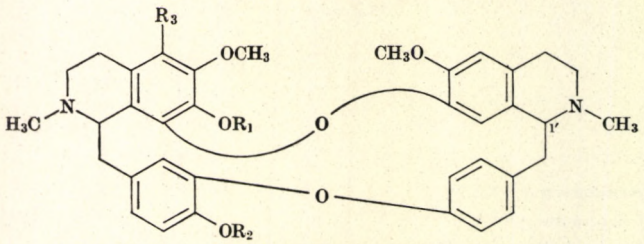
No	Name	Frequency (cm^{-1}) —OH group
1.	Armepavine (XLIV)	3605
2.	Coclaurine (XLV)	3540, 3585
3.	Thalifendlerine (XII)	3600
4.	Berberine (XLVI)	3400
5.	Thalmethine (XLII)	3560
6.	Thalmelatine (XLVII)	3550
7.	Dehydrothalmelatine (XLI)	3550
8.	Foetidine (XLVIII)	3400
9.	Thalicberine (XLIX)	3560
10.	Thalicrine (L)	3400, 3530
11.	Homothalicrine (LIII)	3540
12.	Thalmine (XXXVa)	3545
13.	Thalidezine (XXVIII)	3535
14.	Obamegine (LII)	3350, 3450
15.	Berbamine (XXXII)	3560
16.	Thalfoetidine (XXXIV)	3560

after acetylation and the appearance of bands for the acetyl group are indications of their presence.

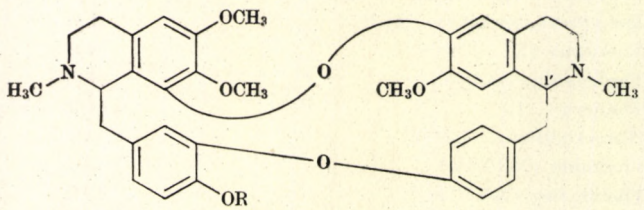
Thirdly, changes in the intensity and the frequency of the bands may give valuable information about the electronic and the steric interactions, both in the molecule itself and intermolecularly. For example, in the spectrum of the phenol base (XIV) obtained by cleavage of thalfoetidine with Na/liquid NH_3, the hydroxy group at C-6 absorbs at 3530 cm^{-1}. This displacement of the absorption is due probably to the formation of an intramolecular hydrogen bond with the oxygen atom of the neighbouring methoxy group.



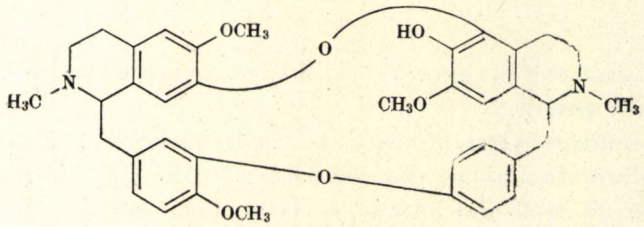
XLIV $R_1=R_2=CH_3$; $R_3=R_4=H$;
XLV $R_1=R_2=R_3=R_4=H$;
XII $R_1=R_2=CH_3$; $R_3=H$; $R_4=OCH_3$,



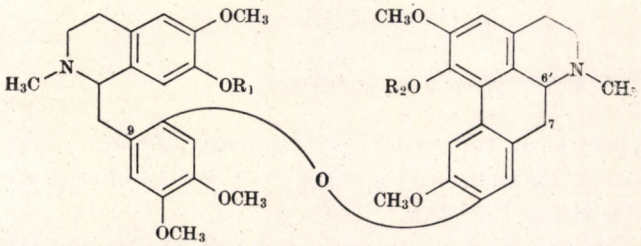
XLIII $R_1=R_2=CH_3$; $R_3=OCH_3$; ($C_{1'}=N-$);
XXXII $R_1=CH_3$; $R_2=R_3=H$;
LII $R_1=R_2=R_3=H$;
XXVIII $R_1=R_2=CH_3$; $R_3=OH$;



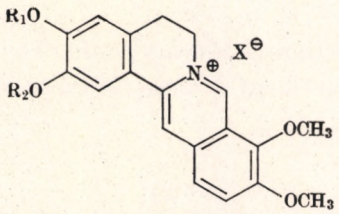
XLIX R=H;
XLII ($C_{1'}=N-$); R=H;
XXXV ($C_{1'}=N-$); $R=CH_3$;



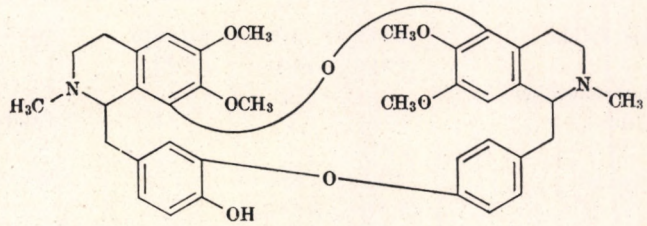
XXXVa



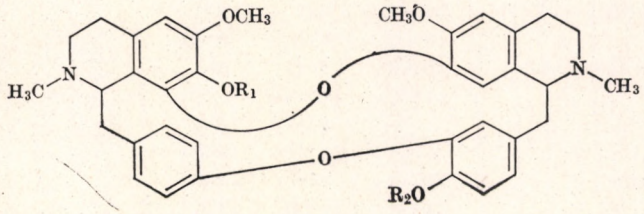
XLI $R_1=H$; $R_2=CH_3$; ($C_{6'}=C_7$);
XLVIII $R_1=CH_3$; $R_2=H$;
XLVII $R_1=H$; $R_2=CH_2$:



XLVI $R_1=R_2=-CH_2-$,
XLVIa $R_1=H$; $R_2=CH_3$;



XXXIV



L $R_1=R_2=H$;
LIII $R_1=H$; $R_2=CH_3$;

4. *Ultraviolet Spectroscopy*

(i) Benzyltetrahydroisoquinoline Alkaloids

The UV spectra of the benzyltetrahydroisoquinoline alkaloids display three absorption maxima, characteristic of the aromatic nucleus, about 204, 232 and 283 mμ [19, 25].

Thalictrum alkaloids of this series include takatonine and thalifendlerine. To the same group belong the monoderivatives which are formed by the cleavage of the bisbenzylisoquinoline alkaloids by a basic metal in liquid ammonia.

The UV spectra of all these bases are similar to one another and exhibit absorption maxima at wavelengths close to those expected (Table X and Fig. 3).

Table X

No.	Name	λ_{max}(mμ)[lg ε]			Solvent	Reference
1.	Takatonine iodide	220 (4·54)	265 (4·54)	315–320 (3·75]	MeOH	[11]
2.	Tetrahydrotakatonine hydrochloride	280 (3·57)			EtOH	[11]
3.	Thalifendlerine	226 (4·10)	282 (3·40)		MeOH	[2, 20]
4.	Laudanosine	207sh (4·89)	232 (4·17)	282 (3·77)		[19]
5.	Laudanosine hydrochloride	207sh (5·03)	232 (4·30)	281 (3·80)		[19]
6.	Laudanosine methiodide	207sh (4·95)	232 (4·30)	281 (3·76)		[19]
7.	Armepavine		228 (4·37)	282 (3·82)		[19]
8.	Norarmepavine	207sh (4·75)	228 (4·23)	283 (3·83)		[19]
9.	Non-phenolic base from thalsimine (or hernandezine)*		225 (4·14)	280 (3·56)	EtOH	[20]
10.	Phenolic base from thalfoetidine*	228sh (4·09)	280 (2·46)		MeOH	[20]

* This base is obtained after sodium–liquid ammonia cleavage of the alkaloid.

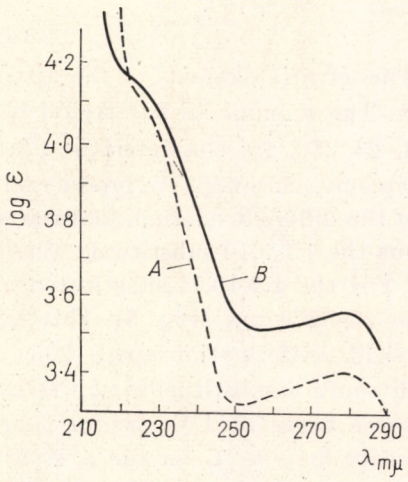


Fig. 3. UV spectra of: *A* — Thalifendlerine (in MeOH); *B* — Non-phenolic base of thalsimine (in EtOH)

A hypsochromic shift of the absorption of the nucleus, from 283 mμ to 265 mμ, is observed in the spectrum of takatonine iodide. This effect is due probably to the additional aromaticity of its molecule [25, 29]. A similar maximum in the same region is observed in the spectrum of papaverine methiodide [29] (Fig. 4).

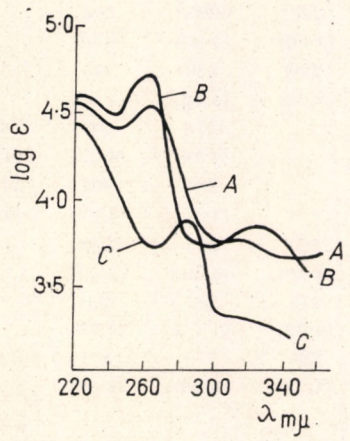


Fig. 4. A — Takatonine iodide (in MeOH); *B* — Papaverine methiodide (in EtOH); *C* — 1,2-Dehydrolaudanosine iodide (in EtOH)

(ii) Aporphine Alkaloids

The UV spectra of Thalictrum alkaloids of the aporphine group exhibit regularities in all cases. The maxima in the region between 268–282 and about 300 mμ [22, 23, 24, 25] are characteristic (Table XI). Differences in substitution of the biphenyl chromophore produce substantial differences both in the intensity of the different maxima and the wavelength at which these are observed. Thus the 1,2,9,10-substituted alkaloids exhibit a maximum around 282 mμ. For the 1,2,10,11-substituted aporphines the same maximum is shifted to 268–270 mμ (Fig. 5). This hypsochromic shift is probably due to the rigid conformation (page 295, XXI and XXII) of these aporphines which contain substituents at C-1 and C-11 [22, 23, 24, 25]. Moreover, both these types of UV spectra contain a maximum at 303–310 mμ, with intensity $\log \varepsilon \leqslant 4$, for the 1, 2, 10, 11-substituted and $\log \varepsilon \sim 4{\cdot}2$ for the 1, 2, 9, 10-substituted alkaloids (Table XI) [23].

Table XI

No.	Name	λ_{max}(mμ)[log ε]				Solvent	Ref.
1.	Thalicmine		283 (4·25)	302 (4·25)		EtOH	[25]
2.	Thalictuberine*	261 (4·84)	285 (4·50)	310 (4·32)	345 (3·50)	EtOH	[26]
3.	Glaucine	218 (4·58)	281 (4·18)	302 (4·16)		EtOH	[25]
4.	Isocorydine	220 (4·60)	268 (4·20)	302 (3·80)			[23]
5.	Thaliporphine	220 (4·52)	280 (4·12)	305 (4·12)		EtOH	[3]
6.	Preocoteine		278 (4·16)	302 (4·22)	312 (4·22)	EtOH	[3]
7.	O-Methylpreocoteine		281 (4·07)	302 (4·03)	311sh (3·98)	EtOH	[3]
8.	Isocorydine hydrobromide	220 (4·60)	268 (4·18)	302 (3·80)		MeOH	[25]
9.	Isocorydine methochloride	217 (4·62)	267 (4·16)	300 (3·76)		MeOH	[25]
10.	Norisocorydine	222 (4·60)	270 (4·18)	305 (3·82)		EtOH	[25]

* It possesses a phenanthrene structure and is biogenetically related with the aporphine alkaloids.

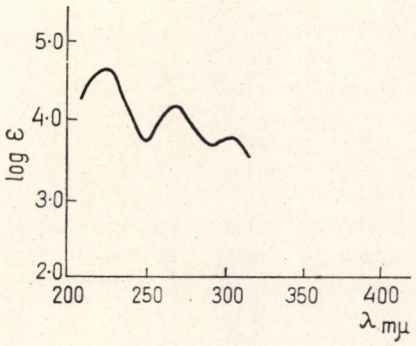


Fig. 5. UV spectrum of isocorydine hydrobromide (in MeOH)

In addition to the UV spectra, optical rotation can also be used in estimating the substituent pattern. Shamma made an interesting correlation between the type of substitution and the optical rotation. He pointed out that the aporphines which have substituents at C-1 and C-11 exhibit a considerably higher optical rotation than do the corresponding alkaloids where C-11 is unsubstituted (up to now all discovered aporphines in nature are substituted at C-1 and C-2). Data about some Thalictrum alkaloids exhibiting this optical phenomenon are given in Table XII [23].

Table XII

No.	Name	Positions of substituents	Optical rotation (solvent)	Ref.
1.	Isocorydine	1, 2, 10, 11	+195° ($CHCl_3$)	[23]
2.	Thalicmine	1, 2, 10, 11	+225° (Ethanol)	[23]
3.	Glaucine	1, 2, 9, 10	+113° (Ethanol)	[23]
4.	Thalicmidine	1, 2, 9, 10	+ 84° (Ethanol)	[23]

(iii) Protoberberine Alkaloids

The UV spectra of Thalictrum alkaloids of this group contain maxima around 230, 267 and 347 mμ (Table XIII).

A characteristic representative of this group of alkaloids is berberine (XLVI). The spectrum of its hydrochloride (in water or alcohol solution) exhibits three maxima at 267, 347 and 426 mμ.

The UV spectrum of berberine can be influenced by the addition of alkali. In alcoholic solution even a small concentration of KOH results in

Table XIII

No.	Name	λ_{max}(mμ)[log ε]				Solvent	Ref.
1.	Berberine chloride	267 (4·45)	347 (4·42)	426 (3·75)		EtOH	[25]
2.	Berberine iodide	226·5 (4·61)	263 (4·41)	344 (4·36)	423 (3·73)	Bz	[25]
3.	Tetrahydroberberine	209 (4·45)	284 (3·71)	230sh (4·07)		EtOH	[25]
4.	Thalifendine	231 (4·17)	269 (4·15)	348 (4·10)		EtOH	[2]
5.	Thalidastine chloride	233 (4·17)	269 (4·16)	348 (4·10)		EtOH	[4]
6.	Deoxythalidastine chloride	247 (4·24)	270 (4·18)	278 (4·18) 348 (3·71)	308 (4·08) 463 (3·68)	EtOH	[4]

changes of the absorption curve (*B* in Fig. 6) owing to the formation of carbinol (LIV) and aminoaldehyde (LV) forms. It has been suggested that

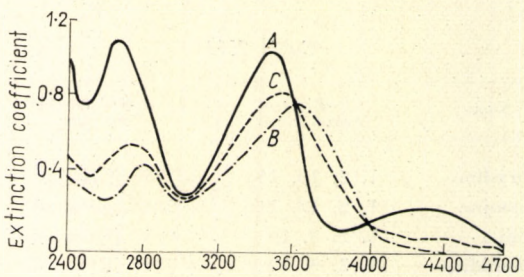
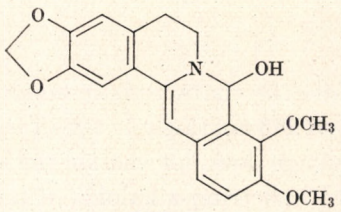


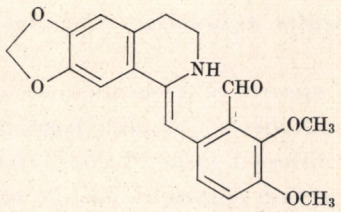
Fig. 6. UV spectra of *A* — Berberine hydrochloride (in alcohol); *B* — *A* + + $7{\cdot}8\times10^{-4}$ N KOH ; *C* — *A* + 0·25 N KOH

both forms are in equilibrium [27], or that they exist simultaneously side by side [43]. In aqueous solution, a low concentration of alkali does not influence the absorption curve (*A* in Fig. 6). A higher concentration has an influence, but here more complicated processes are involved [44].

Berberinol shows two maxima at 280 and 362 mμ. Curve *C* in Fig. 6 corresponds to a mixture of 25% berberine and 75% berberinol, which is formed after alkalization with 0·25 N alcoholic KOH. This curve exhibits two maxima at 272 and 353 mμ [25, 27].



LIV



LV

A similar study on the UV-absorption of jatrorrhizine (XLVIa) was made also [45].

Two maxima at 209 and 284 mμ and a shoulder at 230 mμ are observed in the spectrum of tetrahydroberberine (LVI). This spectrum is typical of tetrahydro derivatives of the alkaloids of this group (Fig. 7) [25].

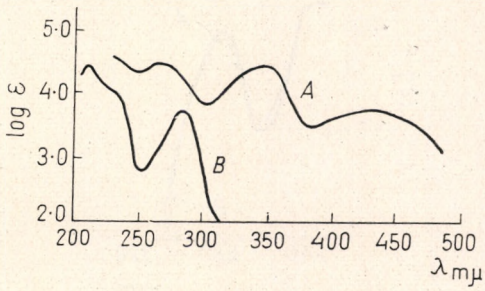
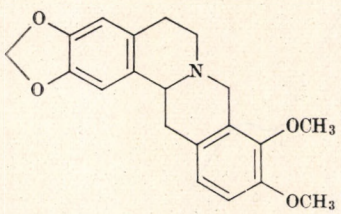
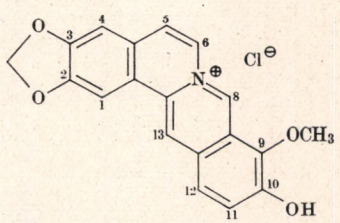


Fig. 7. A—Berberine hydrochloride (in EtOH); *B*—Tetrahydroberberine (in EtOH)

The UV spectrum of deoxythalidastine chloride (LVII) is complex and contains a large number of maxima (Table XIII). Thus, it differs essentially from the hitherto examined spectra of the protoberberine series.



LVI



LVII

On the basis of their UV spectra it is possible to distinguish the 2, 3, 9, 10- from the 2, 3, 10, 11-substituted 5, 6-dehydroberberine bases. The first contain maxima in the regions around 348–358 and 460–490 mμ, while the second contain maxima at 322–332 and 413–422 mμ [4, 28].

(iv) Bisbenzylisoquinoline Alkaloids

The UV spectra of these alkaloids contain the characteristic maximum for benzyltetrahydroisoquinoline bases around 283 mμ ($\log \varepsilon \sim 3{\cdot}8$) (Table XIV) [25]. The different form of their ether linkage does not change the shape of their absorption curves. Some spectra of Thalictrum alkaloids of this series are shown in Fig. 8. The alkaloids thalsimine and O-methylthalmetine, which contain in their molecules a $>C=N-$ function, show an additional maximum in the region between 312 and 314 mμ (Fig. 9)*

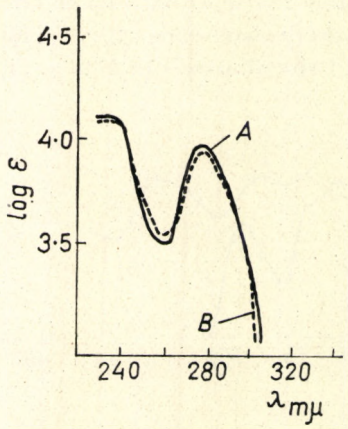


Fig. 8. A—Thalicberine; *B*—*O*-Methylthalicberine

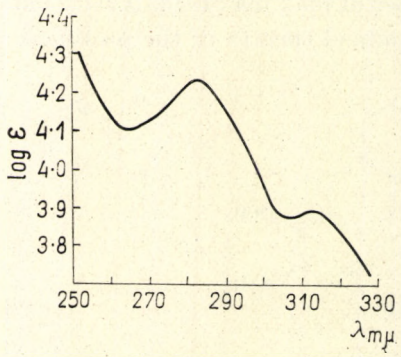


Fig. 9. UV spectrum of thalsimine (in MeOH)

* See the spectrum of 1,2-dehydrolaudanosine in Fig. 4.

Table XIV

No.	Name	λ_{max}(mμ)[log ε]			Solvent	Ref.
1.	Berbamine	206 (4·97)	238sh (4·38)	282 (3·84)		[21]
2.	Hernandezine	205 (5·03)	239sh (4·42)	284 (3·92)	EtOH	[20]
3.	Thalisamine			284 (4·06)	MeOH	[8]
4.	Thalidezine		283 (4·02)		EtOH	[3]
5.	Thalicberine		240 (4·17)	280 (3·92)	EtOH	[30]
6.	O-Methylthalicberine		240 (4·13)	280 (3·86)	EtOH	[30]
7.	Thalfoetidine	207 (5·02)	275 (3·87)	285 (3·87)	EtOH	[31]
8.	O-Methyltalfoetidine (thalidasine)	206 (4·99)	275 (3·77)	281 (3·75)	EtOH	[20]
9.	Thalicrine			285 (3·88)	EtOH	[25]
10.	Thalisopine			284 (3·65)		[32]
11.	Thalsimine		282 (4·23)	312 (3·90)	MeOH	[20]
12.	O-Methylthalmethine		280 (4·13)	314 (3·87)	MeOH	[33]

(v) Aporphine-benzylisoquinoline Alkaloids

The representatives of this group of Thalictrum alkaloids exhibit in their UV spectra (Fig. 10) maxima at 283 and 300–305 mμ, which are typical

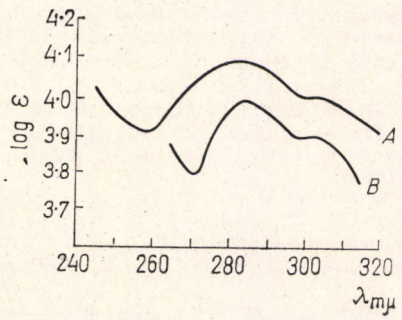


Fig. 10. UV spectra of *A* — Thalicarpine (in EtOH); *B* — Thalmelatine (in EtOH)

of the simple aporphine bases which do not contain a substituent at C-11 (Table XV).

Table XV

No.	Name	λ_{max}(mμ)[log ε]			Solvent	Ref.
1.	Thalicarpine		283 (4·09)	303 (4·01)	EtOH	[25]
2.	Thalmelatine		283 (4·00)	303 (3·90)		
3.	Foetidine	220 (4·80)	280 (4·36)	305 (4·24)	EtOH	[34]
4.	Dehydrothalicarpine		268 (4·82)	331 (4·34)	MeOH	[35]
5.	Dehydrothalmelatine		277 (4·64)	300 (4·56)	MeOH	[35]

Dehydrothalicarpine, whose aporphine system is partially dehydrogenated, exhibits two maxima at 268 and 331 mμ.

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IV. STEREOCHEMISTRY

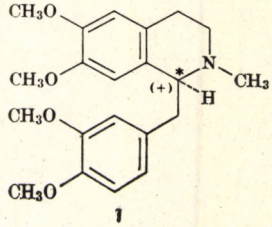
(N. M. Mollov and V. St. Georgiev)

1. Benzyltetrahydroisoquinoline Alkaloids

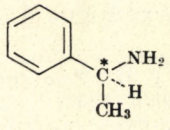
From a study of the optical activity of (+)-laudanosine (I) and (−)-α-phenylethylamine (II) in solvents with different polarity, Leithe [1] has reached the conclusion that these two compounds have the same absolute configuration. Further, (−)-α-phenylethylamine is converted to L-alanine

(III) by oxidative degradation without affecting the asymmetrical carbon atom. The L-alanine in turn is linked configurationally with D-glycerinealdehyde [2].

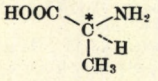
Later Corrodi and Hardegger proved the absolute configuration of (+)-laudanosine by means of oxdidative degradation to an optically active derivative of asparaginic acid. They obtained optically active N-methyl-N-β-carboxyethyl-asparaginic acid (IV) by the ozonolysis of (+)-laudanosine. But the asparagine acid is unstable and is racemized easily. On account of this Corrodi and Hardegger used (−)-tetrahydropapaverine (V), which on methylation of the nitrogen atom gave natural (+)-laudanosine, and on ozonolysis gave stable N-β-carboxyethyl-asparaginic acid (VI) which stereochemically belongs to the D-glycerinealdehyde series [3].



I



II



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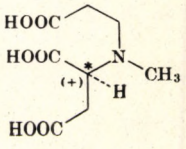

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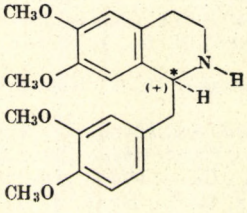
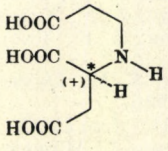
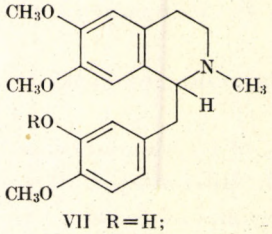


V

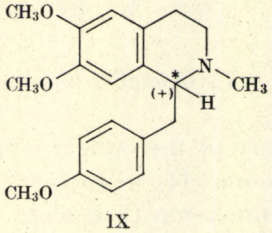


VI

The same authors also proved the absolute configuration of *R*-(−)-laudanidine (VII) converting it to *R*-(−)-laudanosine [3]. The opposite reaction, the conversion of *R*-(−)-laudanosine to *R*-(−)-laudanidine was



VII R=H;
VIII R=Ph;



IX

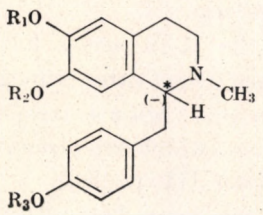
made by Tomita and Kunimoto [4]. They obtained *R*-(−)-laudanidine, without affecting the asymmetrical centre, by the cleavage of *R*-(−)-laudanosine with sodium in liquid ammonia. Later on these authors obtained (from the phenylether of *R*-(−)-laudanidine (VIII) by cleavage, again with alkali metal in liquid ammonia) *l*-O,O,N-trimethylcoclaurine (IX). The *S*-(+)-laudanosine is also transformed into *d*-O,O,N-trimethylcoclaurine in a similar way (Table I).

Table I

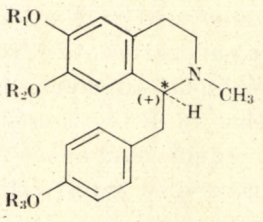
No.	Name	*R*-(D)-series $[\alpha]_D$	*S*-(L)-series $[\alpha]_D$	Ref.
1.	Laudanosine	−103·5° ($CHCl_3$)	+104·1° ($CHCl_3$)	[4]
2.	Laudanidine	− 86·3° ($CHCl_3$)	+ 86·2° ($CHCl_3$)	[4]
3.	Laudanidine phenylether	− 33·7° ($CHCl_3$)	+ 22·2° ($CHCl_3$)	[4]
4.	O,O,N-Trimethylcoclaurine	− 85·7° ($CHCl_3$)	+ 86·3° ($CHCl_3$)	[4]
5.	Non-phenolic base of thalsimine (or hernandezine)*	—	+ 36° (methanol)	[7]
6.	Phenolic base of thalfoetidine*	—	+ 46·8° ($CHCl_3$)	[7]
7.	Thalifendlerine	−108° (methanol)	—	[7, 27]

* This base is obtained after sodium–liquid ammonia cleavage of the alkaloid.

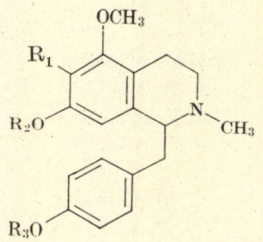
Tomita and Kunimoto, from measurements of the angle of rotation, determined the absolute configuration of some more optically active bases of the coclaurine type (X–XVII) which are obtained by the cleavage of bis-coclaurine alkaloids with sodium in liquid ammonia. A large number of these bases are also obtained by the cleavage of the Thalictrum alkaloids of the bisbenzylisoquinoline series.



X *l*-Armepavine (R_1=R_2=CH_3; R_3=H)
XI *l*-N-Methylcoclaurine (R_1=CH_3; R_2=R_3=H)
XII *l*-O,N-Dimethylcoclaurine (R_1=R_3=CH_3; R_2=H)
XIII *l*-O,N-Dimethylisococlaurine (R_1=H; R_2=R_3=CH_3)



XIV *d*-Coclanoline-A (R_1=CH_3; R_2=R_3=H)
XV *d*-O,N-Dimethylcoclaurine (R_1=R_3=CH_3; R_2=H)
XVI *d*-Armepavine (R_1=R_2=CH_3; R_3=H)
XVII *d*-N-Methylisococlaurine (R_1=R_3=H; R_2=CH_3)



XVIII R_1=H; R_2=R_3=CH_3;
XIX R_1=OH; R_2=CH_3; R_3=H;
XX R_1=OCH_3; R_2=CH_3; R_3=H

In the last few years the method of optical rotatory dispersion has been successfully used to determine the absolute configurations of optically active alkaloids. In order to determine the absolute configuration of an alkaloid, it is necessary to compare its dispersion curve with the dispersion curve of another similar alkaloid, whose configuration is known in advance, and which, in addition, contains an identical chromophore in the same stereochemical and conformational surroundings.

It is found that the dispersion curves of the benzyltetrahydroisoquinoline alkaloids of the S-(L)-series exhibit three positive Cotton effects in the region between 200 and 320 mμ.

Their enantiomers of the R-(D)-series have the corresponding mirror curves containing three negative Cotton effects [5, 9, 22] (Fig. 1). The presence of three extrema in this region corresponds to the absorption bands about 280, 230 and 207 mμ, which are observed in the UV spectra of these bases [5] (see Table X, Part III. p. 280).

The dispersion curves of some benzyltetrahydroisoquinoline alkaloids and optically active bases obtained by the cleavage of some bisbenzylisoquinoline Thalictrum alkaloids are shown in Figs 2 and 3. Although (—)-armepavine and (—)-norarmepavine are laevorotary, their curves clearly

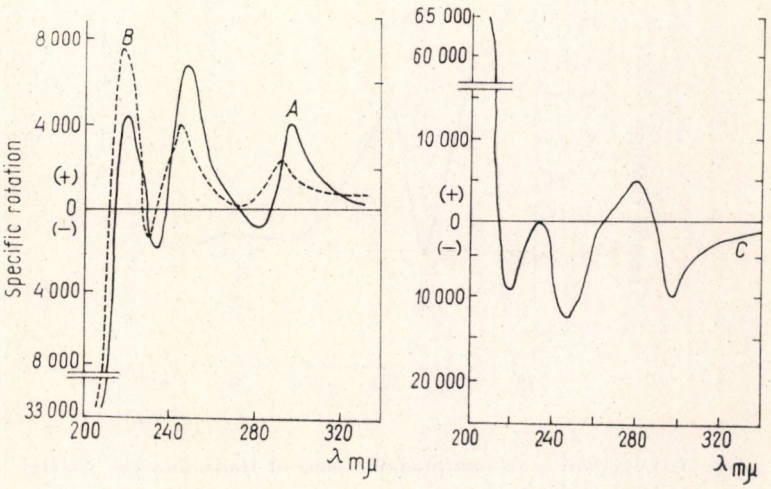


Fig. 1. *A*—*S*-(+)-Laudanosine; *B*—*S*-(+)-Laudanosine methiodide; *C*—*R*-(−)-Laudanidine

point out that the latter belongs to the *S*-series, while the first possesses an *R*-configuration (Fig. 2). This fact is also in agreement with the chemical conversions, which determine the correctness of their absolute configuration [2, 6].

The quaternization of the nitrogen atom does not introduce fundamental changes in the nature of the dispersion curves of these alkaloids (Table II)

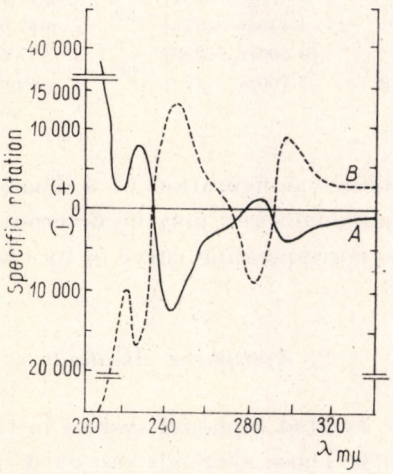


Fig. 2. *A*—*S*-(—)-Norarmepavine; *B*—*R*-(−)-Armepavine

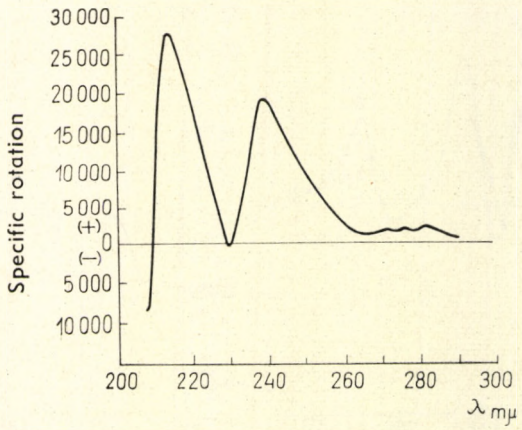


Fig. 3. ORD curve of non-phenolic base of thalsimine (in EtOH)

[5, 8]. For example, the curve of the *S*-(+)-laudanosine methiodide corresponds well to the curve of the initial base (Fig. 1).

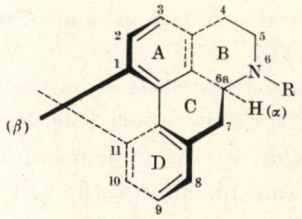
Table II

No.	Compound	First Cotton effect [ø]	$\lambda_{m\mu}$	a	Second Cotton effect [ø]	$\lambda_{m\mu}$	a	Ref.
1.	*S*-(+)-Laudanosine methiodide	+11 500p + 4 500tr	286 267	+70	+25 200p −26 400tr	238 221	− 516	[8]
2.	*S*-(+)-Armepavine-methiodide	+ 9 620p + 4 440tr	287 273	+52	+47 400p −57 000tr	234 219	+1004	[8]
3.	*R*-(−)-O-Methyl-armepavine methiodid	−10 400tr − 3 760p	286 270	−66	−37 600tr +40 000p	234 221	− 776	[8]

Therefore, the absolute configuration of a Thalictrum alkaloid of the benzyltetrahydroisoquinoline series may be determined both by its dispersion curve and by the corresponding curve of its quaternary salt.

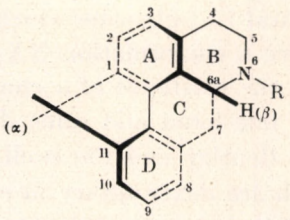
2. *Aporphine Alkaloids*

The presence of the twisted biphenyl system in the aporphine molecule causes its non-planarity. These alkaloids can exist in one of the following two conformations:



XXI

S-series



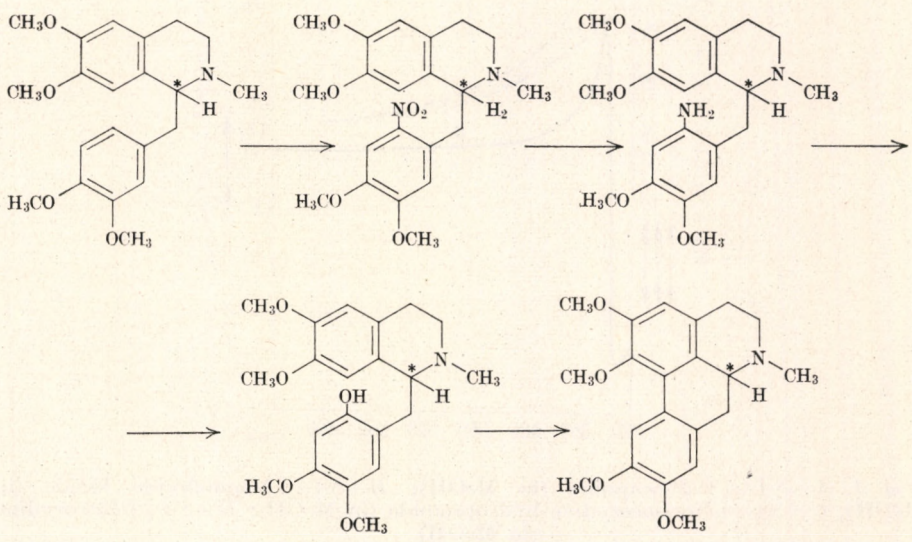
XXII

R-series

All aporphines found in nature so far contain substituents at C-1 and C-2 in the aromatic ring A. In addition they may have substituents in ring D, the 9,10- or the 10,11-substituted are the most frequently met.

The simultaneous presence of substituents at C-1 and C-11 imposes a fundamental limitation on the already strained conformation of these alkaloids. Besides, if the hydrogen atom at the asymmetrical centre is in situation α (below the plane) then the substituents in ring A are in situation β (above the plane), and conversely (XXI and XXII) [16]. The more specific situation of the substituents in this part of the aporphine molecule is also confirmed by studies of the NMR spectra of these alkaloids (see page 267).

The absolute configuration of the aporphine alkaloids was determined for the first time by Faltis and Adler [10]. They used *R*-(−)-laudanosine as an initial compound, its absolute configuration being known. A number of simultaneous transformations gave *R*-(−)-glaucine which must have a conformation **XXII**:



Thus natural (+)-glaucine which is also found in some Thalictrum species will have a conformation XXI [11].

Owing to the existence of a chemical interrelation between S-(+)-glaucine on the one hand and some dextrorotary aporphine alkaloids on the other hand, Bentley and Cardwell came to the conclusion that all aporphines which are dextrorotary in ethanol or chloroform will belong to the S-series (XXI) and conversely [11].

They also mentioned the fact that these bases form isomethine derivatives on Hofmann degradation, which posses a sign opposite to that of the initial base. Thus, dextrorotary aporphines give laevorotary isomethine bases, while laevorotary aporphines yield dextrorotary isomethine derivatives.

Not long ago Tomita and Fukagawa, using alkali metal cleavage in liquid ammonia, determined the absolute configuration of (+)-magnoflorine iodide [12].

Parallel with the study of the conformation of the aporphine molecule, the ORD behaviour of these alkaloids was examined [13–15]. The presence of a twisted biphenyl chromophore in the molecule plays an essential role in every Cotton effect observed in the dispersion curves of these bases. The

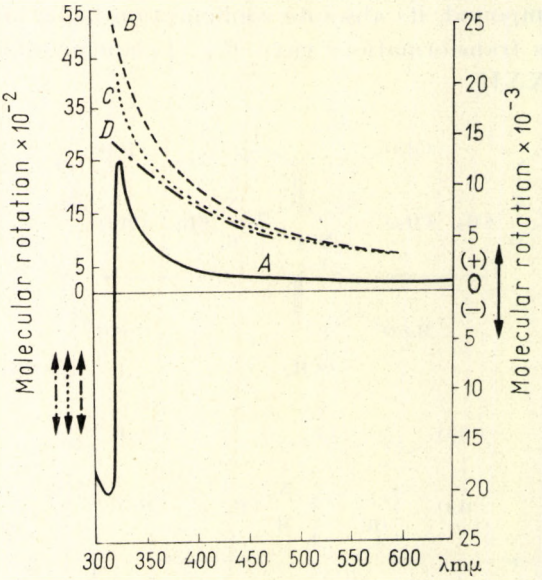


Fig. 4. *A*—*S*-(+)-Bulbocapnine (in MeOH); *B*—*S*-(+)-Magnoflorine iodide (in MeOH); *C*—*S*-(+)-Norisocorydine hydrobromide (in MeOH); *D*—*S*-(+)-Isocorydine (in MeOH)

manner in which the molecule is substituted is also of great importance [13, 14, 15, 17]. Thus all 1,2,10,11-substituted alkaloids have dispersion curves which are continuously positive and show a positive Cotton effect in the region between 310 and 330 mμ. The ORD curves of *S*-(+)-bulbocapnine and some aporphine Thalictrum alkaloids are shown in Fig. 4. The latter are 1,2,10,11-substituted and belong to the corydine group. Their conformation (XXI) can be assigned as a result of the similarity of their curves with that of the *S*-(+)-bulbocapnine [13]. The alkaloids which are 1, 2, 9, 10-substituted (e.g. *S*-(+)-glaucine) have dispersion curves with one negative Cotton effect in the range about 320 mμ, while their smooth curve has a positive sign in the visible region (Fig. 5).

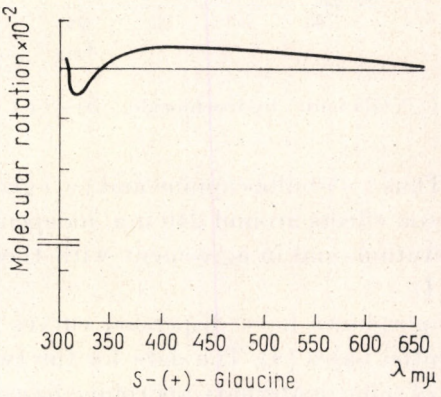


Fig. 5

Since the absolute configuration of some of these alkaloids is determined in advance by chemical means (see p. 295) the conclusion may be drawn that all of them have conformation XXI.

An increased amplitude of the long-wavelength Cotton effect is observed (in the region about 320 mμ) in the ORD curves of the aporphine alkaloids containing a methylenedioxy group. This peculiarity can be used as a distinguishing feature for the methylenedioxy group. Nevertheless this must be done with some caution, because some alkaloids which do not contain such a group (e.g. laurifoline chloride) can also have an increased amplitude of the long-wavelength Cotton effect [13].

Cymerman Craig and Roy [15] have proposed that the Cotton effect which is observed between 235 and 245 mμ may be employed for the determination of the absolute configuration of the aporphine alkaloids, because it does not depend on the way in which the aporphine molecule is substi-

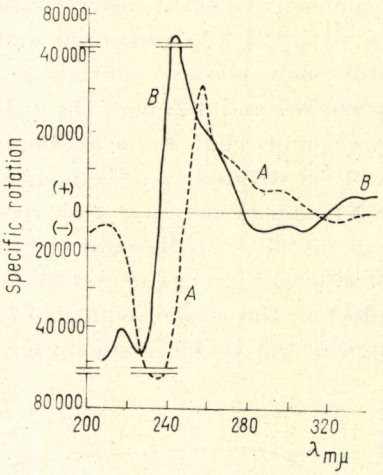


Fig. 6. *A*—*S*-(+)-Glaucine hydrochloride; *B*—*S*-(+)-Bulbocapnine

tuted (see p. 283). Thus (+)-bulbocapnine and (+)-glaucine (Fig. 6) exhibit large positive Cotton effects around 240 mμ, independently of the differences in their substitution, and in agreement with their identical absolute configurations (XXI).

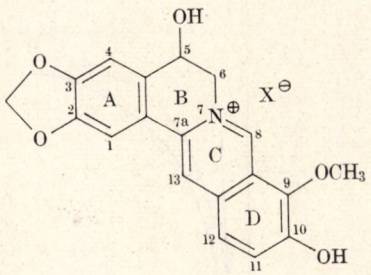
The quaternary aporphines have dispersion curves which differ a little from those of the initial bases [8]. The data for the two Cotton effects observed in the curves of some quaternary aporphine bases isolated from plants of Thalictrum species are given in Table III. All of them have a well known absolute configuration and belong to the *S*-series [8]. The second Cotton effect, as in the case of the benzyltetrahydroisoquinoline alkaloids, does not change its sign in passing from tertiary to quaternary base. Evidently, this Cotton effect is connected with the intensive UV absorption band at 220–227 mμ, and the wavelength at which it is observed depends on the presence of a substituent at C-11. When this carbon atom is substituted, it appears in the region about 240 mμ, and when there is no substituent at C-11 at around 250 mμ. The Cotton effect at 310–330 mμ corresponds to the UV absorption band at 300–320 mμ. Analogously with the case of the tertiary aporphine bases [13, 16, 18], its character depends to a great extent on the dihedral angle between the two aromatic rings of the biphenyl system. This effect has a negative sign and a large amplitude for the 1,2,9,10-substituted quaternary bases, while the 1,2,10,11-substituted bases, on account of the diminished conjugation between the aromatic ring exhibit a positive Cotton effect with a diminished amplitude.

Table III

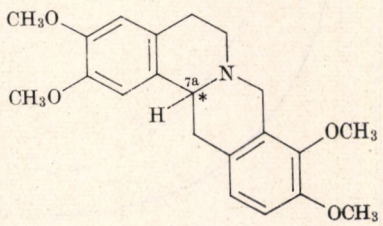
No.	Compound	First Cotton effect [⌀]	$\lambda_{m\mu}$	a	Second Cotton effect [⌀]	$\lambda_{m\mu}$	a	Ref.
1.	*S*-(+)-Magnoflorine iodide	+ 6 200p	341	+134	+108 000p	249	+2160	[8]
		− 7 200tr	306		−108 000tr	222		
2.	*S*-(+)-Isocorydine methiodide	+ 4 320p	321	+ 86	+169 000p	237	+3760	[8]
		− 4 320tr	302		−207 000tr	221		
3.	*S*-(+)-O,O-Dimethyl-magnoflorine iodide	+ 8 820p	318	+151	+180 000p	249	+4300	[8]
		− 6 300tr	294		−250 000tr	224		
4.	*S*-(+)-Glaucine methiodide	−11 000tr	317	−355	+129 000p	251	+4080	[8]
		+24 500p	291		−279 000tr	227		
5.	*S*-(+)-Ocotein methiodide	−14 400tr	318	−330	+ 86 500p	250	+2395	[8]
		+18 600p	292		−153 000tr	224		

3. *Protoberberine Alkaloids*

Of the Thalictrum alkaloids which belong to this group only thalidastine chloride (XXIII) contains an asymmetrical carbon atom at C-5 [26]. The rest of the alkaloids are quaternary bases for which $[\alpha]_D$ is equal to 0°. However they consume two moles of hydrogen and are converted into tetrahydro derivatives on hydrogenation. At the same time a new asymmetrical carbon centre is created, which can possess configuration XXIV or XXV. The known tetrahydroprotoberberine alkaloids with one asymmetric atom have an angle of rotation of about ±300°. The hydroxy and methoxy groups do not cause a substantial change in the optical rotation [3].



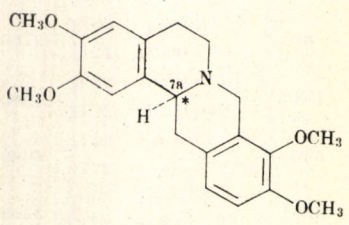
XXIII



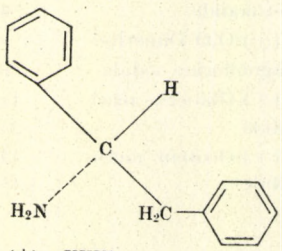
XXIV

The absolute configuration of (+)-tetrahydropalmatine shown in formula XXIV has been determined by Corrodi and Hardegger by chemical means [3]. Its antipode, (−)-tetrahydropalmatine, has a configuration XXV.

Since tetrahydropalmatine and 1,2-diphenylethylamine have a certain structural resemblance (the amine group is similarly situated with regard to the aromatic rings in both compounds), it can be expected that their dispersion curves will be similar (Fig. 7) [21].



XXV



(+)—XXVI

In practice, (—)-tetrahydropalmatine exhibits one negative Cotton effect in the region about 300 mμ, which is in accordance with its absolute configuration [3] and shows that (+)-1,2-diphenylethylamine (XXVI) is its enantiomer.

Quaternization of the nitrogen atom does not change the shape of the curves, and the sign of the Cotton effect is preserved (Fig. 7).

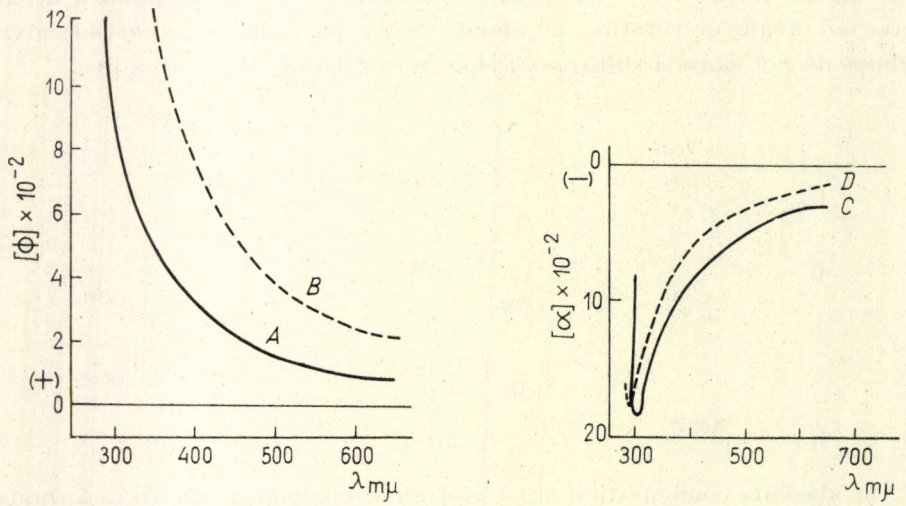


Fig. 7. A—(+)-1,2-Diphenylethylamine; *B*—(+)-1,2-Diphenylethylamine hydrochloride; *C*—(—)-Tetrahydropalmatine; *D*—(—)-Tetrahydropalmatine hydrochloride

4. Bisbenzyltetrahydroisoquinoline Alkaloids

Of the total number of Thalictrum alkaloids the percentage of bisbenzyltetrahydroisoquinoline alkaloids is the greatest. Their molecules contain two asymmetrical carbon atoms. This, and the presence of two ether bridges, lead to complex stereochemical problems.

The determination of the absolute configurations of the asymmetric centres of the two moieties of the molecule is usually done in the following way. The alkaloid is cleaved with an alkali metal in liquid ammonia after which the optical rotations at the D-line of the two benzyltetrahydroisoquinoline moieties obtained are measured. The found $[\alpha]_D$ is compared with the corresponding values of the benzyltetrahydroisoquinoline alkaloids that have determined configurations (Table IV). The bis bases are described as (++), (+−), (−−), and (−+) or correspondingly as (*S*, *S*), (*S*, *R*),

Table IV

No.	Compound	Sign of $[\alpha]_D$ of the component monobases	Absolute configuration
	BISBENZYLISOQUINOLINE ALKALOIDS		
1.	Berbamine	−, +	*R*, *S*
2.	Isotetrandrine	−, +	*R*, *S*
3.	Obamegine	−, +	*R*, *S*
4.	Hernandezine	+, +	*S*, *S*
5.	Thalisamine	+, +	*S*, *S*
6.	Thalicrine	+, −	*S*, *R*
7.	Homothalicrine	+, −	*S*, *R*
8.	Thalicberine	+, +	*S*, *S*
9.	O-Methylthalicberine	+, +	*S*, *S*
10.	Thalfoetidine	+, +	*S*, *S*
11.	Thalisopine	+, −	*S*, *R*
12.	Thalmine	+, +	*S*, *S*
13.	Thalmethine	+	*S*
14.	O-Methylthalmethine	+	*S*
15.	Thalsimine	+	*S*
	APORPHINE–BENZYLISOQUINOLINE ALKALOIDS		
16.	Thalicarpine	+, +	*S*, *S*
17.	Thalmelatine	+, +	*S*, *S*
18.	Dehydrothalicarpine	+	*S*
19.	Dehydrothalmelatine	+	*S*
20.	Foetidine	+, +	*S*, *S*

(*R*, *R*) and (*R*, *S*) depending on the optical rotation of the composite monobases.

Bruchhausen *et al.* make an assumption that the optical rotation at the D-line of a bisbenzylisoquinoline alkaloid is the sum of the corresponding values of the two bisbenzylisoquinoline components [23, 24]. This additive rule is true for the majority of alkaloids of this group and can be used to obtain preliminary information. The observed exceptions from the rule (oxyacanthine, repandine) are due probably to molecular dissymmetry [25].

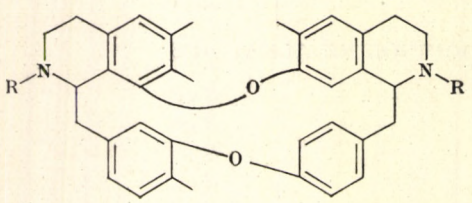
The ORD curves of bisbenzylisoquinoline alkaloids may also serve for configurational estimation of the asymmetric centres of the molecule.

The dispersion curves of the Thalictrum alkaloid of the bisbenzylisoquinoline group are complex and exhibit some extrema in the region about 300–220 mμ [7, 9]. The most characteristic is the Cotton effect observed between 290 and 270 mμ and a large extremum at about 235 mμ. The UV spectra of these alkaloids exhibit two maxima. The first of them is at about 280 mμ and has moderate intensity, while the second has strong intensity and is observed at about 205 mμ with one shoulder between 225–240 mμ [7, 9] (see Table XIV, Part III, p. 287).

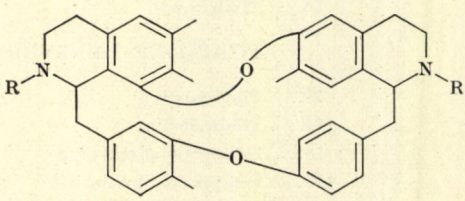
The first Cotton effect can be ascribed to the characteristic aromatic chromophore absorption band at 280 mμ, analogous to the case of the benzyltetrahydroisoquinoline alkaloids. The second effect (only the beginning of which is reached) corresponds to an absorption band at 205 mμ [9].

In considering the dispersion curves of these alkaloids, attention must be paid to the positions of the ether linkages in the molecule. In addition, the various configurations of the two asymmetrical centres are of fundamental importance. Thus conclusions concerning the configurations of bisbenzylisoquinoline alkaloids on the basis of ORD can only be drawn if two alkaloids, a known and an unknown compound, are compared which have identical ether linkages.

The alkaloids of the berbamine type exhibit two types of dispersion curve according to whether the configurations of their asymmetrical carbon atoms



Berbamine type



Thalicberine type

are (*S*, *S*) or (*R*, *S*) Fig. 8 [7, 9]. The *S*,*S*-compounds have curves with two positive Cotton effects, the second peak being much bigger than the first. Hernandezine, which has an additional methoxy group at C-5, exhibits a

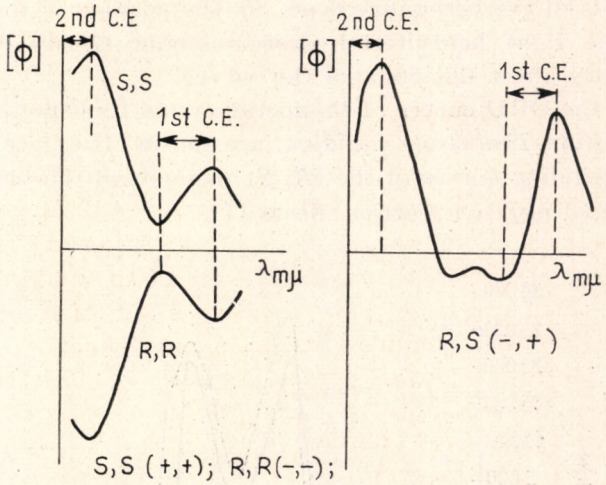


Fig. 8

similar curve. This suggests that the presence of a substituent at C-5 does not exert a great influence and consequently a (*S*,*S*)-configuration can be ascribed to hernandezine [9, 20].

The (*R*, *R*)-compounds have corresponding mirror curves with two negative

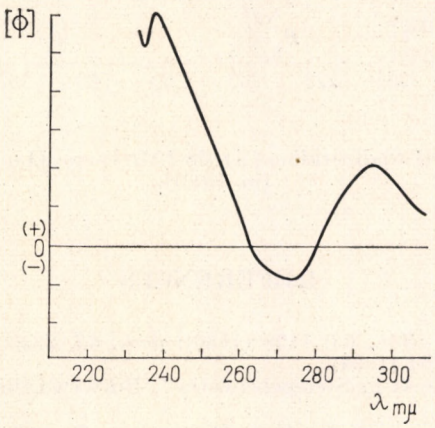


Fig. 9. ORD curve of thalicberine (in EtOH)

Cotton effects. (*R*, *S*)-Alkaloids also exhibit two negative Cotton effects, but the difference between them is small.

Thalicberine [19] with (*S*, *S*-)-configuration has an ORD curve shown in Fig. 9. As may be seen, it does not differ essentially from the curves of the (*S*, *S*)-alkaloids of the berbamine type. So, the position of the upper ether linkage in the right benzyltetrahydroisoquinoline moiety (C-6′ or C-7′) does not greatly affect the shape of the curve.

In Fig. 10 the ORD curves of thalfoetidine and its O-methyl derivative [7], isolated from *Thalictrum foetidum*, are shown. They are the opposite of the corresponding curves of the (*R*, *S*)-alkaloids of the berbamine type and exhibit two negative Cotton effects [7].

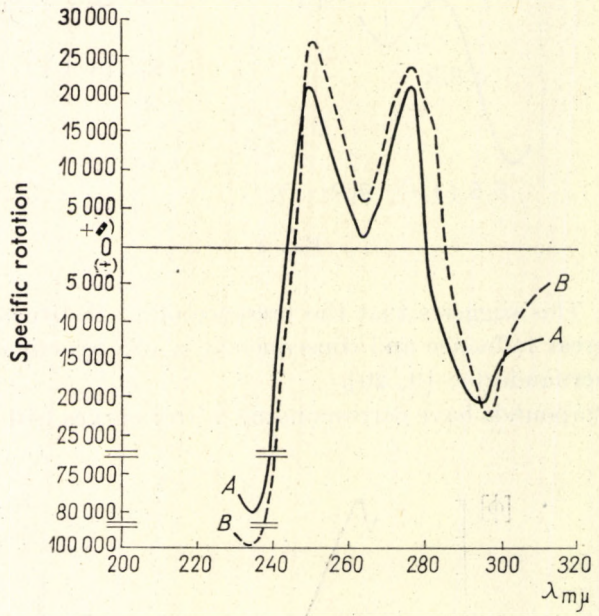


Fig. 10. ORD curves of thalfoetidine (*A*) (in EtOH) and O-methyl-thalfoetidine (*B*) (in EtOH)

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TO

THALICTRUM ALKALOIDS

amply illustrated also by the methods developed by the authors for the preparation of glycosides, oligo- and polysaccharides.

In the introduction of the third monograph, the well-known authors, *N. M. Mollov*, *H. B. Dutschewska* and *V. St. Georgiev* outline the biogenesis and pharmacological activity of Thalictrum alkaloids. The following part deals with the different chemical types of these compounds, such as the isoquinoline, benzyltetrahydroisoquinoline, aporphine, protoberberine, protopine, bisbenzylisoquinoline, pavine, isopavine and other related alkaloids. The discussion includes the spectral (mass, NMR, IR, UV) properties and the stereochemistry of these alkaloids.

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