The Chemistry of Natural Depsipeptides

by
M. M. SHEMYAKIN D.Sc.
and
Yu. A. OVCHINNIKOV D.Sc.

Constituents of the Bulgarian Zdravets Oil

by
I. OGNYANOV D.Sc.
and
D. IVANOV D.Sc.

Structure and Synthesis of Ipecac Alkaloids

by
Cs. SZÁNTAY D.Sc.

AKADÉMIAI KIADÓ, BUDAPEST

RECENT DEVELOPMENTS IN THE CHEMISTRY OF NATURAL CARBON COMPOUNDS

Volume II

This book — the second volume of the series — consists of three monographs on natural organic compounds.

M. M. Shemyakin and *Yu. A. Ovchinnikov* present a truly authentic, very concise and brilliantly composed survey on the chemistry of depsipeptides. Recent developments described in the monograph well illustrate the rapid progress in depsipeptide research; several details of the chemistry of these compounds as given in textbooks published only a few years ago, appear now discredited.

I. Ognyanov and *D. Ivanov* report their pioneering work on the structural elucidation of the constituents of Bulgarian Zdravets oil. The main component, germacrone, has been shown to possess the structure of a sesquiterpene with a formerly unknown carbon skeleton.

Cs. Szántay gives an account of his own researches on ipecac alkaloids within the framework of a lucid monograph that contains reference to all important papers

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VOLUME II

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PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES

BUDAPEST 1967

THE CHEMISTRY OF NATURAL DEPSIPEPTIDES

by

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CONSTITUENTS OF THE BULGARIAN ZDRAVETS OIL

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STRUCTURE AND SYNTHESIS OF IPECAC ALKALOIDS

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THE CHEMISTRY OF NATURAL DEPSIPEPTIDES

Depsipeptides constitute a large and rapidly expanding class of peptide-related compounds, which are built up of hydroxy and amino acid residues joined by amide and ester linkages. Various members of this class are quite frequently encountered among natural products, particularly in substances of microbiological origin. Most of these are cyclodepsipeptides with considerably varying ring size up to 36-membered cyclododecadepsipeptides. Many of the naturally occurring depsipeptides are biologically active. These include an appreciable number of antibiotics, some alkaloids, and, apparently, certain proteins.

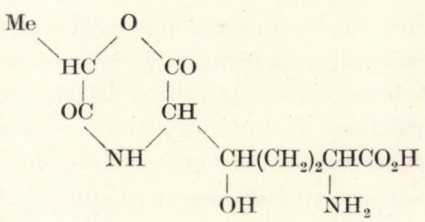
In the past years we devised general methods for the synthesis of optically active linear and cyclic depsipeptides with any desired sequence of the amino acid and hydroxy acid residues. Simultaneously we studied some of their properties, including the stereochemical aspects of their ring closure. Furthermore, new methods for their structural study were developed, among which the mass spectrometric approach deserves special mention.

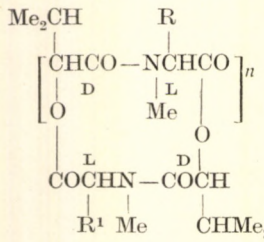
All these studies led to the syntheses in 1962–64 of a number of naturally occurring depsipeptides; in the course of this work it was discovered that formulae proposed for some of them had been erroneous and these were corrected. Parallelly, work was begun on the relations between chemical constitution and physiological action of antibiotic depsipeptides. Probable biogenetic pathways to a number of these compounds were also outlined.

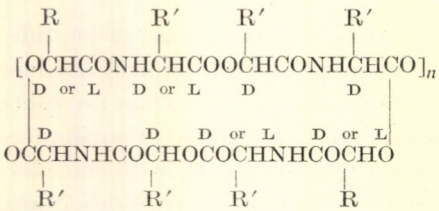
*

The naturally occurring depsipeptides known at present may be divided into a number of groups.

I. Woolley's toxin (1955)







Enniatin A

II. R = R¹ = CHMeEt, $n = 1$; Plattner et al. (1947–48)

III. R = R¹ = CHMeEt, $n = 2$; Shemyakin et al. (1963); Vogler et al. (1963)

Enniatin B

IV. R = R¹ = $CHMe_2$, $n = 1$; Plattner et al. (1947–48)

V. R = R¹ = $CHMe_2$, $n = 2$; Shemyakin et al. (1963); Plattner, Vogler et al. (1963)

Valinomycin

VI. R = CH_3, R′ = $CHMe_2$, $n = 1$; Brockmann et al. (1955–57)

VII. R = CH_3, R′ = $CHMe_2$, $n = 2$; Shemyakin et al. (1963)

Amidomycin

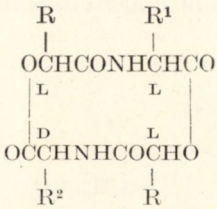
VIII. R = R′ = $CHMe_2$, $n = 1$, Vining, Taber (1957)

Formula VIII is wrong; Shemyakin et al. (1963)

Amidomycin is an artefact; Vining (1963)

To the first group belong cyclodepsipeptides with regularly alternating α-amino and α-hydroxy acid residues. The simplest of these is Woolley's phytopathogenic toxin [191] with the cyclodidepsipeptide formula (I). Here also belong two antibiotics, namely enniatin A and enniatin B, to which Plattner [45, 90–94] had ascribed a cyclotetradepsipeptide structure (II and IV), but which we [152–155] and simultaneously Plattner–Vogler's team [95, 96, 100, 101] have recently shown to be the cyclohexadepsipeptides (III and V). Latterly, mass spectrometric studies have revealed the existence in naturally occurring enniatin mixtures of a "mixed" type of enniatins, in particular enniatin A_1 (R = CHMeEt, R¹ = $CHMe_2$, $n = 2$) and B_1 (R = $CHMe_2$, R¹ = CHMeEt, $n = 2$) [62]. The group further includes the antibiotic valinomycin for which Brockmann had proposed the structure of the cyclooctadepsipeptide (VI) [24, 32], whereas it is actually the cyclododecadepsipeptide (VII) [35], as we have directly shown by its synthesis [130, 131]. Research in this group of compounds was not without its comic turns. For instance with amidomycin, Vining and Taber [170, 171, 183] had suggested for this substance the cyclooctadepsipeptide structure (VIII). On synthesizing the compound with this formula we discovered that it had properties entirely different from those attributed to amidomycin [158, 159], only to find out afterwards from Vining in a private communication that this compound was an artefact. Finally, this group of depsipeptides. includes also a metabolite of certain species of *Pithomyces*, angolide [13, 103]

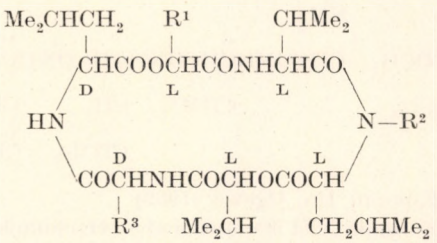
This compound is a cyclotetradepsipeptide, its structure (IX) being elucidated by Russell [106] and confirmed mass spectrometrically both by us [60, 61] and by Shannon [74], and then through its total synthesis [61].



Angolide

IX. $R = CHMe_2$, $R^1 = CHMeEt$
$R^2 = CHMeEt$-*allo*
Russell (1965)
Shemyakin (1964)

To the second group of depsipeptides belong substances with irregular amino acid and hydroxy acid sequences. This group contains compounds with both α- and β-hydroxy acid residues. Among its members there are four cyclohexadepsipeptides, sporidesmolides I, II, III and IV, biologically inactive metabolites isolated by Russell from the fungus *Pithomyces chartarum* and *Pithomyces maydicus* [11, 14, 104, 105, 107]. To sporidesmolide I Russell assigned the formula X, whose validity we demonstrated soon after by direct synthesis [82, 142, 145, 146]. In the case of sporidesmolide II, Russell who had proposed the structure XI, did not elucidate the stereoisomerism of the isoleucine residue. We found it to be *allo*isoleucine, so that the final structure is represented by XII as we confirmed it by the synthesis of sporidesmolide II [89, 143, 146]. The structure of sporidesmolide III (XIII) was elucidated by Russell and Shannon mass spectrometrically [108] and confirmed by us synthetically [58]. Russell also established the formula of sporidesmolide IV (XIV) [13, 14], whose total synthesis we carried out [59].



Sporidesmolide I

X. $R^1 = R^3 = CHMe_2$, $R^2 = Me$;
Russell (1960); Shemyakin et al. (1962)

Sporidesmolide II

XI. $R^1 = CHMe_2$, $R^2 = Me$, $R^3 =$
$=$ CHMeEt; Russell (1960)

XII. $R^1 = CHMe_2$, $R^2 = Me$, $R^3 =$
CHMeEt-*allo*; Shemyakin et al. (1963)

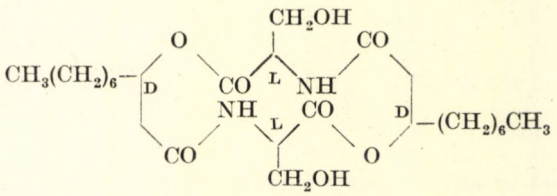
Sporidesmolide III

XIII. $R^1 = R^3 = CHMe_2$, $R^2 = H$;
Russell (1964); Shemyakin et al. (1965)

Sporidesmolide IV

XIV. $R^1 = CH_2CHMe_2$, $R^2 = Me$, $R^3 =$
$CHMe_2$; Russell (1964); Ovchinnikov et al. (1965)

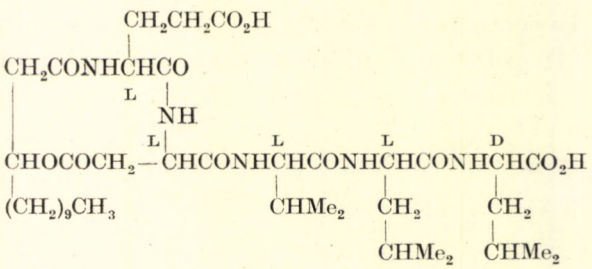
To this group of depsipeptides, but containing β-hydroxy acid residues, belong two antibiotics, serratamolide and esperin. For the former Wasserman [187] proposed the structure of a cyclotetradepsipeptide (XV), which we have recently confirmed by the synthesis of diacetylserratamolide [139, 140] and serratamolide itself [5, 63].



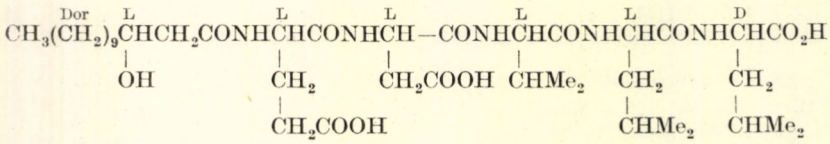
Serratamolide

XV. Wasserman et al. (1961)
Shemyakin et al. (1964–1965)

As for esperin Ito and Ogawa [54] formulated its structure (XVI), leaving open the question of the configuration of the hydroxy acid residue. On subjecting esperin to alkaline hydrolysis, they isolated esperinic acid (XVII). We synthesized two stereoisomers corresponding to this formula, one containing L- and the other D-β-hydroxytridecanoic acid. However, both stereoisomers differed considerably from esperinic acid, which thus places questionable the correctness of the formula (XVI) proposed for esperin [85].

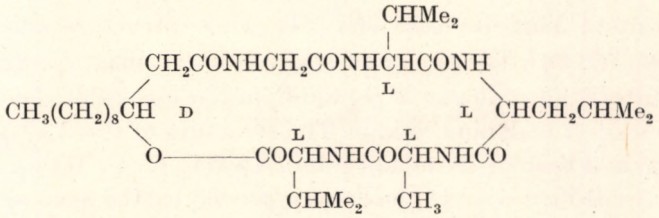


XVI. Esperin; Ito, Ogawa (1959)
Formula XVI is questionable; Ovchinnikov et al. (1966)



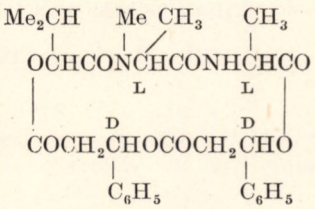
XVII. Esperinic acid; Ito, Ogawa (1959)
Formula XVII is wrong; Ovchinnikov et al. (1966)

This type of depsipeptides also includes isariin, a metabolite of the fungus *Isarea cretacea*, described by Vining [184],who also established its structure (XVIII) mass spectrometrically [190].



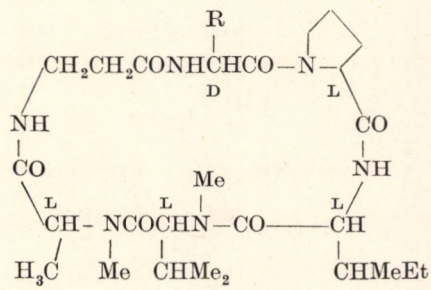
XVIII. Isariin; Vining (1966)

Still another depsipeptide called pithomycolide was found among the metabolic products of *Pithomyces chartarum*. It was established by Briggs [20] that pithomycolide is a 17-membered cyclopentadepsipeptide (XIX), containing two D-β-hydroxy-β-phenylpropionic acid residues.



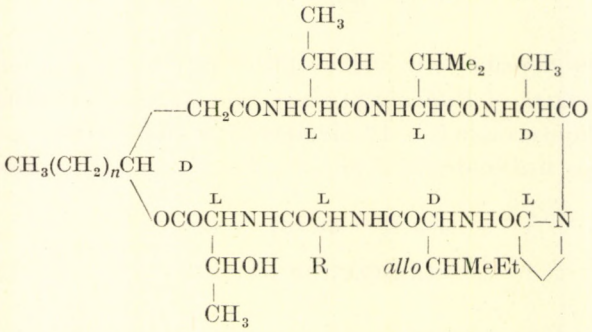
XIX. Pithomycolide; Briggs (1964)

To depsipeptides of the second group belong also the destruxins, possessing insecticide properties. These were studied by Japanese workers [64, 172], who proposed the structure XX for destruxin B [173], confirmed by its total synthesis [67] and recently structure XXI for destruxin A [169].



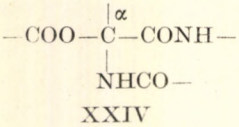
XX. Destruxin B; R = CH_2CHMe_2; Tamura (1964)
XXI. Destruxin A; R = $CH_2CH=CH_2$; Tamura (1966)

Finally, this group of depsipeptides also includes the quite recently discovered compounds related to the lipoproteins whose molecules contain residues of the higher hydroxy acids as well as amino acid residues. To date compounds of this type best known chemically are peptidolipin NA, isolated from a culture of *Nocardia asteroides* [51], whose structure was formulated as XXII [49, 50, 68]. The structure was confirmed mass spectrometrically [10]. Just latterly an analogue of peptidolipin NA was isolated containing an L-valine instead of L-alanine residue. The structure of this Val^6-peptidolipin NA (XXIII) and that of its homologues (XXIII; R = $CHMe_2$, $n = 17$ and XXIII; R = $CHMe_2$, $n = 18$) have been proved by the mass spectrometric method [52].

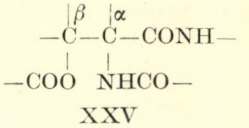


XXII. Peptidolipin NA; R = CH_3, $n = 16$; Lederer (1964–1965)
XXIII. Val^6-Peptidolipin NA; R = $CHMe_2$, $n = 16$; Lederer (1966)

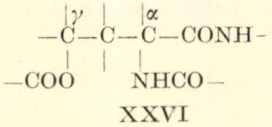
Depsipeptides of the third group are characterized by the presence of one or more hydroxyamino acid residues with both the hydroxyl and amino functions in the same molecule. This group may be subdivided into compounds containing α-hydroxy- (XXIV), β-hydroxy- (XXV) and γ-hydroxy- (XXVI) -α-amino acid residues. To the first group belong two new ergot



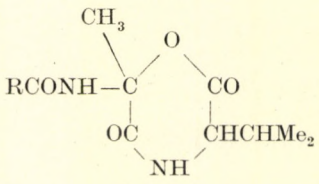
XXIV



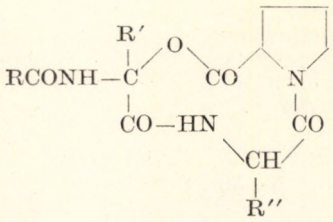
XXV



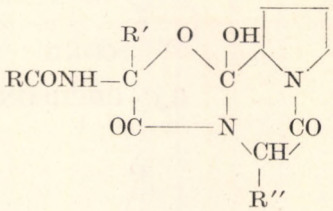
XXVI



XXVII
Ergosecaline
Ergosecalinine



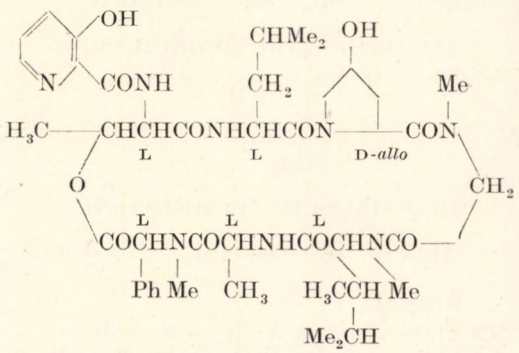
XXVIII



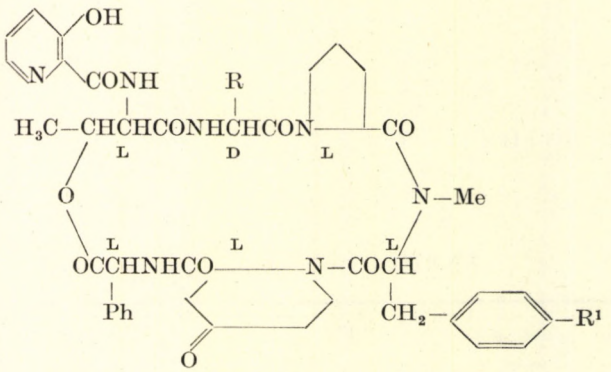
XXIX

R = Lysergic acid	R′	R″	R = Isolysergic acid
Ergocristine	$CHMe_2$	CH_2Ph	Ergocristinine
Ergotamine	Me	CH_2Ph	Ergotaminine
Ergokryptine	$CHMe_2$	CH_2CHMe_2	Ergokryptinine
Ergocornine	$CHMe_2$	$CHMe_2$	Ergocorninine
Ergosine	Me	CH_2CHMe_2	Ergosinine

alkaloids of the type XXVII, to which Japanese investigators have ascribed a cyclodidepsipeptide structure [1], as well as the long-known ergot alkaloids of the ergotamine and ergotoxine groups, which are cyclols (XXIX) isomeric with the corresponding cyclotridepsipeptides (XXVIII) [53, 166]. The second subgroup (XXV) embraces a large number of antibiotics whose molecules contain one or two serine or threonine residues incorporated in the depsipeptide ring by means of an amide and ester bond. The best known compound of this series is etamycin (XXX) [9, 124, 125] and also the compounds structurally related to it, staphylomycin S (XXXIa) and S_1 (XXXIb) [180—182], ostreogricin B (XXXIc), B_1 (XXXId) and B_2 (XXXIe) [41, 174, 175, 188] also called, respectively, vernamycins B_α, B_γ and B_β [16], as well as vernamycin B_δ (XXXIf) [16] and doricin [17].

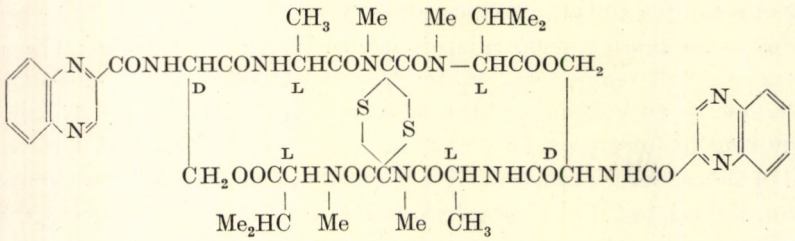


XXX. Etamycin; Sheehan et al. (1958)

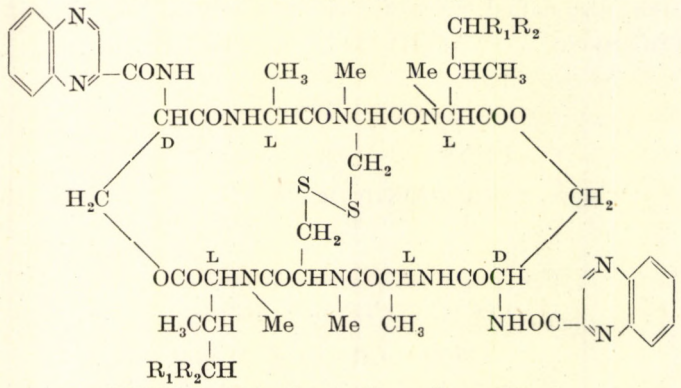


XXXIa. Staphylomycin S; R = Et; R^1 = H; Vanderhaeghe et al. (1960)
XXXIb. Staphylomycin S_1; R = Me; R^1 = H; Vanderhaeghe et al. (1965)
XXXIc. Ostreogricin B; R = Et; R^1 = NMe_2; Todd (1960)
XXXId. Ostreogricin B_1; R = Me; R^1 = NMe_2; Todd (1962)
XXXIe. Ostreogricin B_2; R = Et; R^1 = NHMe; Todd (1962)
XXXIf. Vernamycin B_δ; R = Me; R^1 = NHMe; Bodanszky et al. (1963)

To this subgroup should also be referred telomycin [122, 123], echinomycin (XXXII) [40, 56, 57] and the closely related quinomycins B and C [77, 80, 179], the triostins A, B and C (XXXIIIa—XXXIIIc) [77—79, 179],

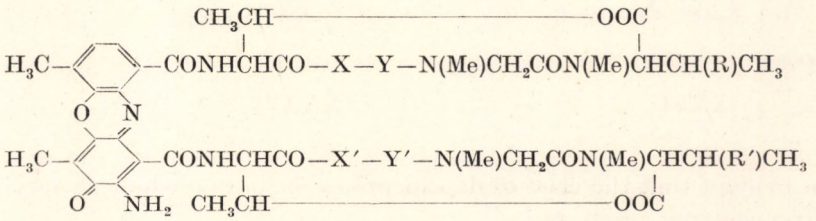


XXXII. Echinomycin; Prelog (1957)



XXXIIIa. Triostin A; R_1 = R_2 = H
XXXIIIb. Triostin B; R_1 = H; R_2 = Me
XXXIIIc. Triostin C; R_1 = R_2 = Me

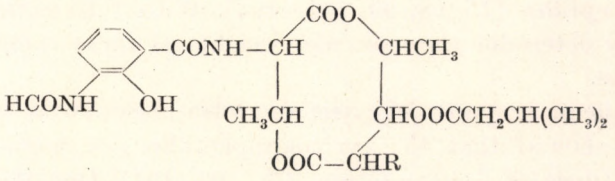
and a large group of actinomycins of the general formula (XXXIV) [21, 138].



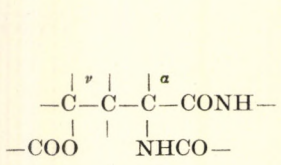
XXXIV. Actinomycins

Actinomycin	X and X′	Y and Y′	R and R′	Actinomycin	X and X′	Y and Y′	R and R′
I (X_0)	Val	Pro; HyPro	CH_3	E_1	*a*Ileu	Pro	CH_3; C_2H_5
II (F_8)	Val	Sar	CH_3	E_2	*a*Ileu	Pro	C_2H_5
III (F_9)	Val	Sar; Pro	CH_3	F_1	*a*Ileu; Val	Sar	CH_3
IV (C_1)	Val	Pro	CH_3	F_2	*a*Ileu; Val	Sar; Pro	CH_3
V (X_2)	Val	Pro; *γ*-Opro	CH_3	F_3 F_4	*a*Ileu *a*Ileu	Sar Sar; Pro	CH_3 CH_3
VI (C_2)	*a*Ileu; Val	Pro	CH_3	X_1	Val	Sar; *γ*-Opro	CH_3
VII (C_3)	*a*Ileu	Pro	CH_3	X_0	Val	Pro; HyPro	CH_3

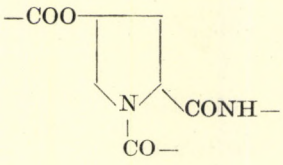
A somewhat special position is occupied by the antimycins A_1 and A_3 (XXXV) in which there are three ester bonds to each peptide bond [12, 167, 189]. The type XXVI subgroup of depsipeptides is of particular interest, since it includes the connective tissue proteins, procollagen and collagen. Besides peptide bonds, these contain ester linkages formed by means of the hydroxyl group of hydroxyproline (XXXVI) [36].



XXXVa. Antimycin A_1; R = $(CH_2)_5CH_3$
XXXVb. Antimycin A_3; R = $(CH_2)_3CH_3$



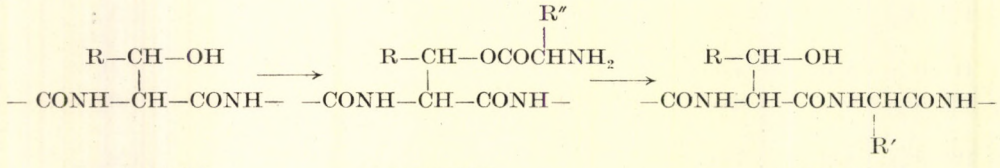
XXVI



XXXVI

It is thus evident that the class of depsipeptides includes a whole variety of naturally occurring products.

Depsipeptide structures may possibly also arise during biochemical reactions, as for instance in the conversions postulated by Brenner: XXXVII → XXXVIII → XXXIX for certain peptide and protein molecules [18, 19].



XXXVII XXXVIII XXXIX

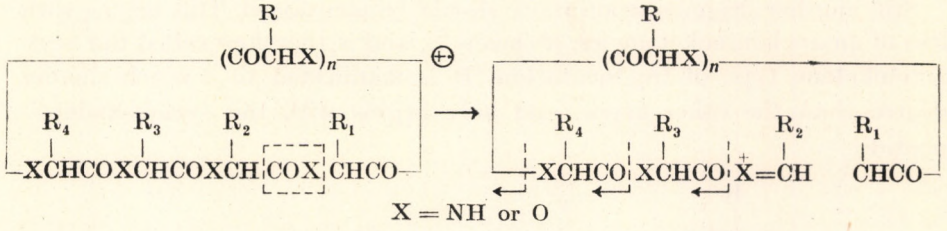
Progress in depsipeptide chemistry is obviously intimately connected with the existence of reliable methods for studying the structure of these compounds. In the early stages, molecular weight determination proved to be quite an obstacle and since the values obtained were too low, sometimes erroneous formulae were derived (enniatins, valinomycin). We found in this case the thermoelectric and mass spectrometric procedures to be the most reliable.

A certain advantage in the structural investigation of depsipeptides, in comparison with peptides, is that alkaline hydrolysis, attacking the ester bond selectively, allows one to pinpoint the places of rupture. However, in the more complicated cases a mere combination of this procedure and acid hydrolysis is insufficient.

Much information can be obtained by mass spectrometric fragmentation of cyclodepsipeptides [15, 98, 99, 192—194]. With this method one can simultaneously determine the molecular weights of these compounds and their structures.

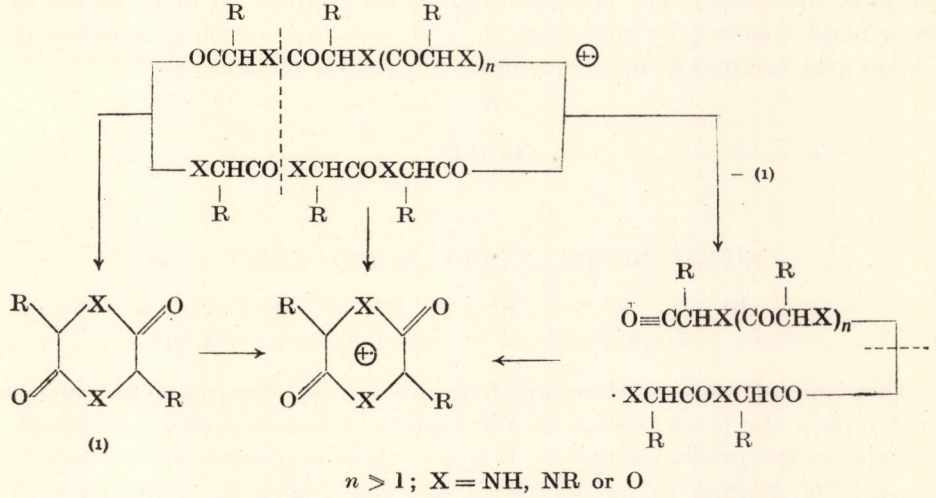
A study of the mass spectra of cyclic di-, tetra-, hexa-, octa- and dodecadepsipeptides showed that the cyclodepsipeptides are characterized by certain basic types of fragmentation [98, 193, 194]. One that we have termed the COX-type of fragmentation, begins with loss of the

elements of an ester or amide group, leading to formation of a linear ion radical, which then eliminates amino and hydroxy acid residues stepwisely by successive rupture of the amide and ester bonds (Scheme 1). This type of fragmentation is very clearly manifested in the strained cyclotetradepsipeptides, but it is slightly, if at all, expressed in the higher membered compounds of this class, beginning with the cyclohexadepsipeptides.



Scheme 1. Fragmentation of the COX type

Another type of cyclodepsipeptide fragmentation, which has been called the morpholinic type, is characterized by direct or stepwise conversion of the molecular ion radical to 2,5-dioxomorpholine (Scheme 2), which then eliminates one or both substituents and finally suffers opening of the hetero ring. If different 2,5-dioxomorpholines can arise, the one with larger molecular weight is formed preferentially [194].

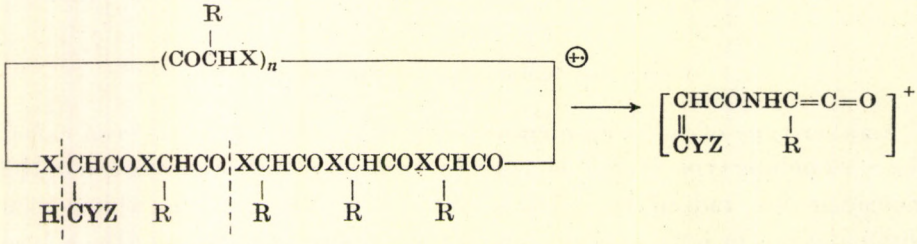


Scheme 2. Fragmentation of the morpholinic type

The morpholinic type of fragmentation is typical of all unstrained cyclodepsipeptides beginning with 18 and higher membered rings. It is highly

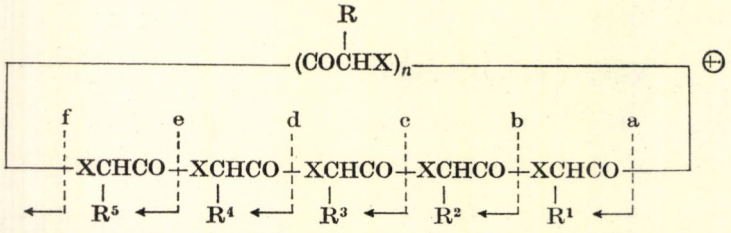
dependent on the temperature, increasing sharply with increase in the latter, since not only the molecular ions but the cyclodepsipeptides themselves are capable of thermal conversion directly into dioxomorpholines, which then undergo electron impact. Injection of the sample directly into the ion source, allowing much lower temperatures to be used, can greatly lower the intensity of this type of fragmentation [99].

Still another fragmentation route should be mentioned. This begins with loss of an acylaminoketene ion (Scheme 3), and is therefore called the acylaminoketene type of fragmentation. It is manifested to a much smaller degree than the other types, and only begins with the cyclohexadepsipeptides.



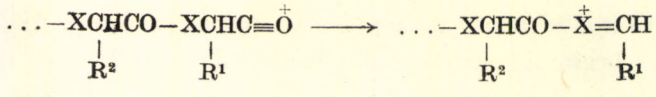
Scheme 3. Fragmentation of the acylaminoketene type

Of particular interest is the specific type of cyclopeptide fragmentation we have discovered that involves preliminary rupture (a) of an amide or ester bond, followed by successive (b, c, d, e, f) elimination of hydroxy or amino acid residues from the resulting ion radical (Scheme 4).



Scheme 4. Fragmentation of the amino (hydroxy) acid type

This type of fragmentation which we have called the amino (hydroxy) acid type is the most general for the various cyclodepsipeptides, although not always the predominant one. It is sometimes accompanied by elimination of CO, leading to the formation of imine fragments (imine type of fragmentation):



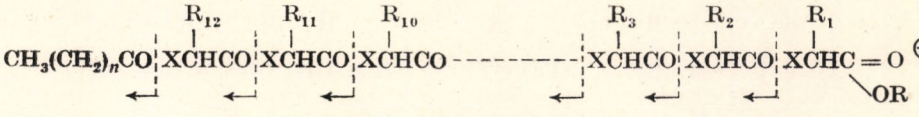
Naturally, in the case of cyclic depsipeptides containing different hydroxy and amino acid residues, the picture can be quite complicated since the molecular ion can undergo cleavage at different amide and ester bonds and thereby form several ion radicals with different acid residue sequences. Nonetheless, the subsequent fragmentation of each of the ion radicals by the amino (hydroxy) acid route may often be followed, as it is seen in the example of one of the sporidesmolide analogues (Scheme 5), whose mass spectrum shows all the three degradation routes due to rupture of two amide and one ester bonds in the cyclic molecular ion.*

$$\begin{array}{l} \qquad\qquad\qquad\qquad\nearrow \text{MeIle}(525) \rightarrow \text{Val}(426) \rightarrow \text{HyIv}(326) \rightarrow \text{MeIle}(199) \rightarrow \text{Val}(100) \\ (\text{MeIle-Val-HyIv})_2 \rightarrow \text{M}^+(652) \rightarrow \text{Val}(553) \rightarrow \text{HyIv}(453) \rightarrow \text{MeIle}(326) \rightarrow \text{Val}(227) \rightarrow \text{HyIv}(127) \\ \qquad\qquad\qquad\qquad\searrow \text{HyIv}(552) \rightarrow \text{MeIle}(425) \rightarrow \text{Val}(326) \rightarrow \text{HyIv}(226) \rightarrow \text{MeIle}(99) \end{array}$$

Scheme 5. Degradation routes of a sporidesmolide analogue

The information that one may obtain from the amino (hydroxy) acid and imine type of fragmentation is of particular value, since it may lead to establishment of the nature and simultaneously of the sequence of the hydroxy and amino acid residues in the ring, i.e. may give a complete knowledge of the cyclodepsipeptide structure under investigation.

The mass spectrometric method proved especially fruitful in structural studies of linear depsipeptides. These should be acylated at the N-(O)terminus and esterified at the C terminus, not only to augment their volatility for the mass spectrometric determination, but also because it facilitates their stepwise fragmentation (beginning with the C-terminus after it has acquired a positive charge). As a result, all the hydroxy and amino acid residues are consecutively eliminated by rupture of the ester and amide bonds, giving information on both the structure and sequence of their hydroxy and amino acid residues (Scheme 6).



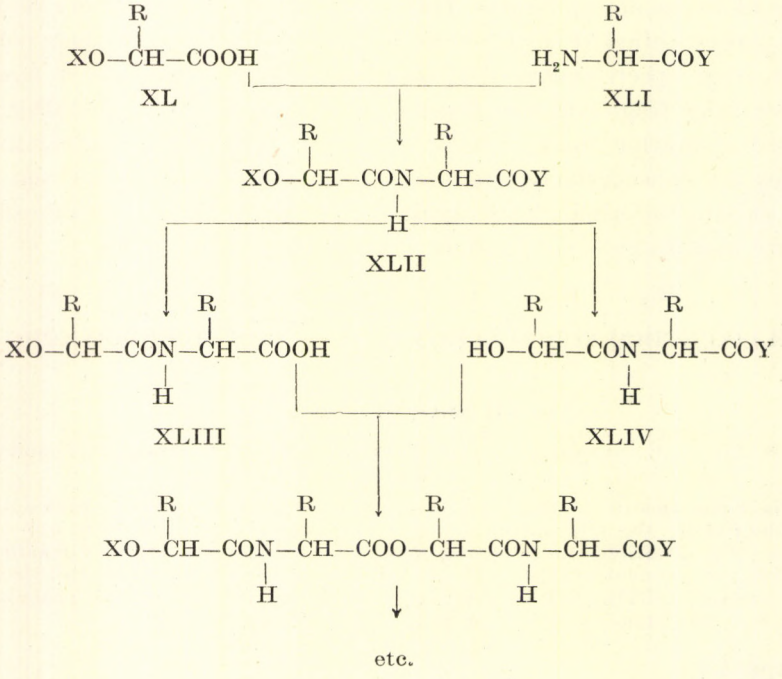
Scheme 6. Amino (hydroxy) acid type of fragmentation in the depsipeptide and peptide molecule

* This paper uses the symbols and designations for amino acids, peptides and proteins adopted by the Committee of the International Union of Pure and Applied Chemistry; in addition, the following designations are used; HyIv = residue of α-hydroxyisovaleric acid $-OCH(CHMe_2)CO-$; HyIc = residue of α-hydroxyisocaproic acid $-OCH(CH_2CHMe_2)CO-$; β-HyDec = residue of β-hydroxydecanoic acid $-O-\underset{\displaystyle (CH_2)_6CH_3}{\underset{|}{CH}}-CH_2CO-$; Lac = residue of lactic acid $-O-CH(CH_3)CO-$.

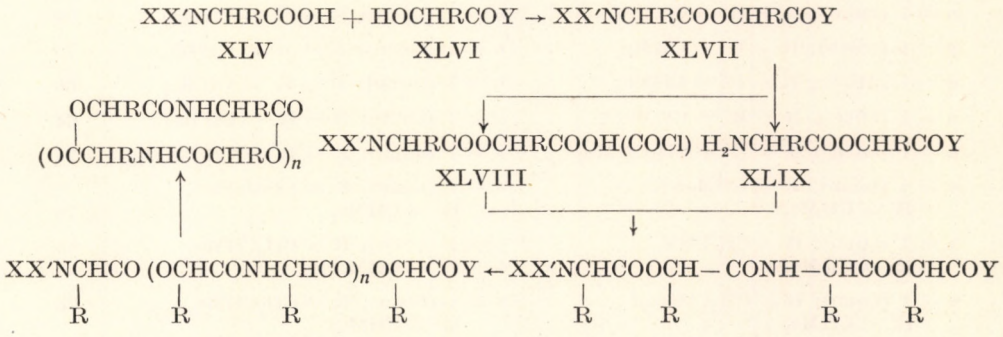
Since this type of fragmentation proved also to be characteristic of N-acylated peptide esters, the mass spectrometric method can be used also for determining the amino acid sequence in oligopeptides [88, 156].

Synthetic studies of depsipeptides which we began in 1958, gradually led to the development of general methods for preparing linear and cyclic representatives of this class of compounds [55, 58–61, 63, 75, 81–85, 89, 102, 117–121, 126–129, 131, 138–145, 147, 150–155, 158–160, 163] see also [2, 11, 22, 23, 25–31, 33, 34, 37, 39, 42, 44, 46–48, 65, 66, 69–73, 95, 96, 100, 101, 109–112, 115, 116, 164, 168, 174, 176–178, 186].

Two different methods were investigated. In one, the suitably protected hydroxy acid and amino acid residues (XL and XLI) were linked by an amide bond, and then from the resulting amide (XLII) either one (X) or the other (Y) of the protective groups was removed; the fragments (XLIII and XLIV) were then linked by an ester bond. In this case further building up of the depsipeptide chain and cyclization is achieved only through ester linkages. This route was found to be not very promising since establishing of the ester linkage required considerable activation of the carbonyl group in the terminal amino acid, which was always accompanied by increased racemization, moreover, ring closure in general could not be effected [159, 161, 163].



The second approach, on the contrary, proved to be very fruitful. In this case the substituted amino and hydroxy acids (XLV and XLVI) are linked together by an ester bond, utilizing the anhydride or acid chloride method; one of the protective groups (X or Y) of the resulting ester (XLVII) is then removed, and the fragments (XLVIII and XLIX) are united by an amide bond. Here, further building up of the depsipeptide chain and then cyclization is achieved through only amide bonding, which can be accomplished at all stages by the acid chloride method, because the terminal hydroxy acid residues show no tendency towards racemization. The most suitable protective groups were found to be benzyloxycarbonyl, nitrobenzyloxycarbonyl, *t*-butyloxycarbonyl or *o*-nitrophenylsulphenyl for the amino group, and benzyl or *t*-butyl for the hydroxyl group which could be easily removed either simultaneously or selectively.

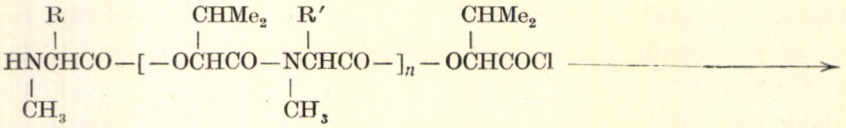


$X + X' = C_6H_4(CO)_2$; $X = H, X' =$ Cbo or O_2NCbo; $X = X' = H$

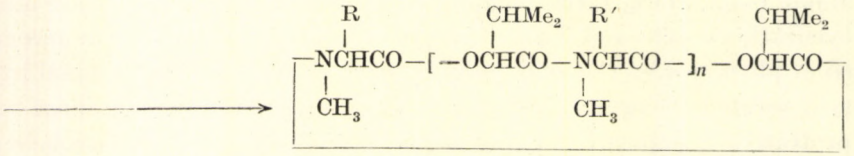
$Y = OCH_2Ph$, OBu—*t*, OCH_2CN, Cl, OSO_2Ar

Using this method we synthesized the most varied regular and irregular linear depsipeptides, up to hexadecadepsipeptides inclusively, yields for most stages reaching a value of 75–95%.

Ring closure, usually carried out in highly diluted benzene or tetrahydrofuran solution of the N-alkylated tetra-, hexa- and octadepsipeptides (L, $n = 1, 2, 3$), ordinarily proceeds in the direction of the expected cyclodepsipeptides (LI) which are obtained in 50–75% yields. On the other hand, when tetra-, octa-, and dodecadepsipeptides (LII, $n = 1, 2, 3$) with non-alkylated amide nitrogen are used, the normal cyclization products (LIII, $n = 1, 2, 3$) are obtained in only 5–10% yields, rarely

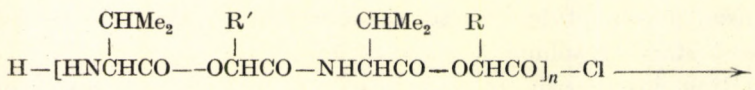


L

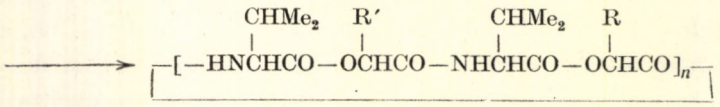


LI

Linear depsipeptide	Cyclodepsipeptide	Yield, %
$n = 1$ (LDLD); $R = R' = CHMe_2$	$n = 1$ (LDLD); $R = R' = CHMe_2$	70
$n = 1$ (LDDD); $R = R' = CHMe_2$	$n = 1$ (LDDD); $R = R' = CHMe_2$	75
$n = 1$ (DDLD); $R = R' = CHMe_2$	$n = 1$ (DDLD); $R = R' = CHMe_2$	60
$n = 1$ (LDLD); $R = R' = CHMeEt$	$n = 1$ (LDLD); $R = R' = CHMeEt$	64
$n = 1$ (LDLD); $R = R' = CH_2CHMe_2$	$n = 1$ (LDLD); $R = R' = CH_2CHMe_2$	74
$n = 1$ (LDLD); $R = CHMeEt$; $R' = CHMe_2$	$n = 1$ (LDLD); $R = CHMeEt$; $R' = CHMe_2$	75
$n = 1$ (LDLD); $R = CH_2CHMe_2$; $R' = CHMeEt$	$n = 1$ (LDLD); $R = CH_2CHMe_2$; $R' = CHMeEt$	65
$n = 1$ (LDLD); $R = CH_2CHMe_2$; $R' = CHMe_2$	$n = 1$ (LDLD); $R = CH_2CHMe_2$; $R' = CHMe_2$	76
$n = 2$ (LDLDLD); $R = R' = CHMe_2$	$n = 2$ (LDLDLD); $R = R' = CHMe_2$	52
$n = 2$ (LDLDLD); $R = R' = CHMeEt$	$n = 2$ (LDLDLD); $R = R' = CHMeEt$	50
$n = 2$ (LDLDLD); $R = CHMe_2$; $R' = CHMeEt$	$n = 2$ (LDLDLD); $R = CHMe_2$ $R' = CHMeEt$	49
$n = 3$ (LDLDLDLD); $R = R' = CHMe_2$	$n = 3$ (LDLDLDLD); $R = R' = CHMe_2$	55



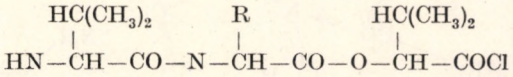
LII



LIII

	Linear depsipeptide		Cyclodepsipeptide	Yield, %	
				C_6H_6	THF
I	$n = 1$ (DDDD); R = R′ = $CHMe_2$	Ia	$n = 1$ (DDDD); R = R′ = $CHMe_2$	5	
	,,	Ib	$n = 2$ (DDDDDDDD); R = R′ = $CHMe_2$	5	
II	$n = 1$ (LDLD); R = R′ = $CHMe_2$	IIa	$n = 1$ (LDLD); R = R′ = $CHMe_2$	6	26
	,,	IIb	$n = 2$ (LDLDLDLD); R = R′ = $CHMe_2$	19	18
	,,	IIc	$n = 3$ (LDLDLDLDLDLD); R = R′ = $CHMe_2$	8	9
III	$n = 1$ (DLLD); R = $CHMe_2$; R′ = Me	IIIa	$n = 1$ (DLLD); R = $CHMe_2$; R′ = Me	0	3
	$n = 1$ (DLLD); R = $CHMe_2$; R′ = Me	IIIb	$n = 2$ (DLLDDLLD); R = $CHMe_2$; R′ = Me	9	10
IV	$n = 2$ (DLLDDLLD); R = $CHMe_2$ R′ = Me	IIIb	,,	3	
V	$n = 3$ (DLLDDLLDDLLD); R = $CHMe_2$; R′ = Me	Va	$n = 3$(DLLDDLLDDLLD); R = $CHMe_2$; R′ = Me	10	

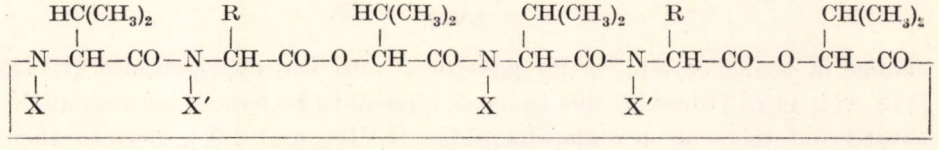
reaching 20–25% (compounds Ia–Va), due to concurrent formation of polymeric compounds and of cyclodepsipeptides with doubled and tripled molecular weight (compounds Ib, IIb, IIc and IIIb). The tridepsipeptides (LIV) do not give the normal cyclization products at all, instead cyclohexadepsipeptides (LV) are formed in yields up to 40% [83].



LIV

(DDL); X = X′ = H; R = *i*-Bu
(LLL); X = X′ = Me; R = *i*-Bu
(DLD); X = H; X′ = Me; R = *sec*-Bu

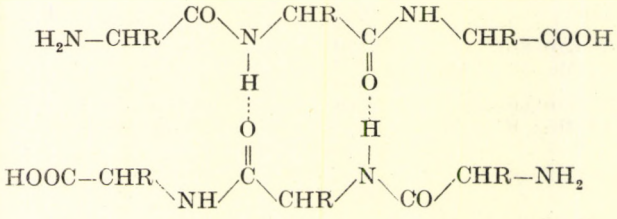
(LLD); X = H; X′ = Me; R = *sec*-Bu
(DLL); X = H; X′ = Me; R = *i*-Bu
(LLL); X = H; X′ = Me; R = *i*-Bu



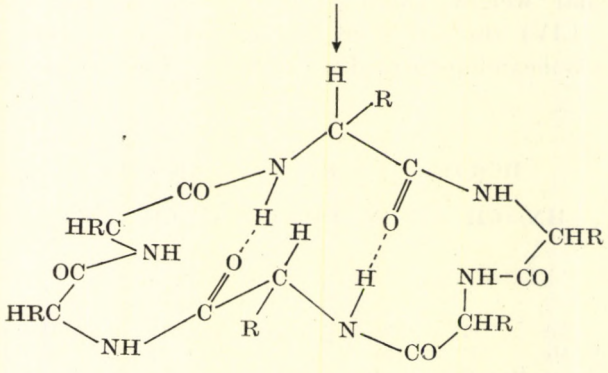
LV

Cyclodepsipeptide	Yield%	M.P.°C	$[\alpha]_D^{20}$ in $CHCl_3$
(DDLDDL); X = X′ = H; R = *i*-Bu	32	297°	+112° (c 0·9)
(LLLLLL); X = X′ = Me; R = *i*-Bu	21	oil	−156° (c 1·1)
(DLDDLD); X = H, X′ = Me; R = *sec*-Bu	29	216°	+71° (c 1·7)
(LLDLLD); X = H, X′ = Me; R = *sec*-Bu	28	223°	−179° (c 3·1)
(DLLDLL); X = H, X′ = Me; R = *i*-Bu	33	246°	−6° (c 1·6)
(LLLLLL); X = H, X′ = Me; R = *i*-Bu	41	238°	−268° (c 1·4)

Schwyzer regarded the doubling reaction, which he was the first to observe during the cyclization of peptides, as the outcome of hydrogen bonding of the linear molecules into the antiparallel associates (LVI); these without



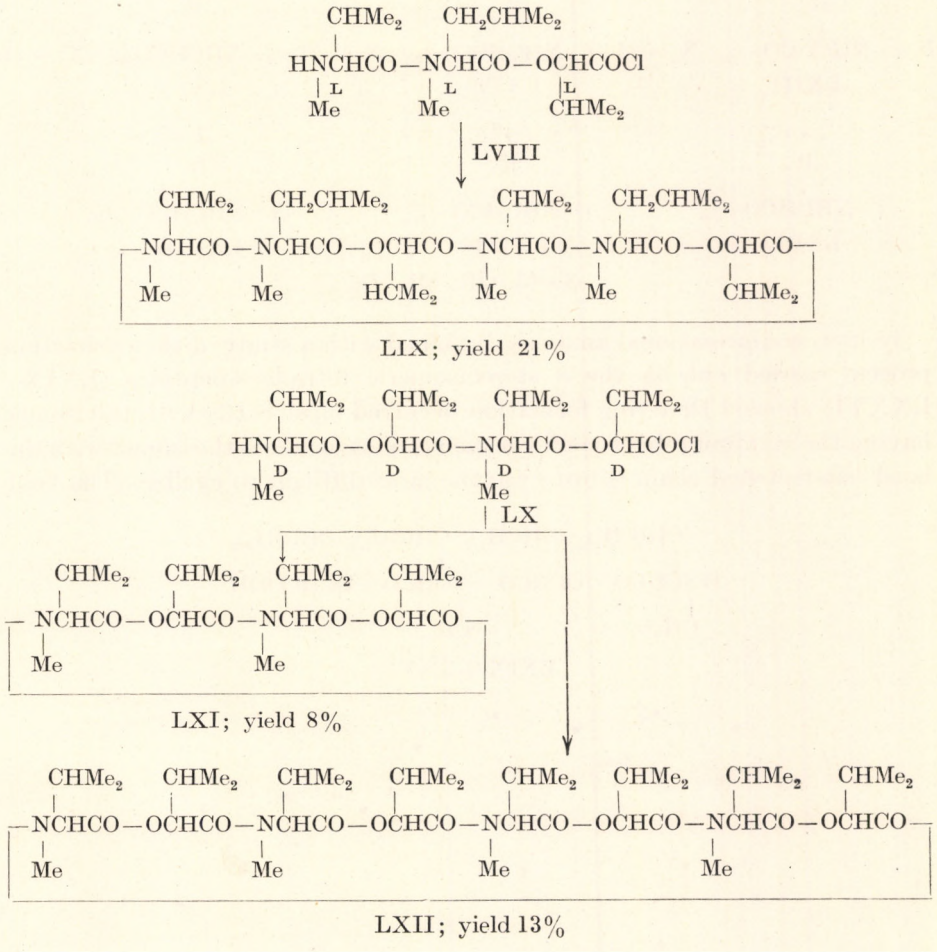
LVI



LVII

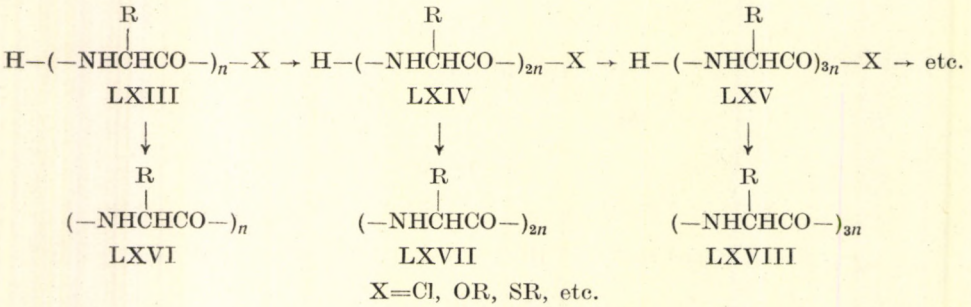
change in conformation, would pass over into the cyclopeptides (LVII) [113, 114, 116]. However, this concept appears to be wrong, because all-N-substituted tri- and tetradepsipeptides (LVIII and LX), despite their inability to form hydrogen bonded associates, turned out to be quite prone

to double (LIX and LXII). Moreover, peptides can double during cyclization also in polar solvents (for instance, in water), i.e., under conditions excluding the formation of the hydrogen bonded associates [83].

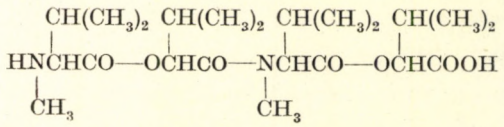


In our opinion the doubling (or tripling) reaction mechanism is radically different from that proposed by Schwyzer. The basic competitive process accompanying cyclization of a peptide (depsipeptide) is linear polycondensation leading to the formation of polymer homologues of increasing degrees of polymerization. However, under conditions of high dilution, the initial and resulting linear peptides (LXIII–LXV) can also undergo intramolecular condensation to the corresponding cyclopeptides (LXVI–LXVIII). The contribution of either of these processes (polycondensation and

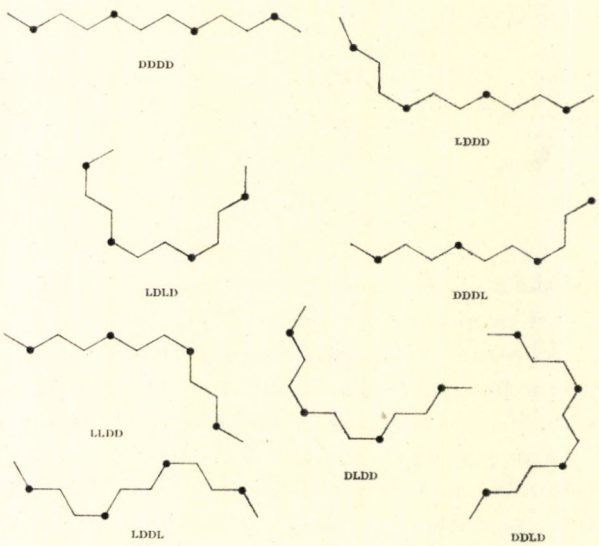
cyclization) is determined mainly by two factors: the preferred conformation of the initial depsipeptide (peptide) and the degree of strain in the cycle to be formed.



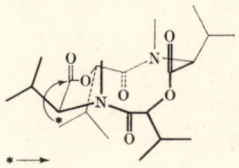
In fact, conformational analysis combined with a study of the cyclization process carried out on the 8 stereoisomeric tetradepsipeptides (LXIX–LXXVI) showed that ring formation occurred most easily with the isomer having the maximum bent conformation (LDLD), whereas the isomer with the most outstretched chain (DDDD) was the most difficult to cyclize. (The bent

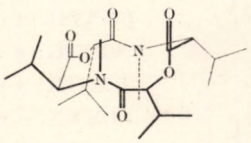


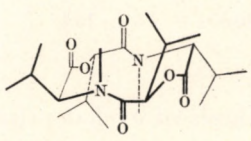
LXIX–LXXVI

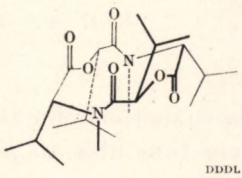


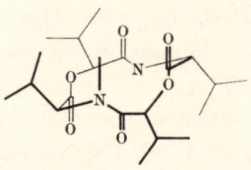
and stretched conformations can be very clearly observed on Stuart models.) This conclusion is in agreement with the fact that the least strained cyclodepsipeptide (LDLD) is formed in high yield, and the most strained (DDDD)

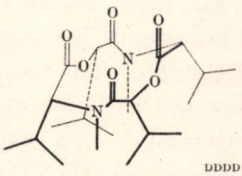




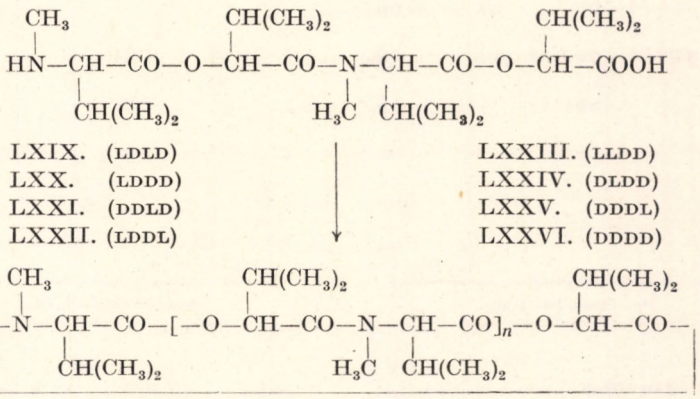








in very low yield. The above statements are illustrated by the experimental data listed for all the 8 stereoisomeric N-methyltetradepsipeptides (LXIX–LXXVI). The LDLD isomer (LXIX) affords the cyclotetradepsipeptide (LXXII) in 70% yield, whereas the DDDD isomer (LXXVI) gives the cyclotetradepsipeptide (LXI) in only 8% yield, together with a 13% yield of the cyclooctadepsipeptide (LXII) [84, 87].

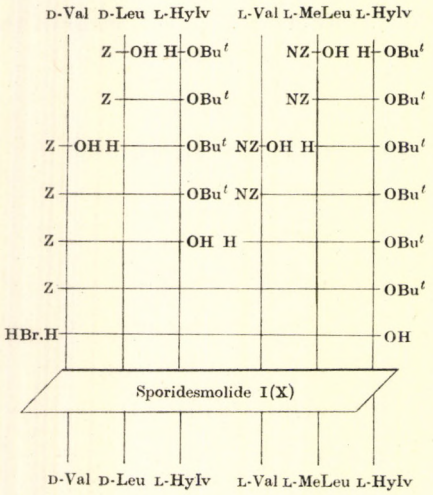


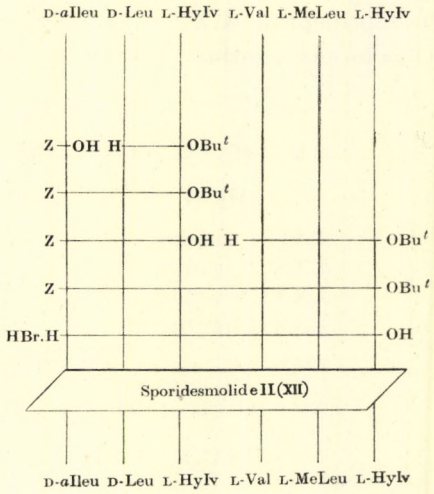
Linear depsipeptide	Cyclodepsipeptide	Yield, %	M.p., °C	$[\alpha]_D^{20}$ (in $CHCl_3$)
LXIX. (LDLD)	IV. $n = 1$ (LDLD)	70	229°	+4·8° (c 0·9)
LXX. (LDDD)	LXXVII. $n = 1$ (LDDD)	75	144°	+218° (c 0·7)
LXXI. (DDLD)	LXXVII. $n = 1$ (LDDD)	60	144°	+218° (c 0·7)
LXXII. (LDDL)	LXXVIII. $n = 1$ (LDDL)*	70	227°	0°
LXXIII. (LLDD)	LXXIX. $n = 1$ (LLDD)*	40	227°	0°
LXXIV. (DLDD)	LXXX. $n = 1$ (DDDL)	46	129°	+66° (c 0·4)
LXXV. (DDDL)	LXXX. $n = 1$ (DDDL)	45	129°	+66° (c 0·4)
LXXVI. (DDDD)	LXI. $n = 1$ (DDDD)	8	158°	+61° (c 1·0)
,,	LXII. $n = 3$ (DDDDDDDD)	13	184°	+124° (c 0·8)

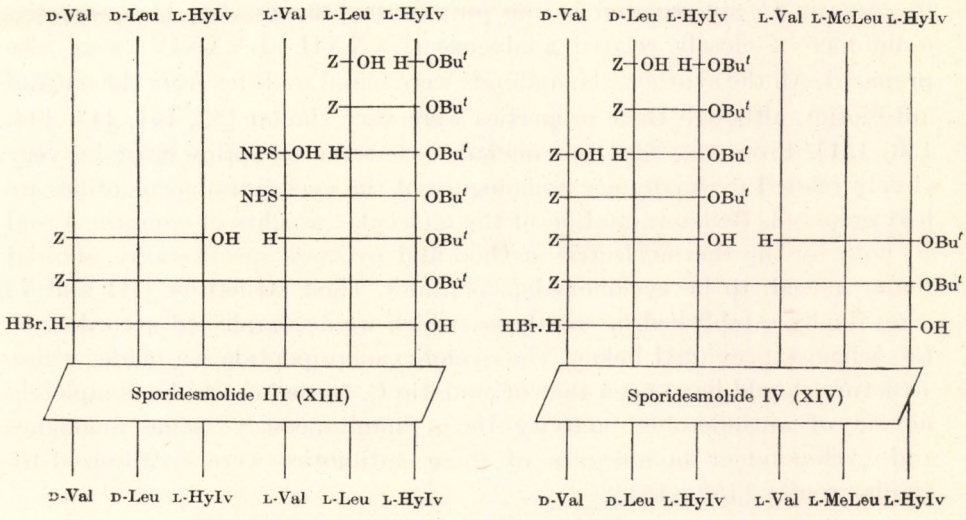
* meso-form

Having theabove methods for the synthesis of cyclodepsipeptides at our disposal, we were thus able to prepare a number of naturally occurring members of this class of compounds. As a result, the structures which had been proposed for them could be verified, or corrections made where necessary. Simultaneously, a number of analogues of the naturally occurring cyclodepsipeptides were produced.

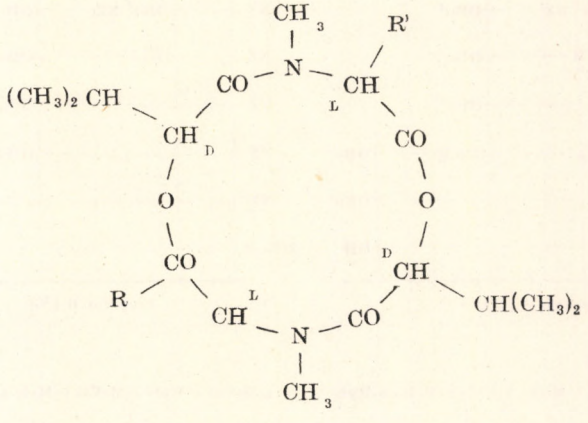
The first compound to be synthesized according to the scheme outlined above was sporidesmolide I (X) [82, 142, 145, 146]. This was followed by the synthesis of sporidesmolide II (XII) after the corresponding structural corrections had been made [89, 143, 146]. In a similar way the synthesis was achieved of sporidesmolides III (XIII) and IV (XIV) [58, 59]. The syntheses confirmed the structures postulated for these cyclohexadepsipeptides.







We then prepared the cyclotetradepsipeptides (II, IV, LXXXI) whose formulae had been ascribed by Plattner to enniatin A and B, and also



II. R = R′ = $CH(CH_3)C_2H_5$ (enniatin A)

IV. R = R′ = $CH(CH_3)_2$ (enniatin B)

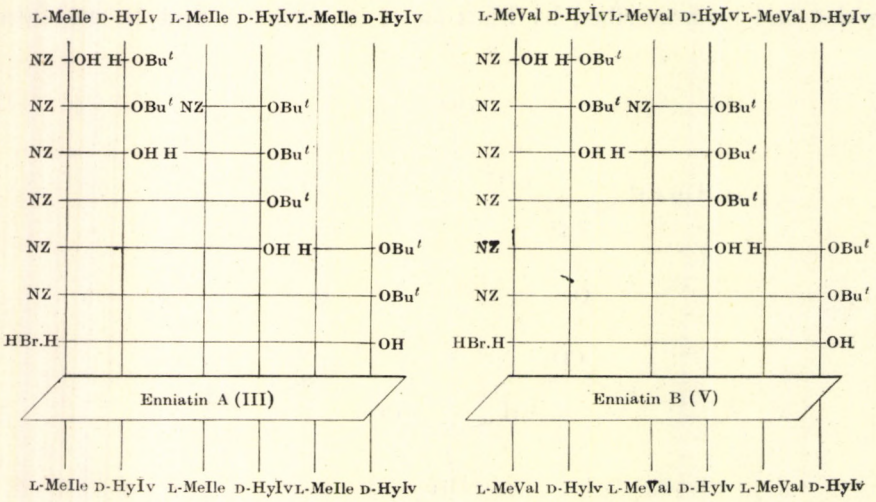
LXXXI. R = R′ = $CH_2CH(CH_3)_2$ (enniatin C)

LXXXII. R = $CH(CH_3)C_2H_5$; R′ = $CH(CH_3)_2$

LXXXIII. R = $CH_2CH(CH_3)_2$; R′ = $CH(CH_3)C_2H_5$

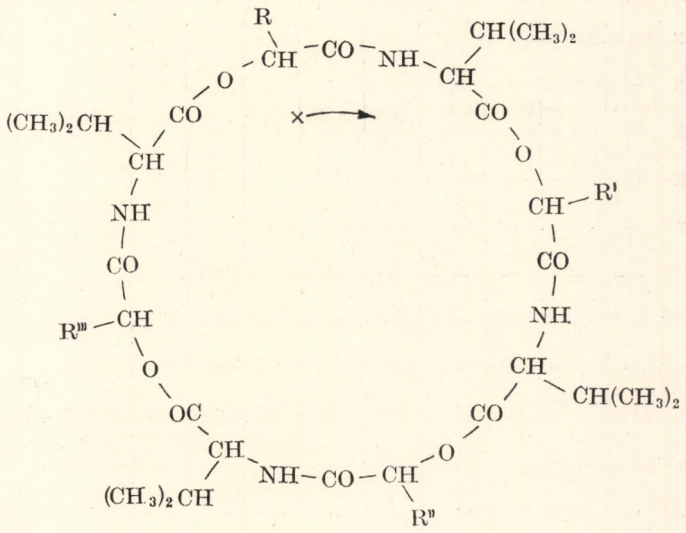
LXXXIV. R = $CH_2CH(CH_2)_2$; R′ = $CH(CH_3)_2$

to enniatin C not isolated in the pure state [45, 90—94]. Concurrently, a number of closely related analogues (LXXXII—LXXXIV) were also prepared. All the synthetic compounds were found to differ from the natural antibiotics, although their properties were very similar [82, 141, 143, 144, 150, 151]. From this fact we concluded that the enniatins must be very closely related cyclopolymer homologues of the cyclotetradepsipeptides we had prepared. Redetermination of the molecular weights of enniatin A and B, both by the thermoelectric method and by mass spectroscopy, showed them, indeed, to be cyclohexadepsipeptides. Their structures (III and V) were finally established by synthesis, which we accomplished according to the schemes presented below. The cyclohexadepsipeptide we made, whose structure should have been that of enniatin C, turned out to be completely devoid of antimicrobial activity [86]. Simultaneously, some analogues and cyclopolymer homologues of these antibiotics were synthesized by similar routes [152—155].



When we attempted to confirm synthetically the structure of the antibiotics valinomycin and amidomycin, for which Brockmann [24, 32] and Vining [170, 171, 183] had proposed the cyclooctadepsipeptide structures VI or LXXXV and VIII, respectively, we found that all the three synthetic compounds had considerably different properties from those of the natural products [82, 158, 159, 161]. Regarding amidomycin, Vining soon after

came to the conclusion that under this name an insufficiently purified sample of valinomycin had been described (cf. constants in the table below).



VI. (LLDDLLDD), $R = R'' = CH_3$, $R' = R''' = CH(CH_3)_2$

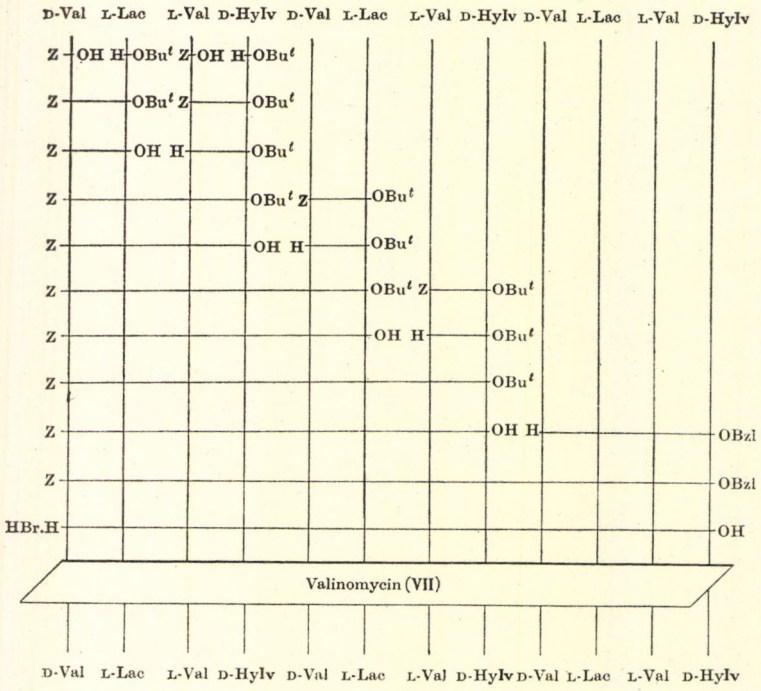
LXXXV. (LLLLDDDD), $R = R' = CH_3$, $R'' = R''' = CH(CH_3)_2$

VIII. (all D), $R = R' = R'' = R''' = CH(CH_3)_2$

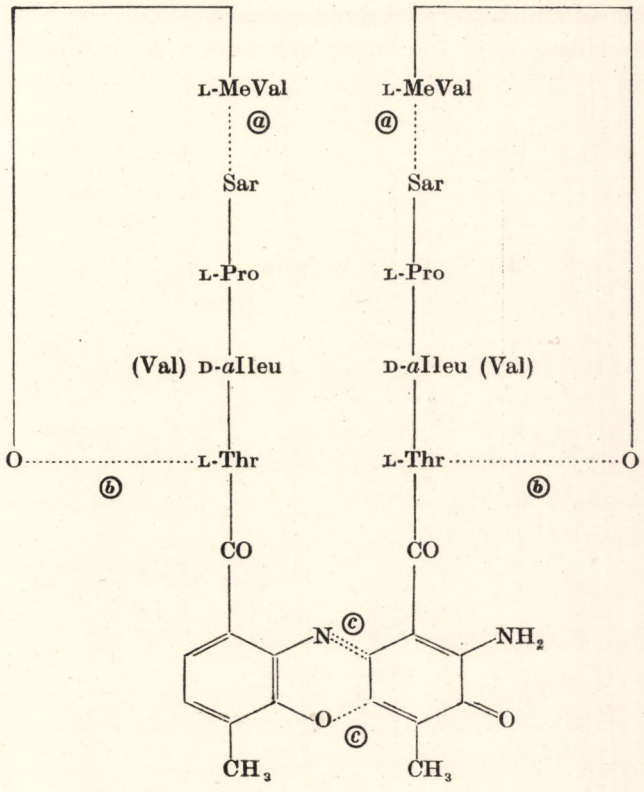
Antibiotic	Formula	M.P.°C	$[\alpha]_D^{20}$		Structure
Valinomycin	$C_{36}H_{60}O_{12}N_4$	190°	+31° (c 1·6, C_6H_6)	H. Brockmann et al. (1955)	VI or LXXXV
Amidomycin	$C_{40}H_{68}O_{12}N_4$	192°	+19·2° (c 1·2, EtOH)	L. C. Vining, W. A. Taber (1957)	VIII

As for valinomycin itself, new determinations of its molecular weight, which we carried out by the thermoelectric and Brockmann by the sedimentation method, showed that the compound was probably the cyclododecadepsipeptide VII, rather than a cyclooctadepsipeptide. Indeed, when VII was

prepared by us according to the above scheme, it was found to be identical with the natural substance [35, 55, 130, 131].



In the very last years Brockmann and his collaborators [22, 25—31, 33, 34] carried out the total synthesis of a number of actinomycin antibiotics — actinomycins C_1, C_2, C_3 and others. The actinomycin molecule can be made in three different ways represented schematically by the general formula (XXXIV), where the dotted lines show the bonds (*a*, *b* and *c*) formed in the final stage of the synthesis. The first route used, for instance, in the synthesis of actinomycins C_1 and C_3, involves preliminary formation of the tricyclic chromophore, bearing two open depsipeptide or peptide chains and this is followed by two ring closures through formation of an amide (*a*) or ester (*b*) bond. The second route (actinomycin C_2) is based on the initial synthesis of a cyclopentadepsipeptide containing an *o*-amino-*m*-hydroxy-*p*-toluic acid residue, followed by the oxidative condensation of two such molecules with the formation of the heterocyclic chromophore system (*c*).

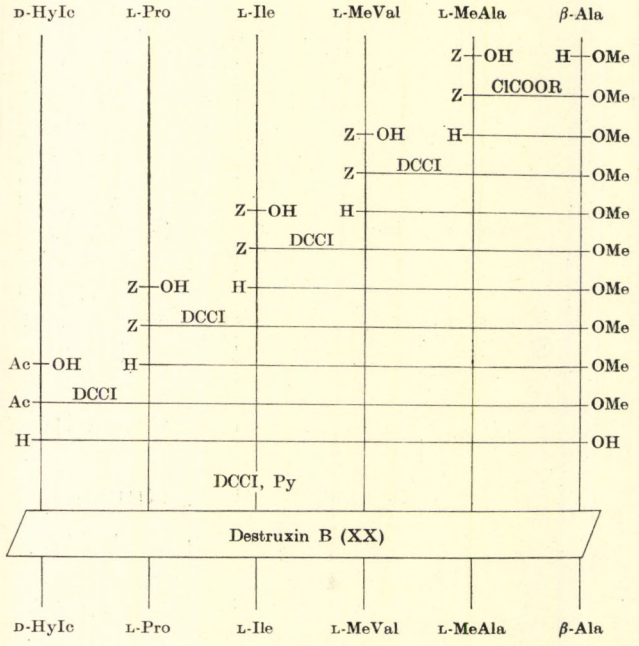


(XXXIV)

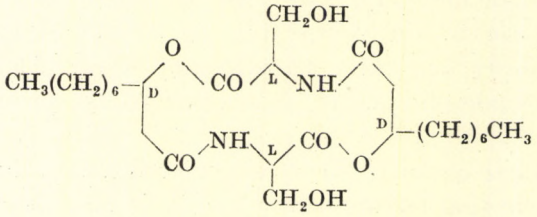
Regarding synthesis of the intermediate linear peptides, formyl protection was used as a rule for the N-terminus and benzyl esters for the C-terminus. In order to make the amide bonds, the carbodiimide method was used in the majority of cases, an exception being the bond between threonine and *allo*-isoleucine or between threonine and valine, for which the most efficient proved to be Woodward's reagent. It is noteworthy that in all cases the imidazolide method was used for the formation of the ester bond; when this bond served for closing the ring, this step could be accomplished satisfactorily only with a mixture of acetylimidazole and acetyl chloride as the condensing agent, and even then the yield did not exceed 15–20%.

Recently Japanese chemists [67] have achieved the synthesis of destruxin B (XX). An interesting feature of this synthesis was the successful use of carbodiimide in practically all the stages of the synthesis, including the

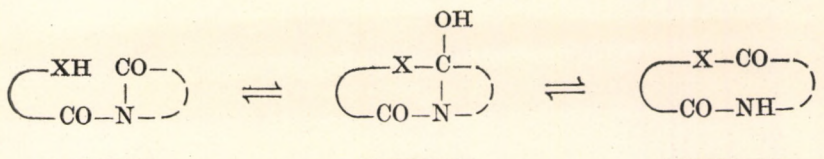
making of the difficultly formed amide bond between the N-methylamino residues and cyclization of the linear hydroxyacylpentapeptide by way of an ester bond.



An entirely different route to cyclodepsipeptides was used by us in the synthesis of serratamolide (XV) and some other natural depsipeptides and their analogues. The basis of this route was laid by our recently discovered reaction of incorporating hydroxy- and amino-acid residues into the peptide chain or ring [3, 4, 6–8, 132–137]. It was found that owing to the strong activation of the carbonyl group in acylamides of type LXXXVI, these compounds easily rearrange, often spontaneously, *via* the cyclol intermediates (LXXXVII) into linear or cyclic depsipeptides or peptides (LXXXVIII).



XV. Serratamolide

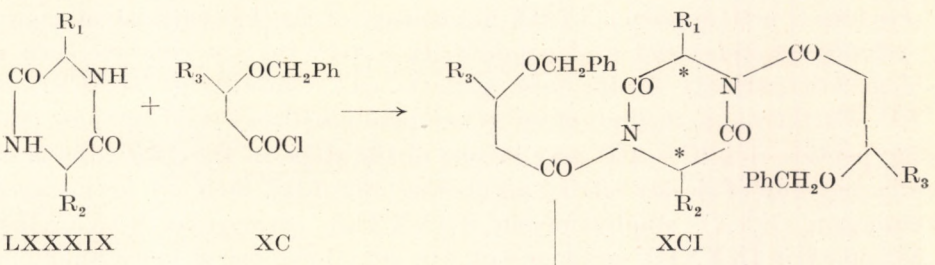


LXXXVI LXXXVII LXXXVIII

X=O, NH

The application of this reaction to the diacyldiketopiperazines (XCI), readily obtainable by reacting a benzyloxyacyl chloride (XC) with a diketopiperazine (LXXXIX), permits the synthesis of various analogues of serratamolide of type (XCIII) in high yields and in only two steps, without the isolation of the intermediate cyclols (XCII) [4, 6, 133, 134].

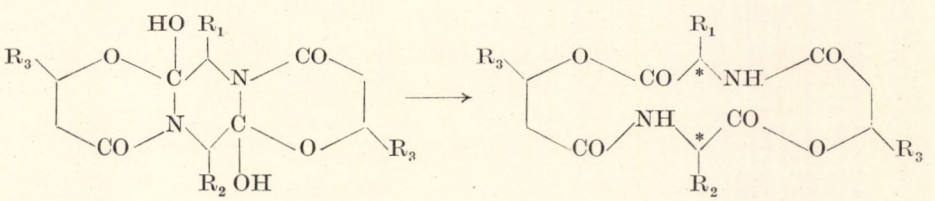
Serratamolide itself, was prepared as the diacetate (XCVI) from the corresponding diacetyldiketopiperazine (XCIV) after the hydrogenolytic removal of two benzyl groups from the intermediate tetraacyldiketopiperazine (XCV). Compound (XCVI) was then converted by careful hydrolysis into serratamolide (XV) itself [5, 63, 139, 140].



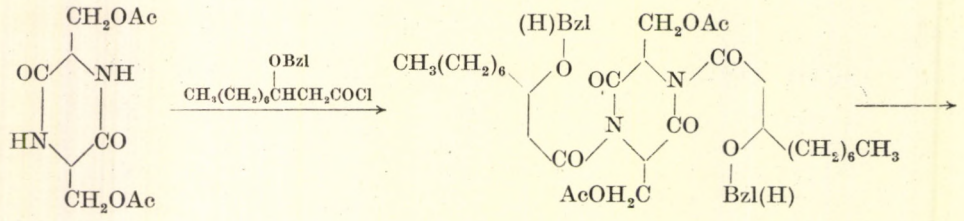
LXXXIX XC XCI

R_1=H, CH_3
R_2=H, CH_3, CH_2OH
R_3=H, CH_3

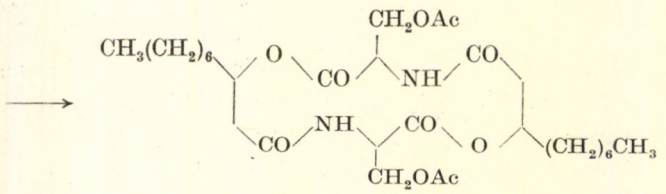
H_2/Pd



XCII XCIII

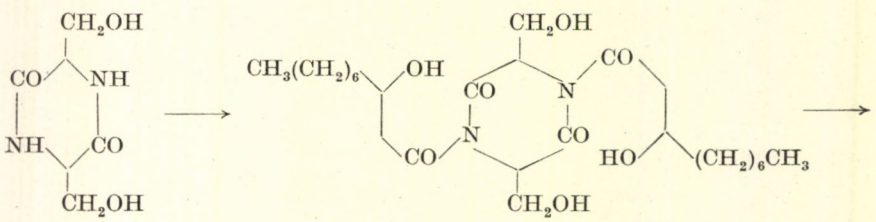


XCIV XCV

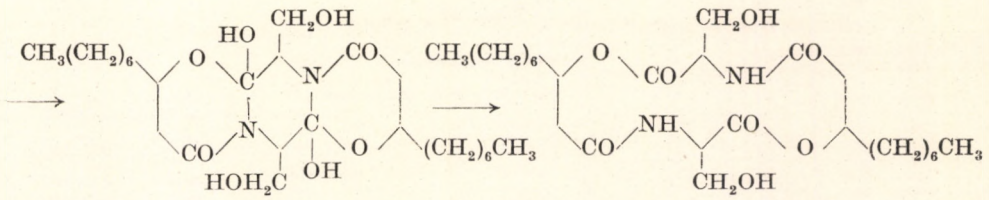


XCVI

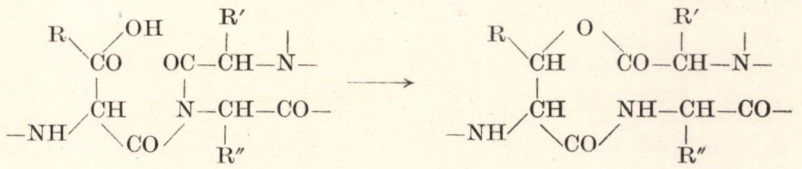
The incorporation of hydroxy acid and amino acid residues into the peptide chain or ring is not only a reaction of preparative importance, but also provides a new approach to the mechanism of the biosynthesis of many cyclodepsipeptides and cyclopeptides. It is very easy to conceive such a biogenetic pathway, for instance, in the case of serratamolide *via* XCVII → XV. Furthermore, incorporation of α-amino-β-hydroxy acid residues into the peptide ring (C → CI) may be one of the steps in the biosynthesis of a large group of closely related antibiotics [133, 134]. Such are for instance etamycin (XXX), staphylomycin S (XXXIa), ostreogricin B (XXXIb), echinomycin (XXXII), and many others, containing one or two α-amino-β-hydroxy acid residues in the molecule. The biogenesis of these compounds is at present one of the objectives of our studies.



XCVII XCVIII



XCIX XV



C CI

In conclusion, it may be of interest to discuss briefly the structure–activity relation of depsipeptide antibiotics and the closely related problem of their mode of action [75, 147, 162, 163].

Here again the study was facilitated by the simplicity of the synthetic methods for depsipeptides, making available a large number of their analogues. The antibiotic activity of the compounds synthesized was investigated on a number of gram-positive, gram-negative and acid-fast bacteria, yeasts and phytopathogenic fungi. Tables I—IV list some of the analogues and microorganisms which have been studied.

The results of studies of numerous enniatin, valinomycin and sporidesmolide analogues, compared with the activities of serratamolide, angolide and other depsipeptides, led to the following conclusions.

Table I

Linear Analogues

Compound	Minimal growth inhibiting concn (μg/ml)		
	Staphylococcus aureus UV-3	*Candida albicans*	*Sclerotinia libertiana*
CII. H–(L-MeIle–D-HyIv)$_{2-4}$–OH	> 100	> 100	> 100
CIII. H–(L-MeVal–D-HyIv)$_{2-4}$–OH	> 100	> 100	> 100
CIV. H–(D-Val–L-Lac–L–Val–D-HyIv)$_{1-4}$–OH	> 100	> 100	> 100

Table II

Cyclic Analogues

Compound	Minimal growth inhibiting concn (μg/ml)		
	Staphylococcus aureus UV-3	*Candida albicans*	*Sclerotinia libertiana*
IX. $\overline{\text{D-}a\text{Ile}\rightarrow\text{L-HyIv}\rightarrow\text{L-Ile}\rightarrow\text{L-HyIv}}$ Angolide	> 100	> 100	> 100
XV. $\overline{(\text{L-Ser}\rightarrow\text{D}-\beta\text{-HyDec})_2}$ Serratamolide	4·5	> 100	9
II. $\overline{(\text{L-MeIle}\rightarrow\text{D-HyIv})_2}$	> 100	> 100	> 100
III. $\overline{(\text{L-MeIle}\rightarrow\text{D-HyIv})_3}$ Enniatin A	1·5	9	> 100
IV. $\overline{(\text{L-MeVal}\rightarrow\text{D-HyIv})_2}$	> 100	> 100	> 100
V. $\overline{(\text{L-MeVal}\rightarrow\text{D-HyIv})_3}$ Enniatin B	9	37	10
CV. $\overline{(\text{L-MeVal}\rightarrow\text{D-HyIv})_4}$	1·5	> 100	> 100
VI, CVI. $\overline{(\text{D-Val}\rightarrow\text{L-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv})_{2,4}}$	> 100	> 100	> 100
VII. $\overline{(\text{D-Val}\rightarrow\text{L-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv})_3}$ Valinomycin	0·8	0·8	0·8

1. All linear analogues of the depsipeptide antibiotics completely lack activity (Table I), a prerequisite for manifestation of the latter being a ring structure.

2. The activity of the antibiotics is determined by the size of the ring, which may vary within broad limits from 14 to 36 members. No activity has as yet been found below or above these limits (Table II). Each group of antibiotics has its own optimal ring size. Thus, the optimum in the case of the enniatins is the 18-membered ring and in the case of valinomycins, the 36-membered ring.

3. The structure of the amino acid residues in the antibiotic molecules may suffer considerable change (with the enniatins, in some cases over the entire length of the chain; with valinomycin, over restricted areas) with retention of activity. In the case of valinomycin, it was even found possible to change

Table III

Analogues with Modified Amino and Hydroxy Acid Residues

	Compound	Minimal growth inhibiting concn. (μg/ml)		
		Staphylococcus aureus UV-3	*Candida albicans*	*Sclerotinia libertiana*
CVII.	└L-MeVal→D-HyIv(L-MeIle→D-HyIv)$_2$┘	2	9	—
CVIII.	└L-MeAla→D-HyIv(L-MeVal→D-HyIv)$_2$┘	75–100	> 100	—
CIX.	└L-MeVal→D-Lac(L-MeVal→D-HyIv)$_2$┘	> 100	> 100	—
CX.	└L-Val→D-HyIv(L-MeVal→D-HyIv)$_2$┘	25	75	—
CXI.	└L-MeVal→L-HyIv(L-MeVal→D-HyIv)$_2$┘	> 50	> 50	—
CXII.	└D-MeVal→D-HyIv(L-MeVal→D-HyIv)$_2$┘	> 50	> 50	—
CXIII.	└L-MeVal→D-HyIc(L-MeVal→D-HyIv)$_2$┘	> 100	> 100	—
CXIV.	└L-MeLeu→D-HyIv(L-MeVal→D-HyIv)$_2$┘	9	18	—
CXV.	└L-MeVal→D-HyIv(L-MeLeu→D-HyIv)$_2$┘	4–6	37	—
CXVI.	└L-MeLeu→D-HyIv(L-MeLeu→D-HyIv)$_2$┘	> 50	> 50	—
CXVII.	└D-Ala→L-Lac→L-Val→D-HyIv(D-Val→ →L-Lac→L-Val→D-HyIv)$_2$┘	0·7	0·8	—
CXVIII.	└D-Ala→L-Lac→L-Ala→D-HyIv(D-Val→ →L-Lac→L-Val→D-HyIv)$_2$┘	8	8	1
CXIX.	└L-Val→L-Lac→L-Val→D-HyIv(D-Val→ →L-Lac→L-Val→D-HyIv)$_2$┘	8	8	9
CXX.	└(L-Val→L-Lac→L-Val→D-HyIv)$_3$┘	> 100	> 100	> 100
CXXI.	└D-Val→L-HyIv→L-Val→D-HyIv(D-Val→ →L-Lac→L-Val→D-HyIv)$_2$┘	> 100	> 100	> 100
CXXII.	└D-Val→D-Lac→L-Val→D-HyIv(D-Val→ →L-Lac→L-Val→D-HyIv)$_2$┘	> 100	> 100	> 100
X.	└D-Val→D-Leu→L-HyIv→L-MeLeu→L-HyIv┘ Sporidesmolide I	> 100	> 100	> 100

the configuration of the amino acid residues, within definite limits (Table III, compounds CVII, CVIII, CX, CXIV, CXV, CXVII–CXX).

4. An opposite picture holds for the hydroxy acid residues, in which case it was shown (e.g., in valinomycin) that any change in the structure of the radical or in the configuration always leads to complete obliteration of activity (Table III, compounds CIX, CXIII, CXXI and CXXII).

5. Particularly noteworthy is the fact that the ester group may be replaced by the amide group without change in the antibiotic efficacy, as we found in the valinomycin analogues (Table IV).

Table IV

Analogues with an Ester Group Substituted by an Amide Group

Compound	Minimal growth inhibiting concn (μg/ml)		
	Staphylococcus aureus UV-3	*Candida albicans*	*Sclerotinia libertiana*
CXXIII. $\lfloor$—D-Val→L-Ala→L-Val→D-HyIv(D-Val→→L-Lac→L-Val→D-HyIv)$_2$—$\rfloor$	1	4·5	2
CXXIV. $\lfloor$—D-Val→L-Val→L-Val→D-HyIv(D-Val→→L-Lac→L-Val→D-HyIv)$_2$—$\rfloor$	0·7	15	2
CXXV. $\lfloor$—D-Val→L-Lac→L-Val→D-Val(D-Val→→L-Lac→L-Val→D-HyIv)$_2$—$\rfloor$	1	3	2

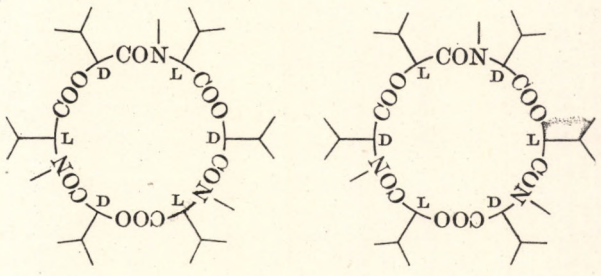
These findings are particularly interesting in the light of observations that the replacement of some amide groups in bradykinine and glutathione has little bearing on their biological properties [102, 117, 120, 121]. Hence, we have shown the possibility in some cases of mutual exchangeability between ester and amide groups in the molecules of biologically active peptides and depsipeptides [157].

The fact that the highest antimicrobial activity among the enniatin cyclodepsipeptides that we had investigated was displayed by the cyclohexadepsipeptides (for instance compounds CVII, CXIV, CXV), and moreover by those of the latter whose molecules contained a certain sequence of hydroxy and amino acid residues of a given nature and a given configuration (compounds CXIV and CXV, but not compounds CXI and CXII) was very convincing evidence of the essential role played by steric factors in the manifestation of biological action by compounds of this series. This led to the

surmise that the mode of action of these antibiotics is associated with a biological act such that the molecule as an integral whole must be congruous with a certain region of the receptor. However, the interaction of a biologically active substance with a selective receptor (for instance, substrate and enzyme) to cause a certain physiological effect is chemically complex, requiring not only a certain minimum of steric congruity of the molecules but also a given electron density distribution in the interacting functional centres. If either of these requirements is not satisfied, the reaction will either not take place at all, or its effectiveness will be diminished by several orders of magnitude. It is thus important to discover the limits of steric and electronic matching, both in the rational search for substances with similar, modified or antagonistic action, and to understand the underlying mechanism.

In order to define these limits it is not always necessary to employ the usual technique of changing restricted areas of the molecule step by step. In the case of cyclic peptides and depsipeptides it is possible to change the whole molecule in such a way that the resultant analogues are still very similar to the parent compound, both in the overall spatial arrangements, that is topologically, and also with respect to the electronic nature of the functional groups. The topochemical approach to the structure–activity relation then becomes particularly fruitful [148, 149].

We began to study the structure–activity problem from the topochemical standpoint with the enniatin antibiotics, in which the essential part played by steric factors (size of the depsipeptide ring, configuration of the hydroxy- and amino acid residues, etc.) in biological activity had already been shown. The antipode of enniatin B, enantio-enniatin B with the configurations of the amino- and hydroxy acid residues opposite to those of enniatin B, was chosen as one of the first objects and was therefore synthesized [148].



Enniatin B Enantio-enniatin B

Fig. 1

Since enantio-enniatin B is the mirror image of the natural product, the stereoselective receptor should behave quite differently towards these two stereochemically non-equivalent molecules. One would not, therefore, have expected enantio-enniatin B to show biological activity; it would have been as groundless as looking for it in antipodes of such naturally occurring peptides as bradykinin [165], angiotensin [185], oxytocin [43], etc. [33], compounds that *ipso facto* should be biologically inactive, provided, of course, they do not manifest activity by participation in some fundamentally different reaction (as, for instance, in the case of the L-isomer of cycloserine [38]). We considered it very likely, however, that enantio-enniatin B would possess biological activity equal or close to that of enniatin B. Indeed, if one turns one of the formulas depicted in Fig. 1 by 60° in the plane of the figure, all the like asymmetric centres coincide, while each ester group will take the place of the N-methyl amide group and vice versa. Consequently, relative to the receptor these two antipodes differ only structurally. At the same time the ester group models the amide group both spatially and in the electron density distribution. There should therefore be a close matching of both these topochemically similar antipodes to the same stereoselective receptor and they should possess similar activities. Another ground for such an assumption was the exchangeability found in a number of cases of amide and ester groups in naturally occurring peptides and depsipeptides without affecting their biological activity (see above).

We synthesized enantio-enniatin B by a scheme analogous to that for the total synthesis of enniatin B. The product was found to be identical with the natural one in all physical and chemical properties except the optical

Table V

Antimicrobial Activity of Enniatin B and Enantio-Enniatin B

Minimal growth inhibiting concentration (μg/ml)

Compound	*Staph. aureus 209 P*	*Staph. aureus UV-3*	*Sarcina lutea*	*Bac. mycoides*	*Bac. subtilis*	*E. coli*
Enniatin B	18	9	18	25–37	35–50	> 50
Enantio–enniatin B	18	9	18	25–37	35–50	> 50

Compound	*Mycob. phlei*	*Mycob. tuberc.*	*Cand. albicans*	*Sacch. cereviseae*	*Botritis cinereae*	*Nigrospora orysae*
Enniatin B	9–12	4·5–6	9–12	9–12	9	9
Enantio–enniatin B	9–12	4·5–6	9–12	9–12	9	9

properties (the rotatory dispersion curves were of the same shape but of opposite signs). A test of the antimicrobial behaviour of enantio-enniatin B showed it to possess exactly the same activity both qualitatively and quantitatively towards the organisms investigated as enniatin B (Table V).

This is noteworthy as the first case when a naturally occurring compound and its antipode display absolutely the same biological properties along the entire antimicrobial spectrum, which virtually excludes the possibility of their having different modes of action.

It should be mentioned that enniatin B and its antipode are an example in which two molecules are very similar, both topologically and in the character of the functional centres. If these centres are retained while the effective volume of the radicals in the depsipeptide molecule is changed, one may easily determine the limits of its topological correspondence to the receptor. Thus we have recently shown that the antipode of enniatin A, enantio-enniatin A, has also exactly the same activity as the parent compound.

At the same time, one may synthesize analogues of some biologically active cyclopeptides that are identical topologically to the parent molecules, but differ considerably from them in the nature or position of the atoms in the functional centres. The topochemical investigation of such analogues, which we are at present engaged in, might shed light not only on the nature of the interaction between the biologically active substance and receptor, but also on the structure of the receptor itself, which is of particular importance in studies of enzyme systems.

Our discovery of the biological activity of enantio-enniatin B and enantio-enniatin A led to the conclusion that the mechanism of action of enniatin antibiotics depends on the correspondence of their molecules as a whole to the receptor, which according to the latest data seems to belong to the group of mitochondrial lipoproteins, controlling the active cation transport in the mitochondria.

Recently, Pressman [97] showed that valinomycin causes strong active transport of K^+, but not of Na^+, in animal mitochondria and also in *Azotobacter*. In a subsequent cooperative study by Pressman and us of a number of synthetic valinomycin and enniatin analogues, the conclusion was drawn that a parallelism exists between the antimicrobial activity of the depsipeptide antibiotics and their ion transport induction properties. Among the valinomycin analogues, this parallelism is not only qualitative (active K^+, but no Na^+ transport), but also quantitative, as can be seen by comparing Tables II—IV with Table VI [97, 163].

Table VI

Potassium Ion Transport Induction Properties of Cyclodepsipeptides

Compound	*Relative induction activity*
VII. $\overline{\text{D-Val}\rightarrow\text{L-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv})_3}$ (Valinomycin)	100
CXIX. $\overline{\text{L-Val}\rightarrow\text{L-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv(D-Val}\rightarrow\text{L-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv})_2}$	13
CXX. $\overline{(\text{L-Val}\rightarrow\text{L-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv})_3}$	0·00
CXXII. $\overline{\text{D-Val}\rightarrow\text{D-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv(D-Val}\rightarrow\text{L-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv})_2}$	2·2
VI. $\overline{(\text{D-Val}\rightarrow\text{L-Lac}\rightarrow\text{L-Val}\rightarrow\text{D-HyIv})_2}$	0·00
III. $\overline{(\text{L-MeIle}\rightarrow\text{D-HyIv})_3}$ (Enniatin A)	0·61
V. $\overline{(\text{L-MeVal}\rightarrow\text{D-HyIv})_3}$ (Enniatin B)	1·3
CVII. $\overline{\text{L-MeVal}\rightarrow\text{D-HyIv(L-MeIle}\rightarrow\text{D-HyIv})_2}$	0·46
IV. $\overline{(\text{L-MeVal}\rightarrow\text{D-HyIv})_2}$	0·00
CV. $\overline{(\text{L-MeVal}\rightarrow\text{D-HyIv})_4}$	0·23
X. $\overline{(\text{D-Val}\rightarrow\text{D-Leu}\rightarrow\text{L-HyIv}\rightarrow\text{L-Val}\rightarrow\text{L-MeLeu}\rightarrow\text{L-HyIv}}$ (Sporidesmolide I)	0·01

Thus, the antimicrobial activity and the ion transport induction capacity of compound CXIX are both approximately one-tenth that of valinomycin (VII), and both properties are either completely or almost completely absent in compounds XI and CVI, and CXXII. A similar picture is to be seen in the enniatin analogues. Compounds III, V and CVII, differing comparatively little in antimicrobial properties, have also closely related ion transport induction properties. Compound CV, with a narrow antimicrobial spectrum, also shows diminished ion transport induction activity; in compound IV, both of these properties are completely absent. Finally, all cyclohexadepsipeptides of the sporidesmolide series lack antimicrobial activity, and the results of the tests carried out on sporidesmolide I (X) by

Pressman have shown that it is practically devoid of the ability to induce ion transport.

Apparently, the depsipeptide antibiotics are inducers of K^+ transport as the result of interaction with a specialized mitochondrial receptor site controlling this transport [76].

Our efforts are now to a considerable extent being directed to elucidation of such structural details in the depsipeptide antibiotics which determine their interaction with this receptor surface.

It should be stressed that the depsipeptide antibiotics are now being transformed from an object of investigation *per se* into a tool for the study of certain biochemical processes taking place in the cellular membranes, just as chloramphenicol and puromycin have become important biochemical tools for the study of protein biosynthesis.

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I. OGNYANOV and D. IVANOV

CONSTITUENTS OF THE BULGARIAN ZDRAVETS OIL

I. INTRODUCTION

The zdravets *(Geranium macrorrhizum)* belongs to the family of *Geraniaceae*. Several members of this family are essential oil-yielding plants. Foremost in this respect is the genus *Pelargonium*, indigenous to South Africa. An essential oil is derived from its various species, transplanted and disseminated along the Mediterranean, which is thus incorrectly called geranium oil. Among the genera belonging to *Geraniaceae*, zdravets is the only *Geranium* species which is used for obtaining an essential oil, known as 'zdravets oil' or as 'Bulgarian geranium oil'.

The zdravets (Fig. 1) (in Bulgarian the word 'zdravec', from which the name of the plant is derived, means 'health') is a grass-like perannial plant growing in the highlands. In Bulgaria it grows at altitudes of 800–1700 metres above sea level. The plant forms long stems in the soil which branch out. Numerous green leaves grow out from the overground vegetative tip, containing an essential oil with a typical, pleasant and refreshing odour.

Bulgarian zdravets oil is obtained by distilling the above-ground green parts of the wild-grown zdravets with steam. Production started in Bulgaria in 1926. Small quantities may have been obtained prior to that by some small producers of rose oil. Some 500 kg of standard oil are now produced. annually. However, this quantity is by no means a ceiling figure, and it could be considerably increased.

Production is usually begun late in September, and is continued until snowfall. The locality is in the Rose Valley. The plant material is placed loosely in a still, and five parts of warm water are poured over it. Heating is done by means of indirect steam or direct fire. Distillation lasts from 3½ to 4 hours and during that time 2·2–2·5 litres of distillate is collected from 1 kg of zdravets. The distillate is transferred into a Florentine flask maintained at a temperature of 50–55 °C and there the oil is separated The aqueous layer of the distillate contains 0·02–0·03 % of dissolved oil,

and it is cohobated. The oil obtained by cohobation amounts to about an eighth of the total oil. The commercial product is a mixture of both oils. One kg of oil is obtained from 800–1200 kg of zdravets.

At ordinary temperatures zdravets oil is a mixture of crystals and of a liquid portion. When rapid cooling is applied, fine crystals are obtained, with the liquid part included among them, while on slow cooling the solid

Fig. 1

portion is deposited in the form of large, long, flat and prismatic crystals occupying about two-thirds of the volume of the total oil. When heated, the solid part begins to melt at about 32 °C and is completely dissolved in the oil at 45–52 °C. The liquefied oil is green to greenish brown in colour, while the cohobated oil is more yellowish brown. It has a strong and pleasant odour with a slight resinous note, differing considerably from the odour of the plant.

The fragrant principles are contained in the liquid part. The properly purified solid part is odourless. Exposed to air and light, the oil slowly turns from green into brownish green, there is an increase in the acid number, and the odour assumes a pronounced resinous note.

Some physical and chemical properties of the oil are given in Table I.

Haegi's [4] assertion that the plant is used as an aphrodisiac in Bulgaria is not true. Nor is the statement [4] correct that Bulgarian rose oil is adulterated with zdravets oil.

Table I

	Whole oil	Water oil (only for 1957)
Specific gravity	0·9390–0·9532	0·9559
Refractive index at 40°C	1·5024–1·5135	1·5075
Optical rotation at 40°C	—4·8° – —8·6°	—18·0°
Acid number	1·20 – 1·87	0·43
Ester number	4·97 – 7·91	8·78
Combined alcohol content (as geraniol)	1·35 – 2·18%	2·41%
Ester number after acetylation	20·75 – 49·78	53·43
Free alcohols (as geraniol)	5·80 – 11·88%	12·70%
Total alcohol content (as geraniol)	7·70 – 14·05%	15·11%
Germacrone * (crude)	47·75 – 50·13%	48·11%

* Determinated by the test for determination of stearoptenes content of rose oil.

II. THE COMPOSITION OF ZDRAVETS OIL

The authors of the present article have carried out systematic studies on the composition of zdravets oil [18]. The physical and chemical data of the sample of oil investigated were within the limits given in Table I [7]. The solid part of the oil, germacrone, was removed by filtration of the crystals deposited at ordinary temperatures; cooling of the filtrate to —15°C, and second filtration gave another crop of crystals [7]. The second mother liquor still contained dissolved germacrone.

The germacrone which was isolated at —15°C, was isomerized by treatment with alcoholic sulphuric acid [16, 28]. The reaction product was distilled under reduced pressure; recrystallization of the distillation residue from ethanol gave a mixture of paraffins with m. p. 63·5–64·5°C.

By extracting the liquid part of the oil with a 3% solution of sodium hydroxide, 0·3% of a substance with acid character was isolated; this product has not been studied further.

1. *Terpene Compounds* [16]

A fraction boiling up to 120°/7 mm and containing mainly terpene hydrocarbons and their oxygenated derivatives was isolated from the liquid portion of the oil. It was carefully fractionated with a 30-theoretical-plate column, and 19 fractions were collected. The first seven fractions consisted of terpene hydrocarbons, the remaining ones contained terpene oxygen compounds mixed in the final fractions with sesquiterpene hydrocarbons.

Extensive use was made of chromatography on a neutral active aluminium oxide in order to separate the substances contained in the various fractions; identification of the components was achieved by means of determining the physical constants, by evaluating the infra-red spectra, and by preparing chemical derivatives. The following substances have been identified in this manner: dipentene, *p*-cymene, *γ*-terpinene and terpinolene. Two unidentified terpene hydrocarbons have also been isolated — an aliphatic one and a monocyclic one. The presence of a small quantity of borneol was established with certainty in the fraction containing oxygenated monoterpene compounds (about 1% of the oil), furthermore, a primary monocyclic alcohol ($C_{10}H_{16}O$) was isolated.

2. *Sesquiterpene Compounds*

Sesquiterpene compounds are contained in the residue (about 39% of the oil) obtained after distilling the terpene fraction. This residue was roughly fractionated, using a column of about 10 theoretical plates, to give four crude fractions [15]. The first fraction, b.p. 79–100°/1 mm (11% of the oil), contains sesquiterpene hydrocarbons. These were purified from the oxygenated derivatives through chromatographic filtration on alumina. The eluate obtained with petrol ether was fractionated with a colum of 30 theoretical plates, and a total of 14 fractions were collected. The substances contained in these fractions were separated by chromatography on aluminium oxide, and, after purification, the following compounds were identified: ar-curcumene (main component), a hydrocarbon with an elemane skeleton whose infra-red spectrum is very close to that of α-elemene [25], as well as traces of what seemed likely to be α-selilene.

The second sesquiterpene crude fraction [17] (100–106°/1 mm) contained mainly sesquiterpene carbonyl compounds. The principal component isolated here was the optically inactive ketone $C_{15}H_{22}O$ which gave a 2,4-dinitrophenylhydrazone with m.p. 134–136°C. The infra-red spectrum

showed that this was an α,β-unsaturated ketone which we denoted as β-elemenone. The establishment of its structure will be dealt with further on. In addition to this compound, two more sesquiterpene ketones were isolated, but they could not be studied in detail on account of the minute quantities in which they were obtained.

When allowed to settle, fraction four yielded a crystalline substance, m.p. 165·5–166·5 °C, which proved to be identical with the sesquiterpene alcohol known as 'juniper camphor' isolated from the oil of *Juniperus communis* [5]. From the liquid part of the same fraction, after chromatographing on alumina of activity II and eluting with benzene, it was possible to isolate a liquid sesquiterpene alcohol. Its infra-red spectrum showed absorption maxima characteristic of the alcohol elemol. On dehydrogenation with sulphur guaiazulene was obtained, identified as its adduct with trinitrobenzene. It has been established that elemol yields guaiazulene upon dehydrogenation. The presence in the zdravets oil of an alcohol which yields guaiazulene had been established by other authors, too [21].

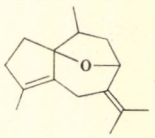
III. STRUCTURAL STUDIES

1. *Germacrone*

The most interesting compound among the constituents of zdravets oil is undoubtedly its solid part, both because it represents about 50% of the oil, and in view of the subsequently established fact that it proved to be the first isolated substance with a new medium ring carbon skeleton, and intriguing chemical behaviour.

The solid part of the oil was first called 'germacrol,' but this name does not correspond to the functional character ascertained subsequently. It was first examined by Rovesti [22] who assumed it to be tricosan on the basis of the molecular refraction. Later on Wienhaus and Scholz [29] assigned the molecular formula $C_{16}H_{24}O$ to the compound, and assumed that it was an oxide. Naves [11] found the correct composition of this compound to be $C_{15}H_{22}O$ and assumed it to be a bicyclic sesquiterpenoid oxide with two double bonds. By hydrogenation he obtained a liquid product $C_{15}H_{28}O$, which he regarded as a saturated alcohol; dehydrogenation over palladium charcoal at high temperatures gave cadalene (1,6-dimethyl-4-isopropylnaphthalene). Shortly afterwards 'germacrol' was studied by Treibs [28] who obtained guaiazulene by dehydrogenation with iodine.

On the basis of a series of experiments he assigned structure I to the compound. The location of the double bonds was inferred chiefly from the formation of acetone on ozonization, and from the formation of laevulinic acid on oxidation with potassium permanganate.



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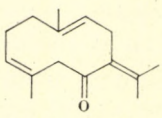
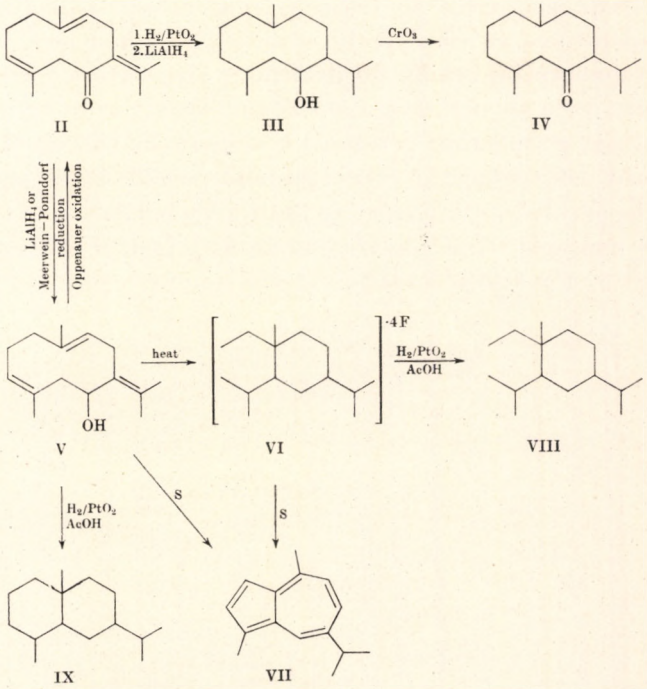


II

Several years later structure I was re-examined by Ognyanov, Ivanov, Herout, Horák, Pliva and Šorm [19] who pointed out that germacrone was the first isolated representative of a new group of sesquiterpene compounds with a ten-membered carbon ring, and suggested structure II. This was done on the basis of the following facts and properties of germacrone: The infra-red spectrum which has an absorption band at 1675 cm^{-1} showed that the compound had a ketonic carbonyl group conjugated with a double bond, i.e., that germacrone was not an oxide, but a ketone. The Raman spectrum, too, revealed the corresponding band at 1670 cm^{-1}, as further evidence of the ketone character. The presence of an α,β-unsaturated ketone was proved by the ultra-violet absorption spectrum which had maxima at 315 mμ (log ε 2·55) at 240 mμ (log ε 3·47) and an end absorption at 213 mμ (log ε 4·10). In view of this, the suggestion was made to change the name to the more precise one of germacrone. The carbonyl group of germacrone is only slightly reactive, apparently as a result of steric hindrance, which had been the cause of the former erroneous views about its structure.

The hydrogenation of germacrone in the presence of platinum oxide in glacial acetic acid yielded a liquid product the chief constituent of which, according to the infra-red spectrum, was a substance possessing a ketone carbonyl group (band at 1704 cm^{-1}); a small amount of a material with a hydroxyl group was also present. This hydrogenated product was reduced with lithium aluminium hydride to yield the saturated alcohol III having the molecular formula $C_{15}H_{30}O$. Oxidation of this product with chromic acid in glacial acetic gave the liquid ketone IV of the molecular formula $C_{15}H_{28}O$. Like the initial germacrone, this ketone failed to yield a semicarbazone, or a 2,4-dinitrophenylhydrazone. An analysis of these saturated derivatives of germacrone indicated that they possessed a monocyclic carbon skeleton, and that three C=C double bonds had been present in the original molecule of germacrone.

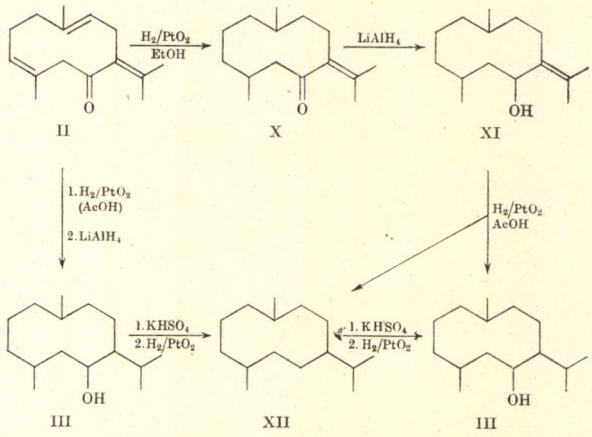
The reduction of germacrone with aluminium isopropoxide or lithium aluminium hydride led to a highly unstable liquid alcohol, germacrol (V), which could readily be dehydrated, either by heating with formic acid, or, in better yield, by heating up to the boiling point. This gave the unstable



hydrocarbon VI which readily underwent polymerization. The dehydrogenation of alcohol V or of hydrocarbon VI with sulphur gave guaiazulene (VII) in yields of 15–20%. These results confirmed the view suggested by Treibs [28] about the guaiane skeleton of germacrone; on the other hand they could not be readily interpreted in terms of the above-established monocyclic structure.

The hydrogenation of hydrocarbon VI in the presence of platinum oxide in glacial acid resulted in the uptake of nearly four equivalents of hydrogen, giving the saturated hydrocarbon elemane (VIII). On the other hand, considerable hydrogenolysis took place when germacrol (V) was hydrogenated under the same conditions. The main product isolated was the saturated bicyclic sesquiterpene hydrocarbon selinane (IX). Finally, when germacrol (V) was oxidized according to Oppenauer, it was possible to recover germa-

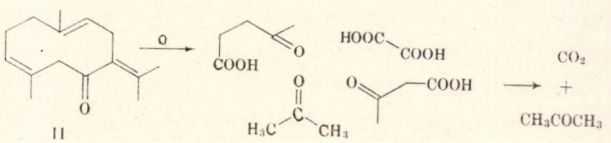
crone in satisfactory yields. Consequently, depending on the particular conditions, germacrone can yield three different compounds, elemane (VIII), selinane (IX), and guaiazulene (VII), possessing three altogether different carbon skeletons. It is necessary, in this connection, also to take into account the data obtained by Naves [11] and Treibs [28] concerning the formation of cadalene from germacrone by dehydrogenation with palladized charcoal at high temperatures. In view of the results which had been obtained so far, it was therefore impossible to determine the correct carbon skeleton of germacrone, even though only the elemane type was in agreement with the findings that germacrone possessed a monocyclic carbon skeleton. The strikingly easy conversion to other sesquiterpenoid types could have a bearing on the peculiar arrangement of the double bonds and of the carbonyl group in the molecule. That was the reason why further studies comprised a careful and systematic elimination of the double bonds.



When germacrone was hydrogenated in the presence of platinum oxide in ethanol, two equivalents of hydrogen were absorbed and tetrahydrogermacrone (X) was obtained having the molecular formula of $C_{15}H_{26}O$. The remaining double bond was in conjugation with the ketone group, as indicated by the infra-red and ultra-violet spectra. Reduction with lithium aluminium hydride converted tetrahydrogermacrone into tetrahydrogermacrol (XI) of the molecular formula $C_{15}H_{28}O$. In agreement with the allylic position of the double bond with respect to the hydroxyl group, this alcohol underwent hydrogenolysis to the extent of 70% when it was hydrogenated in glacial acetic acid. Chromatography of the hydrogenated product gave two compounds, the saturated hydrocarbon XII having the molecular

formula $C_{15}H_{30}$, and hexahydrogermacrol (III) identical with the product obtained by the direct hydrogenation of germacrone in acetic acid followed by reduction with lithium aluminium hydride. The properties and the composition of the saturated hydrocarbon XII corresponded to a monocyclic saturated sesquiterpene; its infra-red spectrum, however, differed from all known spectra of fully saturated monocyclic sesquiterpenic hydrocarbons. In addition to the absorption bands due to the isopropyl group, the spectrum also showed a maximum at 722 cm^{-1} corresponding to the grouping $-CH_2-CH_2-CH_2-$. That is why this hydrocarbon was called germacrane. This same hydrocarbon (XII) was also obtained in the dehydration of hexahydrogermacrol (III) with potassium bisulphate, followed by hydrogenation of the produced unsaturated hydrocarbon. The molecular refraction of germacrane showed a negative exaltation of about —0·7 which has been observed in compounds possessing a ring of medium size. The suggested structures have been further confirmed by the absorption maximum at 1704 cm^{-1} for the carbonyl group in hexahydrogermacrone (IV).

Oxidative degradation of tetrahydrogermacrone (X) and of the initial germacrone provided information as to the location of the carbonyl group and of the double bonds. The quantitative determination of acetone by means of Kuhn and Roth ozonization [8] showed that ketone X gave one equivalent of acetone. This indicates that the double bond conjugated with the carbonyl group is of the isopropylidene type. The ozonization of germacrone, which was carried out in the same manner, gave rise to 1·5–1·6 equivalents of acetone, whereas the quantitative determination of the methylenic double bond according to Naves [10] detected only traces of formaldehyde. By repeating the oxidation of germacrone with potassium permanganate as described by Treibs [28], we were also able to demonstrate the formation of oxalinic acid and of laevulinic acid.



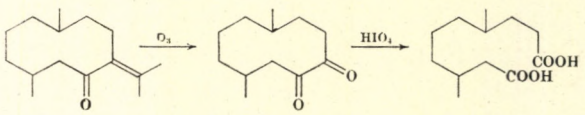
II

The formation of more than one equivalent of acetone is due to the intermediery formation of acetoacetic acid which undergoes partial decarboxylation.

Structure II can very well explain these results of the oxidative degradation of germacrone. It also explains very satisfactorily the formation of all reaction products mentioned above, belonging to the different sesquiter-

pene types, if three different ways of transannular cyclization are assumed. Ring closure between $C_{(1)}$ and $C_{(6)}$ leads to derivatives of selinane, between $C_{(4)}$ and $C_{(9)}$ to those of cadalene, and finally the cyclization betweeen $C_{(6)}$ and $C_{(10)}$ to the guaiane derivatives. The reaction leading to the formation of derivatives of the elemane type probably proceeds via the cyclization to an intermediate selinane derivative which is converted to an elemane derivative by fission of the ring between $C_{(8)}$ and $C_{(9)}$. A similar reaction mechanism for the formation of compounds of the elemane type had been suggested by Ružička [22a]. Finally, the absence of optical activity in germacrone and in its derivatives is also in agreement with the proposed formula II.

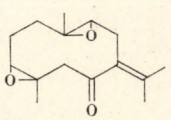
The object of further studies was to obtain direct proof of the structure suggested. Suchý and Šorm [27] synthesized the hydrocarbon germacrane whose physical constants and infra-red spectrum completely coincide with those of hydrocarbon XII. In view of the fact, however, that the formation of germacrane could likewise be the result of some preliminary isomerization of germacrone, Herout and Suchý [6] made a thorough study of the ozonolysis of tetrahydrogermacrone (X). In the course of this work, the main product obtained was the diketone XIII, which on further oxidation with periodic acid afforded β,γ-dimethylsebacic acid (XIV).



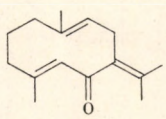
X XIII XIV

The ultra-violet spectrum of germacrone [24] exhibits a maximum at 213 mμ (log ε 4·10), a shoulder at 240 mμ (log ε 3·47) and another maximum at 315 mμ (log ε 2·55). Whereas the latter two are due to the conjugation of the double bond of the isopropylidene group with the carbonyl group, the first value is indicative of two endocyclic double bonds. This can be seen from the fact that tetrahydrogermacrone (X), just as well as diepoxygermacrone (XV), in which these two double bonds have been eliminated, possess normal spectra in the ultra-violet region with maxima characteristic of a ketone group conjugated with an exocyclic isopropylidene group, as it is seen from the following values: 254 mμ (log ε 3·59) and 308 mμ (log ε 2·12) for X, and 250 mμ (log ε 3·60) and 316 mμ (log ε 2·23) for XV. Normal is likewise the ultra-violet spectrum of isogermacrone (XVI), a compound obtained by the isomerization of germacrone with alcoholic potassium hydroxide at room temperature [19]. The position of the double bonds has been established [26] in this compound by comparing the products

of its oxidative degradation with those obtained from germacrone. In connection with these anomalies in the ultra-violet spectrum of germacrone, Ohloff [20] allowed for the presence of a cyclopropane ring in the molecule of germacrone and suggested a structure with a maalianic skeleton. Bater and Gale [2] confirmed structure II on the basis of the nuclear magnetic resonance spectrum.

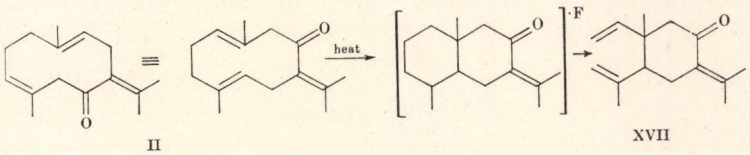


XV



XVI

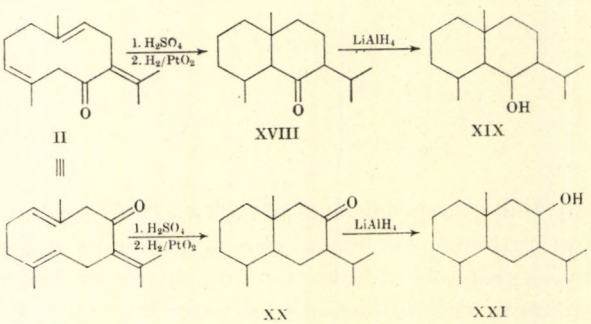
It was found that short heating of germacrone to the boiling point (about 260 °C) at ordinary pressures gave β-elemenone (XVII) quantitatively. The process probably follows a course analogous to the transformation of the germacrane skeleton into the elemane structure during the pyrolysis of germacrol (V) (p. 55). With germacrone the reaction may take place in the way shown below:



II XVII

A study was also made of the acid isomerization of germacrone which had been first investigated by Treibs [28]. He found that the treatment of germacrone with sulphuric acid in alcoholic solution resulted in profound isomerization yielding a ketone as the main product. Treibs suggested structure XVIII for this compound. However, when the same experiments were repeated [19], a complex mixture of various reaction products was obtained. A crystalline alcohol, $C_{15}H_{28}O$, with selinane carbon skeleton has been isolated from the mixture after hydrogenation over platinum dioxide catalyst in glacial acetic acid followed by reduction with lithium aluminium hydride. This shows that isomerization of germacrone in acid medium leads to a bicyclic ketone of the selinane type, which is obtained as the main product of transannular cyclization. The latter can take place both between the $C_{(1)}$ and $C_{(6)}$ atoms and between $C_{(2)}$ and $C_{(7)}$ atoms. The compounds obtained in either case differ in the position of the carbonyl group [12] (XVIII and XX).

The saturated alcohol $C_{15}H_{28}O$ is not identical with the alcohol XIX described by Motl, Herout and Šorm [9], therefore it must possess structure XXI. Consequently, cyclization has occurred between $C_{(2)}$ and $C_{(7)}$. This deduction is also confirmed by the infra-red spectrum of the ketone XX which contains inflexion at 1420 cm^{-1}, characteristic of a methylene group

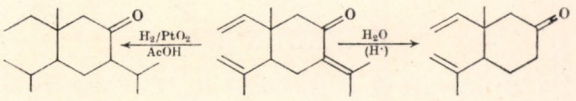


adjacent to a carbonyl group. Actually, by means of quantitative bromination of ketone XX according to Barnes [1] the presence of three hydrogen atoms in α-position was established which is in agreement with structure XX.

2. *β-Elemenone*

The structure of this sesquiterpene ketone has been established by Ognyanov, Herout, Horák and Šorm [14]. The thermal isomerization of germacrone to β-elemenone was observed in the course of the foregoing studies. The infra-red spectrum of β-elemenone (XVII) contains maxima characteristic of methylene- (898 cm^{-1}) and vinyl- (917 cm^{-1}) double bonds, and of a carbonyl group in a six-membered ring conjugated with a tetra-substituted double bond (1683 cm^{-1}). The latter is also confirmed by the ultra-violet spectrum of the ketone (max. 255 mμ, log. ε 3·82) and of its semicarbazone (max. 260 mμ, log. ε 3·95). Besides that, the infra-red spectrum of β-elemenone and of the saturated ketone (β-elemanone, XXII) obtained by hydrogenation contains an inflexion at 1427 cm^{-1} which indicates the presence of a methylene group adjacent to the carbonyl group. Elemane is obtained when the oxygen atom of β-elemanone (XXII) is eliminated by condensation with ethanedithiol followed by hydrogenolysis with Raney nickel. Finally, quantitative ozonization according to the method of Kuhn and Roth [8] gives one equivalent of acetone which corresponds

to the presence of an isopropylidene group, while ozonization by the method of Naves [10] reveals the presence of two terminal methylene groups in XVII. Quantitative bromation of XXII according to Barnes [1] indicate that there are three hydrogen atoms in α-position to the carbonyl group. All these facts are in good agreement with the structure XVII.



XXII XVII XXIII

One of the phenomena observed in the course of the above studies was the ready hydrolytic splitting of the isopropylidene group of XVII in the form of acetone. This hydrolysis, which had long been known to occur in the case of pulegone and compounds of analogous structure, is effected not only by acidic agents, but it takes place even under very mild conditions, such as chromatographing β-elemenone on Brockmann aluminium oxide [3] of activity III–IV [27], or upon boiling the compound in petroleum ether of low b.p. with aluminium oxide of the same activity. The structure of the ketone ($C_{12}H_{16}O$) XXIII obtained in this way has been established in an analogous manner.

The thermal isomerization of germacrone to β-elemenone cast some doubts on the original presence of the latter in the zdravets oil. It appeared quite possible that β-elemanone is not synthesized in the plant at all, but it is a secondary product formed from germacrone by thermal isomerization either during the steam distillation of the oil, or—even more probably—during the laboratory rectification of the liquid part of the oil which still contains dissolved germacrone. The examination of the substances obtained by extraction with petrol ether from the green parts of the plant and from some industrial samples of essential oils, using the technique of chromatography over a thin layer of aluminium oxide showed [13] that none of the samples contained any β-elemenone, and germacrone was present alone. The spot due to germacrone disappeared upon heating the samples to their boiling point, and a spot of β-elemenone appeared instead. These facts can be accepted as unequivocal proof to show that β-elemenone is not contained in the zdravets oil as a natural component but it is a secondary product formed from germacrone during the laboratory investigation of the oil.

The studies on the composition of the Bulgarian zdravets oil, described above, have finally led to the establishment of the structure of germacrone,

and showed it to be a sesquiterpene compound with a new — germacrine — ten-membered carbon skeleton. Closer investigation of the properties of germacrone enriched our knowledge on the particlar features of compounds possessing a ring with ten carbon atoms. It was found later [23] that a series of natural substances possess the same carbon skeleton.

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Cs. SZÁNTAY

STRUCTURE AND SYNTHESIS OF IPECAC ALKALOIDS

I. INTRODUCTION

The roots of various species of Ipecacuanha, belonging to the Rubiaceae family, native mainly in South America, contain several alkaloids significant also from a therapeutical point of view. Particularly *Cephaelis ipecacuanha* Rich. *(Psychotria ipecacuanha* Stokes, *Uragoga ipecacuanha* Baill., commercial name Brasil or Rio ipecac) and *Cephaelis acuminata* Karsten *(Psychotria granadensis* Benth., *Uragoga granatensis* Baill., commercial name Nicaragua or Panama ipecac) are remarkable for their high alkaloid contents.

Table I gives, in chronological order of isolation, a summary on bases of proved structure, isolated so far from the above plants.*

Besides the alkaloids listed in Table I there are literary references on the isolation of further bases. Thus Hesse [77] denominated two compounds isolated by him, ipecamine and hydroipecamine, respectively, however his substances were not pure [126]. Recently Battersby et. al. [19, 20] isolated a compound, 'Alkaloid A', whose structure is, however, unknown. Bellet reported the isolation of a glycoside named ipecoside [39]; this is a compound of non-basic character which has a structure [25a] considerably different from that of other derivatives, though a genetic connection is probable.

Recently [52b] emetine, cephaeline and psychotrine have been isolated from the roots of *Alangium Lamarckii* Thw., a plant belonging to the family of Alangiaceae, and the alkaloid known previously as alamarckin has been identified as N-methylcephaeline. These facts show that these bases are more wide-spread in the plant kingdom than assumed earlier.

The following discussions will be restricted to the chemistry of compounds enlisted in Table I.

A genetic relationship among the ipecac bases of known structure can be brought forth, as presented in Table II.

* For a more detailed summary of the various derivatives of compounds 1–5 see [80] and [117].

Table I

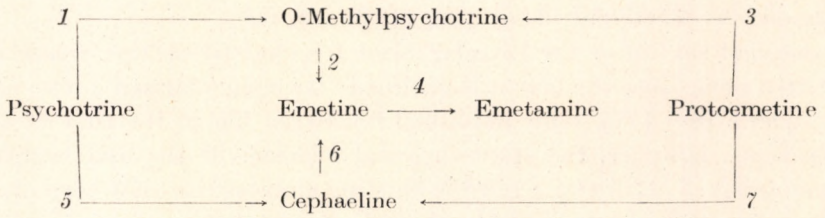
The Ipecac Alkaloids

Name	Empirical formula	Crystal form and m. p.	Optical rotatory power	Optical rotatory power and m.p. of salts
Emetine [121, 122]	$C_{29}H_{40}O_4N_2$	amorphous* 74°C [57, 81]	$[\alpha]_D^{25} = -49{\cdot}2°$ (c = 3·56, $CHCl_3$) [33]	*B*.2HCl.3H_2O (from methanol–ethyl acetate–ether), 235–250°C; $[\alpha]_D^{3} = +46°$ (c = 1·07, $CHCl_3$) [11]
Cephaeline [120]	$C_{28}H_{38}O_4N_2$	needles (from ether) 115–116°C (dried at 100°C: 120–130°C)[57]	$[\alpha]_D = -43{\cdot}4°$ (c = 2, $CHCl_3$) [57]	*B*.2HCl.7H_2O (from dilute aqueous hydrochloric acid 245–275°C; $[\alpha]_D = +25°$ (c = 1·68, H_2O)[57]
Psychotrine [121]	$C_{28}H_{36}O_4N_2$	yellow needles (from aqueous acetone), *B*.4H_2O 120–138°C [57, 45]	$[\alpha]_D^{15} = +69{\cdot}3°$ (c = 2, ethanol) [77]	*B*.H_2SO_4.3H_2O (from water) 214–216°C (anhydrous); $[\alpha]_D = +39{\cdot}2°$ (c = 1, H_2O) [57]
O-Methylpsychotrine [126]	$C_{29}H_{38}O_4N_2$	prisms (from ether) 122–123·5°C [33]	$[\alpha]_D^{25} = +42{\cdot}8°$ (c = 1·87, ethanol) [33]	*B*.2HBr.4H_2O (from H_2O), 190–200°C; $[\alpha]_D = +46°$ (c = 2, H_2O) [126]
Emetamine [126]	$C_{29}H_{36}O_4N_2$	needles (from ethyl acetate) 155–156°C [126]	$[\alpha]_D^{20} = +11{\cdot}2$ (c = 6·1, $CHCl_3$) [126]	*B*.2HBr.7H_2O (from H_2O), 210–225°C; $[\alpha]_D = -22°$ (c = 4·15, H_2O) [126].
Protoemetine [19, 20]	$C_{19}H_{27}O_3N$	resinous		*B*.$HClO_4$ (from aqueous ethanol and after drying), 193–195°C; $[\alpha]_D^{25} =$ $= -10{\cdot}9$ (c = 3·13, ethanol) [20]

* Foster and Nargrove [75] described emetine as a crystalline base, melting at 104–105°C. This was cited also by Boit [42]. However, thorough physical examinations made by Brossi et. al. [47] could not confirm the existence of a macroscopic crystal lattice.

Table II

Genetic Relationship among the Ipecac Alkaloids



Note:

1 — O-methylation [81], *2* — H_2 (126, 127], *3* — several steps [26], *4* — Raney Ni (dehydrogenation) [60], *5* — H_2 [56, 57], *6* — O-methylation [56, 57], *7* — β-(3-hydroxy-4-methoxyphenyl)-ethylamine [141]

Among ipecac alkaloids, considerable practical importance is attached primarily to emetine which is the base occurring in greatest amount in the plant. It has been used in medicine since 1912, as the specific medicament for amoebic dysentery and amoebic hepatic ulcer [129, 130]. For this reason it has been a stimulating scientific project to complete in the first place the synthesis of emetine, and on basis of the existing genetic relationship, that of the other related alkaloids. Particularly during the last fifteen years — since the elucidation of the constitution of alkaloids — serious efforts have been made to attain a rational synthesis of these bases of fairly complicated structure. Learning about the more exact steric structure progressed parallel with the synthetic work.

More recently emetine proved to have virostatic properties [75b], and its potential antitumour and antifungal properties have also been discussed [75a].

II. THE STRUCTURE OF IPECAC ALKALOIDS

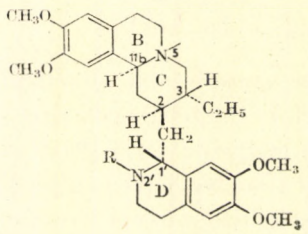
1. Structure of Emetine (I, R = H)

(i) *Constitution*

The first correct suggestion on the constitution of the emetine molecule was made by Robinson [128] in 1948, on basis of biochemical considerations, making use of the biogenetic mechanism introduced by Woodward [162]. This conception was, of course, based on earlier chemical experiments carried out mainly by Pyman and coworkers [45, 57, 126, 127], Hesse [77], Keller [85, 86, 87, 88], Windaus and Hermanns [160], Karrer [81, 82], Späth and Leithe [134], Staub [137], Kunz-Krause [93, 94, 95, 96], Paul and Cownley [120, 121], and Ahl and Reichstein [1].

The correctness of the formula suggested by Robinson was unambiguously proved by the chemical investigations of Späth and Pailer [135], Pailer [115, 116], Pailer and Porschinski [119], and independently from the aforementioned, by Battersby et. al. [20, 27, 28, 31, 32].

A detailed survey of the investigations for elucidating the constitution is omitted here, since the matter has already been summarized in two excellent reviews [80, 117], both published bookwise. Up to the time of these publications, however, the stereochemical relations at the four centres of asymmetry (2, 3, 11b, and 1′) in the emetine molecule had not been cleared up, therefore this question will be fully dealt with here.



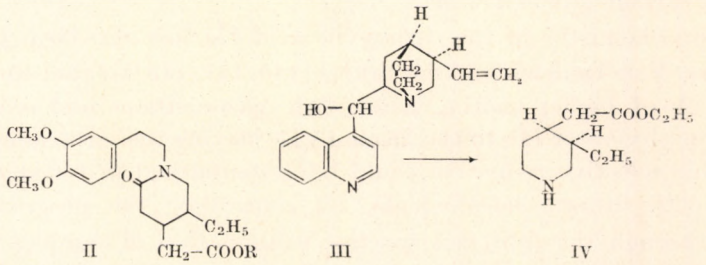
(ii) *Stereochemistry of the Centres of Asymmetry at $C_{(2)}$ and $C_{(3)}$.*

The centres of asymmetry represented by carbon atoms 2 and 3 in emetine can be most reasonably studied by investigating a molecule containing only these two centres of asymmetry, from which emetine itself can unambiguously be derived. A very suitable model for this purpose is compound II, that has two racemates; emetine can be synthesized from one of them [11, 71, 72, 124, 147] by methods to be discussed later.

In the course of the synthesis of the carboxylic acid IIa, two isomers could be isolated, the one with higher melting point (A; m. p. 154–6 °C) and the other with lower melting point (B; m. p. 152–3 °C) [11, 50, 147]. The two isomers obviously differ from each other in the relative orientation of the carboxymethyl and ethyl groups, which is *cis* in the one isomer and *trans* in the other. Emetine can be derived from the A isomer, hence elucidation of the configurational relations of this isomer means the unambiguous solution of the problem.

The biogenetic relationship presumably existing between cinchona and ipecac alkaloids [144] led Brossi and coworkers [50] to the idea of trying to find a chemically unequivocal correlation between compound II and cinchonine; the stereochemistry of the latter had been known in this respect. Cin-

chonine (III*) * may be converted in a known way [123, 84] to cincholoiponate (IV*), which contains the ethyl and carboxymethyl groups in *cis* position to each other [123].

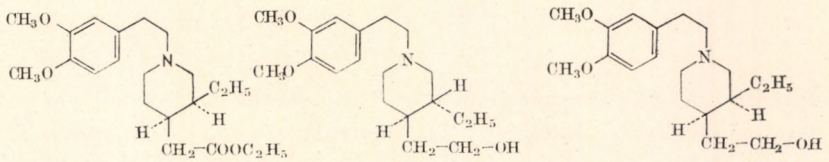


II III IV

R = a H, b CH_3, c C_2H_5

Condensation of base IV* with β-(3,4-dimethoxyphenyl)-ethyl iodide gives rise to the piperidine derivative V*, in which, therefore, the substituents in question are in *cis* position.

The ethyl esters of the carboxylic acid IIa (IIc) — both of the A and B isomers — were reduced with $LiAlH_4$, to remove the oxygen of the carboxamide function and to establish a direct correlation with the derivative V* obtained from cinchonine. Reduction of the carboxamide group, however, was accompanied by the reduction of the ester group, affording the corresponding alcohols (VI A and VI B, respectively).



V VI a VI b

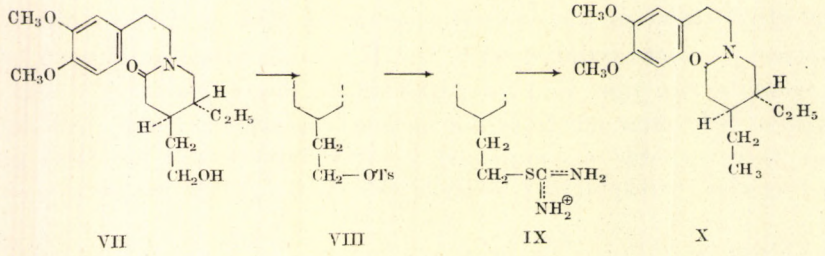
To establish a direct relationship, the ester V* had also to be reduced to alcohol. Comparison of the infra-red spectra of the two racemic alcohols (VI A and VI B) with that of the dextrorotatory alcohol of *cis* configuration derived from cinchonine, led to the conclusion that the spectrum of the alcohol obtained from the natural compound was identical with that of alcohol VI B and differed significantly from the spectrum of VI A. Since alcohol

* In the recent paper only one of the antipodes of racemic compounds are represented in the formulae. For this reason — to avoid misunderstanding — the Roman numerals are marked with asterisk when the given formula actually and exclusively represents the optically active compound in question.

VI A was derived from the A modification of the carboxylic acid IIa, and the latter substance is the starting material for emetine synthesis, this compound as well as emetine must have *trans* configuration of the substituents at carbon atoms 2 and 3.

The stereochemistry of the carboxylic acid IIa has also been proved in other ways. Van Tamelen and coworkers [145, 147, 149] treated the carboxylic acid A of higher melting point with diazomethane and reduced the obtained methyl ester IIb to the alcohol VII, leaving the carboxamide group intact. This selective reduction could be accomplished in tetrahydrofuran solution with lithium borohydride, by controlling the progress of the reaction through the infra-red spectral examination of samples taken at regular intervals, and stopping the reaction when optimum.

The alcohol VII could be tosylated in pyridine; the obtained compound (VIII) was convertible into the iso-thiuronium salt (IX) by means of heating with thiourea, which gave then the diethyl derivative (X) on heating with Raney nickel.



VII VIII IX X

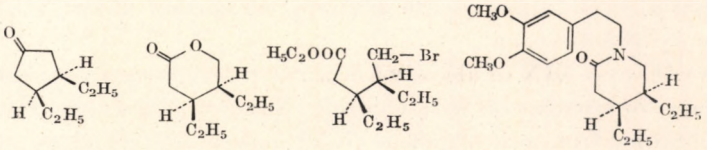
Compound X was prepared then by the authors mentioned [147, 148], and independently by Battersby and Garratt [18, 25] — inspired by the procedure used in the stereospecific synthesis of alloyohimban [138] and yohimban [151] — in the following controlled way.

Reaction of *cis*-3,4-diethylcyclopentanone (XI) of known stereochemistry [92] with perbenzoic acid results in the formation of *erythro*-β,γ-diethyl-δ-hydroxyvaleroic lactone (XII), which yields ethyl *erythro*-β,γ-diethyl-δ-bromovalerate (XIII) on treatment with hydrogen bromide in ethanol. The bromo-derivative XIII gives rise to the lactam XIV when treated with β-(3,4-dimethoxyphenyl)-ethylamine, which is not identical with lactam X deducible from the carboxylic acid IIa (A), the substance applicable to the synthesis of emetine.

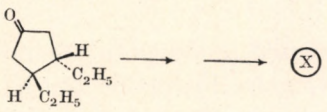
On the other hand, starting with *trans*-3,4-diethylcyclopentanone (XV) [92] and following the same procedure as used with the *cis* compound, a

product identical in all respects with lactam X is obtained through the intermediate *threo*-β,γ-diethyl-δ-bromovaleroic ester, which is further proof for the *trans* orientation of the substituents.

The above conclusions are confirmed also by the reactions which can be carried out with lactams XIV and X, respectively. Ring closure takes place

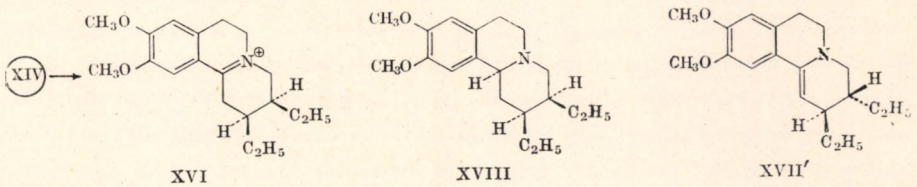


XI XII XIII XIV

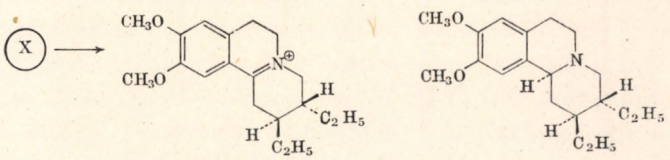


XV

with both compounds on treatment with $POCl_3$, to give rise to the immonium salts XVI and XVII, respectively. (It is to be noted that these immonium salts may be converted to enamines, for example XVII′, when acted upon by bases). Catalytic or sodium borohydride reduction of both immonium salts yields the corresponding saturated derivative.



XVI XVIII XVII′



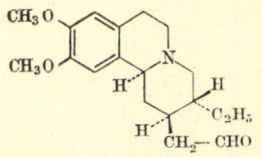
XVII XIX

Generally, the addition of hydrogen at catalytic hydrogenation takes place preferentially at the sterically less hindered side of the molecule [64, 105], therefore the structure of the product formed in the catalytic hydrogenation of the perchlorate of the immonium ion XVI is presumably XVIII.

Owing to the *trans* position of the ethyl groups in XVII it is hard to differentiate between the two sides of this molecule from the above viewpoint; however, hydrogen is likely bound also in this case in the thermodinamically more stable position (cf. p. 80 (iv)), consequently the structure of the molecule is probably XIX. The word 'probably' refers here of course exclusively to the spatial orientation of the asymmetric carbon atom (11b) being in α-position to the nitrogen).

Now XVIII and XIX of already known stereochemistry provide a further opportunity for correlation with emetine. Namely, it is known that emetine may be obtained from protoemetine (XX*) in well-controlled steps (cf. Table II). Once a correlation can be established between protoemetine on the one hand, and XVII or XIX, respectively, on the other, the above conclusions may be confirmed.

Battersby and Garratt [25] treated protoemetine semicarbazone at 155 °C in anhydrous ethylene glycol with dry hydrazine and KOH, when reaction took place yielding the corresponding diethyl derivative.



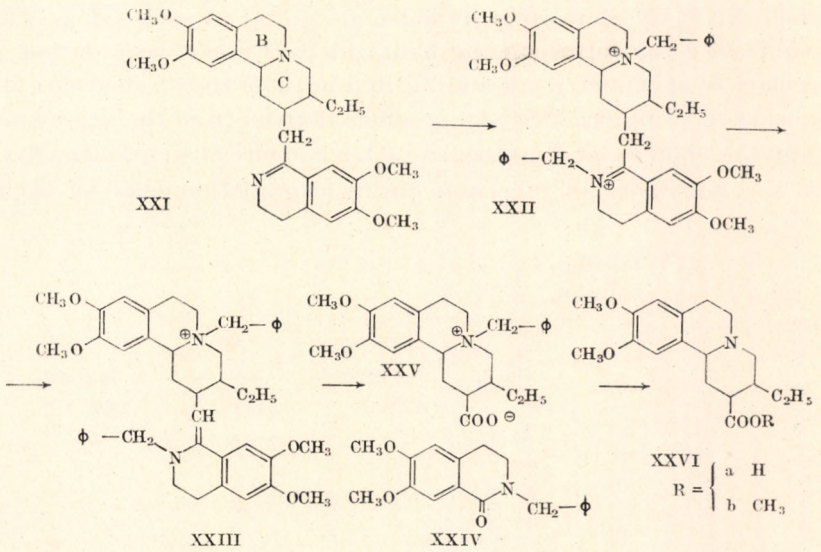
Naturally, in such energetic circumstances there is a risk of a possible inversion: therefore, emetine itself was subjected — as a control — to the same conditions under which the Wolff–Kishner reduction of protoemetine had been accomplished. The alkaloid was recovered without any inversion, hence it is highly probable that no inversion had occurred in the case of protoemetine either.

The product obtained from protoemetine is naturally optically active, while products XVIII and XIX are racemates, thus also in this case, comparison was made on basis of the infrared patterns. According to the spectra, the product obtained from natural protoemetine was identical in structure and configuration with XIX, containing the ethyl groups in *trans* position, while the spectrum of the *cis* compound (XVIII) was considerably different. Comparison of the spectra of the XVI and XVII immonium salts, obtained from the above products by means of oxidation with mercury(II) acetate, with the analogous immonium salt derived from protoemetine also led to the same conclusion.

A study of the spatial orientation of the substituents linked to carbon atom 2 of the emetine molecule may be effected not only in the above detailed

'synthetic' ways, but also by direct degradation, carried out by Battersby and others [15].

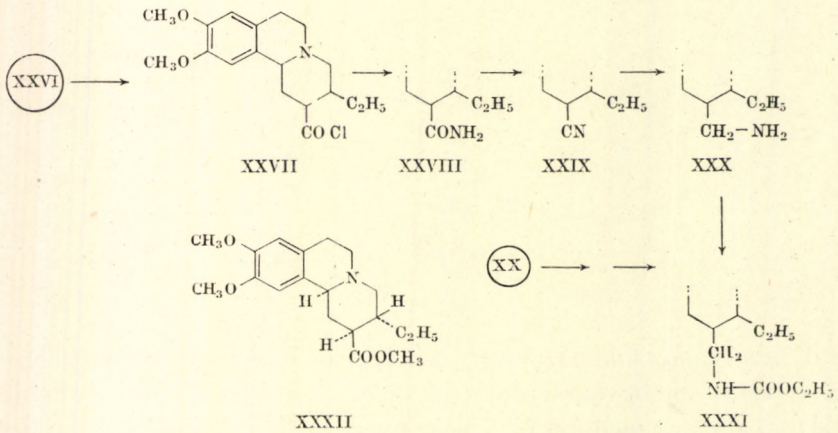
O-Methylpsychotrine (XXI*) served as the starting material for degradation. Since this substance is convertible to emetine by reduction (cf. Table II), rings B, C, and their substituents must have identical configura-



tion in both compounds. O-Methylpsychotrine reacted with benzyl chloride to give rise to the bis-quaternary salt XXII*, which could be oxidized with $KMnO_4$ in alkaline medium to N-benzylcorydaldine (XXIV) and the betaine XXV*. The intermediate product is the iso-base XXIII*. Hydrogenation of the betaine in the presence of palladium gave the carboxylic acid XXVIa. To decide the configuration of carbon atom 2, the ester XXVIb* was heated in methanol with sodium methoxide under much more energetic conditions than, for instance, sufficient to invert the axial methoxycarbonyl group of ecgonine methyl ester [74]. Since the ester XXVIb was subsequently recovered unchanged, the conclusion might be drawn that the methoxycarbonyl group was equatorial and the molecule existed in the thermodynamically favoured conformation. Assuming that the preferred annelation of rings B and C is *trans* (cf. p. 80 (iv), the configuration of carbon atom 2 is given according to formula XXXII.

Since ester XXVIb* possesses the stable orientation at carbon atom 2, it should be decided whether the originally unstable configuration became inverted during the degradation or not.

The sodium salt obtained from the hydrolysis of the ester XXVIb*, when reacted with oxalyl chloride gave the acid chloride XXVII*, which could be dehydratated through the carboxamide (XXVIII*) to the nitrile (XXIX*). Reduction of the latter afforded the amine (XXX*), which was converted to the urethane XXXI* by treatment with ethyl chlorocarbonate. On the other hand, the same urethane could be obtained from protoemetine (XX*) by converting its aldehyde group to ester (cf. p. 112 (f)) and subjecting the corresponding hydrazide to Curtius degradation. Since the centres of asymmetry are not affected in these transformations, it may be considered as proved that the products derived from the degradation of O-methylpsychotrine and protoemetine have identical stereochemistry, and hence the possibility of inversion during degradation may be excluded.

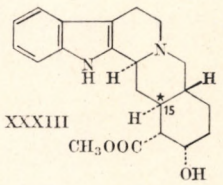


Having thus deduced the *trans* orientation of the substituents at carbon atoms 2 and 3, the steric structure of the ester in question can be represented as shown in formula XXXII*, or the mirror-image thereof. (As to the arguments concerning the configuration of carbon atom 11b, see p. 80 (iv).

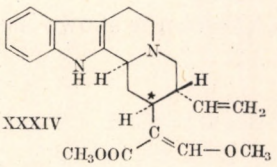
The only problem that remains to be solved is to determine the absolute configuration, which, in case of the centre of asymmetry at carbon atom 2, was accomplished by Battersby and coworkers [24].

In deciding the question, we may as well rely upon our knowledge concerning the biogenesis of alkaloids. It has been ascertained, namely, with indole alkaloids that all derivatives of known steric structure originating from tryptophane, phenylalanine, or any equivalent, have identical absolute configuration at a given carbon atom [43, 44, 128, 131, 155, 156, 162], for example at the sites starred in the formulae of yohimbine (XXXIII*)

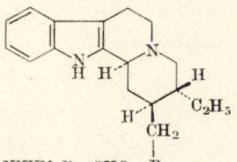
[91], or corinanteine (XXXIV*) [148, 155, 156]. Presumably, this centre preserves its configuration during the biosynthesis of the alkaloid from the assumed precursor. If we accept Robinson's conception [128], according to which emetine is formed in the plant by the same type of synthesis as indole alkaloids, then carbon atom 2 can be assumed to possess the same absolute configuration as the one indicated in the above alkaloids.



XXXIII

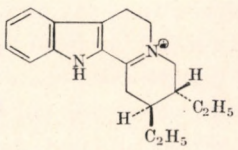


XXXIV



XXXV R =CHO R

XXXVI R= CH_3



XXXVII

In support of these concepts a comparison has been made of the optical properties of some corinanteine and protoemetine derivatives. Corinanteine (XXXIV*) is convertible to dihydrocorinanteale (XXXV*) and further to dihydrocorinanteane (XXXVI*) perchlorate of known absolute configuration in a known way [148, 155]. Oxidation of the perchlorate by means of mercury(II) acetate yields 3-dehydro-dihydrocorinanteane (XXXVII*). The structures of protoemetine (XX*) and of the derivatives XVII* and XIX*, obtainable therefrom as described above, are quite analogous to these compounds.

The values of the molecular optical rotatory power of the above substances (at room temperature, in aqueous ethanol) are shown below:

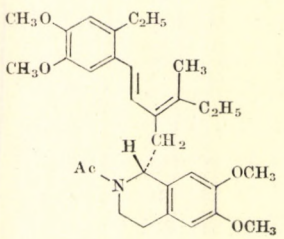
Perchlorate	$(M)_D$		$(M)_D$
XXXV*	+ 63°		
XXXVI*	− 69°	XXXVI*−XXXV*	−132°
XXXVII*	+318°	XXXVII*−XXXVI*	+387°
XX*	− 43°		
XIX*	−165°	XIX*−XX*	−122°
XVII*	+317°	XVII*−XIX*	+482°

From these data the conclusion may be drawn (cf. [66] and [110]) that the two types of compounds have similar absolute configurations, and hence formula XXXII* indicates also the absolute configuration of the molecule; it means at the same time that the absolute spatial orientation of carbon atoms 2 and 3 in emetine has been ascertained.

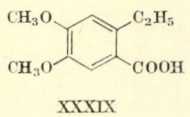
Upon the suggestion of Bose [43] carbon atom 15 of (+)-yohimbine (XXXIII*) is regarded as the reference standard of representation, and the hydrogen in question is shown as if it were under the plane of paper.

(iii) *Stereochemistry of the 1'Position.*

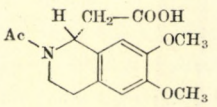
In order to perform an unambiguous and thorough study of the stereochemistry of the centre of asymmetry at position 1' in the emetine molecule, it is advisable to isolate it from the rest of the molecule. This isolation was accomplished by Battersby et. al. [16, 17] by subjecting 2'-acetylemetine (I*, R = Ac) to three subsequent Hofmann degradations — inserting a hydrogenation prior to the second step — when the diene XXXVIII* was obtained. Oxidation of this diene with $KMnO_4$ in pyridine gave only 6-ethylveratric acid (XXXIX) as the isolable product, but when the diene was



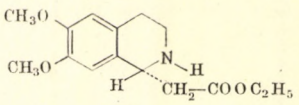
XXXVIII



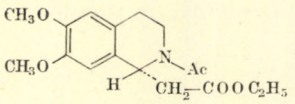
XXXIX



XL



XLI



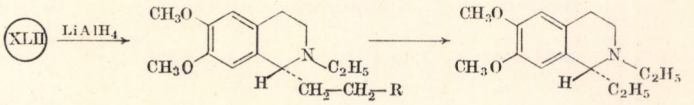
XLII

treated with ozone and then with peracetic acid the carboxylic acid XL* could be obtained, which, consequently has retained the configuration of the $C_{(1')}$ atom of emetine. The compound is strongly laevorotatory ($[\alpha]_D$: —144°, in ethanol).

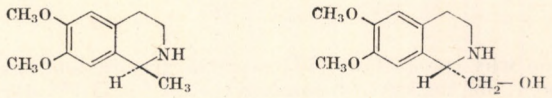
Ester XLI having a structure analogous to carboxylic acid XL* can also be readily prepared by synthesis [32, 58], and it is resolvable with (—)-O, O-dibenzoyl tartaric acid. The ester (+)-XLI* obtained thus afforded on acetylation (XLII*) and hydrolysis performed under mild conditions a laevorotatory product identical in all respects with compound (—)-XL*, enabling authors to obtain higher quantities required for further examinations.

Further work involved the reduction of (+)-XLII* with $LiAlH_4$ to the alcohol (XLIII*, R = OH) and acetylation of the latter to give XLIII* (R = OAc). On the other hand, XLIII* (R = OH) was converted with PCl_5 into the chloride and reduced to yield the diethyl derivative XLIV*. The products were subjected to optical study.

On investigating the optical rotatory power of various optically active bases, Leithe found [98, 99] that in case of derivatives with identical absolute configuration an increase in the polarity of the solvent changed the rotatory power in the same direction.



XLIII XLIV



XLV XLVI

It should be noted, however, that this empirical rule is by no means of general validity. Thus, for instance in the case of more complicated bases examined by Beckett and Casy [34] the above rule did not hold good hence these authors cast doubt on its practical applicability.

On examining the absolute configuration of (—)-salsoline (XLV*) and (+)-calycotomine (XLVI*), Battersby and Edwards [23] pointed out that the configuration of the asymmetric carbon atom of salsoline is identical with that of natural L-alanine, while the asymmetric carbon atom in calycotomine has just the opposite absolute configuration. In spite of this, the optical rotatory power of both salsoline and derivatives and calycotomine changes in the positive direction when the polarity of the solvent is increased. The phenomenon is explained by the above authors by the suggestion [23]

that in calycotomine two highly polar groups are attached to the asymmetric carbon atom and hence the character of both groups is strongly influenced by the polarity of the solvent. This statement supports the essential general principle that only closely analogous molecules should be used for optical comparison. Nevertheless, in the light of literary data the Leithe-rule seems to be applicable provided that the base in question belongs either to the group of α-methylbenzylamines, or to that of 1-alkyl-1,2,3,4-tetrahydroisoquinolines, and the nitrogen atom is the only highly polar centre in the molecule. In this respect the methoxyl group is not to be regarded as a highly polar grouping.

Taking all these facts into consideration, when comparing derivatives prepared from isoquinoline type fragments obtained during the degradation of emetine, compound XLIII* (R = OH) should be disregarded because of its content of a polar group. The specific optical rotatory power of the three other derivatives in solvents of different polarity are summarized below:

Base	Benzene	Chloroform	Ethanol	N HCl
(+)-XLI*	+21·3°	+18·3°	+15·2°	−15°
XLIII* (R = OAc)	− 5·8°	− 9·7°	−10·7°	−20·6°
XLIV*	+ 2·6°	− 3.1°	− 4·3°	− 9·5°

The reason why the authors [17] gave the specific and not the molecular rotatory power is that — for the sake of economy — a (+)-XLI* ester, not fully resolved, was used in the experiments, which however, perfectly served the purpose, namely the evaluation of the shift in rotatory power.

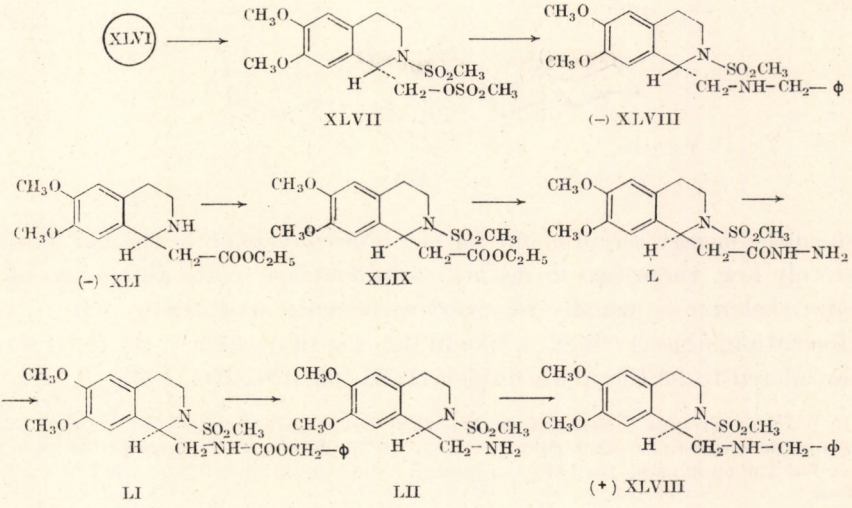
It is seen that with an increase in the polarity of solvent all the three compounds investigated change their optical rotatory power markedly in the negative direction. This is in conformity with the behaviour of compounds of analogous structure having the absolute configuration corresponding to formula XL*.

However, due to the reservations made above, the foregoing experiments do not give fully convincing proof. The latter can be provided only through chemical correlation.

(+)-Calycotomine (XLVI*) of already known absolute configuration [23] appeared to be a suitable basis for comparison. On treatment with methanesulphonyl chloride, the latter compound yielded the N,O-dimethanesulphonyl derivative (XLVII*), which then afforded through substitution

of the methanesulphonyl group with benzylamine base XLVIII*, a compound of negative rotatory power.

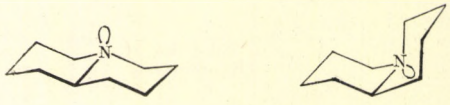
On the other hand, the laevorotatory ester XLI* was obtained by resolving the synthetically prepared ester XLI. (The fact that it had a configura-



tion opposite to that of XL* — the product of the degradation of emetine — was shown by preparing XL* from the enantiomer, that is from the dextrorotatory XLI* ester.) The laevorotatory ester XLI* could be converted through the corresponding N-methanesulphonyl derivative (XLIX*) to hydrazide (L*), the Curtius-rearrangement of which, in the presence of benzyl alcohol, gave the urethane derivative LI*, that was catalitically hydragenated to the amine LII*. Condensation of the latter with benzaldehyde, followed by hydrogenation of the resulting azomethine compound gave the dextrorotatory enantiomer of XLVIII*, consequently our compound has a configuration opposite to that of calycotomine. The above assumptions are also supported by the fact that a mixture of equal proportions of base (—)-XLVIII* obtained from calycotomine, and base (+)--XLVIII* prepared as described above yielded a product identical in all respects with the independently synthesized racemate. Thus, the conclusion may be drawn that ester (+)-XLI* has a configuration identical with that of calycotomine, and hence the absolute configuration of carbon atom 1′ of emetine has become known, as represented in the formula I*, or indicated as configuration *R* according to the Cahn—Ingold—Prelog convention [55].

(iv) *Determination of the Annelation of Rings B/C and the Configuration of Carbon Atom 11b*

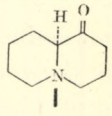
The basic skeleton of the B/C rings of the emetine molecule, the quinolizine ring system, may practically assume either a *trans* (LIIIa) or a *cis* (LIIIb) form.



a LIII b

Since the energy requirement for the interconversion of the two forms is relatively low, these two forms are — in contrast to the isomerism of the decalin skeleton — usually regarded as formations differing only in their conformation, among which — like in the case of decalin — the *trans* isomer is considered to be the more stable [41, 61, 89, 101, 107, 108].

On basis of circular dichroism studies with 1-oxo-quinolizidine (LIV*) and taking into consideration the octant rule (p. 427 in [66]), Mason and others [106] have also arrived at the conclusion that the compound exists predominantly in the *trans* conformation.



LIV

Measurements revealed a difference of about 1·9 kcal/mole in the energy of the two forms, thus the equilibrium mixture may contain about 3% *cis* derivative at 20°C.

In some respects, however, a distinction should still be made regarding the stability of the *trans* modification, as compared with the decalin skeleton. Namely, if one accepts the statement of Aroney and Le Fèvre [4] — made on basis of the electric polarizability of some bases measured in benzene solution — according to which the space requierement of the unshared electron pair of nitrogen is higher than that of a hydrogen atom in covalent bond and approaches the space requirement of a methyl group, then quinolizine should rather be compared with a decalin containing an angular methyl group.

The entalpy of the *cis*-decalin $\rightleftarrows$ *trans*-decalin interconversion is $\Delta H = -2{\cdot}7$ kcal/mole [3]. Now, if an angular effect of about 0·8 kcal/mole is attributed to the unshared electron pair of the nitrogen, this value being obtained as the half of the angular effect of a methyl group ($0{\cdot}5 \times 2 \times 0{\cdot}8$ kcal/mole *syn*-staggered interaction), moreover taking into consideration the generally accepted hypothesis that substitution of a carbon atom by nitrogen does not practically alter the geometry of the molecule, the entalpy of the LIIIb $\rightleftarrows$ LIIIa interconversion may be estimated as $\Delta H = -1{\cdot}9$ kcal/mole [89], and thus quinolizine in equilibrium must contain more of the form with *cis* geometry than decalin.

Regarding the problem of *cis–trans* conversion, or the equilibrium state, respectively, it should also be taken into consideration that in the case of several alkaloids contain-

ing the quinolizine ring system, there are substituents the orientation of which *(axial* or *equatorial* position) changes with the *cis–trans* interconversion. Thus, for example, if there is a methyl group in the *axial* orientation in the *trans*-conformer, e.g. as in the case of cevine [97], its destabilization value may be estimated to be 2·4 kcal/mole (0·8 kcal/mole being due to the effect of the unshared electron pair of nitrogen, and 1·6 kcal/mole to that of the *axial* methyl group), and thus the *cis*-conformer will be 0·5 kcal/mole more stable than the *trans* compound [89].

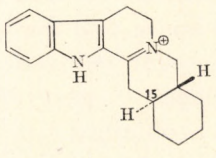
Conformational analysis by infrared and NMR spectroscopy of quinolizine derivatives containing methyl substituents at different positions have led Moynehan and others [107, 108] to the conclusion that — in contrast to the findings of Aroney and Le Fèvre — the space requirement of the unshared electron pair of nitrogen is less than that of a covalently bound hydrogen.

Recently on basis of experiments accomplished on sterically greatly hindered systems containing the quinolizine skeleton, the conclusion has been drawn by Pumphrey and Robinson [125] that the space requirement of the unshared electron pair of nitrogen must be less than that of the N—H bond. Recent publications [2a, 39a, 52a] express views to the same effect. On the other hand, newly published investigatigations by Lambert and Keske [97a] seem to substantiate the order CH_3 > unshared electron pair > H.

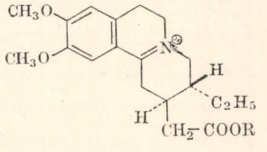
Accordingly, it can be stated that the presented problem cannot as yet be considered as solved and a satisfactory explanation of the contradictory results requires further thorough studies.

In the case of emetine, the conformation of the B/C qunolizine ring-system bears closely upon the configuration of carbon atom 11b. In the thermodynamically more stable space orientation of ring C, the hydrogen attached to carbon atom 11b assumes a *cis* orientation relative to the hydrogen atom of carbon 2 (see for example [154]).

This thermodynamic stability manifests itself also in the preferred formation of this configuration on catalytic hydrogenation of the corresponding unsaturated compounds. Thus, for instance, the reduction of 3-dehydro-yohimban (LV*) affords the C–3, C–15 *cis*-type derivative, and the case is similar in products obtained by the hydrogenation of 3-dehydro-dihydro-corynantean (XXXVII*) [148, 151, 152].



LV



LVI

Catalytic hydrogenation of the immonium salt XVII likewise yields exclusively compound XIX, which had been proved to possess a configuration corresponding to emetine [25, 147]. Reduction with sodium and alcohol in alkaline milieu affords again only that particular epimer. In alkaline medium — as mentioned above — compound XVII exists in form XVII′, hence in this case the reaction is the saturation of the carbon—carbon double bond, which is known to yield the thermodynamically more stable product [12],

when the reduction is accomplished with an alkali metal and alcohol. The same substance is the only isolable product of the reduction carried out by means of $NaBH_4$.

Investigations performed with ester LVI are particularly significant from the point of view discussed above, since this compound is convertible to emetine through hydrogenation and further reactions. Reduction of the ester performed either catalytically with PtO_2, or with $NaBH_4$, sodium dithionite, or formic acid, gave equally only one isolable epimer [11, 14, 33]. Therefore, it is highly probable that a thermodynamic control is in operation at these reactions, and thus the hydrogen enters from the *cis* direction as related to the hydrogen of carbon atom 2.

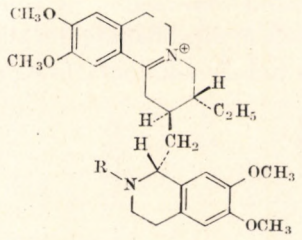
The same conclusion may be drawn from observations made during the catalytic hydrogenation of 2′-benzoyl-11b-dehydroemetine (LVII*, R = benzoyl). Hydrogenation of the compound in methanol in the presence of platinum afforded N-benzoylemetine (I*, R = benzoyl) in 78·8% yield. This product is isolable in epimer-free form also in the reduction completed with Zn + 50% acetic acid [6, 7, 143]. Since under the latter conditions, the analogous indole alkaloid derivatives yielded the thermodynamically less stable products, e. g. pseudoyohimbin [159], or reserpine [90], it may be reasoned that if the 11b epimer is formed at all, it is so unstable that an immediate isomerization takes place.*

The above conclusions concerning the relative configuration of carbon atom 11b are supported also by epimerization experiments, carried out with N-benzoylemetine (I*, R = benzoyl) [143]. Namely, if the unstable form were present, then — similarly to the pentacyclic indole alkaloids [79] — it would presumably epimerize under the effect of acids to the more stable form. However, the starting material could be recovered in 45% yield after refluxing the substance in acetic acid for 8 hours, and the formation of the epimer could not be detected. Epimerization with inorganic acids — which experiments would have been more convincing — gave unfortunately no information, because a resinous material was obtained.

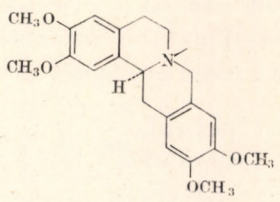
Concerning the absolute configuration of carbon atom 11b, a basis has been established by means of optical comparison with (—)-tetrahydroprotoberberine derivatives (LVIII*, LIX*, LX*) [143].

Corrodi and Hardegger [62, 63] have determined the absolute configuration of (—)-*nor*-coralidine (LVIII*), tracing it back to (—)-tetrahydropapa-

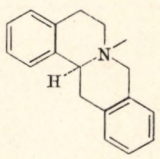
* It should be noted, however, that in the reduction of the quaternary salt XVII by means of Zn + acetic acid, a small amount of the epimer could also be isolated in addition to compound XIX [25]. Furthermore, Evstigneeva [67, 72] succeeded in isolating different isomers (cf. p. 93 (a) when the ester LVI was reduced in the presence of different catalysts and at various pH values.



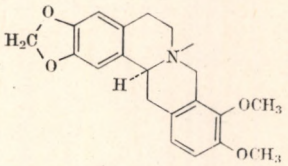
LVII



LVIII



LIX



LX

verine, and through that compound to L-aspargic acid. According to experience, all alkaloids of the (–)-tetrahydroberberine type containing only one asymmetric carbon atom possess almost the same molecular rotatory power, $(M)_D = \sim 1000°$, and their absolute configurations are identical with that of (—)-*nor*-coralidine [98, 99].

Now comparison of the optical rotatory power of (—)-2′-acylemetines (I*, R = acetyl and I*, R = benzoyl) with that of the corresponding unsaturated compounds (LVII*, R = acetyl and LVII*, R = benzoyl) containing no centre of asymmetry at site 11b, show a difference in molecular rotatory power $(M)_D$ of about the same order of magnitude as can be observed with (—)-*nor*-coralidine and its analogues — (—)-tetrahydroprotoberberine (LIX*) and (—)-canadine (LX*) — as it is seen below:

	$(M)_D$		Solvent	$\Delta(M)_D$
I* (R = benzoyl)	— 387·1°	[143]	pyridine	—862·6°
LVII* (R = benzoyl)	+ 475·5°	[143]	pyridine	
I* (R = acetyl)	— 401·4°	[143]	chloroform	—1026·9°
LVII* (R = acetyl)	+ 625·5°	[143]	chloroform	
LVIII*	— 983·4°	[63]	chloroform	
LIX*	— 1072°	[98]	pyridine	
LX*	— 1265°	[98]	pyridine	

This fact leads to the conclusion that the significant shift in the optical rotatory power towards the negative direction caused by the emergence of the centre of asymmetry represented by carbon atom 11b, may be attributed to the fact that its absolute configuration is identical with that of (—)-*nor*-

coralidine. This method — as well as the result obtained — is similar to the process followed and the observations made in establishing the absolute configuration of carbon atom 3 of analogous indole alkaloids (see, e.g. [91]).

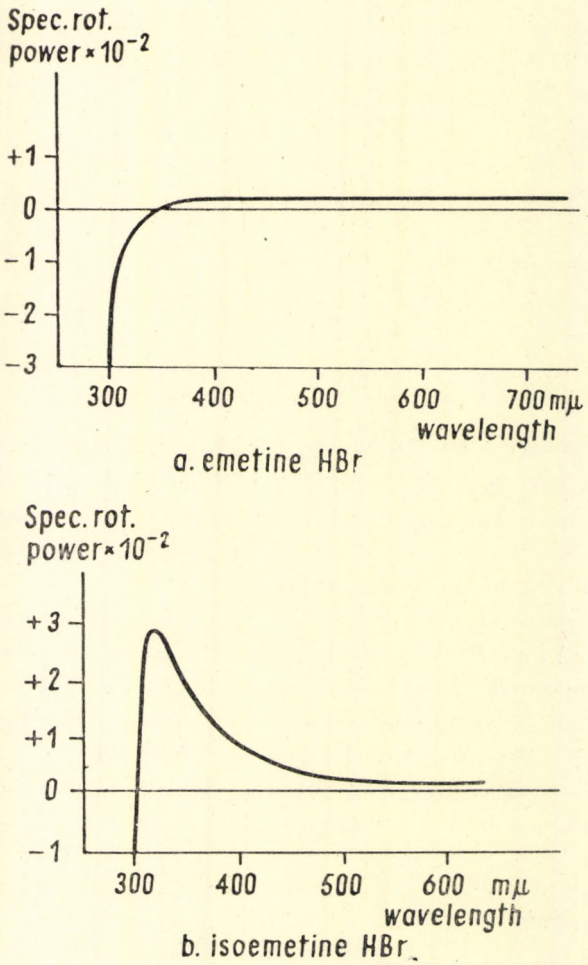


Fig. 1

Optical rotatory dispersion of emetine and isoemetine [147] (in water; c = 0·189 (700–305 mμ); c = 0·0378 (300–295 mμ); c = 0·277 (700–400 mμ); c = 0·0554 (375–300 mμ))

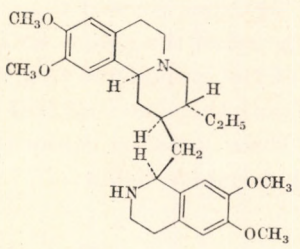
Correlation with carbon atom 1′ of already proved absolute configuration gives further proof concerning the spatial arrangement of the carbon atom in question. In this connection the optical rotatory dispersion [65] of emetine may be mentioned as one of the methods available. These investigations have been carried out by Van Tamelen and others [147].

In emetine only the asymmetric carbon atoms 11b and 1′ are attached to chromophoric groups absorbing strongly in the ultra-violet region. Therefore, if we observe the response of the molecule to polarized light of different wave lengths, the result presumably reflects the behaviour of these two individual centres. Moreover, assuming that identical absolute configurations of two centres implie similar optical behaviour, then — as may be expected — in case of the opposite configurations of the two centres ('antipodes') this opposite behaviour would — in ideal case — compensate each other in the 300–700 mμ region, resulting in the absence of a change in rotation in that range of the spectrum. If, however, the absolute configurations are identical, the individual effects of the respective centres are added, and a normal dependence of rotation on the wave length ought to result.

Dependence of the optical rotation of the emetine molecule on the wave length is shown in Fig. 1, diagram *a*. Hence on basis of the foregoing arguments the two centres must possess opposite absolute configurations.

However, in order to support this presumption, it is reasonable to study also the behaviour of the compound containing the centres of asymmetry of opposite configurations, i.e. isoemetine (**LXI***); the optical rotatory dispersion of the latter is fully in accordance with the above conclusions, as shown in Fig. 1, diagram *b*.

Since the absolute configuration of carbon atom 1′ of emetine is known (cf. p. 76 (iii)), furthermore since isoemetine differs from emetine only in

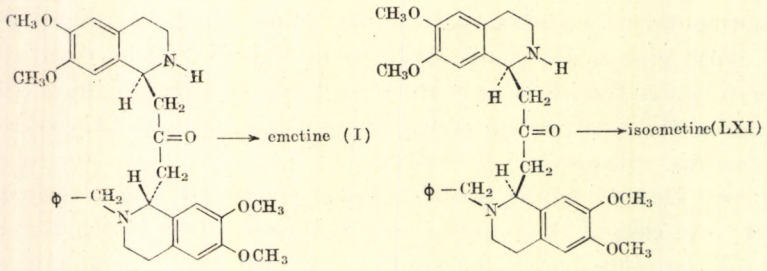


LXI

the configuration of this carbon atom (the two compounds are formed simultaneously in the reduction of O-methylpsychotrine (XXI*) (cf. p. 109 (d)), the procedure proves the absolute configuration of the centre of asymmetry 11b.

Correct as the deductions based on the optical behaviour may be, they cannot be considered fully exact.

The unambiguous and exact correlation of carbon atoms 1′ and 11b has been achieved in a preparative way by Chappman and others [58, 59, 60].



LXII LXIII

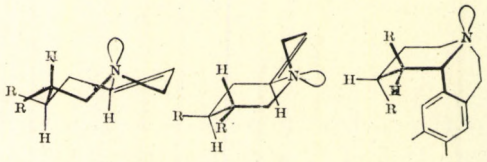
Using compounds LXII and LXIII, respectively, they pointed out that starting with the substance containing centres of asymmetry of opposite configurations (LXII) emetine could be obtained, whereas the substance possessing carbon atoms of identical configurations (LXIII) gave isoemetine. The preparation of the starting materials, the elucidation of their steric structure and the procedures by which they are convertible to emetine will be dealt with in detail later (cf. p. 114 (v)).

On basis of this synthesis it can unambiguously be ascertained that car. bon atom 11b has an absolute configuration as indicated in structure I*-

It is to be noted that contrary opinions regarding the configuration of carbon atom 11b have also been published in literature [10, 49]. However, these arguments were mainly based on erroneous experimental data and were corrected first by Battersby [14], later the authors themselves who had represented contrary opinions revised their viewpoints [46, 114].

With the knowledge in mind of the above, let us now revert to the question of the conformation of the quinolizine ring B/C.

It can be stated that if only the chair and half-chair forms are taken into consideration, there are three possible conformations (LXIV, LXV and LXVI) [15] shown in the formulae below:



LXIV LXV LXVI

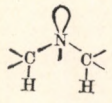
Among these conformations, in LXIV the unshared electron pair of the nitrogen and the hydrogen attached to carbon atom 11b are in *trans-axial* position to each other, while this does not hold true in the two other conformations.

One way of distinction among such types of conformations is dehydrogenation of the bases with mercury(II) acetate [103, 104]. Namely, according to experience this oxidation proceeds satisfactorily if the unshared electron pair of the nitrogen is *trans-axial* to the hydrogen in the α-position.

This method could be applied with good results in the field of indole alkaloids to clear up the conformational relations [73, 157, 159]. For example yohimbine and alloyohimbine could be well dehydrogenated by this method (the C/D rings, i.e. the quinolizine system is *trans*-connected in both), while pseudoyohimbine (*cis* C/D ring junction) could not. On the other hand, further investigation revealed that unambiguous results could be expected only if both stereoisomers were accessible for the purpose of direct comparison of the rates of dehydrogenation. Namely it frequently occurs that under the conditions of oxidation inversion takes place, therefore the result may not be evaluated exactly.

This method of determining the conformation cannot be applied directly in the case of emetine, since during mercury(II) acetate oxidation it is oxidized at both nitrogen atoms [29, 113]. For this reason Ban and others [9] subjected 2′-acylated derivatives (I*, R = benzoyl or acetyl) to dehydrogenation. Though a number of model compounds were examined, no unequivocal result could be attained, because the stereoisomer compound for comparison was not available.

Recently infra-red spectroscopy has proved to be a highly effective tool in examining the conformational relations of quinolizine rings. Wenkert and Roychaudhuri [158] have pointed out that in the spectrum of indole alkaloids containing the hydrogen in 'α'-position at carbon atom 3 — for instance in yohimbine and alloyohimbine — characteristic bands are to be found in the 2700–2800 cm^{-1} region, while the phenomenon cannot be observed in the case of isomers containing a 'β'-oriented $C_{(3)}$—H bond.



LXVII

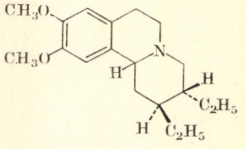
Bohlman [40, 41] has further improved the observation, extending its scope to simpler quinolizine derivatives and stating that this characteristic band or set of bands is actually due to a C–H vibration which appears only if there are at least two hydrogens in *trans-axial* orientation at the position *adjacent* to the unshared electron pair of nitrogen (LXVII). In this way, the conformation can be unambiguously elucidated on basis of the spectra.

If the lone electron pair of nitrogen becomes somehow shared (for example through salt formation, or N-oxide formation), or if a strong mesomeric effect is in operation (for example the nitrogen takes part in a carboxamide bond) the band will be missing. This phenomenon can probably be explained by sigma-conjugation similar to hyperconjugation.

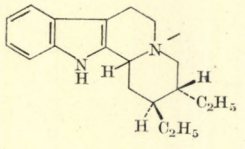
Several years of experience since the recognition of the phenomenon have amply supported the applicability of the method (see, e.g., [132]), although exceptions have also been reported [80a].

The infra-red spectrum of 2-acetylemetine (I*, R = acetyl) recorded in chloroform solution exhibits a characteristic absorption band at 2740 cm^{-1}, which disappears on sharing the lone electron pair by perchlorate formation [143]. This proves the *trans* annelation of rings B/C in emetine, and that its conformation corresponds to LXIV. Quite recently it has been shown [144a] that inferences concerning the stereochemistry of the discussed system can also be drawn by a study of the NMR spectra. The characteristic signal of the angular proton of the *trans* form LXIV is foundabove τ 6·2, while the same proton of the two *cis* conformations gives signals below this value. The character of the splitting of the signals permit differentiation even between the two alternative *cis* forms.

It is noteworthy that derivatives corresponding to LXV *cis* conformation have only seldom been found so far among compounds of the benzo(a)quinolizine type [144a]. For instance, in the reduction of derivative XVII by means of zinc and acetic acid, an epimer could also be isolated besides the thermodynamically stable product XIX, the mercury(II) acetate oxidation of which led to the starting material; hence, it can be only the 11b isomer of XIX, represented by structure LXVIII. The characteristic absorption band at 2750 $\pm$ 10 cm^{-1} is present in its infra-red spectrum, indicating again the *trans* annelation of the quinolizine ring system, in spite of the fact that in this case the two ethyl groups have to assume *axial* positions. The compound could not be epimerized to XIX even by heating with potassium *tert.*-butoxide in *tert.*-butanol [25].



LXVIII

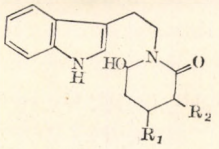


LXIX

On the other hand, the corresponding indole analogue (LXIX) — prepared from cinchonine — being the C–3 epimer of dihydrocorinanteane (XXXVI) contains a *cis*-fused quinolizine ring [111], from which the conclusion may be drawn that *cis* conformation is formed with indole-type compounds more easily, than with benzo(a)-quinolizines.

The same observation is supported also by the fact that ring closure of a LXX-type lactam leads to a *cis*-fused compound, as pointed out by Van Tamelen and others [153] in the course of their investigations on the synthesis of yohimbine.

On the other hand the analoguous reaction in the benzo(a)quinolizine series yielded a single product containing rings B/C in *trans* annelation (cf. p. 101 (ii)), Burgstahler and Bithos attributed this difference to the fact that the indole ring is more reactive than the dimethoxyphenyl ring when attacked by an electrophilic agent. This may account — at least in part — for the dissimilar behaviour. If, however, the above phenomena observed with compound LXVIII are also taken into consideration, it is



LXX

highly probable that the main difference between the two systems should be attributed to other as yet not clarified factors.

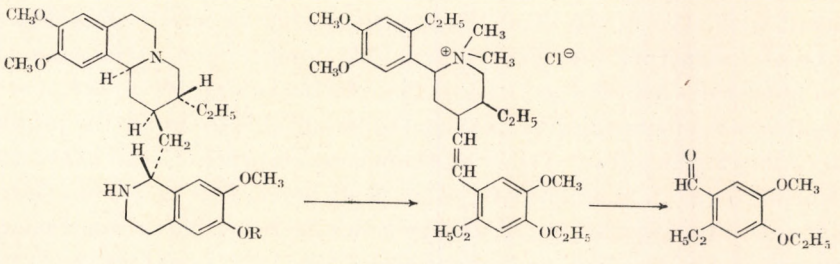
However, it is to be noted that in the case of pentacyclic hydrobenzo(a)quinolizine derivatives — similarly to the corresponding indole derivatives — products possessing *cis* annelation at the points in question are again readily formed [80b].

Summarizing the conclusions drawn from the accumulated experimental data concerning the stereochemistry of (—)-emetine, it may be unequivocally stated that the absolute configuration of its centres of asymmetry are 2*S*, 3*R*, 5*R*, 11b*S*, 1′*R*, and thus emetine is represented by formula I* (R = H).

2. *Structure of Minor Alkaloids*

(i) *Cephaeline*

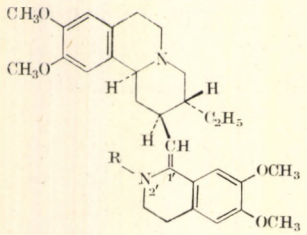
Among the minor alkaloids, cephaeline (LXXI*, R = H) may be considered to be the most important product. This compound contains three methoxy groups and one phenolic hydroxyl function. O-Methylation of the latter leads to emetine (cf. Table II) (the significance of cephaeline lies exactly in this reaction), thus the only doubtful point of the structure is the position of the phenolic hydroxyl group. Pailer and Porschinski [119] degraded O-ethylcephaeline (LXXI*, R = ethyl) to compound LXXII, the further degradation of which with ozone gave 2-ethyl-4-ethoxy-5-methoxybenzaldehyde (LXXIII), which decided the position of the hydroxyl group as shown by formula LXXI* (R = H).



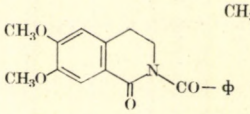
LXXI LXXII LXXIII

(ii) *Psychotrine*

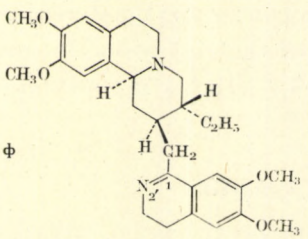
Psychotrine can be reduced to a mixture of cephaeline and isocephaeline (the 1′-epimer of cephaeline) (cf. Table II); O-methylation converts the compound into O-methylpsychotrine, which yields on reduction a mixture of emetine and isoemetine. The above reactions fix the position of the hydroxyl group in psychotrine, furthermore they prove that the double bond is connected to carbon atom 1′ both in psychotrine and O-methylpsychotrine. This double bond was for long considered to be an exocyclic bond (LXXIV*, R = H) and earlier reviews [80, 117] reported this structure based upon reactions such as the acylation of the compound on the nitrogen atoms. In the products obtained in this reaction, obviously only an exocyclic double bond can be found, which is proved among others by the oxidation of the N-benzoyl derivative (LXXIV*, R = benzoyl), to N-benzoylcorydaldine (LXXV) by means of perphthalic acid [83].



LXXIV



LXXV



LXXVI

On the basis of a spectroscopic study of O-methylpsychotrine oxalate in the ultra-violet region Openshaw and Wood [113] declared the double bond to be in the 1′–2′ position, in other words, a dihydro-isoquinoline structure (LXXVI*) was suggested.

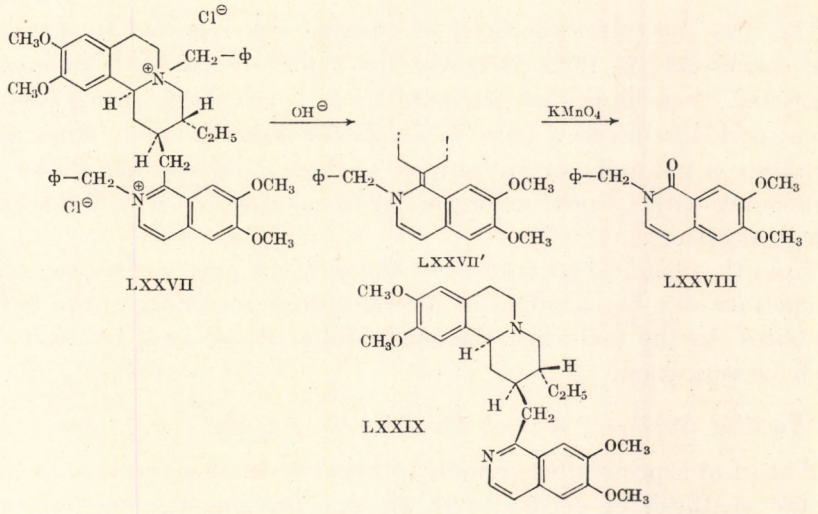
However, no deduction regarding the structure of bases may be drawn merely from the immonium ion character of salts, since in such cases isomerization to enamine structure is a frequent phenomenon (cf. for example [102, 161]). The ultra-violet spectrum of the base, on the other hand, was identical with that of the N-acyl derivatives [72] — which obviously can only be of a structure with exocyclic double bond — but at the same time it was identical with the spectrum of the oxalate as well; therefore, a study of the ultra-violet spectra does not seem to be suitable to decide the problem.

According to the infra-red spectra, there is no absorption band in the range of 3500–3100 cm^{-1}, characteristic of an N–H bond. This fact is in contrast with the structure containing an exocyclic double bond. No proton exchange proceeded in the presence of heavy water either, nor could an N–D bond

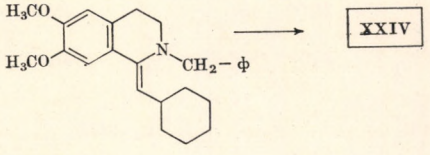
be detected, which again supports the hypothesis of an endocyclic structure. Accordingly, the structure of O-methylpsychotrine is correctly represented by formula LXXVI*, however, the substance is capable of prototropic rearrangement, and thus for instance under the effect of acylating agents its structure is shifted towards form LXXIV* [69, 72]. The 6′ position for the free hydroxyl group in psychotrin [142b] has also been established by an unequivocal synthesis.

(iii) *Emetamine*

Battersby et. al. [13, 21] oxidized the bis-chlorobenzylate of emetamine (LXXVII*) in alkaline medium — through the anhydrobase LXXVII* — to N-benzyl-6,7-dimethoxyisoquinolone (LXXVIII), and thus proved that the material contained an aromatic isoquinoline ring.



That the double bond of isoquinolone in the 3,4-position was not formed during the oxidation is proved by the fact that under similar conditions the anhydrobase LXXX became converted into N-benzylcorydaldine (XXIV).



LXXX

Furthermore, emetamine can be reduced to isoemetine (LXIX) [45] by means of sodium and alcohol, hence the substitution of the tetrahydroiso-

quinoline ring in isoemetine by an isoquinoline ring gives the structure of emetamine: LXXIX*.

(iv) *Protoemetine*

Protoemetine is a representative of the probably very rare type of alkaloids containing an aldehyde group. It shows characteristic aldehyde reactions — for instance the Tollens reaction [26]. Its structure as represented by XX* is further supported by the fact that emetine may be synthesized from this compound in well-controllable steps [26, 141].

III. SYNTHESIS OF IPECAC ALKALOIDS

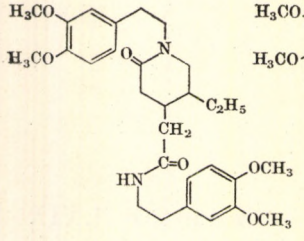
1. Synthesis of Emetine

The first successful synthesis of emetine was reported by Evstigneeva and others [71] in 1950. However, this synthesis had little stereoselective character, thus numerous attempts have been made during the subsequent period to devise a more rational and possibly highly stereoselective synthesis. A not unimportant purpose of these syntheses was the search for compounds with a construction similar to emetine, but with better pharmacological effects.

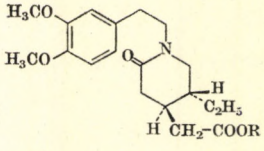
From the chemical point of view, the methods proposed for the synthesis of emetine may be classified in several groups according to the key intermediates. In the following, the successful syntheses will be dealt with in such classification.

(i) *Emetine Syntheses through the Diamide LXXXI*

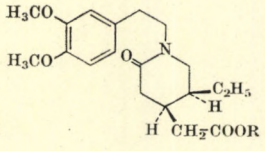
Historical aspects offer grounds to begin discussing the various methods of the synthesis of emetine with the one utilizing the key intermediate LXXXI. The diamide in question can be prepared through the piperidone derivative LXXXII; the first synthesis of emetine, too, was achieved in this way [71].



LXXXI



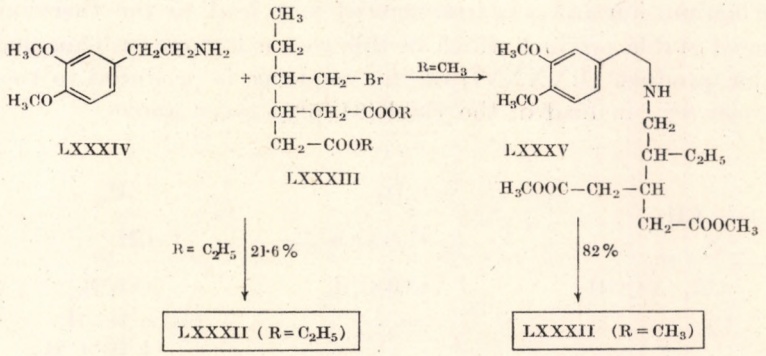
a



b

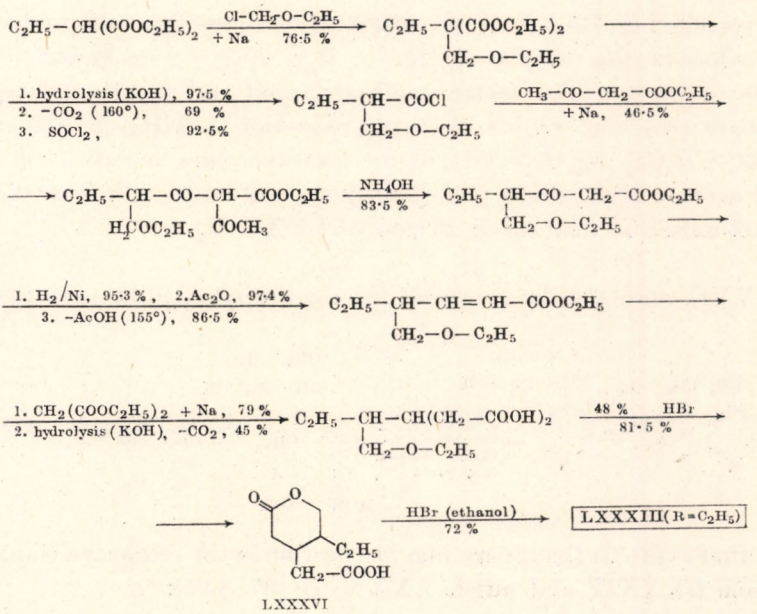
LXXXII

(a) *Preparation of the piperidone derivative LXXXII.* – One of the procedures serving for the synthesis of the piperidone LXXXII is the reaction of the glutaric ester derivative LXXXIII with β-(3,4-dimethoxyphenyl)-ethylamine (LXXXIV).

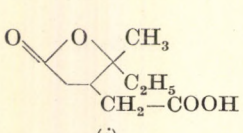


The reaction can be accomplished in one step in 21·6 % yield by refluxing the reactants in toluene [164], or in two steps, preparing first compound LXXXV in isopropyl ether, then heating it at 190–200 °C. The latter method gives a yield of 82 % [120].

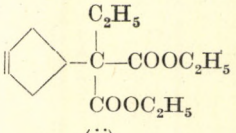
Ester LXXXIII, required for the reaction, can be prepared from α-ethyl-malonic diethyl ester, through the following series of reactions [163, 164]:



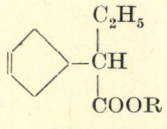
Recently Weisbach and others [154a] have found that the above reaction leading to lactone LXXXVI is not unambiguous. During the acid cleavage of the primary alkyl ether one carbon is removed from the tertiary centre. This type of reaction is often known to occur *via* the tertiary carbonium ion and a rearrangement may lead to the thermodynamically most stable product which in this case is lactone (*i*). Thus the reported major product (LXXXVI) of this sequence is produced to the extent of at most 8%, instead of the yield (81·5%) given above.



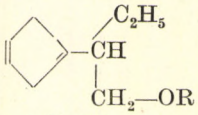
(*i*)



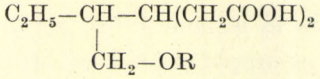
(*ii*)



(*iii*) *a* R=H
b R=C_2H_5



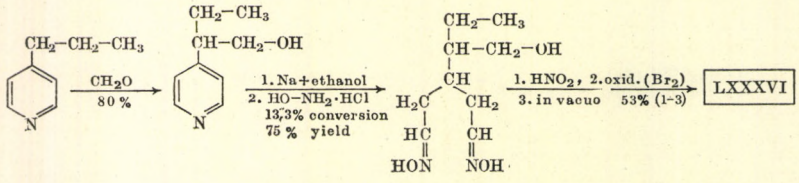
(*iv*) *a* R=H
b R=$COCH_3$



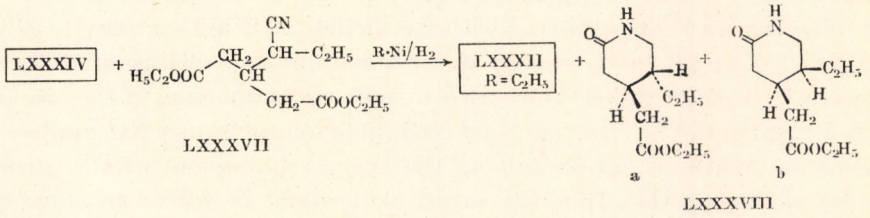
(*v*) *a* R=$COCH_3$
b R=H

For producing LXXXVI an alternative route was devised. Diethyl ethylmalonate was alkylated (Na—C_6H_6) with $\triangle^3$-cyclopentyl tosylate to give (*ii*) (80%). The latter was hydrolyzed and decarboxylated to the mixture of (*iii*)*a* and (*iii*)*b*, which upon reduction ($LiAlH_4$) gave (*iv*)*a*. Oxidation (O_3–CH_2Cl_2; Ag_2O–NaOH) of the corresponding acetate (*iv*)*b* gave the diacid acetate (*v*)*a*, which was directly saponified to (*v*)*b*. The diacid (*v*)*b* was lactonized by heating in xylene to LXXXVI.

LXXXII may also be prepared from γ-propylpyridine [118]:

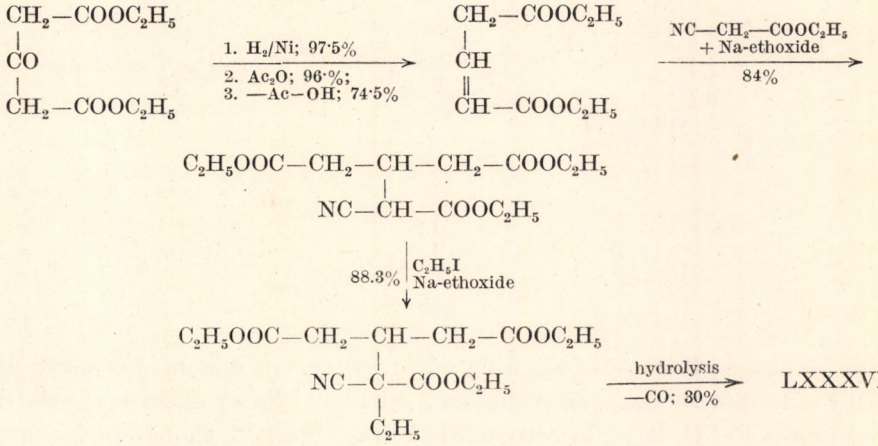


Another route to the piperidone in question is the reductive condensation of amine LXXXIV and nitrile LXXXVII [67, 147]:



LXXXVII LXXXVIII

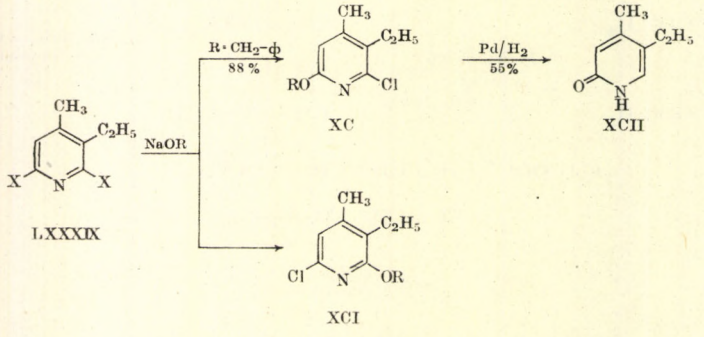
Compound LXXXVII is, in turn, accessible through the method shown below [70]:



On performing the reductive condensation with the ethyl ester (LXXXVII), the yield was 34%, while use of the analogous methyl ester yielded 43·7% [67]. As a by-product 8–9% of α-piperidone (LXXXVIII) was formed. Another isolated by-product was di-homoveratrylamine.

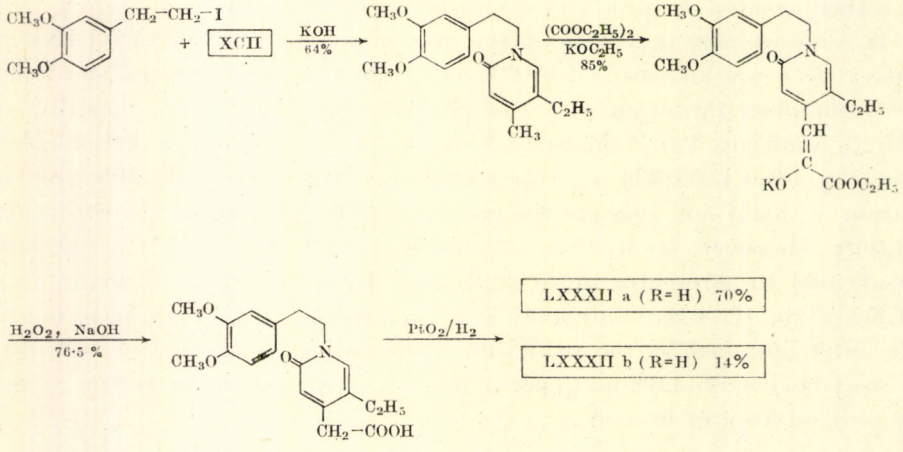
It is noteworthy that while in the case of compound LXXXVIII Evstigneeva [67] was able to isolate the two expected stereoisomers, she failed to accomplish the same with the piperidones LXXXII (R = methyl or ethyl). Similarly, Van Tamelen and others [147] — who succeeded in isolating ethyl ester LXXXII in 29% yield as the result of the reductive condensation — could not separate the two isomers even by thorough chromatography. However, on hydrolyzing the ester and subjecting the obtained crude acid to chromatography, 33% *trans* (LXXXIIa, R = H) and 6% *cis* (LXXXIIb, R = H) compound were isolated (cf. p. 68 (ii)). Accordingly, the ester obtained by the reductive condensation — which had been previously believed to be homogeneous [67, 72] — turned out to consist actually of a mixture of isomers.

Barash and others [11] prepared the piperidone derivative LXXXII in the following way. They started with 2,6-dichloro-3-ethyl-4-methylpyridine (LXXIX, X = Cl), and reacted it with sodium alcoholates of different types in 1 : 1 molar ratios. They tried to shift the proportion of the two isomers formed in the reaction (XC and XCI) in favour of isomer XC, applicable in emetine synthesis, by increasing the space requirement of the alcohol employed, hoping that thus the substituent would be forced to enter the sterically less hindered site. In the case of isopropyl alcoholate the yield of the undesired isomer (XCI, R = isopropyl) was more about 20%; the



ratio became still more favourable when benzyl alcoholate was used, and with the sodium compound of diphenyl carbinol almost exclusively the desired product (XC, R = diphenylmethyl) was obtained, though in the latter case, the yield was decreased.

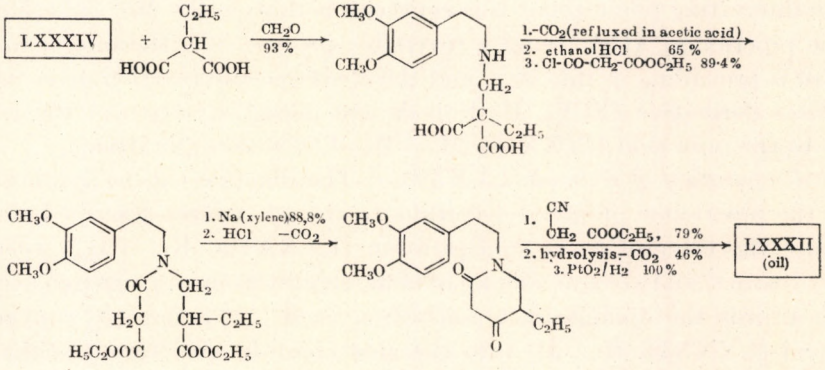
Hydrogenolysis of compound XC afforded directly the pyridone XCII.



It should be mentioned that preparation of compound XCII was also attempted from β-collidine (LXXXIX, X = H) by reacting the latter with sodium amide to give the corresponding amino derivative and then pyridone. However, in this case the undesired isomer was preferentially formed, due to the prevailing electron displacement effects.

Pyridone XCII was converted into compound LXXXII (R = H) in the way outlined below; fractionated crystallization afforded the two diastereoisomers.

An apparently more convenient synthesis of the isomers LXXXII was suggested by Ban [5], consisting of the steps shown below:

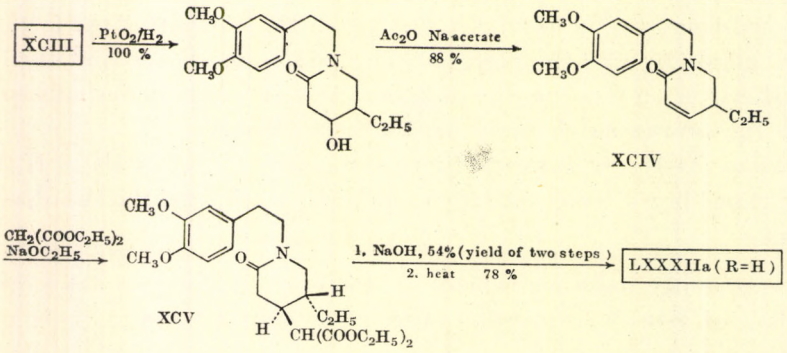


XCIII

With the above method Ban obtained the mixture of isomers in the form of an oil and he did not proceed to separate it. Barash et. al. [11] found on repeating the sequence of reactions described by Ban that condensation of ketone XCIII with cyanoacetate ester, followed by converting the compound formed (LXXXII, R = H) to the carboxylic acid, yielded only traces of crystalline product. However, when cyanoacetic acid was used instead of its ester as the condensation partner, decarboxylation, conversion of the cyano group into ester, catalytic hydrogenation and hydrolysis of the ester group gave rise to crystalline *trans* (LXXXIIa, R = H) and crystalline *cis* (LXXXIIb, R = H) compounds in good yields.

XCIII
1. CN
CH_2—COOH
2. ethanol HCl → LXXXIIa (R=H), 56·5%
3. PtO_2/H_2 4. hydrolysis (KOH) → LXXXIIb (R=H), 12%

Battersby and Turner [33] prepared the desired compound from dioxopiperidine (XCIII) in another way.



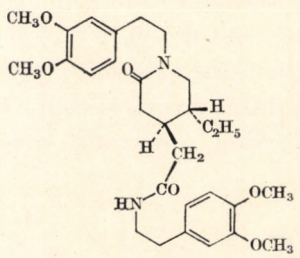
The interesting point about this synthesis is that — the Michael addition to the piperidone XCIV being a reversible process — a thermodynamical control is prevailing in this step, and the stereospecific reaction gives solely the *trans* derivative (XCV). Hydrolysis and decarboxylation of the latter leads to the pure acid LXXXIIa (R = H) of *trans* configuration.

(b) *Preparation of diamide LXXXI.* — The diamide can be synthesized from the piperidone prepared according to the procedures described above. Heating the ester of *trans* configuration (LXXXIIa, R = CH_3) with an excess (more than two equivalents) of homoveratrylamine in nitrogen atmosphere affords the diamide in quantitative yield [147]. Ban [5] converted the acid (LXXXII, R = H) into the acid chloride with thionyl chloride, and condensed it with homoamine, but the mixture of isomers was obtained as an oil only.

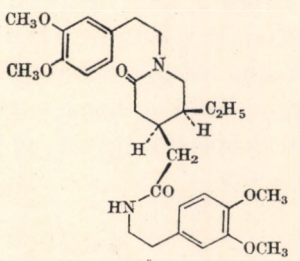
Barash et. al. [11] started with the stereochemically pure *trans* LXXXIIa (R = H) acid which was converted into the mixed anhydride by means of alkyl chlorocarbonate in dimethylformamide solution in the presence of triethylamine. The next step consisted in the acylation of homoveratrylamine (LXXXIV) by means of the obtained mixed anhydride. The yield was almost quantitative, however the diamide LXXXIa could not be crystallized.

Evstigneeva and others [70] reduced the nitrile LXXXVII in the presence of a large excess (1 : 6 molar ratio) of homoveratrylamine (LXXXIV) to obtain again a mixture of the stereoisomers LXXXII in 86% yield, from which two fractions could be isolated by virtue of their different solubilities. The *cis* compound (LXXXIb) (30%) was soluble in toluene, while the *trans* (LXXXIa) (56%) was not. Accordingly, the authors accomplished the separation of isomers at this stage.

(c) *Preparation of emetine from diamide LXXXIa.* — A simultaneous double Bischler–Napieralski type ring closure of diamide LXXXIa gives



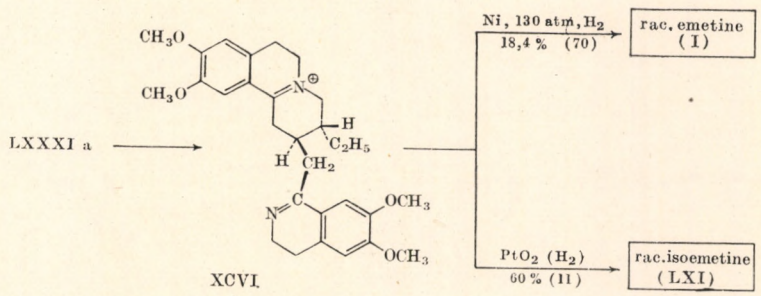
LXXXI a



LXXXI b

rise to dehydro-O-methylpsychotrine (XCVI). Evstigneeva and others [70], furthermore Van Tamelen et al. [147] accomplished the ring closure with $POCl_3$ in toluene, and worked up the crude, oily product.

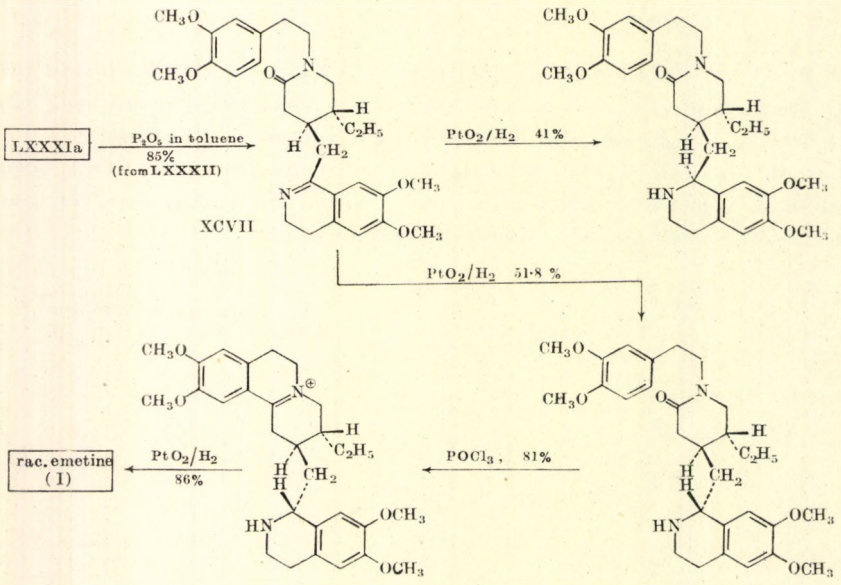
Barash and others [11] found that the cyclization with $POCl_3$ occurred most readily in the absence of a solvent (75% yield); the product was isolated as the crystalline iodide-hydroiodide.



Hydrogenation of the chloride-hydrochloride of XCVI at 70–80°C and 130 atm., in the presence of Ni catalyst, gave the crude emetine base in 18·4% yield [70]. On catalytic hydrogenation of the compound in the presence of platinum oxide, as well as in the reduction of the iodide-hydroiodide with $LiAlH_4$ in tetrahydrofuran solution, Van Tamelen et al. [147] obtained a mixture of emetine and isoemetine; the products, however, were not separated.

The only product isolated in about 60% yield by Barash and others [11] from the hydrogenation of the chloride-hydrochloride of XCVI in the presence of PtO_2 at atmospheric pressure was isoemetine (LXI), and though the presence of emetine could be detected by paper chromatography (the relative R_f values of the two substances in hydrochloric acid–methyl ethyl ketone mixture, using ascending technique were 1 (emetine): 0·62 (isoemetine)). A preparative separation could not be effected. It was found, however,

that better results were obtained if the cyclization of the diamide LXXXI was carried out in two steps. Though reduction of the amide XCVII obtained in the first step led to a heterogeneous product, the substance isolated from the second cyclization, being a 11b-N dehydro derivative, could be hydrogenated stereospecifically (cf. p. 80 (iv)), yielding practically homogeneous racemic emetine.

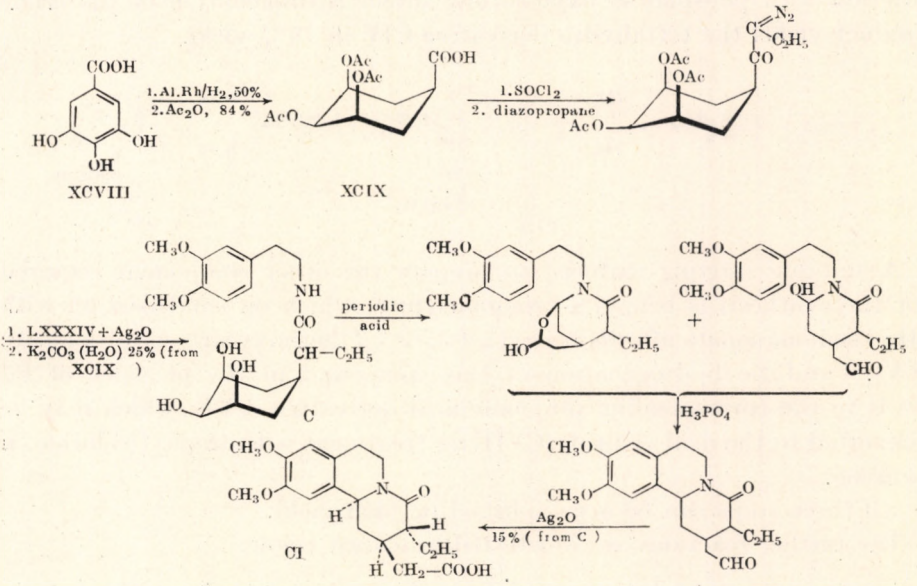


Racemic emetine (I) and racemic isoemetine (LXI) can readily be separated through their oxalates. The hydrogen oxalate of racemic emetine crystallizes well from methanol, while that of racemic isoemetine does not [47]. The compounds can also be purified through the salts formed with camphor-10-sulphonic acid [70].

The resolution of synthetic racemic emetine can be accomplished with (+)-O,O-dibenzoyltartaric acid, by dissolving the components in a mixture of methanol and ethyl acetate; on standing, the salt of (—)-emetine (I*) corresponding to the natural substance is deposited first [11]. When the resolution of the antipodes is made with N-acetyl-L-leucine, it is again the salt of (—)-emetine to crystallize first from methanol–ether mixture [47, 60]. The resolution may be carried out also by the use of (+)-camphor-10-sulphonic acid [72].

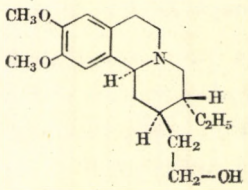
(ii) *Synthesis of Emetine through Lactam CI*

Burgstahler and Bithos [53, 54] devised an ingenious synthesis starting with gallic acid (XCVIII). Hydrogenation of this substance in the presence of aluminium catalyst containing 5% rhodium gave hexahydrogallic acid in 45–50% yield, with each substituent in *cis* orientation. The further course of the synthesis is readily understood from the series of equations shown below. The drawback of this synthetic route is that all intermediary products from triacetylhexahydrogallic acid (XCIX) to the end-product (CI) — with the exception of the acid amide C — are of oily consistence, and the overall yield hardly exceeds 1·5%.

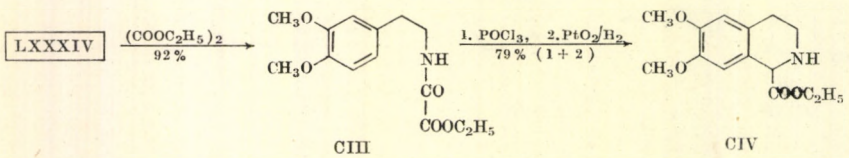


In the course of the last step two stereoisomers (m. p. 223–225 °C and 201–202 °C, respectively) could be isolated besides the lactam CI, the melting point of which was 190–191 °C. The steric structure of lactam CI shown in the formula could be proved by the fact that reduction of its methyl ester with $LiAlH_4$, tosylation of the resulting alcohol and simultaneous reductive removal of the tosyloxy group and the oxygen of the lactam group gave XIX (cf. p. 68–71 (ii)). The steric structure of compound CI is supported furthermore by the fact that reduction of its methyl ester with $LiAlH_4$ affords CII, which is identical with the alcohol prepared by reduction of ester XXXII whose steric structure had already been proved (cf. p. 74 (ii)).

The lactam acid CI has also been synthesized by the essentially different method given below [76].



The amine LXXXIV was acylated by means of ethoxalyl chloride in 35% yield [22], and with diethyl oxalate in 92% yield [76], respectively. The resulting carboxamide (CIII) can be cyclized with phosphorus pentoxide in 48% yield [22]. However, it is more advantageous to carry out the reaction with phosphorus oxychloride; direct hydrogenation of the crude product yields the tetrahydro derivative CIV in 79% yield.

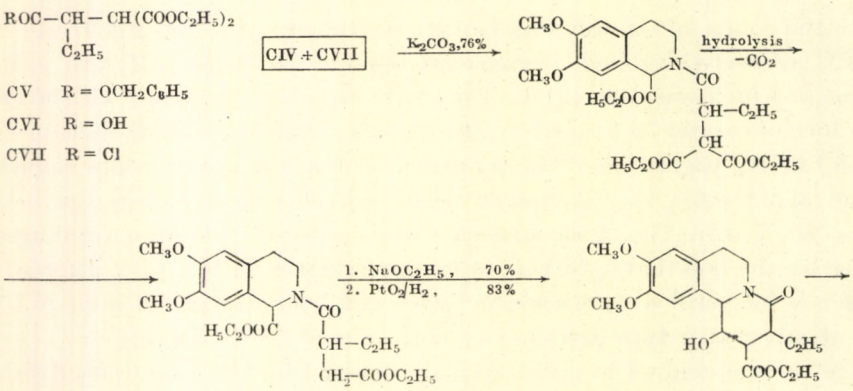


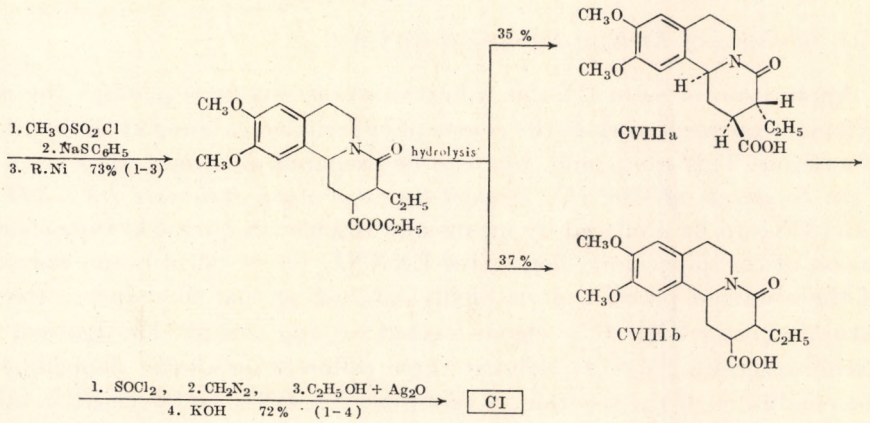
CIII CIV

A suitable starting material to prepare the other component required for the synthesis is benzyl α-bromobutyrate, which on condensation with ethyl sodiomalonate affords benzyl α-ethyl-β,β-diethoxycarbonylpropionate (CV). Catalytic hydrogenation of this compound in the presence of Pd leads to the corresponding propionic acid derivative (CVI), which may be converted to the acid chloride (CVII) by treatment with thionyl chloride in benzene.

All three steps can be accomplished in good yield.

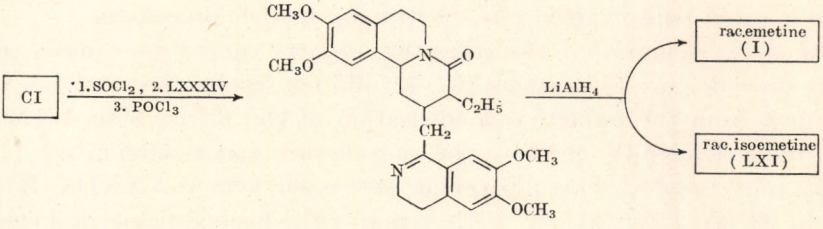
The further reactions are illustrated as shown below:





Refluxing of the carboxylic acid CVIIIb in alkali converts this compound into a mixture of isomers, of which CVIIIa, the compound with the desired steric structure, can be isolated; in this way CVIIIb becomes gradually converted to the product applicable in the further synthesis. The configuration of compound CVIIIb has not been elucidated as yet, but it is notable that none of the four possible 2,3-*cis* emetine isomers prepared later by Brossi and Schnider [52], was identical with the emetine isomer deduced from compound CVIIIb. Therefore, it would be interesting to clear up the question, especially in view of the problems mentioned in connection with the conformational relations of the benzo(a)quinolizine ring.

In the course of the further synthesis towards emetine, the acid CI is converted to the oily carboxamide by reacting its ester [54] or chloride [76] with homoveratrylamine (LXXXIV). The Bischler–Napieralski ring-closure of the product gives CIX. This compound could not be crystallized either, in spite of thorough chromatographic purification. Reduction of the lactam by means of $LiAlH_4$ leads to a mixture of racemic emetine and racemic isoemetine, from which the former can be isolated in 12% yield, calculated for the starting lactam acid (CI).

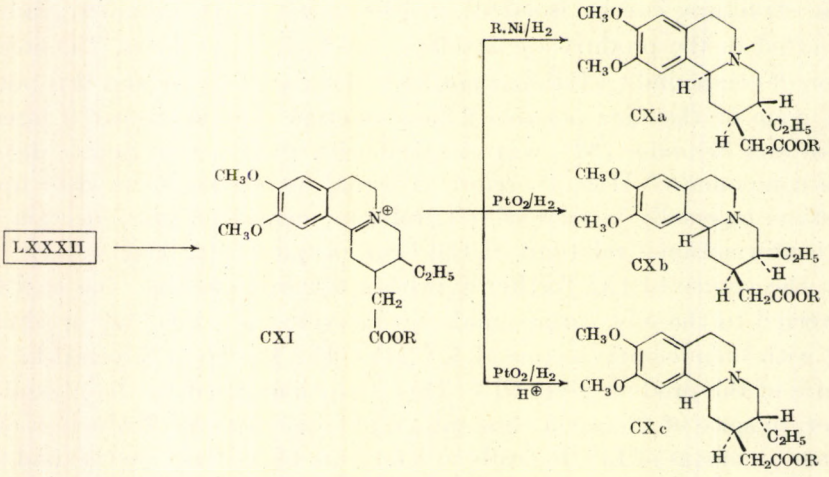


CIX

(iii) *Synthesis of Emetine through Ester CXa*

Application of ester CXa as a key substance induced perhaps the most extensive research work in the course of experiments aiming at the synthesis of emetine. This compound, too, can be prepared in several ways.

(a) *Synthesis of ester CX through the piperidone derivative LXXXII.* — Ester CX can be prepared by means of a Bischler–Napieralski type condensation of the piperidone derivative LXXXII (cf. p. 93 (a)), and reduction of the obtained product. It is highly interesting that in reducing the unsaturated ester CXI (R = ethyl) formed by ring closure, Evstigneeva and Preobrazhenskii [67, 72] isolated three different products, depending on the conditions of the reaction. Structures CXa, CXb, and CXc (R = ethyl) have been assigned to the products obtained by catalytic reduction in acid medium in the presence of Pt, by hydrogenation in neutral medium,



and by reduction in neutral medium in the presence of Raney nickel, respectively. The structure of compound CXIa has been unequivocally proved, since it could be converted into emetine by further procedures.

The steric structure of the other two isomers cannot be considered entirely proved, since the authors [67, 72] did not resolve the ester LXXXII, obtained from the reductive condensation of the nitrile ester LXXXVII and amine LXXXIV, prior to the ring closure and reduction (cf. [147]).

The pure isolated *trans*-piperidone carboxylic acid (LXXXIIa, R = H) (cf. p. 93 (a)) afforded on methylation with diazomethane, followed by condensation with $POCl_3$ in toluene, and hydrogenation of the product in

ethanol in the presence of platinum, the crystalline ester CXa (R = methyl) in 80·5% yield [147].

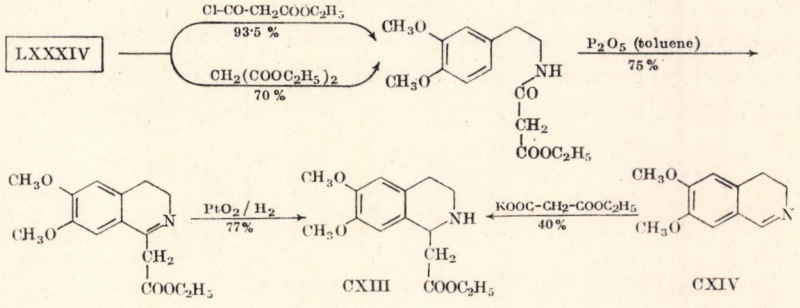
Barash and others [11] esterified carboxylic acid LXXXIIe (R = H) with ethanol and hydrogen chloride, and subjected the product to ring closure, to obtain CXI (R = ethyl) in 86% yield. The iodide of the product could be reduced catalytically in methanol in the presence of platinum in 91% yield. Besides this method, a number of other attempts have been reported, in the course of which hydrogenation has been accomplished in the presence of various catalysts under different conditions. The reduction has also been carried out by means of chemical agents, such as sodium borohydride, sodium dithionite, or formic acid, but also these reductions afforded solely ester CXIa without any sign of the formation of isomers.

Similar observations have been reported by Battersby and Turner [33], in connection with the catalytic reduction of the immonium compound CXI (R = ethyl).

(b) *Synthesis of ester CXa from carboxylic acid CI* [54]. —The lactam acid CI (cf. p. 101 (ii)) can be reduced with sodium and alcohol or, more advantageously, in the presence of a copper chromite catalyst. After esterification of the acid-soluble product and purification by chromatography, CXa is obtained in about 15–20% yield.

(c) *Synthesis of ester CXa through the ketone CXII.* — Consideration of the previous two sections may easily lead to the conclusion that preparation of ester CXa by means of the above methods is a rather cumbersome work consisting of too many steps. For this reason, more simple procedures have been looked for to accomplish the synthesis of the compound in question. Application of ketone CXII offered a promising possibility for the solution of the problem.

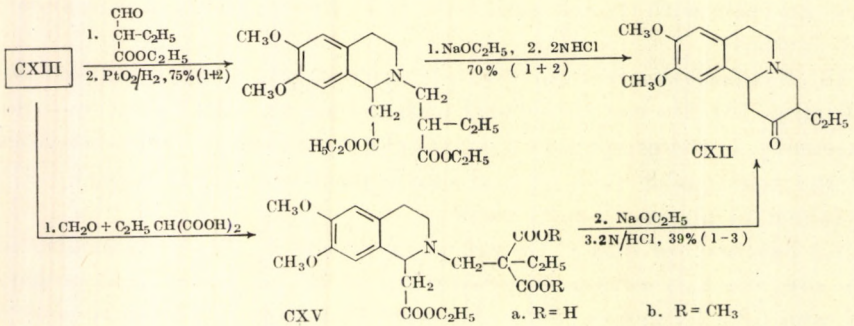
One method for preparing this ketone consists in reacting homoveratrylamine (LXXXIV) either with ethyl malonate chloride, or more conveniently, with an excess of diethyl malonate [32]. Subsequent ring closure and reduc-



tion affords the tetrahydroisoquinoline derivative CXIII. The latter compound may also conveniently be prepared in a single step by the reduction of 6,7-dimethoxy-3,4-dihydroisoquinoline (CXIV) and potassium ethyl malonate; the yield is about 40%.

The desired product (CXII) is then made by reacting with ethyl α-formylbutyrate, catalytic reduction, condensation according to Dieckmann, and refluxing with hydrochloric acid [32].

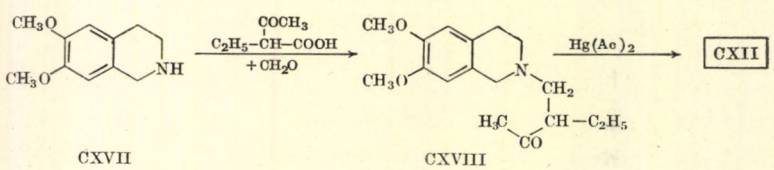
Ketone CXII may be prepared from ester CXIII in another way [51] by reacting it with ethylmalonic acid and formaldehyde. The intermediate dicarboxylic acid (CXV) is methylated with diazomethane or dimethylsulphate to give the ester CXVb. Subsequent Dieckmann condensation and hydrolysis with hydrochloric acid yields CXII (39% from CXIII).



The procedures, in which the dihydroisoquinoline derivative CXIV is transformed to ketone CXII directly in one step, offer a way simpler than those dealt with in the foregoing.

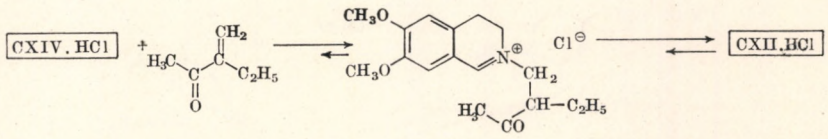
Brossi et. al. [48] prepared ketone CXII in 14% yield by condensing CXIV with 2-ethylbutene-3-one (CXVI) in alkaline medium.

Openshaw and Whittaker [111] synthesized the same product in 68·4% yield by reacting CXIV with 3-dimethylaminomethylpentane-2-one methiodide in alcohol. The same authors prepared the ketone CXII by reacting 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (CXVII) with α-ethylacetoacetic acid and formaldehyde, followed by oxidative cyclization of the product (CXVIII) with mercury(II) acetate; the yield was 54%.



Lénárd and Bite [100] obtained compound CXII in 43% yield by a simple method of reacting α-ethylacetoacetic acid, formaldehyde and 3,4-dihydro-6,7-dimethoxyisoquinoline (CXIV).

Ketone CXII may be prepared most suitably by the reaction of the hydrochloride of CXV with 2-ethylbutene-3-one (CXVI) [37], when the desired product is formed in one step in 90% yield.



CXVI CXIX

In the course of the reaction first a quaternary salt is formed which can be isolated from the reaction mixture by interrupting the process [140]. Under the catalytic effect of an acid, or base, the quaternary salt (CXIX) undergoes ring closure. In more strongly alkaline medium (above pH 8), however, several side reactions occur, for example the base catalyzes the decomposition of the salt CXIX into its components, and the resulting unsaturated ketone may suffer further reactions. Moreover, intermolecular redox processes also take place in strongly alkaline medium [140]. These facts explain the low yields observed by Brossi and others [48], who carried out the reaction in alkaline medium; it is to be noted, however, that good yields can be achieved between pH 7 and 8, notwithstanding the alkalinity of the medium [140].

As to the starting materials, 3,4-dihydro-6,7-dimethoxyisoquinoline (CXIV) can be prepared either from N-formylhomoveratrylamine [133, 136], or — in higher yield — from 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline through the N-chloro compound (oxidation with sodium hypochlorite) [112].

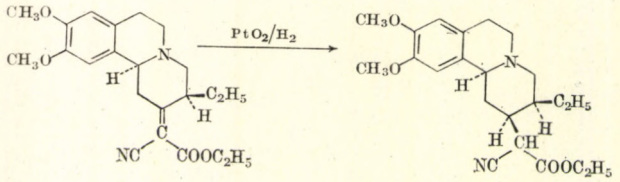
The unsaturated ketone (CXVI) is accessible, e.g., — from α-ethylacetoacetic acid in about 70% yield [139].

The ketone CXII prepared according to the above methods is a homogeneous product; no stereoisomers could be isolated.

Considerable difficulties were observed in converting the ketone CXII into ester CXa, since the presence of the ethyl group in the α-position sterically hinders the reactivity of the oxo group to enter condensation reactions. Battersby and others [32] condensed the ketone with ethyl cyanoacetate, and after hydrolysis hydrogenated the product, catalytically, in the presence of platinum. It is probable that during hydrolysis the exocyclic double bond had migrated to a large extent into endocyclic position, since the compound absorbed only about half of the calculated amount of hydrogen. It is known [46] that an endocyclic double bond cannot be hydrogenated under the given conditions. No crystalline product could be isolated from the reaction.

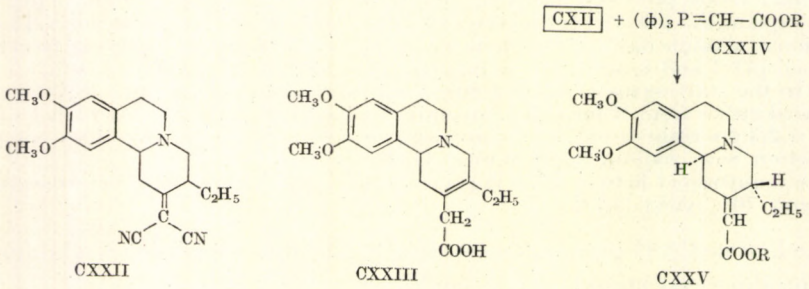
Brossi and Schnider [52] obtained the condensate resulting from the reaction with cyanoacetic acid in crystalline form; the partial hydrogenation of this product was also accomplished (PtO_2/H_2), prior to hydrolysis,

thus eliminating the possibility of the migration of the double bond. It was found, however, that under the conditions of condensation epimerization took place at carbon atom 3 (CXX) [52, 112], and thus hydrogenation, too, afforded a product unusable for further synthesis, containing 2,3-substituents in *cis* orientation (CXXI).



CXX CXXI

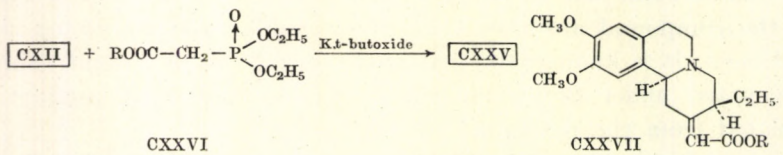
Condensation of ketone CXII may be accomplished in excellent yield also with malonodinitrile [32, 46], but the product (CXXII) cannot be reduced partially, and its hydrolysis affords the caboxylic acid CXXIII containing the double bond in endocyclic position [46].



CXXII CXXIII CXXV

Openshaw and Whittaker [110, 112] prepared the unsaturated ester CXXV in 54% yield by reacting the ketone CXIII in a Wittig reaction with the phosphorane CXXIV at about 150°C.

As compared with the latter reaction, application of the more readily available phosphonic acid esters seems to be preferable; as it is known (see, e.g., [78]), these compounds are also suitable reagents for the conver-



CXXVI CXXVII

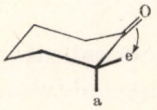
sion of an oxo group into a carbalkoxymethylene group. Szántay, Tőke and Kolonits [142, 142a] prepared the desired ester CXXV in the same

yield as obtainable in the Wittig reaction by reacting the ketone CXII with the ester CXXVI (R = ethyl) at low temperature in dimethylformamide, in the presence of potassium tertiary butylate.

When the reaction was carried out in 1,2-dimethoxyethane, in the presence of sodium hydride, the main product was CXXVII, which is the $C_{(3)}$-epimer of ester CXXV, [112]; accomplishing the reaction in dimethylformamide in the presence of an excess of the latter at elevated temperatures gave the same main product. Openshaw and Whittaker [112] isolated the *cis* and *trans* isomers (in relation to the ethylene bond) of the unsaturated ester CXXVII.

The above reactions of the ketone CXII reveal that substitution of the oxo group by more bulky substituents leads to the preferred formation of $C_{(3)}$-epimeric products, such as CXX and CXXVII.

It has also been observed [16] that the reaction of the ketone CXII with hydroxylamine afforded two isomeric oximes as main products, and two other oximes as by-products, consequently the epimerization of the $C_{(3)}$ atom occurred in this case, too. Though not examined as yet, the adduct prepared from CXXII and malononitrile may also prove to be an epimeric product.



CXXVIII

Presumably, all these phenomena may be traced back to a 2-alkyl effect. As pictured in formula CXXVIII, the substituent of the cyclohexane ring in α-position to the oxo group takes a nearly eclipsed position in relation to the oxygen, if its orientation is equatorial.

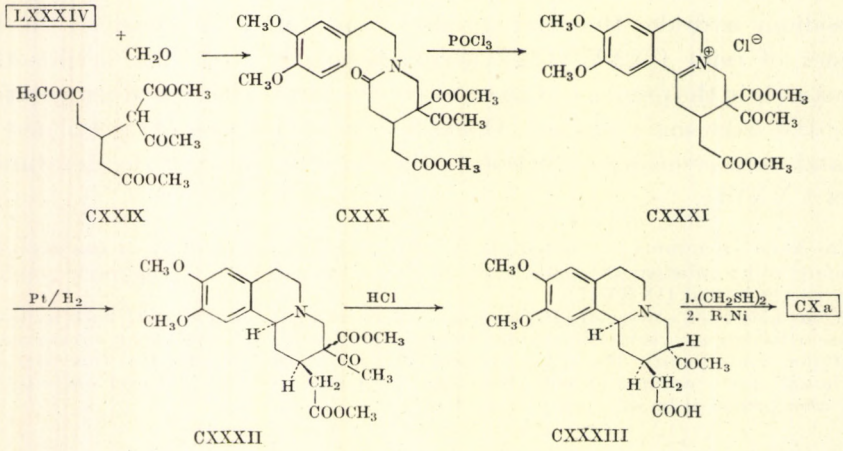
Thus increasing the space requirement of the α-substituent must involve a gradual decrease in the energy difference between the equatorial and axial orientations. According to Allinger and Blatter [2] — for example — if there is an isopropyl group attached to the α-carbon atom, the energy difference between the *a* and *e* positions decreases to 0·4 kcal/mole. Extrapolating these considerations it may be expected that in the presence of more bulky groups attached to a double bond, the substituent occupying the α-position in relation to the former will be more stable in axial than in equatorial orientation. This is the reason why the isomeric compound is easily formed from the above ketone in the presence of a catalyst favouring enolization, and thus the epimerizations discussed in the foregoing are explained.

Hydrogenation of ester CXXV gives preponderantly the ester CXa; for instance, in the presence of palladium catalyst the yield is 74% [112]. Besides this derivative, the $C_{(2)}$-epimeric product is also isolable.

(d) *Preparation of ester CXa according to Van Tamelen et. al.* [150]. — Van Tamelen et. al., starting from biogenetic considerations, elaborated the following procedure for the synthesis of ester CXa.

The Mannich-reaction of homoveratrylamine (LXXXIV), formaldehyde and the triester CXXIX afforded the lactam CXXX in almost quantitative

yield, as an oily product. Treatment with $POCl_3$ in benzene solution gave the immonium salt CXXXI. (The preliminary communication [150] did not give data on yields at this and further steps.)

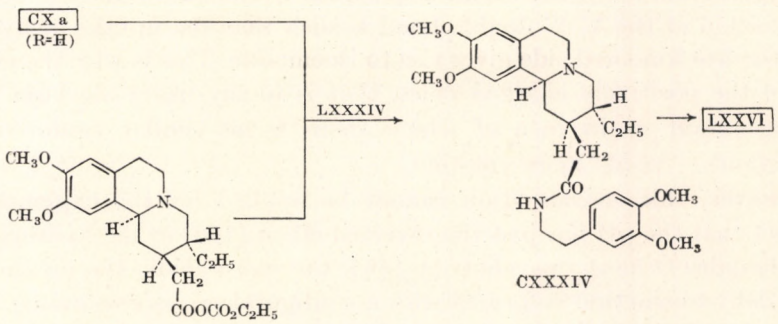


The immonium salt could be crystallized from a mixture of nitrobenzene and petroleum ether. The compound was catalytically hydrogenated in methanol solution in the presence of platinum to give CXXXII. After purification in form of the free base, the β-ketocarboxylic ester was refluxed with 2% hydrochloric acid for a few days, when hydrolysis and decarboxylation occurred. After chromatography and recrystallization from a mixture of chloroform–carbon tetrachloride, the keto-acid CXXXIII could be isolated. The latter was converted into its methyl ester, then to dithioketal by reacting the keto-ester hydrochloride with ethylenedithiol in the presence of boron trifluoride etherate. Desulphuration of the product with Raney nickel in boiling methanol yielded ester CXa.

(e) *Synthesis of emetine from ester CXa.* —The next step in the synthesis of the emetine molecule requires transformation of ester CXa into the amide CXXXIV. This can be accomplished by heating the ethyl ester with homoveratrylamine (LXXXIV) for seven hours at 180–200°C (60·5%) [69], or applying the same procedure to the methyl ester (78%) [114, 147]. According to another procedure, the ester is hydrolyzed, and the resulting acid (CXa, R=H) is refluxed with the amine LXXXIV in xylene (64%) [11], or its triethylamine salt is reacted with alkyl chlorocarbonate, and the obtained anhydride (CXXXV) is treated with the amine (75%) [33].

Ring closure of the amide CXXXIV can be effectuated in 70–90% yield by means of $POCl_3$, to give racemic O-methylpsychotrine (LXXVI). The

reaction is carried out in different solvents, such as chloroform [69], toluene [33, 147], or benzene [11, 47, 112]. Catalytic hydrogenation of the product results in the formation of a mixture of racemic isoemetine and racemic emetine; the purification and resolution have been dealt with in part III (p. 105 (c)).



It is more advantageous, however, to carry out the resolution already in the O-methylpsychotrine stage; it can be best achieved with the aid of (−)-O,O-dibenzoyltartaric acid [33, 112] in ethanol solution, when the salt corresponding to natural (+)-O-methylpsychotrine (LXXVI*) is deposited first in 46·5% (93% of the theoretical) yield [112].

The ratio of emetine (I*) and isoemetine (LXI*) formed in the catalytic reduction of (+)-O-methylpsychotrine (LXXVI*) depends on the pH of the medium. Openshaw and Whittaker [112] separated the isomers by chromatographing the crude product on aluminium oxide; the mixture was applied to the column in trichloroethylene, followed by gradual change of the solvent first to methylene chloride to remove emetine, and then to chloroform, which eluted isoemetine. It was found that reduction with $LiAlH_4$ in ether, or with $NaBH_4$ in methanol yielded the two bases in approximately equal amounts. In the presence of Raney nickel in methanol–triethylamine, or in the presence of platinum in a methanolic solution of KOH, catalytic reduction gave rise to emetine and isoemetine in a ratio of 1·4 : 1. The best result for emetine (a ratio of 1·8 : 1) was obtained by reduction in the presence of platinum and sodium methoxide. In aqueous solution at about pH 5, the amount of isometine formed is 3·5 times as much as that of emetine.

If the mixture obtained after reduction containes a preponderant amount of (—)-emetine, addition of concentrated aqueous hydrogen bromide to the methanolic solution of the mixture is enough to accomplish its isolation, namely, emetine hydrobromide separates in crystalline form, while iso-

emetine hydrobromide and some emetine hydrobromide remain in solution [33, 112].

According to Battersby and Turner [33] the above results experienced during the reduction of O-methylpsychotrine may be explained as follows. The two positive poles — i.e., the two charged nitrogen atoms — tend to keep apart from each other as far as possible. In this conformation, however, the position of the 1′–2′ double bond is such that the uptake of hydrogen from the less hindered side gives rise to isoemetine. This is why the elimination of the positively charged poles, that is to say using the base for the hydrogenation — in case of which there is no similar conformational restriction — yields more emetine.

However, this consideration cannot be applied for the explanation of the fact that the pH has just the reversed effect [140] in the hydrogenation of 2-dehydro-O-methylpsychotrine [46]; the more acidic the medium, the more dehydroemetine is formed with a configuration corresponding to that of emetine, and the formation of isodehydroemetine becomes suppressed.

(f) *Synthesis of the optically active ester CXa**. — The optically active ester CXa* was first prepared semi-synthetically from protoemetine (XX*) [26]. The direct oxidation of protoemetine gives only very poor yields of the acid CXa* (R =H). However, if the oxime is formed first and it is transformed to the nitrile by refluxing with acetic anhydride, subsequent hydrolysis affords the carboxylic acid in good yields; esterification of the acid leads to the optically active compound CXa* (R = methyl or ethyl).

The total synthesis of CXa* can be accomplished by resolving the ketone CXII, and converting the optically active product to the ester CXXV* by means of the above discussed procedure, or through the phosphorane derivative CXXIV [112], or *via* the phosphonic acid derivative CXXVI [141]; subsequent hydrogenation yields the ester CXa.

The problem of the resolution of ketone CXII was very successfully solved by Openshaw and Whittaker [112]. Refluxing the ketone with (—)-camphor-10-sulphonic acid in ethyl acetate gave a 85 % yield (170 % of the theoretical) of the laevorotatory product. Namely, the camphorsulphonic acid salt of the (—)-ketone precipitates due to its slight solubility, while the dextrorotatory isomer in solution suffers gradual racemization under the action of the acid. The small amount of (+)-ketone remaining in the solution can be converted again to racemic ketone CXII by refluxing it with dilute acid.

The advantage of this resolution in comparison to those accomplishing the separation at a later stage (see above) consists in the fact that in the latter cases the undesirable isomers cannot be racemized, consequently their utilization in the synthesis is impossible.

This is probably the first example in literature for a second order asymmetric transformation, which involves the simultaneous inversion of two different centres of asymmetry. The process obviously proceeds through the intermediary formation of the quaternary salt CXIX, in wich the centre of asymmetry at position 11b is no longer present, and in the acid medium the centre at $C_{(3)}$ is racemized through the enol form. This assumption has been supported by the measurements of Szántay and Rohály [140] revealing that a 1 N hydrochloric acid solution of the ketone CXII contains 50 °C about 2–3 % of the quaternary salt in equilibrium with the cyclic form.

The optically active amide CXXXIV* is obtainable from ester CX* in an analogous manner as described in the case of the racemic compound, either by condensing it directly with homoveratrylamine (LXXXIV) [26], or by converting it after hydrolysis into the acid chloride by means of $SOCl_2$, and reacting this product with LXXXIV (84 %) [112]. In another method the sodium salt, obtained by the alkaline hydrolysis of the ester, is converted to acid chloride by treatment with oxalyl chloride, and the latter is reacted with the amine component [26]. The amide obtained can be converted into (+)-O-methylpsychotrine and then into emetine as described in part III (p. 110 (e)).

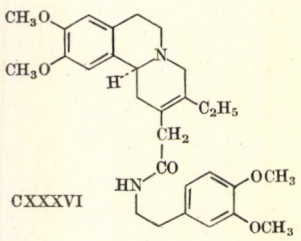
(iv) *Synthesis of Emetine through the Carboxylic Acid CXXIII*

Brossi and others [46] prepared the carboxamide CXXXVI in 77 % yield by reacting the carboxylic acid CXXIII (cf. part III p. 105 (c)) with homoveratrylamine (LXXXIV). Hydrogenation of this amide in methanol solution at 100–120 °C and 100 atm., in the presence of palladium catalyst, led to three isolable amides [47].

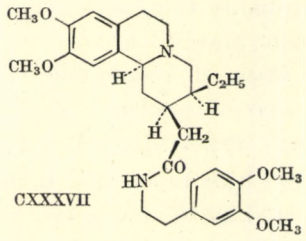
Of these, compound CXXXIV possessing the configuration required for continuing the synthesis of emetine could be isolated in 16·5 % yield. In addition, the isomers CXXXVII, melting at 157–8 °C, and CXXXVIII, melting at 129–30 °C, were also isolated [52].

The surprising feature of the above reaction is the formation of three isomers during the hydrogenation of the unsaturated compound CXXXVI, instead of the expected two. Isomers CXXXVII and CXXXVIII can be oxidized with mercury(II) acetate in good yields to the same 11b-dehydrocompound (CXXXIX), but oxidation of the carboxamide CXXXIV, possessing a configuration identical with that of natural emetine, afforded under the same circumstances a different product; consequently, compounds CXXXVII and CXXXVIII can only be products having *cis* configuration in the 2,3 position.

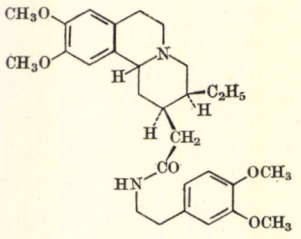
Catalytic hydrogenation of the unsaturated amide CXXXIX gave a mixture containing more than 90 % of compound CXXXVII, and reduction with sodium borohydride yielded exclusively this product; hence, in all probability it is the thermodynamically more stable form thus corresponding to the configuration, given above.



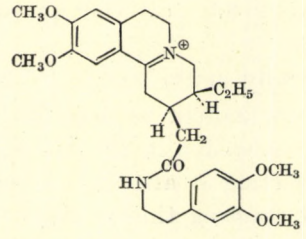
CXXXVI



CXXXVII



CXXXVIII



CXXXIX

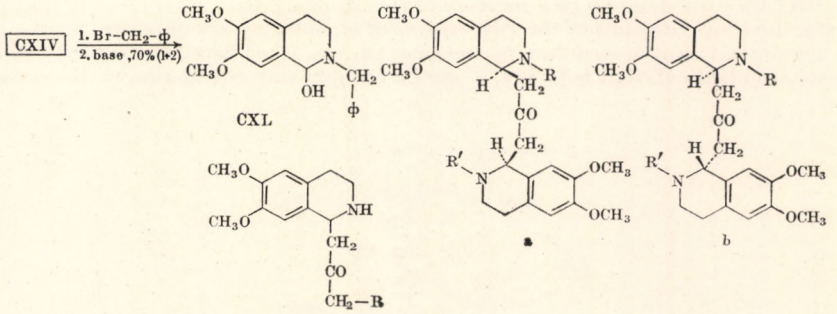
Heating of either amide CXXXVII or amide CXXXVIII in hydrogen atmosphere in the presence of palladium catalyst gives rise to a mixture of epimers containing the two products in about equal amounts; the mixture is easily separable by crystallization. This may account for the appearence of isomer CXXXVIII as a product of the catalytic hydrogenation of the amide CXXXVI.

Amide CXXXIV prepared as above is convertible into emetine through O-methylpsychotrine according to the procedure described in the foregoing (cf. part III (v), below).

(v) *Synthesis of Emetine through the bis-Isoquinoline Derivative CXLIIa*

According to a procedure quite different from the methods dealt with in the preceding chapter [58, 59, 60], the two isoquinoline parts to be found in the emetine molecule are combined first, and the middle part of the molecule is synthesized subsequently. The starting compound of this method, too, is 3,4-dihydro-6,7-dimethoxyisoquinoline (CXIV), which on quaternerization with benzyl bromide followed by treatment with trimethylamine gives the pseudobase CXL in 70% yield. Condensation of the latter with ketone CXLI in ethanol at room temperature, in the presence of sodium carbonate, yields a mixture of compounds CXLIIa and CXLIIb, containing 60% *a* and 40% *b* component. The two isomers are separable by means of fractionated crystallization of their hydrochloride salts.

A mixture of the above two substances (50–50%) is obtained also through the reaction of the pseudobase CXL and the ketocarboxylic acid CXLIII.



CXLI R = H
CXLIII R = COOH

CXLII R = benzyl , R′ = H
CXLIV R = R′ = H
CXLV R = R′ = CO–C_3H_7
CXLVII R = benzyl
R′ = CH_2–CH_2–C(=O)–CH_3

Component CXLII may be prepared by reacting the dihydroisoquinoline CXIV with acetoacetic acid. If the electrophilic activity of carbon atom 1 in the dihydroisoquinoline is increased by means of salt formation at the adjacent nitrogen atom, the reaction can also be accomplished with components of less nucleophilic character. Thus, for example reacting the hydrochloride salt of derivative CXIV with acetone in the presence of pyridine as a base (cf. also [38]), compound CXLI is formed in about 60% yield.

The carboxylic acid CXLIII can be obtained by the interaction of the dihydroisoquinoline CXIV and actonedicarboxylic acid. The substance is rather unstable, spontaneous decarboxylation may easily lead to the derivative CXLI.

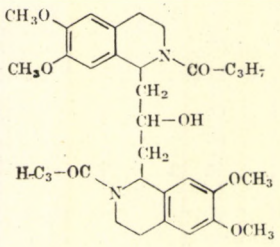
The steric structures of isomers CXLIIa and CXLIIb could be determined as follows.

Reaction of the dihydroisoquinoline CXIV (2 moles) with one mole of acetonedicarboxylic acid in aqueous medium containing some alcohol afforded about 60% yields of a mixture of isomers CXLIVa and CXLIVb (cf. [35, 36]). The two products are mutually interconvertible into each as the result of a process leading to equilibrium in solution. This reaction made possible also the accomplishment of a selective synthesis. On treating the isoquinoline hydrochloride salt with acetonedicarboxylic acid in the presence of some pyridine, the hydrochloride salt of the pure isomer CXLIVa deposited in 60% yield. However, if the condensation was carried out in the presence of aqueous sulphuric acid, the sulphate of the other isomer (CXLIVb) deposited only, in 54% yield. When isomer CXLIVa was allowed to stand with aqueous sulphuric acid under similar conditions, inversion took place and compound CXLIVb deposited in form of the sparingly soluble sulphate, producing a shift of the equilibrium towards this direction.

The two products could be readily identified in the form of the corresponding N,N-dibutyryl derivatives (CXLVa and CXLVb), that have sharp and considerably different melting points.

On reducing the two substances to the alcohol CXLVI, it was found that whilst ketone CXLVa gave rise uniformly to one product only, the alcohol obtained from ke-

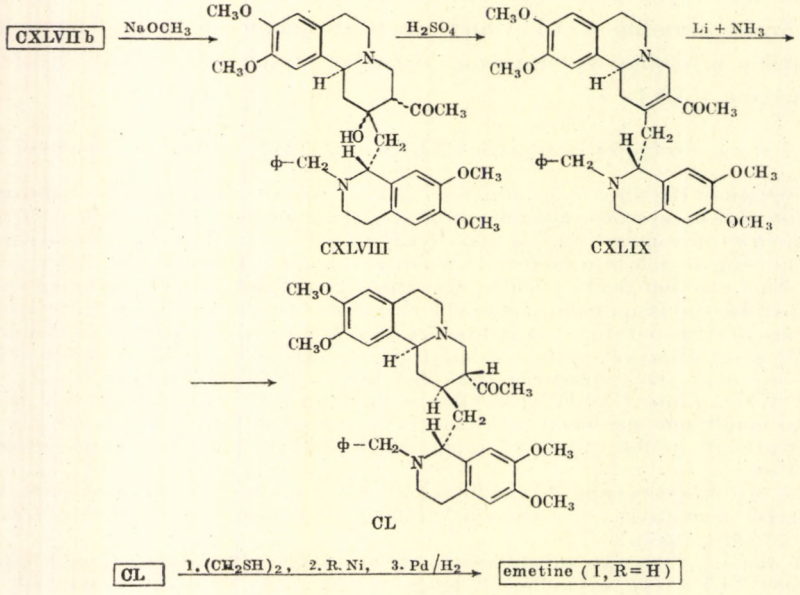
tone CXLVb turned out to be a mixture of two stereoisomers, thus proving that in the *b* series the configurations of the two centres of asymmetry are opposite, consequently this corresponds to the structure of emetine. On the other hand, the configurations in series *a* are identical, that is to say, it can be brought into correlation with isoemetine.



CXLVI

Compounds CXLIIa and CXLIIb could be debenzylated by means of catalytic hydrogenation, affording products CXLIVa and CXLIVb, by which their steric structure has been clarified, too.

When product CXLIIb was reacted with methylvinylketone in benzene, derivative CXLVIIb could be obtained in good yield, which was convertible into CXLVIII by means of intramolecular condensation in benzene, with the aid of sodium methoxide. Dehydratation of the latter by heating in sulphuric acid gave the ketone CXLIX in 60% yield.



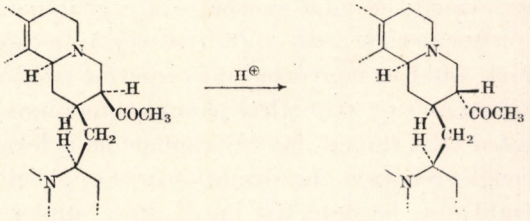
CXLVIII CXLIX CL

CL

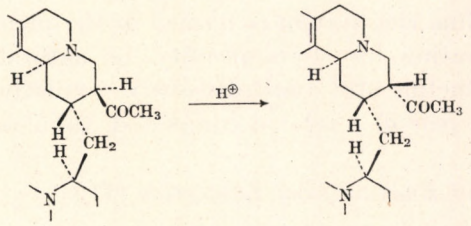
The procedure of reducing the ketone (CXLIX) with Li and ammonia in tetrahydrofuran, followed by refluxing with acid and chromatographing the product, gave the unsaturated ketone CL in about 35% yield.

In connection with the reduction of the ketone CXLIX, the task is the *trans*-diaxial addition of the hydrogen molecule to the double bond in conjugation with the carbonyl group. This type of addition would lead namely to the more stable configuration of the newly formed asymmetric centres at $C_{(2)}$ and $C_{(3)}$ as they are also found in emetine. Barton and Robinson [12] have pointed out that reduction of the olefinic bond of α,β-unsaturated ketones with alkali metals in liquid ammonia usually gives rise to the thermodynamically more stable configuration at the β-carbon atom. Investigations with the 1′ epimeric modification of ketone CL (series *a*) indicated that when the reduction is carried out with calcium metal in ammonia, the product possessing the more stable configuration at the β-carbon atom is formed, indeed, in a ratio of 1 : 6, but at the α-carbon atom a kinetic control is in operation, and thus compounds CLI and CLII are obtained together.

Both can be isomerized to the more stable derivatives CLIII and CLIV, respectively, by means of boiling with acid.



CLI CLIII



CLII CLIV

Presumably the same epimerization proceeding under the effect of acid leads from the above discussed (cf. p. 109 (d) compound (CXXXII), prepared by Van Tamelen and others, to product CXXXIII.

N-Benzylemetine (I, R = benzyl) is obtained from ketone CL in 20–30% yield through the corresponding ethylenethioketal, by desulphurating it with Raney nickel in hot xylene. Reductive debenzylation of the latter compound in the presence of Pd affords racemic emetine.

(vi) *Synthesis of Emetine by Means of the Pictet–Spengler Method*

All syntheses discussed so far — with the exception of that summarized in part III (p. 114 (v)) — applied the Bischler–Napieralski method of ring closure to form ring D of emetine.

The successful isolation of protoemetine (XX*) from plants accomplished recently [19, 20] supported the idea that the biosynthesis of emetine also proceeds through protoemetine as an intermediate [26]. On the basis of this assumption, Battersby and Harper [26] attempted to condense isolated, natural protoemetine with homoveratrylamine (LXXXIV), but they failed in isolating emetine or an emetine-like product.

Szántay and Tőke [141] synthesized protoemetine in 80% yield by the reduction of the ester CXa (R = ethyl) with diisobutyl aluminium hydride in toluene at —60 °C. The reduction could be achieved with the optically active ester (CXa*) without racemization.

The authors performed the total synthesis of cephaeline (LXX*, R = H) by condensing protoemetine with β-(3-hydroxy-4-methoxyphenyl)-ethylamine. The product could be converted into emetine in good yield on treatment with diazomethane or trimethyl-phenylammonium hydroxide [80].

It should be noted that though mainly cephaeline is formed in the course of the Pictet–Spengler reaction, the formation of isocephaeline (the 1′-epimer of cephaeline) could also be detected in all cases, under various reaction conditions. A similar situation is found when protoemetine is condensed with β-(3,4-dihydroxyphenyl)-ethylamine. The presence of isocephaeline, as invariably a byproduct of the Pictet–Spengler reaction, suggests either that the isoquinoline ring system is formed in the plant by some enzymatic reaction accounting for stereospecifity, or naturally occurring isocephaeline and isoemetine still await discovery, similarly as it has occurred with the epimeric pair of a related compound, tubulosine [122a, 138a].

(vii) *Preparation of Emetine from Isoemetine* [112]

Since in the overwhelming majority of the syntheses dealt with so far emetine is accompained by a considerable amount of isoemetine (LXI), it is an attractive task to reoxidize it to O-methylpsychotrine for re-use in the synthesis.

Catalytic dehydrogenation of the substance in mesitylene at 180 °C for 1·5 hours, in the presence of 10% palladium charcoal and nitrobenzene as hydrogen acceptor, yielded O-methylpsychotrine (LXXVI) in 39% yield. Chlorination at the secondary (2′) nitrogen atom — which can readily be effected with aqueous hypochlorite solution — followed by elimination of

hydrochloric acid by means of alkaline treatment, proved to be a much better method. This way isoemetine may be transformed to O-methylpsychotrine in 90—92% yield. The two processes can be accomplished in one step, without isolation of the N-chloro compound, and is applicable both in the case of racemic and optically active compounds. O-Methylpsychotrine is converted then to emetine by reduction, as discussed above (part III. p. 110 (e)).

2. Synthesis of Minor Alkaloids

(i) *and* (ii) *Synthesis of Protoemetine and O-Methylpsychotrine.*

The syntheses of protoemetine (p. 112 (f)) and O-methylpsychotrine (p. 110 (e)) have been discussed in detail in connection with the preparation of emetine. An independent synthesis of psychotrin was achieved by Teitel and Brossi [142b].

(iii) *Synthesis of Psychotrine*

Since on heating natural O-methylpsychotrine with hydrochloric acid at 170 °C partial demethylation takes place, giving rise to psychotrine [45], the total synthesis of the former implies accomplishment of the preparation of the latter compound, too.

(iv) *Synthesis of Cephaeline*

Reduction of psychotrine affords a mixture of cephaeline and isocephaeline [57], thus the syntheses mentioned above ((ii) and (iii)) are formally equivalent to the synthesis of cephaeline as well.

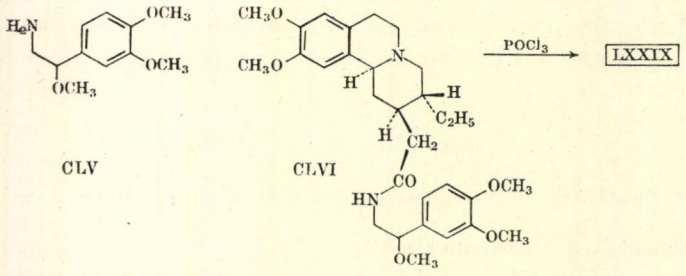
The direct total synthesis of cephaeline was achieved by Szántay and others [141, 142], as outlined above in connection with the synthesis of emetine (cf. p. 112 (f)). The same authors were the first to prepare racemic cephaeline, too.

(v) *Synthesis of Emetamine*

The synthesis of racemic emetamine may be accomplished by condensing the amide CLVI with $POCl_3$ [21, 68, 72]. Amide CLVI is available by heating the phenylethylamine derivative CLVI with the ester CXa ($R = CH_3$) at 200 °C [68], or by means of acylation with the mixed anhydride CXXXV [21].

Ahl and Reichstein [1] obtained emetamine in low yield by heating emetine with palladium charcoal; however on repeating the experiment, Bat-

tersby and others [21] found that though the result was a substance identical in almost all respects with the natural product, the optical rotatory power was different.



Clark and others [60] refluxed natural emetine with Raney nickel in xylene for 24 hours, to obtain emetamine in 33% yield. The product was not compared directly with an authentic sample. The optical rotatory power of the base was identical with that of the natural product, however, there was a significant difference in the optical activity of the hydrogen oxalate salt compared with the value reported in literature. Thus among the ipecac alkaloids discussed, emetamine is the only one whose total synthesis still presents a problem.

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in the field. The monograph deals mainly with the stereochemistry and syntheses of emetine. Less space is allotted to cephaeline, psychotrine, O-methylpsychotrine, emetamine and protoemetamine.

It is hoped that the English speaking reader will find here material of interest that has been heretofore accessible to him only with difficulty.

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