# Approaches to Camptothecin

by
E. WINTERFELDT

# The Pyrrolizidine Alkaloids

by
A. KLÁSEK
and
O. WEINBERGOVÁ

# Advances in the Chemistry of Glycosaminides

by
A. Ya. KHORLIN
and
S. E. ZURABYAN

AKADÉMIAI KIADÓ, BUDAPEST

RECENT DEVELOPMENTS
IN THE CHEMISTRY
OF NATURAL CARBON
COMPOUNDS

Volume 6

The present book in this succession of volumes dealing with natural organic compounds contains three reviews, written by distinguished authors who are themselves active workers in the fields concerned.

The part by *E. Winterfeldt* deals with various synthetic approaches to camptothecin, an alkaloid of highly interesting properties. Discussion of the synthetic methods includes the important contribution of the author himself to this branch of chemistry.

*A. Klásek* and *O. Weinbergová* present a comprehensive new review on pyrrolizidine alkaloids and their components, the necines and necic acids, dealing with the structure, chemistry, stereochemistry of the compounds and with the synthesis and biological effects of the alkaloids, such as the mutagenic, carcinogenic and antitumour actions.

The very readable review by *A. Ya. Khorlin* and *S. E. Zurabyan* gives an up-to-date and well-systematized collection of the methods applicable to the synthesis of glycosaminides, including the stereospecific and enzymic syntheses, as well as the preparation of S- and N-glycosaminides. The extensive list of references will be of great assistance to chemists working in this field of organic chemistry.

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# RECENT DEVELOPMENTS IN THE CHEMISTRY OF NATURAL CARBON COMPOUNDS

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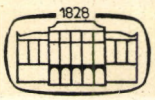
## VOLUME VI



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# APPROACHES TO CAMPTOTHECIN

by

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# THE PYRROLIZIDINE ALKALOIDS

by

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# ADVANCES IN THE CHEMISTRY OF GLYCOSAMINIDES

by

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and

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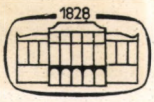


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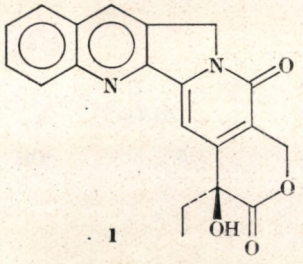
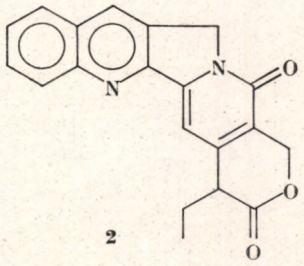
E. WINTERFELDT

# APPROACHES TO CAMPTOTHECIN

In the middle of the sixties, M. Wall and his co-workers isolated an alkaloid with a novel structure from the bark of *Camptotheca acuminata*; the elucidation of the constitution **1** included the use of X-ray diffraction analysis [1, 2].




**1**

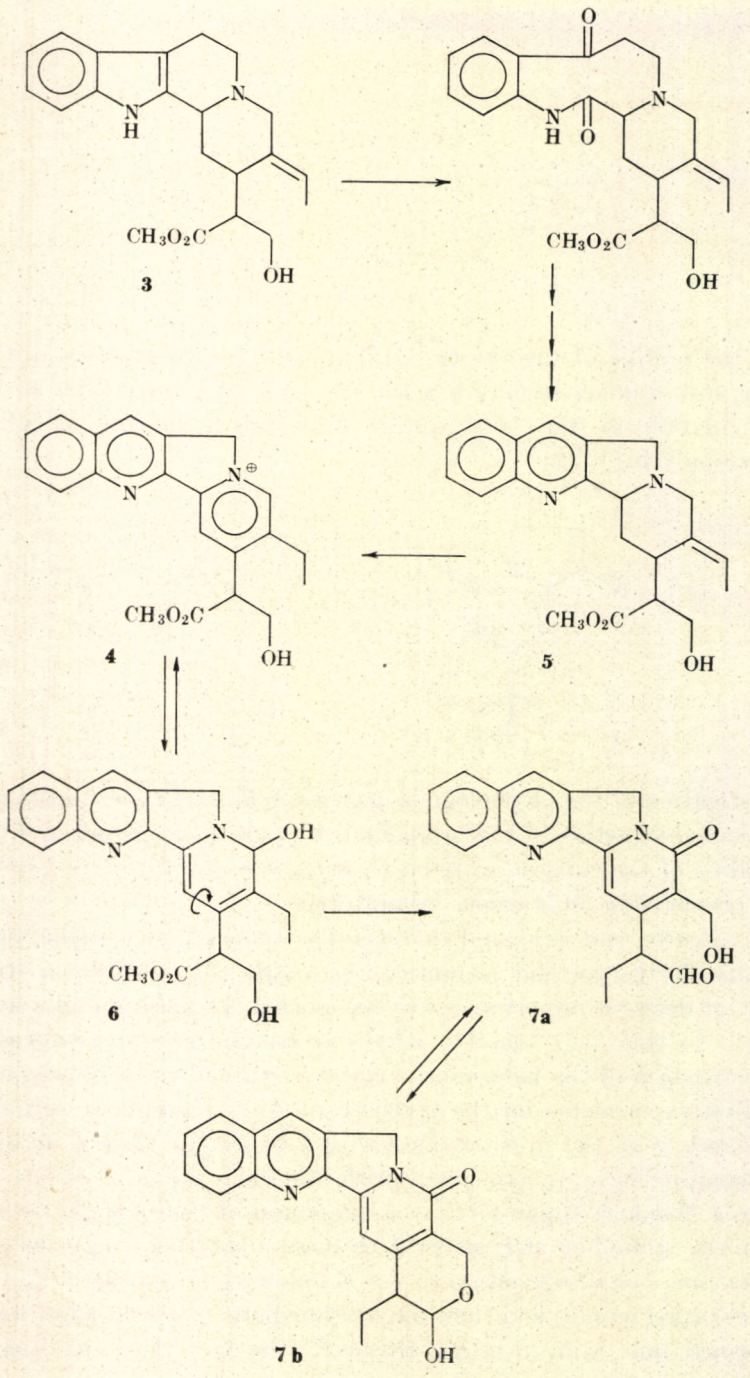



**2**

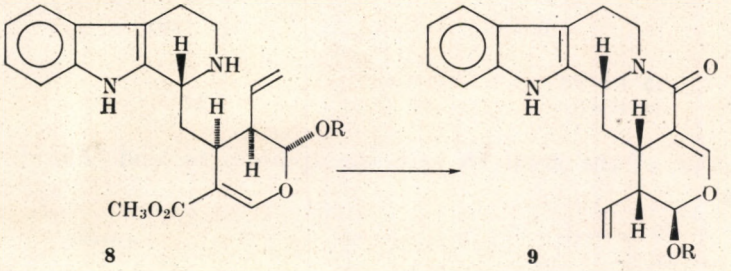
Investigation of the physiological activity in animal experiments revealed an antileukemic action of the compound. In view of this result and the poor availability of the substance, research work was started aiming at its synthetic preparation in various laboratories.

Wall already had prepared deoxycamptothecin **2** by a reduction procedure; this derivative had no anticancer action [3], whereby a structural element of decisive importance was recognized. In addition to a synthetic approach to this very reactive $\alpha$-hydroxylactone group, a procedure for the construction of the heterocyclic ring system had to be developed.

The first experiments for the synthesis of the chromophore were reported by Wenkert *et al.* [4] in connection with a biogenetic theory, according to which camptothecin is associated with the early stage of indole alkaloid biogenesis. Wenkert supposed that a compound of type **3** might be converted into the quinoline derivative **5** through oxidative ring cleavage and recyclization. Dehydrogenation to **4** $\rightleftharpoons$ **6** followed by another ring cleavage and cyclization could yield then the intermediate **7**, which could be simply transformed into camptothecin. However, the fact that vincoside **8**, the

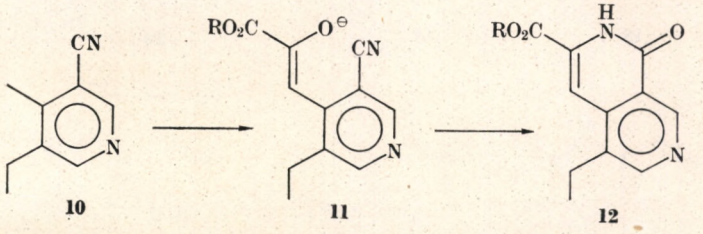

3
4
5
6
7a
7b

immediate biogenetic precursor of indole alkaloids, is very easily converted into the lactam **9**, and this lactam can also be isolated from *Adina Rubescens* [5], indicates that the formation of the lactone takes place at the early stages of the biogenesis, like that of the lactam ring; thus compounds of type **9** very probably transform into camptothecin through the indole-quinoline conversion process. It will be seen below that this conversion of the indole skeleton is a process that readily takes place 'in vitro' also.

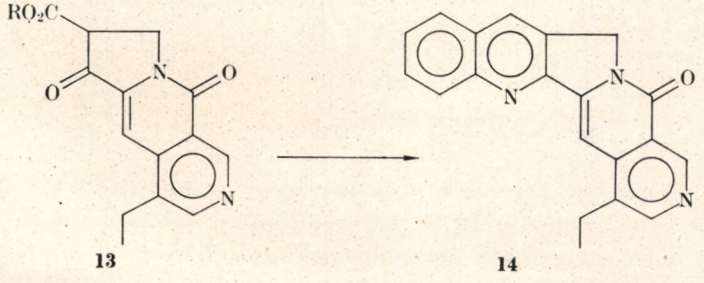



**8** **9**

In the synthesis of the chromophore, Wenkert applied the intermediate **10**, that had been found useful in the synthesis of indole alkaloids.



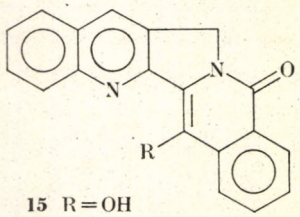

**10** **11** **12**



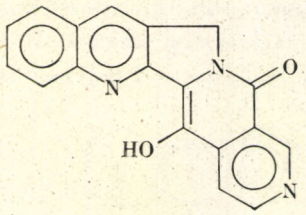

**13** **14**

Condensation with diethyl oxalate gives the enolate **11**, which is converted into **12** by gentle treatment with acid. The keto-ester **13** produced by condensation with ethyl acrylate is decarboxylated and the five-membered cyclic ketone is converted into **14** by Friedländer quinoline synthesis.

The Friedländer reaction has been applied in the synthesis of the heterocyclic system by several research groups. Thus Shamma and Nowak [6] prepared the analogous compounds **15**, **16** and **17** by means of this technique.

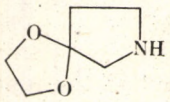


**15** R = OH
**16** R = H

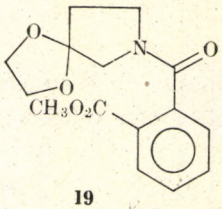


**17**

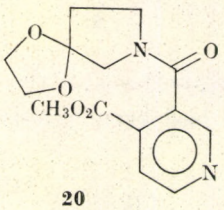
Reaction of the ketal **18** with the appropriate acid chloride yields **19** and **20**.



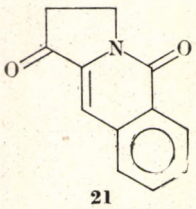
**18**



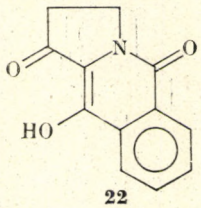
**19**



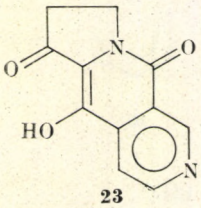
**20**



**21**



**22**



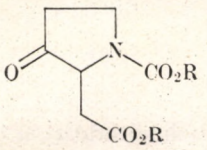
**23**

**16** **15** **17**

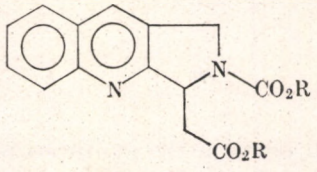
Hydrolysis to the ketones is followed by cyclization to yield **22** and **23**. When the ester group in **19** is first reduced to carbinol then oxidized to aldehyde with manganese dioxide, the non-substituted compound **21** is obtained after hydrolysis of the ketal and cyclization. All the three substances can be converted into the corresponding pentacyclic derivative by Friedländer synthesis.

Wall also applied the Friedländer synthesis in his first experiments aiming at the preparation of this natural product. By condensation of the keto-

ester **24** with anthranilic aldehyde the tricyclic compound **25** is obtainable, which represents an interesting starting material [7].

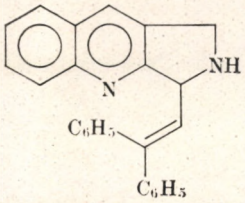


**24**

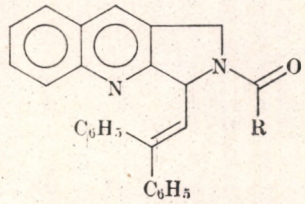


**25**

Wall's experiments for converting **25** into **26** by a Grignard reaction and dehydration gave intermediates of type **27**, which do contain the carbon atom required in the formation of ring E as a masked carbonyl group;



**26**

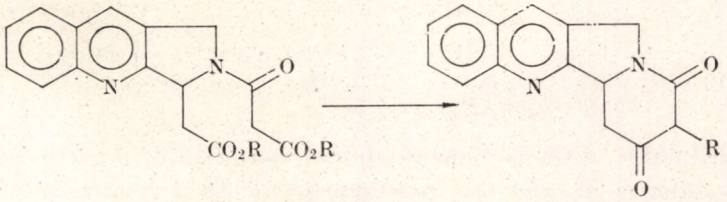


**27**

however, ozonolysis of the trisubstituted double bond to obtain the desired aldehyde could not be achieved.

Stork and Schultz [8] yet succeeded in achieving the first total synthesis of camptothecin using compound **25** as the starting material.

After saponification of the urethane group, subsequent esterification and reaction with malonic semi-ester chloride yielded the amide ester **28**; this was transformed into the tetracyclic $\beta$-dicarbonyl compound **29** by treatment with alkoxide.

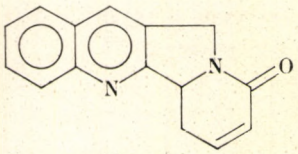


**28**

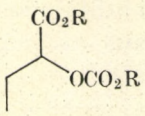
**29** R = $CO_2R$

**29 a** R = H

Saponification and decarboxylation gave the lactam-ketone **29a**; this was reduced to the corresponding lactam-carbinol with borohydride. The carbinol was then treated with acetic anhydride and sodium acetate to obtain the unsaturated lactam **30** with the elimination of water.

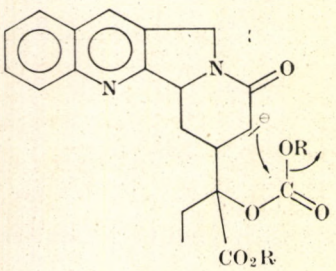


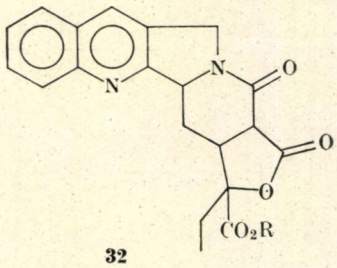
**30**



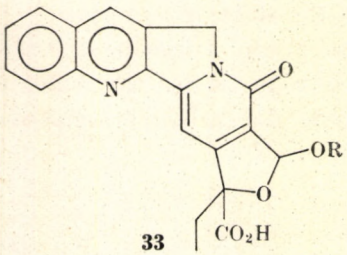
**31**

The crucial point in the synthesis is the Michael addition of the ester **31** to the double bond of the unsaturated lactam. As the lithium salt of compound **31** is unstable, the reaction of the unsaturated lactam with this component must be accomplished at −70°C. Lactone **32** is then formed by Michael addition and interception of the primary enolate by the carbonate group, followed by cyclization.

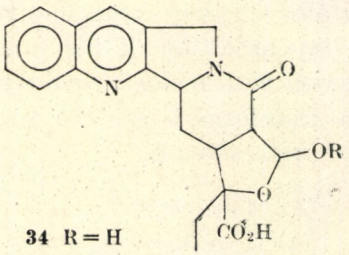




**32**



**33**

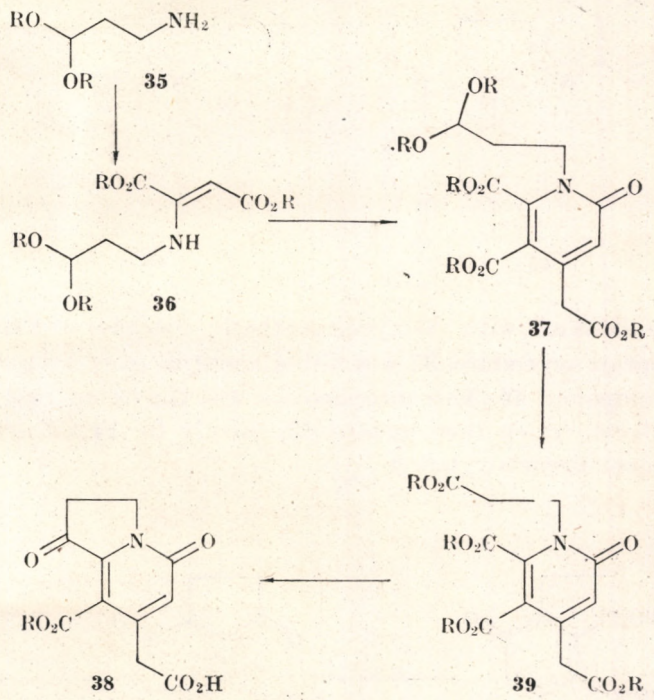


**34** R = H
**34a** R = Ac

The reduction of the lactone group with borohydride is carried out on the corresponding acid, and the cyclo-semiacetal **34** is finally converted into **34a** with acetic anhydride. Dehydrogenation of this compound yields **33**, which on careful saponification liberates the cyclo-semiacetal again. The aldehyde group present according to an equilibrium is reduced with borohydride, when the free carbonyl group reacts immediately with the formation of lactone. Camptothecin synthesized in this way proved to be identical with the natural product.

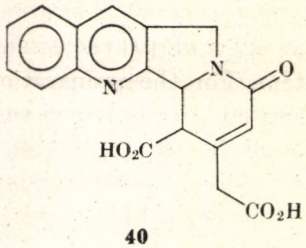
While this synthesis is focussed on the preparation of the hydroxylactone, in the total synthesis proposed by Danishefsky *et al.* [9] the essential point is the formation of the heterocyclic system. For the preparation of this group, a new pyridone synthesis was elaborated [10], in which the decisive step is the addition of an enamine to the allenedicarboxylic ester.

The addition of amine **35** to acetylenedicarboxylic ester yields the enamine **36**. This is converted into the pyridone with **37** allenedicarboxylic ester, by addition and cyclization.




The acetal is subjected to hydrolysis and oxidized to the acid; on subsequent esterification compound **39** is obtained, whose Dieckmann cyclization, saponification and decarboxylation, together with the selective saponification also of the ester group in the side-chain, results in the ester-acid **38**. In the subsequent Friedländer synthesis the second ester group is also saponified and the tetracyclic compound **40** is produced.

Partial esterification yields the monoester; the pyrolysis of this substance results in decarboxylation to afford **41**; this is converted into **43** by alkylation in rather low yields. Condensation of this compound with paraformaldehyde takes place with lactone formation, and the reaction product was

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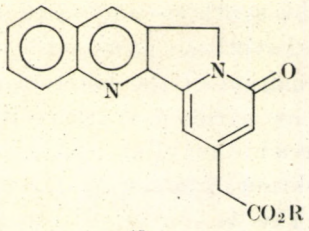


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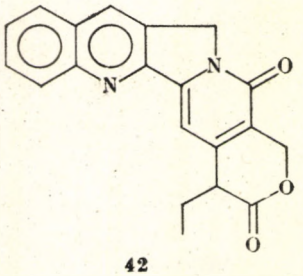


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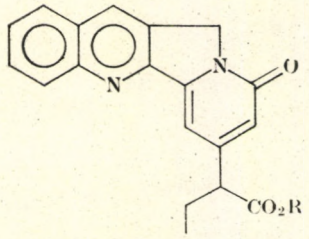
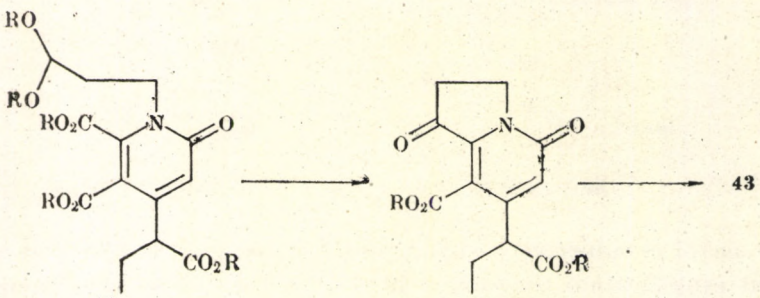



43

found to be identical with deoxycamptothecin. Because of the poor yield in the alkylation procedure, **37** was also allowed to react with ethyl iodide to obtain compound **44**. This intermediate was converted first into **45** as described above, which then yielded **43** directly by Friedländer reaction and subsequent decarboxylation.



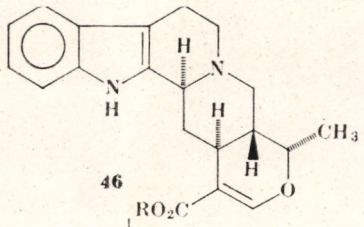

44 45

Deoxycamptothecin proved to be easily oxidizable; however, the preparative application of this observation could not be realized completely, since under the given conditions, in strongly alkaline medium, always the enolate of deoxycamptothecin is present, and owing to the sensitivity of camptothecin towards bases only moderate yields are attainable.

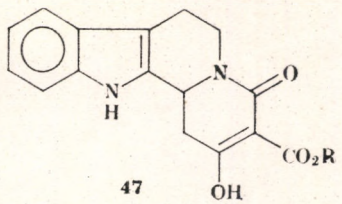
In our biogenesis-orientated total synthesis of camptothecin we started with the fact that the introduction of this hydroxyl group may not give

rise to special problems. As formulae **8** and **9** representing the biogenetic precursors show, neither of them contains this hydroxyl group; it will be attached to the molecule only later, probably after the formation of the pyridone.

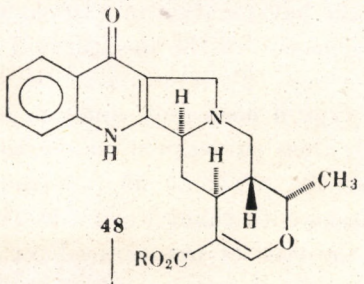
Therefore, we focussed our attention on the indole-quinoline conversion, and examined the behaviour of polycyclic indole derivatives under the conditions of autoxidation [11]. Compounds like ajmalicine **46** and the lactam **47** could be converted into the corresponding quinolones **48** and **49**, respectively, at a high rate and in high yield.



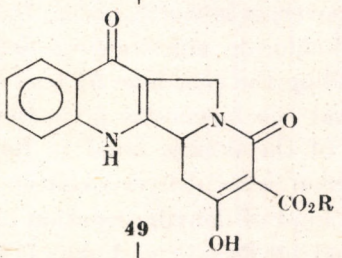
**46**



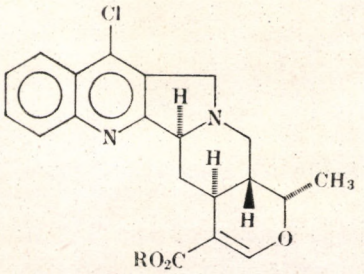
**47**



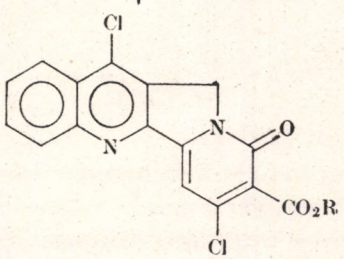
**48**



**49**

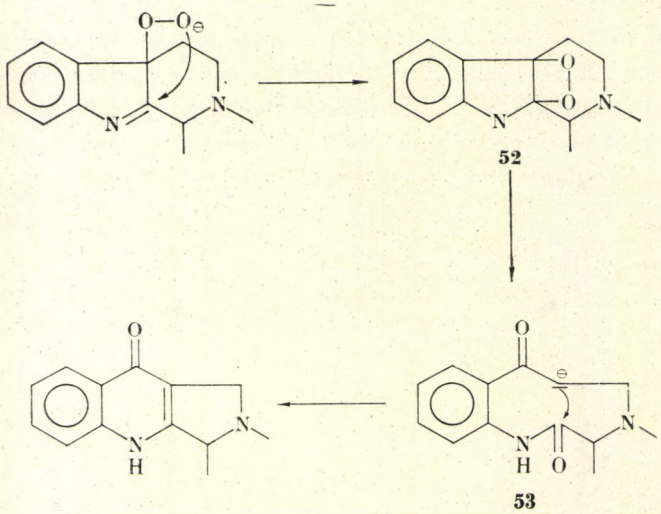


**50**



**51**

On the basis of the mechanism of chemiluminescence of indolyl hydroperoxides, the formation of quinolone can be supposed to take place through the intermediates **52** and **53**.

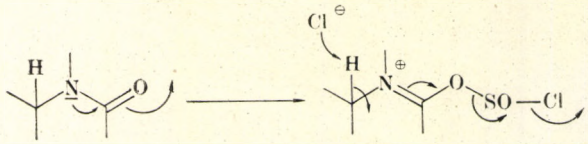


**52**

**53**

Moreover, it is worth noting that in the halogenation of quinolones with thionyl chloride, simultaneous dehydrogenation of the unsaturated lactam **49** yielding the pyridone **51** also takes place [12]. This allows the conclusion that the biogenetic precursors of type **9** need only undergo isomerization of the double bond to form **54**, then subsequent indole-quinoline conversion with dehydrogenation gives the pyridone **55** immediately.

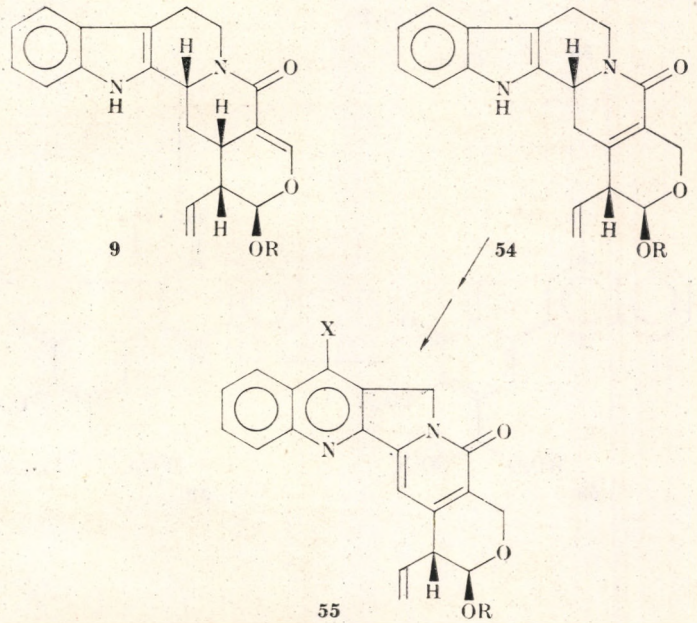
This kind of dehydrogenation of lactams with thionyl chloride had been observed by Büchi and Lukas [13] in the case of unsaturated 5-ring lactams in the course of the total synthesis of holomycin, and its mechanism was also explained.



We gratefully acknowledge Professor Büchi's suggestions regarding the mechanism given here.

In order to utilize this possibility in the total synthesis [14] of camptothecin, **47** was converted into the enol ether **56** with diazomethane; Michael addition with *t*-butylmalonate accompanied by elimination then resulted in the triester **57**. Autoxidation took place in dimethylformamide with

sodium hydride acting as a proton acceptor, and **59** was obtained in a high yield. Subsequent chlorination with thionyl chloride in dimethylformamide, with simultaneous dehydrogenation readily gave **58**.

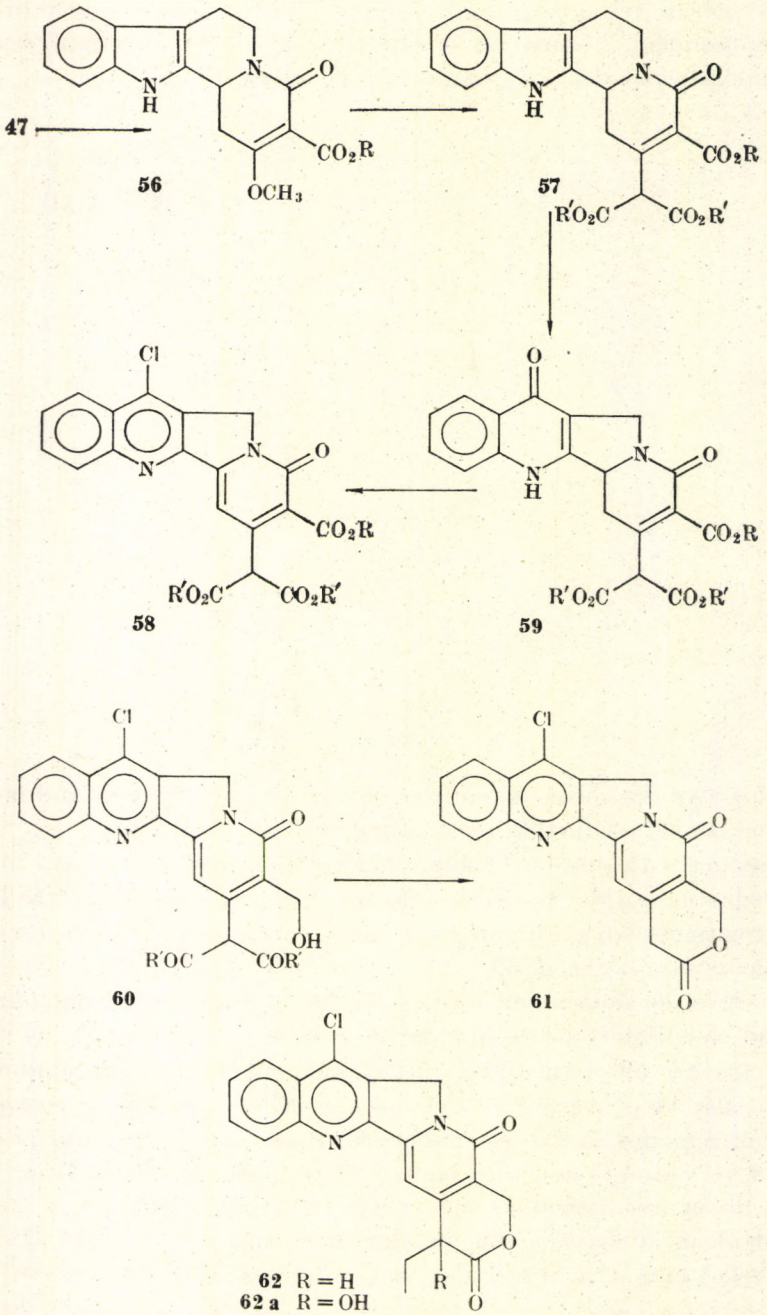



In this way the chromophore was obtained in a few steps, and now this could be converted further in a simple way.

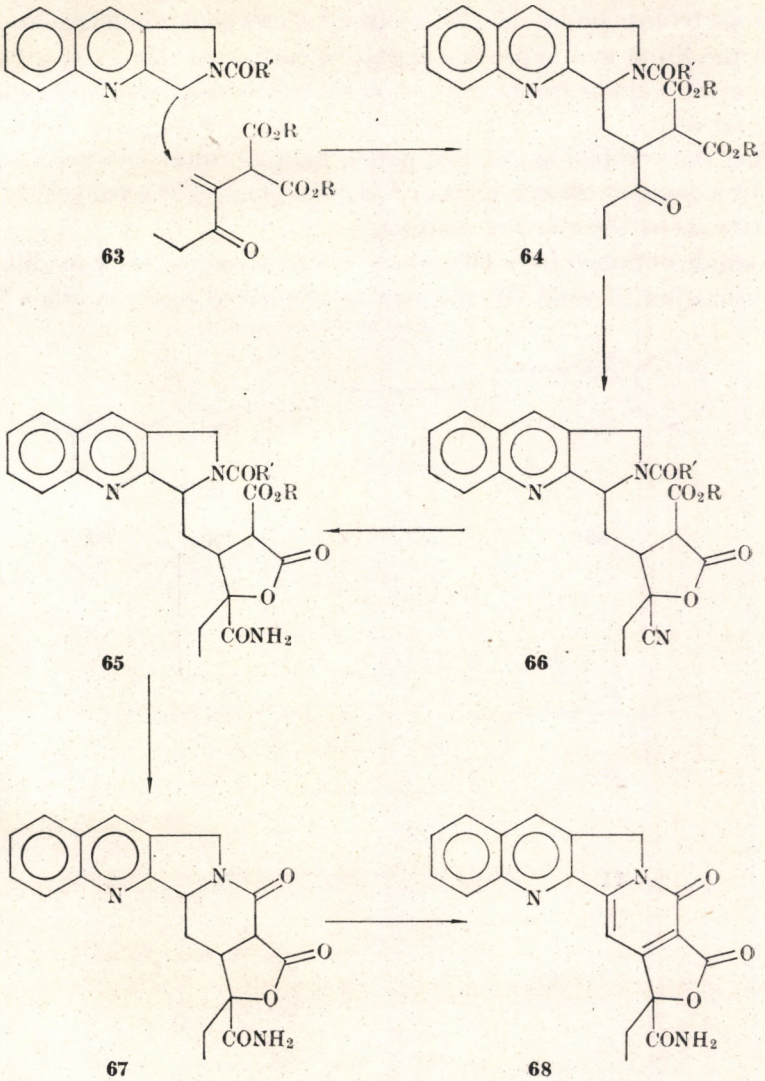
Reduction with diisobutylaluminium hydride and subsequent borohydride reduction yields the carbinol **60**, which is transformed into the lactone **61** on treatment with trifluoroacetic acid. Alkylation of this lactone gives chlorodeoxycamptothecin **62**.

Reductive dehalogenation leading to the non-substituted quinoline system and thus finally to camptothecine can be achieved either by the use of the triester **58** or the deoxychloro compound **62**. Chlorocamptothecin **62a** can also be converted into camptothecin by means of hydrogenation.

The introduction of the tertiary hydroxyl group into the molecule can be effected very simply and in a high yield by aeration of a solution of the actual deoxy compound in the presence of Cu(II) ions and a catalytic amount of an aliphatic amine in dimethylformamide solution. Oxidation with Ce(IV) salts in acid medium, which has been found useful in the case of other compounds [15, 16], may also be applied successfully here, although the yields are very low.


47
56
57
58
59
60
61
62 R = H
62a R = OH

The synthesis proposed by Wall *et al.* [17] involves an interesting Michael addition [18] of **63** yielding **64** as the starting step in the reaction series.



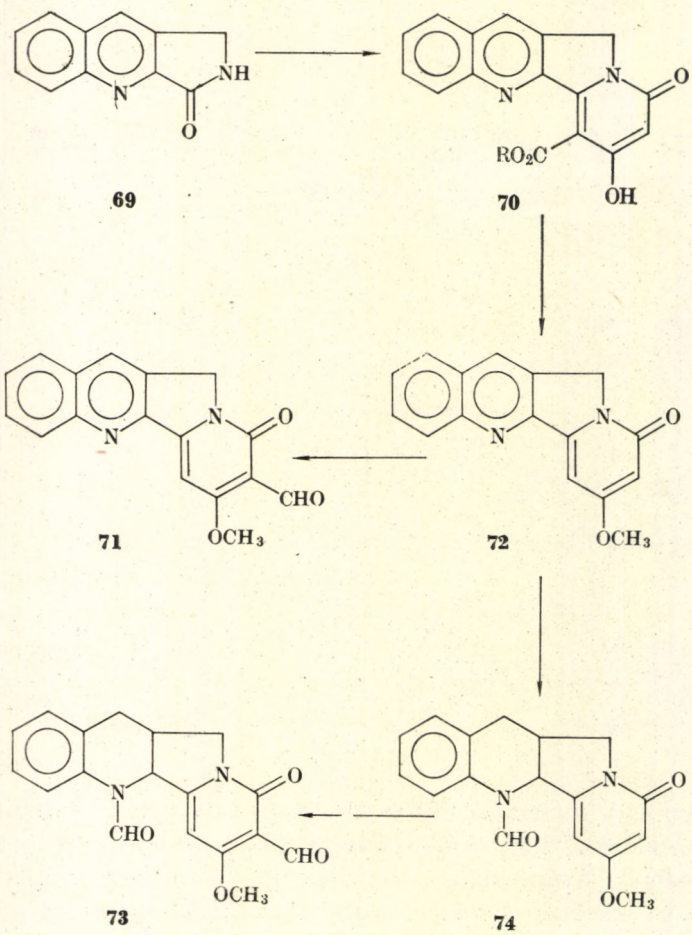

The cyanohydrin reaction of the triester **64** takes place with lactone formation to yield **66**, which can be converted into the amide **65** by treatment with methanolic hydrochloric acid. Reaction with HBr in glacial acetic acid leads to selective urethane hydrolysis, and **67** is formed with simul-

taneous cyclization; the product, however, also contains the non-cyclic hydroxy-ester. This substance can be converted again into **67** by treatment with acid.

Lactone **68** formed by dehydrogenation shows some similarity to the last step in the Stork synthesis, and it can be converted into camptothecin by means of the same reagents, that is, borohydride reduction and subsequent acid treatment.

During the compilation of this paper, another total synthesis was published by a Japanese research group [19]; they also used a tricyclic quinoline derivative **69** as the starting material.

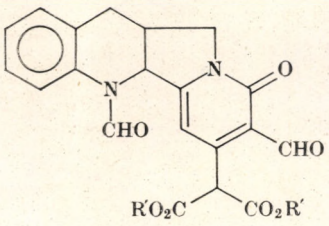
The amide obtained from **69** and acetonedicarboxylic ester readily undergoes cyclization to yield **70**; this can be converted into the ether **72** after



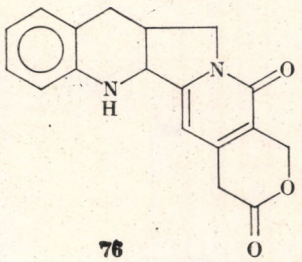

**69** **70** **71** **72** **73** **74**

saponification and decarboxylation. Compound **71** obtained on Vilsmeyer formylation was found unsuitable for Michael addition, although simple pyridone derivatives of this type were successfully used in a Michael elimination procedure involving the replacement of the methoxyl group by a malonate residue. In order to make the intermediate more similar to the model compounds, **74** was prepared by hydrogenation and formylation; this yielded **73** under the conditions of the Vilsmeyer reaction.

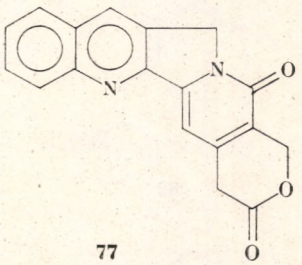
This compound can be, indeed, converted into **75** with sodium hydride and *t*-butylmalonate. Reduction with borohydride and subsequent treatment with acid result in lactone formation and deformylation.




**75**



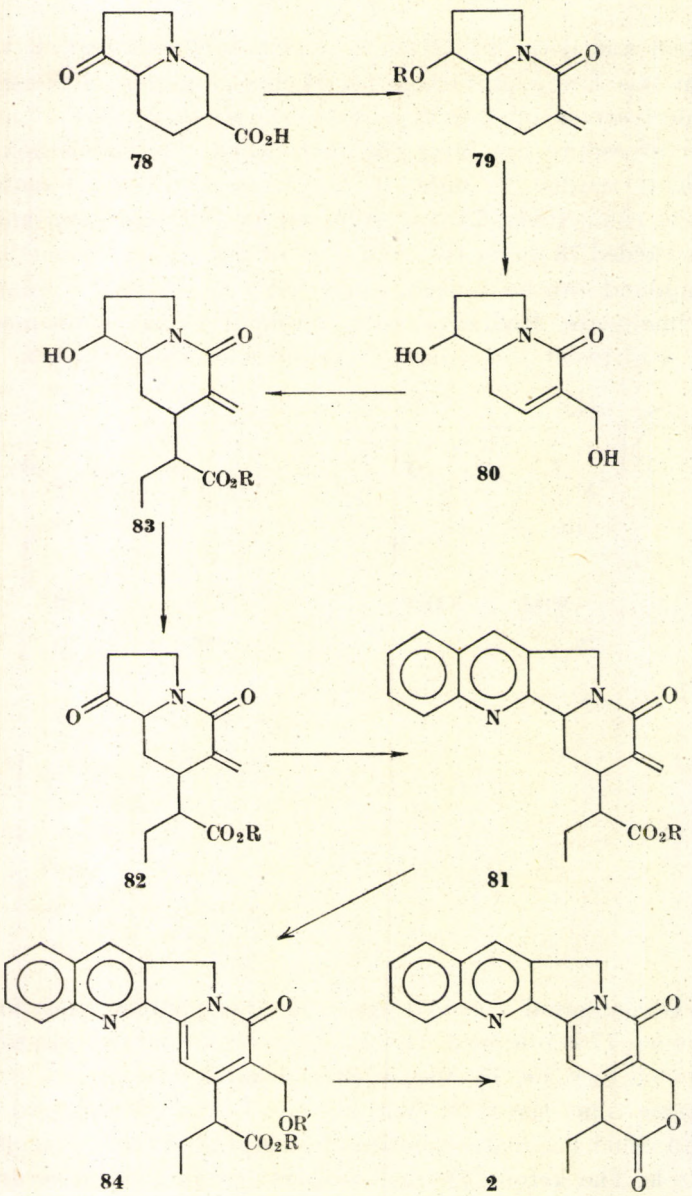

**76**




**77**

When this compound is dehydrogenated, the quinoline is formed again and the lactone **77** is obtained which is then converted to camptothecin in the well-known way, i.e. by alkylation and autoxidation.

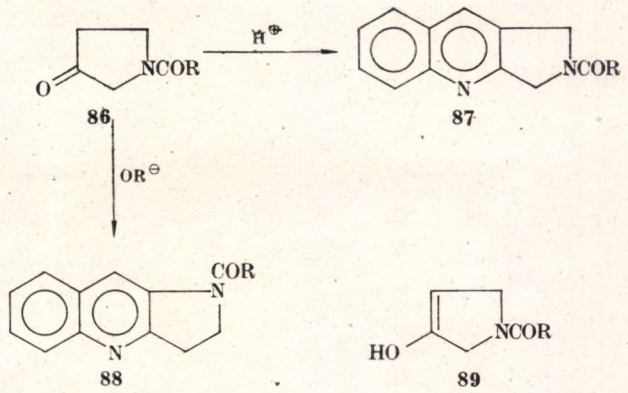
At the same time, the total synthesis by Tang and Rapoport [20] was published, in which the rearrangement of piperidinecarboxylic acids [21] has a decisive role. The ketone **78** can be rearranged into the unsaturated lactam **79** after reduction with borohydride and treatment with acetic anhydride. After saponification, treatment with selenium dioxide yields **80**. When this compound is heated with butyric orthoester, it reacts selectively at the allylcarbinol, and after the elimination of alcohol compound **83** is obtained in a 3.3-sigmatropic rearrangement. This can be oxidized to **82**, then subjected to a Friedländer synthesis to obtain **81**, and this compound




can easily be converted into the camptothecin precursor **84** by repeated treatment with selenium dioxide and simultaneous dehydrogenation; the product yields deoxycamptothecin on treatment with acid. Hydroxylation can be accomplished by the method already described, applying Cu(II) in dimethylformamide medium.

Finally, we have to report on some experiments which, although not leading to a total synthesis, involve the preparation of interesting intermediates.

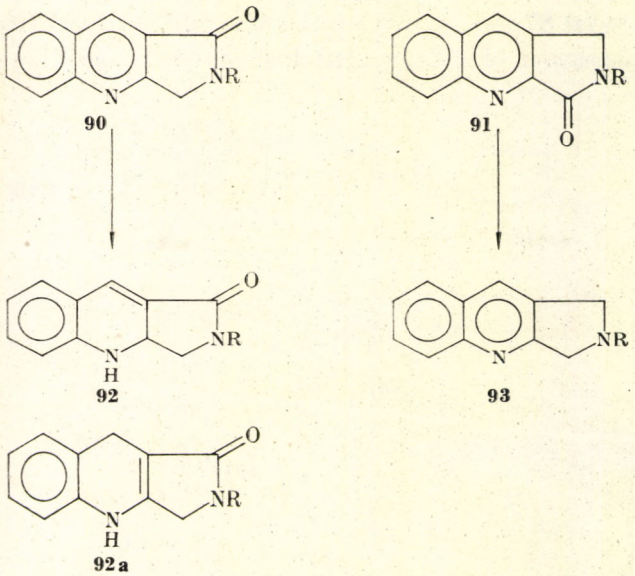
An important contribution to the synthesis of the generally valuable starting material **87** is provided by the very efficient and selective Friedländer condensation of *o*-aminobenzaldehyde with the ketone **86** [22].




When basic catalysts are applied in the condensation, the preponderant main product of the reaction is the non-desired 'false' tricyclic compound **88**, produced in part with the elimination of the N-acyl group. If the conversion is, however, carried out in the presence of an acid catalyst in glacial acetic acid, the ratio of the compound with structure **87** increases considerably, and it is often formed in the same amount as **88** is. Finally, it can be established that optimal conditions are ensured by proton catalysis without solvent. When a mixture of the ketone, *o*-amino-benzaldehyde and *p*-toluenesulfonic acid in 1 : 2 : 0.02 ratio is heated for about 5 minutes in nitrogen atmosphere at 190—195°C, complete conversion takes place, and compound **87** can be isolated in 88% yield; a pure substance is obtained even without the application of chromatography.

In order to elucidate this fact, the following obvious supposition can be forwarded: the enol **89** has high stability under acid conditions, and in addition to this, the exclusion of solvation will prevent the occurrence of trans-enolization. It may also be supposed, however, that the enol **89** is the kinetic end-product of the reaction in acid medium, and the absence of solvation prevents the development of an equilibrium between the different enol forms; thus the reaction proceeds in one way only.

Tricyclic compounds of this type have been prepared in another way by Shamma and Novak [23], using the tricyclic lactams **90** and **91** as suitable intermediates.

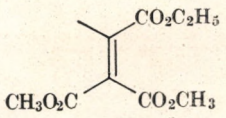



Surprisingly, the reduction of both lactams with hydride yields quite unexpected products. In the case of compound **90**, the carbonyl group is not attacked at all, but the quinoline ring is reduced instead, to obtain the 1,2-dihydro derivative **92**. In compound **91**, the reduction of the carbonyl group can be accomplished in principle, still a very complex mixture of products is formed, from which compound **93** can only be isolated in a poor yield.

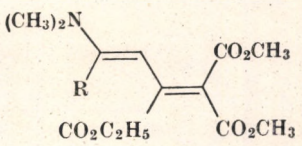
The reduction of lactam **90** was also reported by a Japanese research group [24]. Their data are in accordance, however, only with structure **92a**, i.e. with that of a 1,4-dihydroquinoline. It can be supposed that the structure of the actually isolated product is affected by the conditions of work-up and this may be responsible for the different structures.

A new and more extensive approach was described by Borch *et al.* [25], leading to tetracyclic compounds containing the characteristic camptothecin chromophore, together with a pyridone synthesis. This method was extended to aromatic amides, after it had been established that the enamines **95**, formed from the ester **94** and amidacetals, could be converted into the pyridone **97** with benzylamine, and, in addition to this, the methyl ester group in this compound could be eliminated selectively with lithium

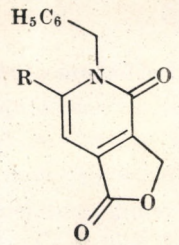
iodide. Thus, after trans-esterification with oxalyl chloride to the acid chloride, and by subsequent reduction lactone **96** can be obtained. The starting materials were chosen so as to ensure the possibility of an intramolecular attack by the nitrogen atom required for the pyridone formation.



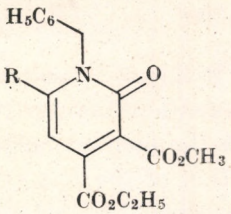

**94**




**95**

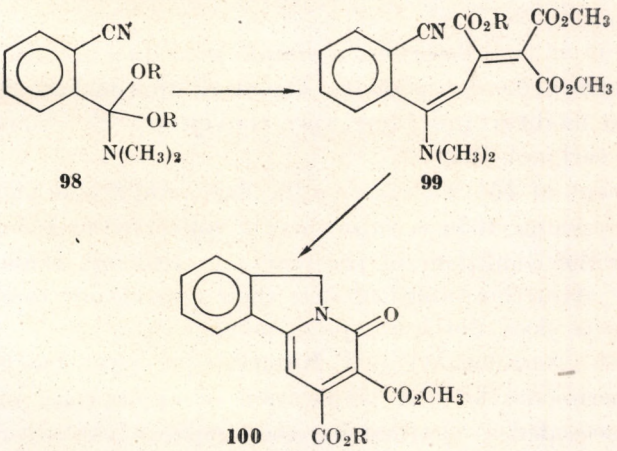



**96**



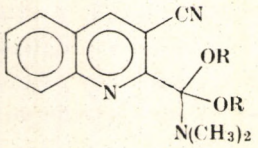

**97**

Thus, the nitrile **98** yields the enamine **99**, which is cyclized immediately to **100** during hydrogenation with Raney nickel.

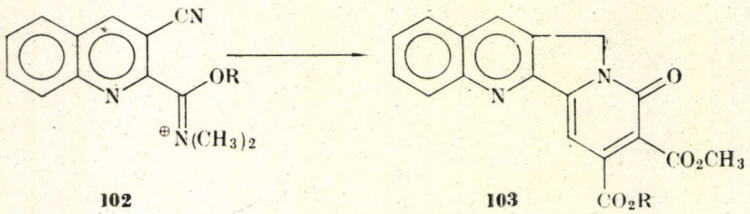



**98** **99** **100**

Some interesting and unexpected observations were also made when the extension of this process to the nitrile **101** was attempted.



**101**



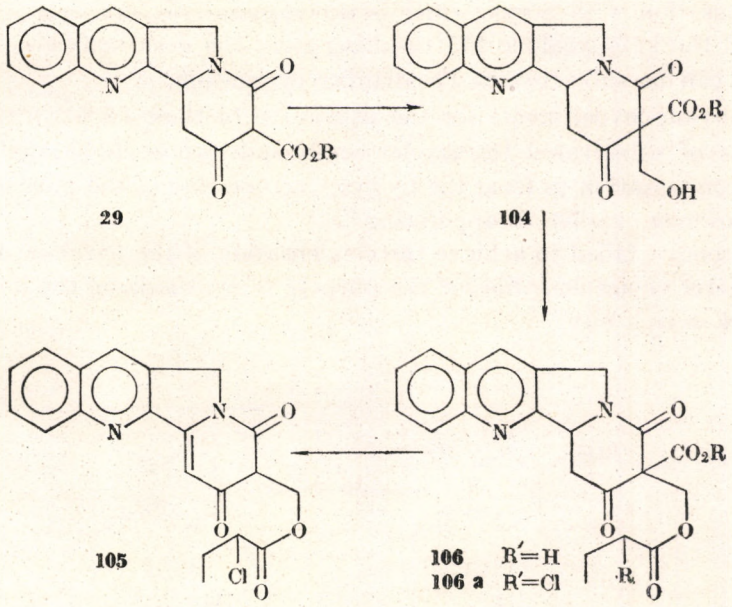
**102** **103**

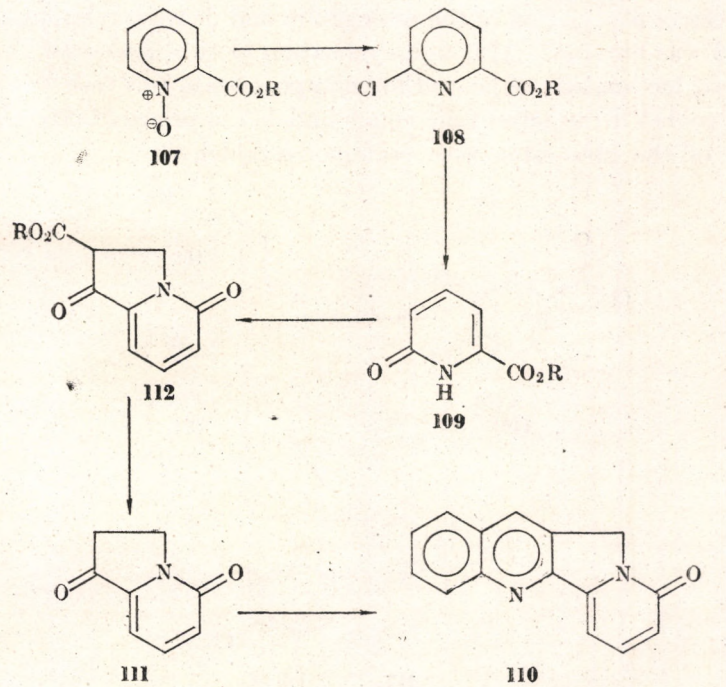
First of all, it should be pointed out that the formation of the amidacetal with triethyloxoniumfluoroborate can successfully be accomplished in this case, although the method failed with pyridine derivatives, because of the interfering action of the N-alkylation. It is a more unfavourable observation, however, that this compound cannot be condensed with **94** into an enamine, in contrast with experiences obtained with several other amidacetals. When, however, the corresponding ammonium salt **102** is applied, condensation and subsequent hydrogenation with Raney nickel yields the very interesting intermediate **103**, which is considered capable of being transformed into the corresponding lactone on the basis of observations discussed above.

T. K. Liao *et al.* [26] described a simple procedure for the preparation of different tetracyclic intermediates. The Stork intermediate **29** is prepared in a somewhat modified procedure, then converted with formaldehyde into the primary carbinol **104**.

The treatment of **104** with carboxylic acid anhydrides yields esters of type **106**. Compound **106a** is subsequently converted to **105** on prolonged stirring under the conditions of the reaction. Although these compounds appear to be attractive intermediates, their ring closure to obtain pentacyclic substances does not seem practicable.

The research group headed by T. Kametani in Japan has carried out a series of experiments for the preparation of appropriate intermediates. They have succeeded in synthesizing the simple non-substituted chromophore **110** [27], starting from the N-oxide **107**. This was first allowed to react with phosphoryl chloride to yield **108**, which was converted by subsequent hydrolysis into the pyridonecarboxylic acid **109**.

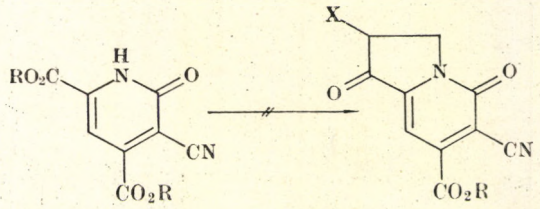

29
104
105
106 R′=H
106 a R′=Cl


107
108
109
112
111
110

Condensation with acrylic ester is accompanied by cyclization to give **112**, and the ketopyridone **111** resulting from the decarboxylation of this product can be subjected to Friedländer cyclization.

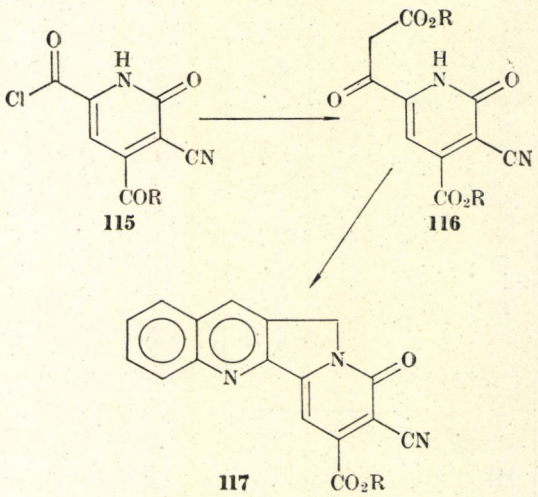
The obvious experiments for the utilization of these experiences in the synthesis of substituted tetracyclic compounds whose functional groups permit condensation to form the hydroxylactone ring of the molecule have been, however, rather disappointing [28].

All attempts failed to achieve the condensation of the pyridone **113** with acrylic ester or acrylonitrile for the purpose of synthesizing the ketones of type **114** in this way.



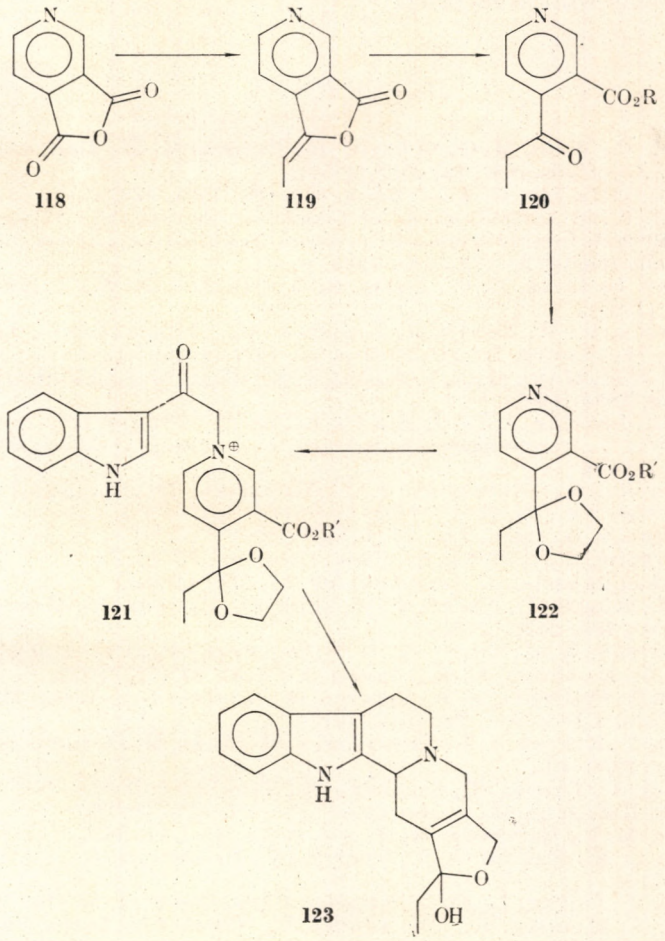

**113** **114**

Finally, the acid chloride **115** was condensed with ethyl *t*-butyl malonate; after selective elimination of the ester group and decarboxylation, the keto-ester **116** was obtained. Although cyclization of this keto-ester to the corresponding lactam-ketone proved to be impossible in this case too, this step can be avoided if the tetracyclic compound **117** is prepared directly by the reaction of the keto-ester with *o*-aminobenzaldehyde.




**115** **116** **117**

Obviously, there are still problems in the further conversion of this compound in the desired manner, as in another publication Kametani *et al.* [29] described the preparation of a preliminary compound containing an indoloquinolizidine ring system, apparently with the purpose of utilizing the oxidative indole-quinolone conversion at a later stage of the synthesis. The starting material is the aldehyde **118**, whose Perkin condensation with propionic anhydride yields **119**. The next step is esterification to obtain **120**.




Condensation of **122** with chloroacetylindole was accomplished after ketalization and the pyridinium salt **121** formed in the reaction was subjected to alanate reduction. Here several reaction steps proceed simultaneously, and probably a pentacyclic, unsaturated ketal-carbinol is

formed, which undergoes hydrolysis during the processing to yield the cyclic hemiacetal **123**.

*

The present review surveys the situation in this field up to about the beginning of 1973; however, in view of the activity of several research groups all over the world investigating these alkaloids, it can be expected that during the time required for the publication of this paper further important results will be reported.

## REFERENCES


1. Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T., Sim, G. A., *J. Am. Chem. Soc.*, *88*, 3888 (1966).
2. McPhail, A. T., Sim, G. A., *J. Chem. Soc.* (B), *1968*, 923.
3. Wall, M. E., in Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin, 1969. 4. Internationales Symposium Biochemie und Physiologie der Alkaloide, Halle (Saale) 25–28 June, 1969, p. 77.
4. Wenkert, E., Dave, K. G., Lewis, R. G., Sprague, P. W., *J. Am. Chem. Soc*,. *89*, 6741 (1967).
5. Blackstock, W. P., Brown, R. T., Lee, G. K., *Chem. Commun.*, *1971*, 910.
6. Shamma, M., Nowak, L., *Tetrahedron*, *25*, 2275 (1969).
7. Kepler, J. A., Wani, M. C., McNaull, J. N., Wall, M. E., Levine, S. G., *J. Org. Chem.*, *34*, 3853 (1969).
8. Stork, G., Schultz, A. G., *J. Am. Chem. Soc. 93*, 4074 (1971).
9. Volkmann, R., Danishefsky, S., Eggler, J., Solomon, D. M., *J. Am. Chem. Soc. 93*, 5576 (1971).
10. Danishefsky, S., Etheredge, S. J., Volkmann, R., Eggler, J., Quick, J., *J. Am. Chem. Soc.*, *93*, 5575 (1971).
11. Winterfeldt, E., *Ann.*, *745*, 23 (1971).
12. Winterfeldt, E., Radunz, H., *Chem. Commun.*, *1971*, 374.
13. Büchi, G., Lukas, G., *J. Am. Chem. Soc. 86*, 5654 (1964).
14. Boch, M., Korth, T., Nelke, J. M., Pike, D., Radunz, H., Winterfeldt, E., *Chem. Ber.*, *105*, 2126 (1972).
15. Trahanovsky, W. J., Young, L. B., *J. Org. Chem. 31*, 2033 (1966).
16. Grover, V. K., Gupta, Y. K., *Austral. J. Chem.*, *23*, 757 (1970).
17. Wani, M. C., Campbell, H. F., Brine, G. A., Kepler, J. A., Wall, M. E., Levine, S. G., *J. Am. Chem. Soc.*, *94*, 3632 (1972).
18. Wani, M. C., Kepler, J. A., Thompson, J. B., Wall, M. E., Levine, S. G., *Chem. Commun.*, *1970*, 404.
19. Sugasawa, T., Toyoda, T., Sasakura, K., *Tetrahedron Letters*, *1972*, 5109.
20. Tang, C., Rapoport, H., *J. Am. Chem. Soc.*, *94*, 8615 (1972).
21. Rueppel, M. L., Rapoport, H., *J. Am. Chem. Soc.*, *94*, 3877.
22. Zalkow, L. H., Nabors, J. B., Frénch, K., Bisarya, S. G., *J. Chem. Soc.* (C), *1971*, 3551.
23. Shamma, M., Novak, L., *Coll. Czechoslov. Chem. Commun.*, 35 (11) 3280 (1970).
24. Tanaka, T., Mashimo, K., Wagatsuma, M., *Tetrahedron Letters*, *1971*, 2803.
25. Borch, R. F., Grudzinskas, C. V., Peterson, D. A., Weber, L. A., *J. Org. Chem.*, *37*, 1141 (1972).
26. Liao, T. K., Nyberg, W. N., Cheng, C. C., *J. Heterocycl. Chem.*, *1971*, 373.
27. Kametani, T., Nemoto, H., Takeda, H., Takano, S., *Tetrahedron*, *26*, 5753 (1970).
28. Kametani, T., Takano, S., Terasawa, H., Takeda, H., *Yakugaku Zasshi*, *92*, 868 (1972).
29. Kametani, T., Takano, S., Takeda, H., *Yakugaku Zasshi 92*, 743 (1972).

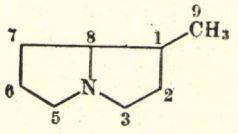
A. KLÁSEK
AND
O. WEINBERGOVÁ

# THE PYRROLIZIDINE ALKALOIDS

# I. INTRODUCTION

In 1867, Theissier [1] was the first to draw attention to the presence of alkaloids in the toxic Mexican plant *Senecio canidicis*. The first crystalline, though not fully identified, alkaloid was obtained [1] from *Senecio tolutanus*. Thirty years later two other alkaloids called senecine and senecionine were isolated by Dalche and Heim [2], who suggested that these substances could be applied as hemostatica; they were also used for a long time in gynaecology. A detailed description of the isolation of these alkaloids is given in the paper of Grandval and Lajoux [3] who were the first to determine the correct empirical formula of senecionine ($C_{18}H_{25}NO_5$). Senecifoline and senecifolidine were isolated [4] in 1909 from *S. latifolius*. The authors of this historically significant paper were the first to discover the ester nature of senecifoline after having obtained senecifolic acid and the amino alcohol senecifolinine by its hydrolysis. Another mention of alkaloids from the plant of the genus *Senecio* was made in 1924 when Müller [5] isolated the alkaloid silva-senecine from *S. silvaticus* and fuchsisenecionine from *S. fuchsii*. He reported that the alkaloids are bound in these plants in the form of tartrates and succinates. Since these alkaloids were isolated from the plants of the genus *Senecio*, they were referred to as Senecio alkaloids and this designation has persisted almost up till now. In 1932 Menshikov [6] isolated the alkaloids heliotrine and lasiocarpine from *Heliotropium lasiocarpum*, and in the years from 1933 to 1937 he elucidated the structure of their basic moiety. For a detailed description of the whole research, see the summarizing report [7]. Menshikov found that the basic components of the alkaloids from the plants of the genus *Heliotropium* and the genus *Senecio* had similar structures, being the variously substituted derivatives of 1-methylpyrrolizidine (I).

In view of this fact, the designation 'pyrrolizidine alkaloids' has generally been introduced for Senecio alkaloids. This designation has found further




I

support by the fact that in the following years substances with a pyrrolizidine skeleton were also found in a series of plants belonging to other genera and families (Table I).

*Table 1*

The Distribution of Pyrrolizidine Alkaloids in Plants

| Family | Genus |
|---|---|
| Apocyanaceae | *Alafia, Anodendron, Urechtites* |
| Asteraceae | *Brachyglottis, Cacalia, Emilia, Erechtites, Eupatorium, Ligularia, Nardosmia, Paracaryum, Petasites, Senecio, Tussilago* |
| Borraginaceae | *Amsinckia, Cynoglossum, Echium, Heliotropium, Lappula, Lindelofia, Macrotomia, Rindera, Solenanthus, Symphytum, Tournefortia, Trachelanthus, Trichodesma* |
| Celastraceae | *Bhesa* |
| Elaeocarpaceae | *Aristotelia* |
| Euphorbiaceae | *Securinega* |
| Gramineae | *Festuca, Lolium, Thelepogon* |
| Leguminoseae | *Adenocarpus, Crotalaria, Cytisus* |
| Orchidaceae | *Chysis, Kingiella, Liparis, Malaxis, Phalaenopsis, Vanda* |
| Rhizophoraceae | *Cassipourea* |
| Santalaceae | *Thesium* |
| Sapotaceae | *Mimusops, Planchonella* |

Much attention has been paid to the chemistry of pyrrolizidine alkaloids since 1930, but the most dramatic development of this research took place after the year 1950 when the majority of the so far isolated pyrrolizidine alkaloids were investigated and their correct structures, including the absolute configurations, were determined.

The pyrrolizidine alkaloids are esters of amino alcohols (necines) derived from 1-methylpyrrolizidine (I) with various organic acids (necic acids) containing 5, 7, 8, 9 or 10 carbon atoms. Free pyrrolizidine bases have also been found in nature. According to the number of carbon atoms and the arrangement of the chain of the acid moiety, the pyrrolizidine alkaloids can be subdivided into several groups; this division was suggested for the first time by Ferles and Bláha [8]. It fundamentally corresponds to the occur-

rence of these alkaloids in plants of the individual genera and families. For the chemistry and the biochemistry of the pyrrolizidine alkaloids and their physico-chemical properties, see the surveys and summarizing reports [9–20].

## REFERENCES


1. Note in: MÜLLER, A., *Heil- und Gewürz-Pflanzen, 7*, 1 (1924).
2. DALCHE, R., HEIM, F., *Therap. Monatsh., 1897*, 275.
3. GRANDVAL, A., LAJOUX, H., *Compt. rend., 120*, 1120 (1895).
4. WATT, H. E., *J. Chem. Soc., 95*, 466 (1909).
5. MÜLLER, A., *Heil- und Gewürz-Pflanzen, 7*, 72 (1924).
6. MENSHIKOV, G. P., *Ber., 65*, 974 (1932).
7. MENSHIKOV, G. P., *Uspekhi khimii, 22*, 1138 (1953).
8. FERLES, M., BLÁHA, K., *Chem. listy, 48*, 284 (1954).
9. LEONARD, N. J., Senecio Alkaloids, in "The Alkaloids", Vol. I (Eds. R. H. F. Manske and H. L. Holmes), Academic Press, New York, 1949, p. 107.
10. LEONARD, N. J., Senecio Alkaloids, in "The Alkaloids", Vol. VI (Ed. R. H. F. Manske), Academic Press, New York, 1960, p. 35.
11. BOIT, H.-G., "Ergebnisse der Alkaloid-Chemie bis 1960", Akademie Verlag, Berlin, 1961, p. 86.
12. WARREN, F. L., *Fortschr. d. Chemie org. Naturstoffe, 12*, 198 (1955).
13. WARREN, F. L., *Fortschr. d. Chemie org. Naturstoffe, 24*, 329 (1966).
14. WARREN, F. L., Senecio Alkaloids, in "The Alkaloids", Vol. XII (Ed. R. H. F. Manske), Academic Press, New York, 1970, p. 245.
15. SAXTON, J. E., The Pyrrolizidine Alkaloids, in "The Alkaloids", Vol. I (Ed. J. E. Saxton), The Chemical Society, London, 1971, p. 59.
16. SAUTON, J. E., The Pyrrolizidine Alkaloids, in "The Alkaloids", Vol. II (Ed. J. E. Saxton), The Chemical Society, London, 1972, p. 59.
17. LIKHOSHERSTOV, A. M., KOCHETKOV, N. K., *Uspekhi Khimii, 34*, 1550 (1965).
18. BULL, L. B., CULVENOR, C. C. J., DICK, A. T., "The Pyrrolizidine Alkaloids", North-Holland Publ. Co., Amsterdam, 1968.
19. ADAMS, R., *Angew. Chem., 65*, 433 (1953).
20. ADAMS, R., GIANTURCO, M., *Angew. Chem., 69*, 5 (1957).

## II. NECINES

### 1. THE STRUCTURE OF NECINES

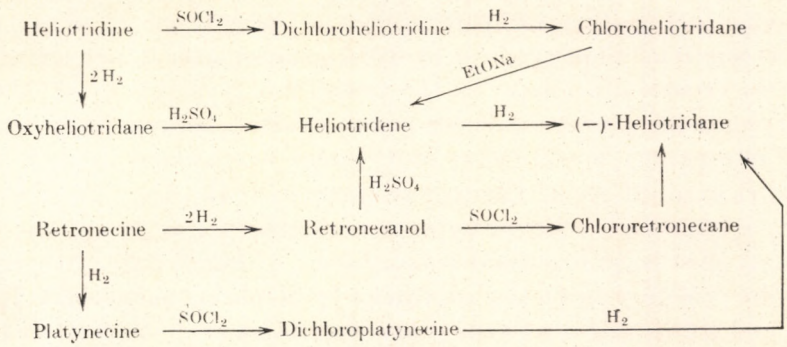
In 1932, Menshikov isolated the alkaloid heliotrine from *Heliotropium lasiocarpum* [1]. By alkaline hydrolysis of this alkaloid, he obtained a substance with the empirical formula $C_8H_{13}NO_2$ and called it heliotridine. The reaction with benzoyl chloride showed that heliotridine had two hydroxyl groups; by reaction with nitrous acid and with methyl iodide the tertiary character of the nitrogen atom was established [2]. Attempt was made to convert heliotridine into the fundamental substance containing no oxygen and no double bond; for that purpose, heliotridine was treated with thionyl chloride to give the dichloro derivative which on hydrogenation over Adams catalyst yielded chloroheliotridane, $C_8H_{14}NCl$. Cleavage of the chlorine atom with sodium ethoxide gave rise to heliotridene, $C_8H_{13}N$, which on hydrogenation resulted in the optically active basic substance (—)-heliotridane $C_8H_{15}N$. (—)-Heliotridane was also prepared from heliotrine [3] by its hydrogenolysis to oxyheliotridane, $C_8H_{15}NO$, which on dehydration with conc. sulfuric acid gave heliotridene and, on subsequent hydrogenation, (—)-heliotridane.

In 1931, Manske [4] isolated the alkaloid retrorsine from *Senecio retrorsus*. Hydrolysis of this alkaloid gave retronecine $C_8H_{13}NO_2$. Retronecine was also obtained by hydrolysis of the alkaloids senecionine [5] and monocrotaline [6]; it was found to be identical [7] with the product of hydrolysis of the alkaloid trichodesmine. Konovalova and Orekhov [8] reported that hydrogenolysis of retronecine afforded the amino alcohol $C_8H_{15}NO$, called retronecanol, which on dehydration with conc. sulfuric acid and subsequent hydrogenation yielded (—)-heliotridane. Later on (—)-heliotridane was also obtained [9] by conversion of retronecanol into the chloro derivative followed by hydrogenation.

In 1945, Orekhov [10] isolated an alkaloid, platyphylline, from *Senecio platyphyllus*, which on hydrolysis gave the amino alcohol platynecine,

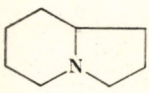
$C_8H_{15}NO_2$. Platynecine was converted [11] with thionyl chloride into the dichloro derivative, which yielded (—)-heliotridane on hydrogenation. Later on [12] it was found that platynecine could be obtained by hydrogenation of retronecine on Raney nickel. Consequently, retronecine, platynecine and heliotridine are derived from the same basic substance, (—)-heliotridane. The experiments illustrated in Table II show that apart from other things, even the relationship between the alkaloids isolated from plants of the genera *Senecio*, *Trichodesma*, *Crotalaria* and *Heliotropium* can be demonstrated.

*Table II*
Conversion of Retronecine, Platynecine and Heliotridine to (—)-Heliotridane




Menshikov undertook to elucidate the structure of (—)-heliotridane. He found that (—)-heliotridane was a tertiary amine which did not contain an $N-CH_3$ group. The empirical formula $C_8H_{15}N$ indicates that the molecule of (—)-heliotridane should contain two rings. Hofmann methylation gave [13] a nitrogen-free product only after the third methylation, suggesting that the nitrogen atom in (—)-heliotridane must be common to both rings. The nature of one of the rings was determined by hydrogenation of des-N-methyl-heliotridane to the dihydro derivative, which on dehydrogenation afforded the substance $C_9H_{15}N$, identified as a pyrrol derivative. It followed that, considering the empirical formula, (—)-heliotridane had to be identical either with indolizidine (II) or it had to be the methyl derivative of the substance III, called pyrrolizidine. Menshikov prepared a series of synthetic substances corresponding to the possible structures of dihydro-des-N-methyl-heliotridane, none of them was, however, identical with it. Therefore, he synthesized 1-methylpyrrolizidine (I) and found it to be identical with (—)-heliotridane on the basis of a comparison of the picrates. For details,

see the summarizing report [14]. Adams and Rogers [9] then synthesized 1,3-dimethyl-2-propylpyrrolidine (IV) and found that it was identical with racemic dihydro-des-N-methylheliotridane.



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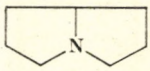

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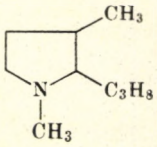
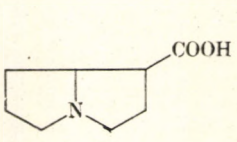



IV

Other papers dealt with the elucidation of the structure of retronecine, platynecine and heliotridine, the most common and longest known amino alcohols. Much attention was paid to the resolution of the structure of retronecine and platynecine particularly by Adams' school. Since retronecine is converted to platynecine by hydrogenation over Raney-nickel [12], these two necines differ only by the presence of a double bond. The two hydroxyl groups of retronecine are not equivalent, because on hydrogenation of retronecine, or still better of its esters, over Adams catalyst one of them is cleaved off giving rise to retronecanol [8, 15]. Retronecanol has a $C—CH_3$ group; since retronecine does not contain this group, one of its hydroxyls must be primary and removable by hydrogenation. The presence of a hydroxymethyl group was confirmed by preparing monobenzoyl platynecine and converting it into the chloro derivative [11], which after reduction, hydrolysis and oxidation yielded pyrrolizidine-1-carboxylic acid (V). The position of the second hydroxyl group was established [16] by application of the following procedure: retronecanol was oxidized to retronecanone by which the secondary nature of the hydroxyl was revealed. Since dehydration of platynecine readily afforded anhydroplatynecine [17], this hydroxyl had to be in the position 6 or 7 to give rise to a five- or six-membered ethereal ring. Reaction of retronecanol with cyanogen bromide gave [18] an adduct which on boiling with alkali yielded a neutral cyanamide ether. The facility of the formation of ether indicated a six-membered ring (VI). The secondary hydroxyl had therefore to be in the position 7. This assumption was confirmed by the synthesis [19] of retronecanone (VII).

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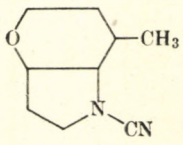
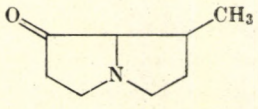
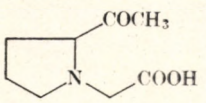



VI

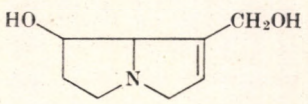



VII

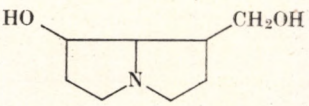
It remained to determine the position of the double bond. Adams and Rogers [12] showed that, in view of the ready hydrogenolysis of retronecine and its esters, the double bond must be in allylic position to the primary hydroxyl group. Confirmatory evidence was provided by Adams and Mahan [20] who converted retronecine by partial hydrogenation to deoxyretronecine ($-CH_2OH \rightarrow -CH_3$); with thionyl chloride this afforded a chloro derivative, whose reduction with chromous chloride gave isoheliotridene ($>CH-OH \rightarrow >CH_2$). Ozonolysis of isoheliotridene gave rise to the keto acid VIII, yielding a positive iodoform reaction characteristic of a methyl ketone. Thus the presence of a double bond in the positions 1,2 could be demonstrated; retronecine and platynecine were assigned the structures IX and X which were confirmed by a series of other reactions.



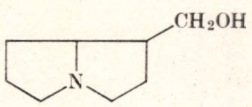

VIII




IX




X



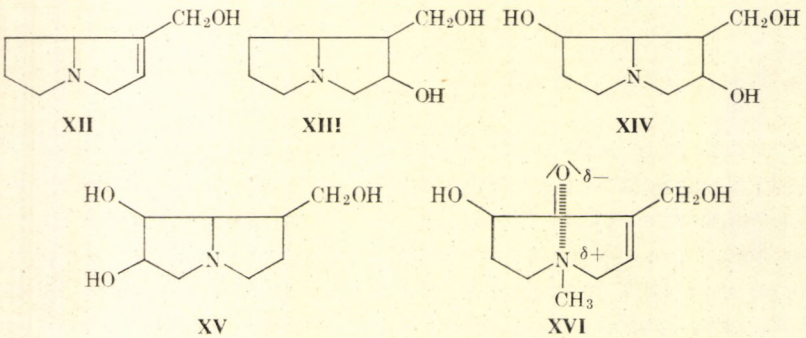

XI

The structure of heliotridine was elucidated by Menshikov *et al.* Oxidation [21] of oxyheliotridane gave a product which was found to be identical with retronecanone (VII). Thus the position of one of the hydroxyls was determined. The position of the second hydroxyl was determined by partial hydrogenation of heliotridine to dihydroxy-heliotridane. The latter was partially benzoylated, the product was dehydrated with thionyl chloride and, finally, hydrogenated and hydrolyzed to an alcohol identical with (—)-isoretronecanol (XI); the latter was prepared in a similar manner [11] from platynecine (X). A comparison of the degradation products of retronecine (IX) and heliotridine showed that the double bond was also located in the positions 1,2; this was confirmed [21] by conversion of the alkaloid heliotrine to the known amino alcohol supinidine (see below). Thus, retronecine and heliotridine are diastereoisomeric substances with the structure IX, and they differ only by their configuration at C-7.

All the known monohydroxynecines have a hydroxymethyl group in the molecule. The longest known substance, not yet found in nature, is (—)-isoretronecanol (XI) which was obtained by degradation of retronecine and

platynecine [16]. A very long known natural substance is (−)-trachelanthamidine prepared [22] for the first time by hydrolysis of the alkaloid trachelanthamine. (−)-Trachelanthamidine was oxidized [23] to the (−)-acid, which on decarboxylation yielded the pyrrolizidine (III); it was also converted to 1-methylpyrrolizidine (I) which, however, was not identical with (−)-heliotridane and was called (−)-pseudoheliotridane. Hydrolysis of the alkaloid lindelofine gave lindelofidine [24] whose structure (XI) resulted from a comparison with (−)-isoretronecanol. The last substance of this group is laburnine which was isolated from *Cytisus laburnum* [25]. Its structure (XI) resulted mainly from a comparison with (−)-trachelanthamidine. Consequently, the above mentioned four monohydroxynecines have the same structure (XI) and they differ from one another by their configuration at the carbon atoms 1 and 8.

There have been found two of the unsaturated monohydroxynecines. Hydrolysis of the alkaloid supinine afforded (−)-supinidine [26]; by hydrogenolysis, this was converted to (−)-heliotridane (I) and by partial hydrogenation to heliotridene, which resulted in structure XII. The enantiomeric (+)-supinidine was isolated only in 1967 [27].




With regard to dihydroxynecines of the empirical formula $C_8H_{15}NO_2$ there were found, apart from platynecine (X), the bases hastanecine from the alkaloid hastacine [28] and turneforcidine from the alkaloid turneforcine [29]. These are the diastereoisomers of platynecine (X) whose structure was determined particularly by spectral methods. Macronecine has the same empirical formula $C_8H_{15}NO_2$. It was isolated from the alkaloid macrophylline [30] and was assigned [31] structure XIII, which was confirmed by conversion to laburnine (XI).

So far, we know two trihydroxynecines. Rosmarinecine $C_8H_{15}NO_3$ arises from the hydrolysis of the alkaloid rosmarinine [32]. Since rosmarinecine is

not oxidized by periodic acid, the two other hydroxyl groups attached to 1-hydroxymethylpyrrolizidine must be located in the positions 2,6 or 2,7. By a series of reactions, rosmarinecine was converted [33] to anhydroplatynecine. This led to the only possible structure of 1-hydroxymethyl-2,7-dihydroxypyrrolizidine (XIV) which was confirmed by synthesis from retronecine (IX). Crotanecine $C_8H_{15}NO_3$ was obtained by hydrolysis of the alkaloids anacrotine and madurensine [34] and its structure XV was determined by NMR spectroscopy.

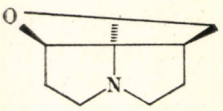
It took a rather long time to elucidate the structure of othonecine which was obtained for the first time [35] by hydrolysis of the alkaloid othosenine. It was assigned the structure XVI on the basis of X-ray crystallography [36]. Confirmatory evidence for this structure was also obtained by oxidative degradation of its dihydrodesoxy derivative [37].

## 2. THE STEREOCHEMISTRY AND ABSOLUTE CONFIGURATION OF NECINES

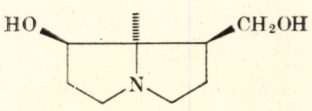
A summarizing report on the stereochemistry of pyrrolizidine alkamines was published in the first volume of this series [38] and therefore it will be dealt with here only briefly and emphasis will be laid on the recent results.

As early as 1935, it was found [17] that platynecine with thionyl chloride gave the anhydro derivative. However, it was only in 1950 that Leonard and Felley [39] interpreted this fact to that effect that in the molecule of anhydroplatynecine an angle is formed by the two five-membered rings of the pyrrolizidine system. The linkage between the hydroxyl group at C-7 and the methylene group at C-1 in a five-membered ethereal ring must be located on the side of the sharp angle of the atoms C-7, C-8 and C-1, and both groups must consequently be in *cis*-position to each other; the hydrogen atom at C-8 must then be in *trans*-position to the two mentioned groups (XVII). Concerning the original configuration of the hydroxyl at C-7, the authors [39] refused to draw conclusions, because they were afraid that during the formation of anhydroplatynecine (XVII) from platynecine (X) inversion might occur. However, Dry *et al.* [33] succeeded to demonstrate that inversion did not take place and that the hydroxyl at C-7 in platynecine was in *trans* position to the hydrogen at C-8. Thus, platynecine could be ascribed structure XVIII except for the reverse absolute configuration at the carbon atoms C-1, C-7 and C-8.

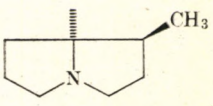
Since platynecine (XVIII) can be converted to (−)-heliotridane, the latter must also have the hydrogen at C-8 and the methyl at C-1 in *trans* position



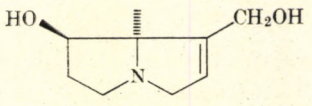

XVII




XVIII



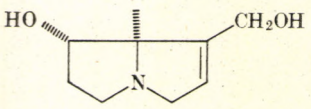

XIX



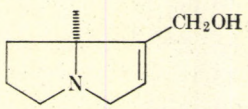

XX

(XIX) to each other. The absolute configuration of (−)-heliotridane (XIX) was determined [40] to be *1S*, *8S* on the basis of its degradation to (+)-3-methylheptane of known absolute configuration. From that followed the assignment of the absolute configurations XVIII and XX to platynecine and retronecine, respectively (hydrogenation of the latter gives rise to platynecine). This absolute configuration was independently confirmed by Adams and Fleš [41] who achieved the synthesis of (−)-retronecanone from (−)-3-methyl-5-aminovaleric acid [41], and effected the degradation of deoxyretronecine to a derivative of *S*-proline [42].

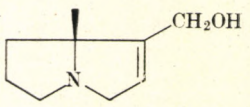
Warren and Klemperer [40] also suggested for heliotridine and supinidine the absolute configuration which logically resulted from the determination of the absolute configuration of (−)-heliotridane. Since heliotridine is diastereoisomeric with retronecine (XX) and since on degradation it also affords (−)-heliotridane (XIX), it must have the reverse absolute configuration at C-7 (XXI). (−)-Supinidine must then have the absolute configuration shown in the formula XXII, because its hydrogenolysis gives rise to (−)-heliotridane (XIX). (+)-Supinidine has the reverse absolute configuration (XXIII) and therefore it belongs to the group of alkaloids of (+)-heliotridane.



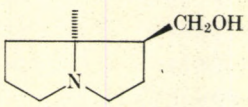

XXI




XXII

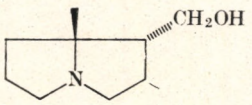



XXIII

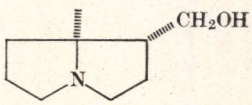



XXIV

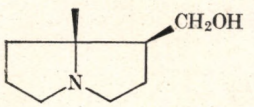
There are known four diastereoisomeric 1-hydroxymethylpyrrolizidines. The absolute configuration for (−)-isoretronecanol (XXIV) has resulted from the degradation of retronecine and platynecine [16]. All four 1-hydroxymethylpyrrolizidines were oxidized [43] to acids. The necines with *trans* arrangement of the hydrogens at C-1 and C-8 yielded only one acid, whereas those with a *cis*-configuration of these hydrogens gave two diasteroisomeric acids (inversion at C-8). The acids were reduced to hydroxymethylpyrrolizidines; a comparison showed the relationship between the mentioned four necines. The absolute configurations, XXV for (+)-isoretronecanol (lindelofidine), XXVI for trachelanthamidine and XXVII for (+)-trachelanthamidine), (laburnine), were inferred from the above-mentioned reactions on the basis of the already known absolute configuration of (+)-isoretronecanol (XXIV).



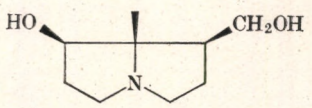

XXV




XXVI

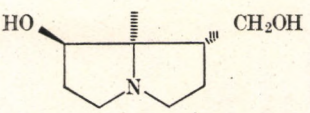



XXVII

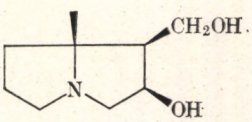



XXVIII

With regard to the saturated dihydroxynecines, the absolute configuration of platynecine (XVIII) was known. The stereochemistry of other three naturally occurring necines of this group — hastanecine, turneforcidine and macronecine — was resolved on the basis of molecular rotation differences [44], the results had, however, later on to be revised. On the basis of mass and NMR spectral studies, hastanecine was assigned [45] to have structure XXVIII, turneforcidine structure XXIX, and macronecine structure XXX. These structures were also confirmed by synthesis [45–47].




XXIX



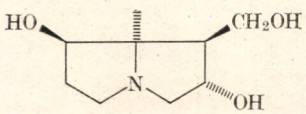

XXX

*Table III*

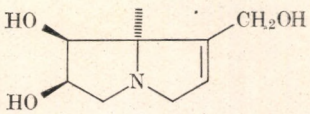
Structure and Physical Constants of Natural Necines

| Necine | Empirical formula | Structure | M.p., °C (or b.p.) | $[\alpha]_D^{EtOH}$ | Parent alkaloid |
|---|---|---|---|---|---|
| (—)-Isoretronecanol | $C_8H_{15}NO$ | XXIV | 39–40 | —78.2 | (—)-Isoretronecanyl tiglate, (—)-Isoretronecanyl *trans*-3-methylthiopropenate |
| Lindelofidine ((+)-Isoretronecanol) | $C_8H_{15}NO$ | XXV | 40–41 | +79.1 | Cynaustraline, Lindelofamine, Lindelofine, Thesine, Thesinine |
| Trachelanthamidine | $C_8H_{15}NO$ | XXVI | (140/15) | —14.9 | Cornucervine, Macrotomine, Phalaenopsine T, Strigosine, Trachelanthamine, Viridiflorine |
| Laburnine ((+)-Trachelanthamidine) | $C_8H_{15}NO$ | XXVII | (134/12) | +15.4 | Laburnine angelate, Laburnine benzoate, Malaxine, Phalaenopsine La, Planchonelline |
| Platynecine | $C_8H_{15}NO_2$ | XVIII | 151–152 | $-57^{CHCl_3}$ | Hygrophylline, Neoplatyphylline, Platyphylline, Sarracine |
| Turneforcidine | $C_8H_{15}NO_2$ | XXIX | 118–120 | —4.3 | Retusine, Turneforcine |
| Hastanecine | $C_8H_{15}NO_2$ | XXVIII | 113–114 | —10.0 | Hastacine |
| Macronecine | $C_8H_{15}NO_2$ | XXX | 126–128 | +49.3 | Macrophylline |
| Rosmarinecine | $C_8H_{15}NO_3$ | XXXI | 171–172 | —116.5 | Angularine, Rosmarinine |
| Supinidine | $C_8H_{13}NO$ | XXII | (159/10) | —10.3 | Amabiline, Heleurine, Supinine |
| (+)-Supinidine | $C_8H_{13}NO$ | XXIII | (85/0.14) | +9.2 | Cynaustine |
| Retronecine | $C_8H_{13}NO_2$ | XX | 121–122 | +50.2 | Acetylindicine, $O^7$-Angelylretronecine, Axillaridine, Axillarine, Bisline, Chlorodesoxysceleratine, Crispatine, Dicrotaline, Echimidine, Echiumine, Erucifoline, Fulvine, Grahamine, Grantaline, Grantianine, Incanine, Indicine, Integerrimine, Intermedine, Isoline, Jacobine Jacoline, Jaconine, Jacozine, Junceine, Latifoline Lycopsamine, Monocrotaline, Mucronatinine, Nilgirine, Retrorsine, Riddelline, Sceleratine, Senecionine, Seneciphylline, Sincamidine, Spartioidine, Spectabiline, Swazine, Symphytine, Trichodesmine, Usaramine |
| Heliotridine | $C_8H_{13}NO_2$ | XXI | 117–118 | +30.4 | $O^7$-Angelylheliotridine, $O^7$-Angelylheliotridine trachelanthate, $O^7$-Angelyl heliotridine viridiflorate, Echinatine, Europine, Heliosupine, Heliotrine, Lasiocarpine, Rinderine |
| Crotanecine | $C_8H_{13}NO_3$ | XXXII | 192 | +39.2 | Anacrotine, Madurensine |
| Othonecine | $C_9H_{15}NO_3$ | XXXIII | — | — | Acetylsenkirkine, Clivorine, Crosemperine, Crotafoline, Emiline, Floricaline, Floridanine, Florosenine, Hydroxysenkirkine, Ligularine, Onetine, Othosenine, Rerusamine, Senkirkine |

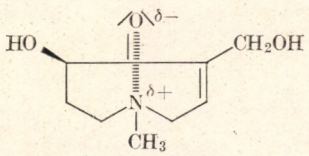
Of the two known natural trihydroxynecines, rosmarinecine (XIV) and crotanecine (XV), the stereochemistry of rosmarinecine was first elucidated. On the basis of a series of reactions and its synthesis from retronecine (XX), rosmarinecine was assigned [33] the absolute configuration expressed by the formula XXXI. The stereochemistry of crotanecine (XXXII) resulted [34] from the interpretation of the mass and NMR spectra. The stereochemistry of othonecine (XVI) was resolved by X-ray analysis [36] as XXXIII.




XXXI




XXXII

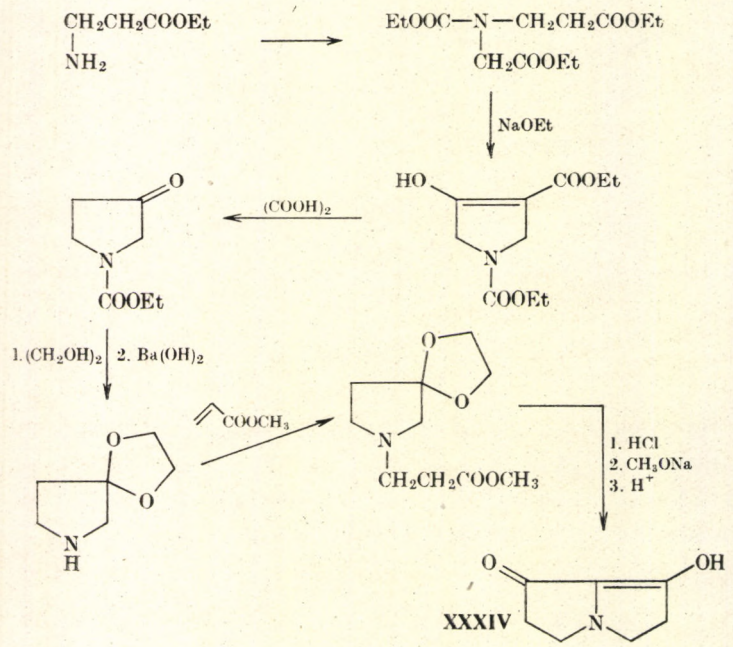



XXXIII

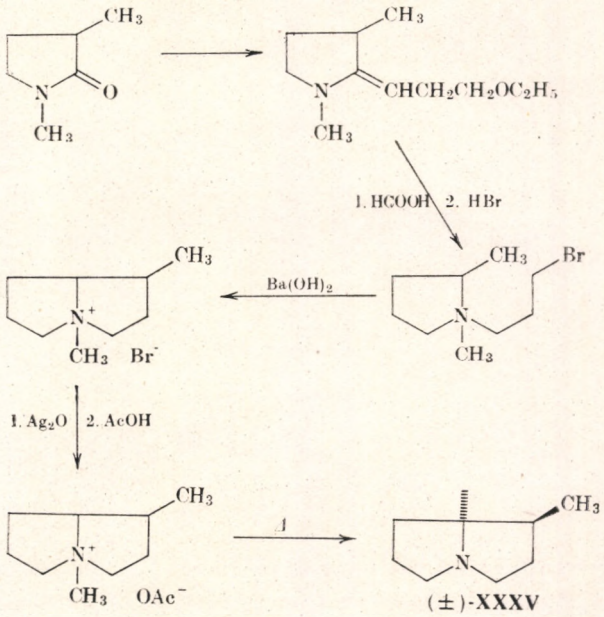
Table III gives a survey of the physical properties of necines isolated after hydrolysis of pyrrolizidine alkaloids. The other pyrrolizidine bases which were found in natural material and were not esterified by acids, have been allocated to the alkaloids in Chapter IV.

## 3. SYNTHESIS OF NECINES

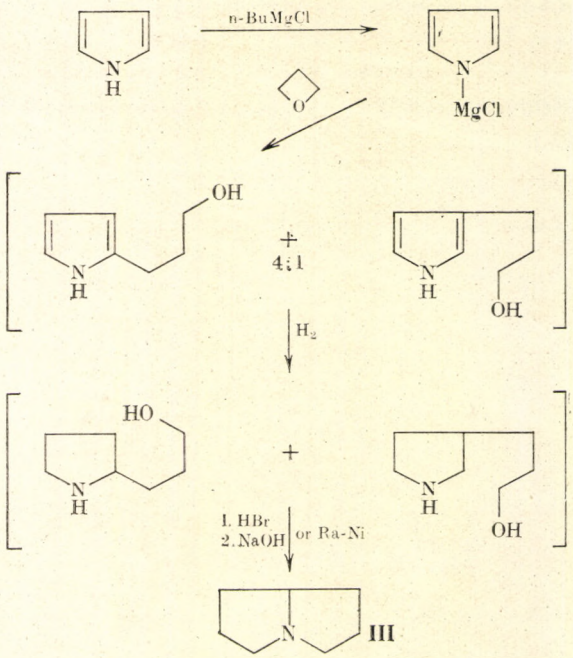
In 1965 Likhosherstov and Kochetkov [48] published a review in which all the known syntheses of the pyrrolizidine system were summarized up to the year 1963. In view of the fact that up to 1963 most of the simple derivatives of pyrrolizidine had been prepared, the number of new papers is not large. Viscontini and Bühler [49] prepared 1,7-dioxopyrrolizidine (XXXIV) along the following pathway:



Dědek and Bárta [50] treated 1,3-dimethyl-2-pyrrolidone with 3-ethoxypropylmagnesium bromide to obtain an enamine, which after reduction with formic acid and cyclization afforded the quaternary bromide derived from (±)-heliotridane; pyrolysis of the quaternary acetate gave (±)-heliotridane (XXXV):



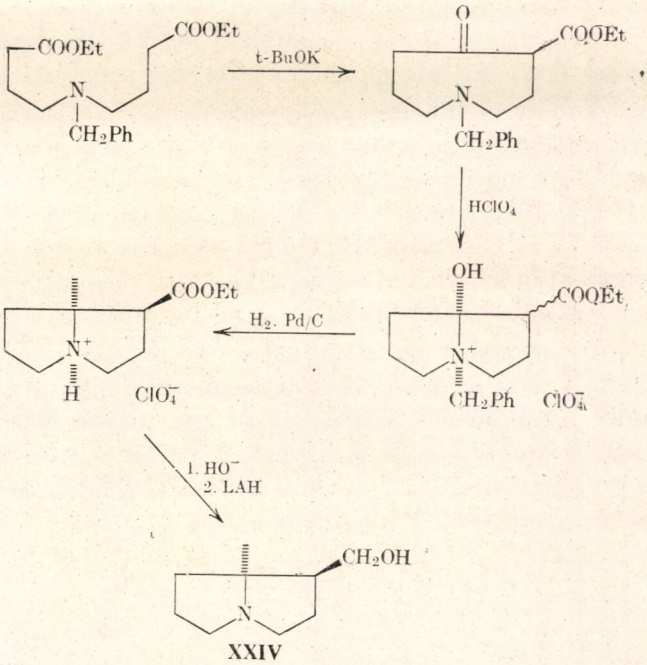

Kray and Reinecke [51] used pyrrol to prepare the pyrrolizidine system. Pyrrol was converted to pyrrylmagnesium chloride which with trimethylene oxide yielded a mixture of pyrrolpropanols. This was then hydrogenated to a mixture of pyrrolidinepropanols, which after cyclization yielded pyrrolizidine (III):



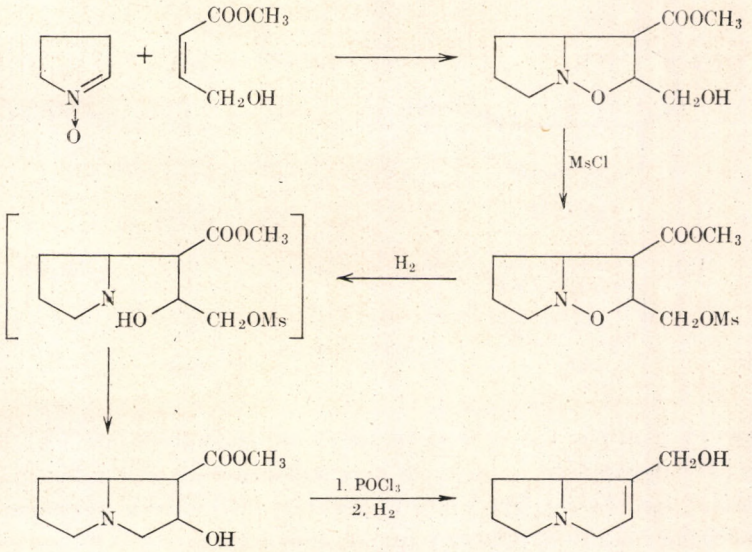

As far as the synthesis of the natural necines is concerned, much attention has been paid to the synthesis of 1-hydroxymethyl-pyrrolizidines. In addition to the syntheses mentioned in the review [48], the synthesis of (±)-isoretronecanol has been carried out [52]. Dieckmann condensation of diethyl 3,3′-benzylimino-*bis*-butyrate gives the β-ketoester, which on treatment with perchloric acid undergoes transannular reaction to a quaternary hydroxyester; the total yield of both steps is 89%. Hydrogenation of the quaternary hydroxyester over Pd/C catalyst affords ethyl (±)-isoretronecanolate perchlorate which on conversion to the free base and reduction with lithium aluminium hydride yields (±)-isoretronecanol (racemate of XXIV); only about 2% of (±)-trachelanthamidine (racemate of XXVI) is formed.

All the four optically pure diastereoisomeric 1-hydroxymethyl-pyrrolizidines were obtained by Kochetkov *et al.* [53]. The racemates synthesized from γ-butyrolactone [54, 55] were resolved by using dibenzoyl-(−)-tartaric acid.

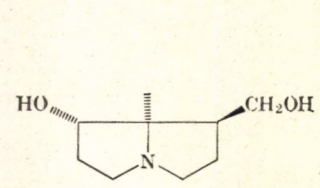
(±)-Supinidine (racemate of XXII) was prepared by Tufariello and Tette [56] from $\Delta^1$-pyrroline-1-oxide. Its 1,3-dipolar addition to methyl γ-hydroxycrotonate gave rise to the isoxazolidine derivative which after



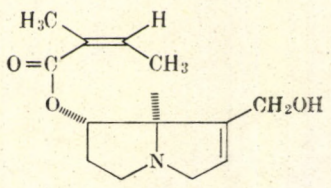

mesylation and hydrogenolysis yielded the methyl 2-hydroxypyrrolizidine-1-carboxylate. Its dehydration and reduction afforded (±)-supinidine:



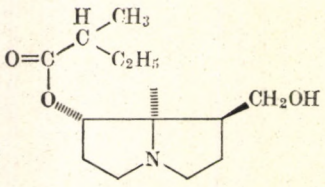
Some partial syntheses were carried out in connection with the determination of the correct absolute configuration of saturated dihydroxynecines. Aasen *et al.* [45] attempted to alter the configuration at C-1 in platynecine (XVIII) or dihydroxyheliotridane (XXXVI). These two diols have the thermodynamically less stable $\beta$-configuration at C-1, and inversion should be achieved readily through a 1-$\beta$-ethoxycarbonyl derivative. Since the C-7 hydroxyl must be protected against oxidation, the initial substance used was $O^7$-angelylheliotridine (XXXVII). Its hydrogenation on Raney nickel afforded the derivative of dihydroxyheliotridane (XXXVIII) which was oxidized with the Jones reagent to give the corresponding 1-carboxylic acid; the latter was esterified to the ester XXXIX. Epimerization of XXXIX was effected by treatment with sodium methoxide to obtain (under simultaneous methanolysis) the diastereoisomer XL. Its reduction with lithium aluminium hydride gave 7-$\alpha$-hydroxy-1-$\alpha$-hydroxymethyl-8-$\alpha$-pyrrolizidine (XLI), identical with natural hastanecine (XXVIII) in all respects except that it was dextrorotatory.



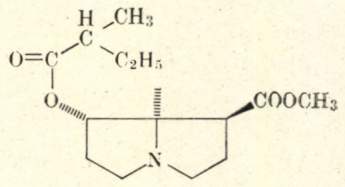

XXXVI



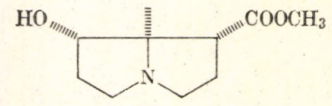

XXXVII



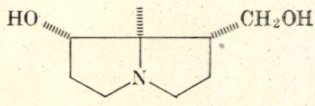

XXXVIII




XXXIX



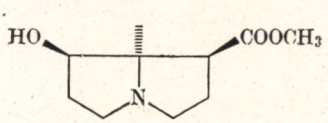

XL



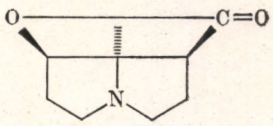

XLI

In order to confirm the stereochemistry of turneforcidine (XXIX), the authors [47] decided to synthesize this substance in a similar manner as hastanecine (see above). The point of greatest difficulty in preparing turneforcidine from readily available pyrrolizidine derivatives is in achieving the

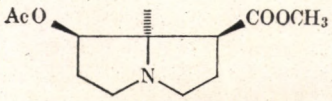
1α-hydroxymethyl-8α-configuration. By oxidation of retronecine (XX) an acid was prepared which was esterified and hydrogenated to the saturated ester XLII having the configuration of platynecine (XVIII). However, on an attempt to epimerize the ester XLII by heating with sodium methoxide, a stable lactone XLIII was obtained whose reduction gave platynecine. The acetyl derivative XLIV prepared from retronecine (XX) could not be epimerized either. Therefore, the same ester (XL) was used for the synthesis of turneforcidine (XXIX) as for that of (+)-hastanecine (XLI). The ester XL was oxidized to give the ketone XLV which on reduction with lithium aluminium hydride was converted to (+)-hastanecine (XLI). Catalytic hydrogenation yielded, however, the 7β-hydroxy-8α-pyrrolizidine-1α-carboxylate (XLVI) which was then further reduced with lithium aluminium hydride to a compound identical with natural turneforcidine (XXIX).



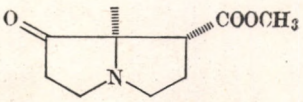

XLII



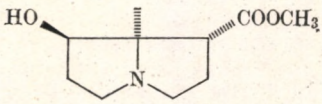

XLIII




XLIV



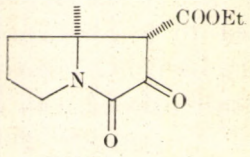

XLV



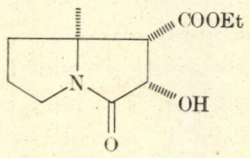

XLVI

From the conversion [31] of the alkaloid macrophylline to laburnine (XXVII), the absolute configuration at C-1 and C-8 of macronecine was inferred. On the basis of the NMR spectra [45], macronecine was assigned the absolute configuration XXX. For synthetic confirmation, the easily available [57] (±)-1α-ethoxycarbonyl-2,3-dioxo-8α-pyrrolizidine (XLVII) was used. Reduction of this substance with zinc in acetic acid gave [46] a mixture of substances XLVIII and XLIX, the first of which (XLVIII) crystallized from the reaction mixture. Its reduction with lithium aluminium hydride gave the corresponding (±)-alcohol L whose constitution is, on the basis of the mass and NMR spectra, identical with that of natural

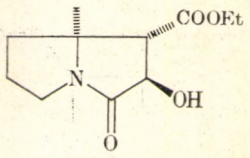
macronecine (XXX). By resolution of this (±)-necine with ammonium α-bromo-D-camphor-π-sulfonate, natural macronecine (XXX) and its (—)enantiomer (L) were obtained. To the other substance obtained in the zinc reduction above (XLIX), the same sequence of reactions was applied, which afforded a mixture of enantiomers with reversed configuration at C-2. By using similar reactions, two other racemates with reversed configuration at C-1 were prepared, so that at present all four possible racemic 1-hydroxymethyl-2-hydroxypyrrolizidines are known.



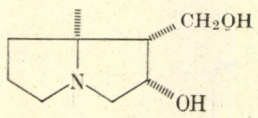

XLVII




XLVIII




XLIX




L

## REFERENCES

1. Menshikov, G. P., *Ber.*, *65*, 974 (1932).
2. Menshikov, G. P., *Ber.*, *66*, 875 (1933).
3. Menshikov, G. P., *Ber.*, *68*, 1051 (1935).
4. Manske, R. H. F., *Can. J. Res.*, *5*, 651 (1931).
5. Barger, G., Blackie, I. I., *J. Chem. Soc.*, *1936*, 743.
6. Adams, R., Rogers, E. F., *J. Am. Chem. Soc.*, *61*, 2815 (1939).
7. Konovalova, R. A., Orekhov, A. P., *Zh. obsch. khim.*, *8*, 396 (1938).
8. Konovalova, R. A., Orekhov, A. P., *Bull. Soc. Chim. France*, (5) *4*, 1285 (1937).
9. Adams, R., Rogers, E. F., *J. Am. Chem. Soc.*, *63*, 228 (1941).
10. Orekhov, A. P., Tidebel, W., *Ber.*, *68*, 650 (1935).
11. Konovalova, R. A., Orekhov, A. P., *Ber.*, *69*, 1908 (1936).
12. Adams, R., Rogers, E. F., *J. Am. Chem. Soc.*, *63*, 537 (1941).
13. Menshikov, G. P., *Zh. obsch. khim.*, *7*, 1632 (1937).
14. Menshikov, G. P., *Uspekhi khimii*, *22*, 1138 (1953).
15. Barger, G., Seshadri, T. R., Watt, H. E., Yabuta, T., *J. Chem. Soc.*, *1935*, 11.
16. Adams, R., Hamlin, K. E., Jr., *J. Am. Chem. Soc.*, *64*, 2597 (1942).
17. Orekhov, A. P., Konovalova, R. A., Tidebel, W., *Ber.*, *68*, 1886 (1935).
18. Adams, R., Carmack, M., Mahan, J. E., *J. Am. Chem. Soc.*, *64*, 2593 (1942).
19. Adams, R., Leonard, N. J., *J. Am. Chem. Soc.*, *66*, 257 (1944).
20. Adams, R., Mahan, J. E., *J. Am. Chem. Soc.*, *65*, 2009 (1943).
21. Menshikov, G. P., Kuzovkov, A. D., *Zh. obsch. khim.*, *19*, 1702 (1949).
22. Menshikov, G. P., Borodina, G. M., *Zh. obsch. khim.*, *15*, 225 (1945).
23. Menshikov, G. P., *Zh. obsch. khim.*, *16*, 1311 (1946).
24. Labenskii, A. S., Menshikov, G. P., *Zh. obsch. khim.*, *18*, 1836 (1948).

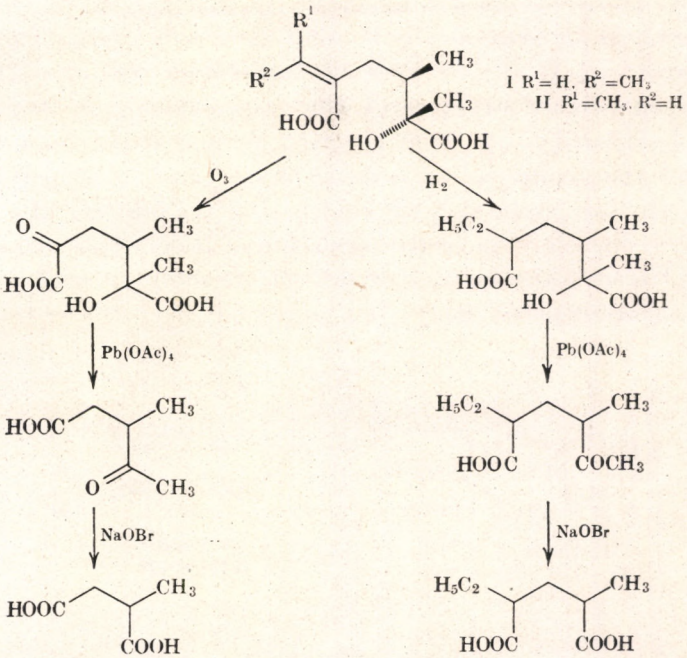
25. Galinowsky, F., Goldberger, H., Pöhm, M., *Monatsh., 80*, 550 (1949).
26. Menshikov, G. P., Gurevich, E. L., *Zh. obsch. khim., 19*, 1382 (1949).
27. Culvenor, C. C. J., Smith, L. W., *Austral, J. Chem., 20*, 2499 (1967).
28. Konovalov, V. S., Menshikov, G. P., *Zh. obsch. khim., 15*, 328 (1945).
29. Menshikov, G. P., Denisova, S. O., Massagetov, P. S., *Zh. obsch. khim., 22*, 1465 (1952).
30. Danilova, A. V., Utkin, L. M., Massagetov, P. S., *Zh. obsch. khim., 25*, 831 (1955).
31. Danilova, A. V., Utkin, L. M., *Zh. obsch. khim., 30*, 345 (1960).
32. Richardson, M. F., Warren, F. L., *J. Chem. Soc., 1943*, 452.
33. Dry, L. J., Koekemoer, M. J., Warren, F. L., *J. Chem. Soc., 1955*, 59.
34. Atal, C. K., Kapur, K. K., Culvenor, C. C. J., Smith, L. W., *Tetrahedron Letters, 1966*, 537.
35. Zhdanovich, E. S., Menshikov, G. P., *Zh. obsch. khim., 11*, 835 (1941).
36. Wunderlich, J. A., *Chem & Ind., 1962*, 2089.
37. Koretskaya, N. I., Danilova, A. V., Utkin, L. M., *Khim. prirod. soed., 1965*, 22.
38. Fodor, G., New Methods and Recent Developments of the Stereochemistry of Ephedrine, Pyrrolizidine, Granatane, and Tropane Alkaloids, in "Recent Developments in the Chemistry of Natural Carbon Compounds", Vol. I, Akadémiai Kiadó, Budapest, 1965, pp. 53—68.
39. Leonard, N. J., Felley, D. L., *J. Am. Chem. Soc., 72*, 2537 (1950).
40. Warren, F. L., Klemperer, M. E. von, *J. Chem. Soc., 1958*, 4574.
41. Adams, R., Fleš, D., *J. Am. Chem. Soc., 81*, 4946 (1959).
42. Adams, R., Fleš, D., *J. Am. Chem. Soc., 81*, 5803 (1959).
43. Labenskii, A. S., Serova, N. A., Menshikov, G. P., *Dokl. Akad. Nauk SSSR, 88*, 467 (1953).
44. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem., 10*, 464 (1957).
45. Aasen, A. J., Culvenor, C. C. J., Smith, L. W., *J. Org. Chem., 34*, 4137 (1969).
46. Aasen, A. J., Culvenor, C. C. J., *J. Org. Chem., 34*, 4143 (1969).
47. Aasen, A. J., Culvenor, C. C. J., *Austral. J. Chem., 22*, 2657 (1969).
48. Likhosherstov, A. M., Kochetkov, N. K., *Uspekhi khimii, 34*, 1550 (1965); *Advan. Heterocyclic Chem., 5*, 315 (1965).
49. Viscontini, M., Bühler, H., *Helv. Chim. Acta, 50*, 1289 (1967).
50. Dědek, V., Bárta, M., *Sborník VŠCHT in Prague, C 8*, 89 (1966).
51. Kray, L. R., Reinecke, M. G., *J. Org. Chem., 32*, 225 (1967).
52. Leonard, N. J., Sato, T., *J. Org. Chem., 34*, 1066 (1969).
53. Kochetkov, N. K., Likhosherstov, A. M., Kulakov, V. N., *Tetrahedron, 25*, 2313 (1969).
54. Kochetkov, N. K., Likhosherstov, A. M., Budovskii, E. I., *Zh. obsch. khim., 30*, 2077 (1960).
55. Likhosherstov, A. M., Likhosherstov, L. M., Kochetkov, Z. K., *Zh. obsch. khim., 33*, 1801 (1963).
56. Tufariello, J. J., Tette, J. P., *Chem. Commun., 1971*, 469.

# III. NECIC ACIDS

## 1. THE STRUCTURE, STEREOCHEMISTRY AND ABSOLUTE CONFIGURATION OF NECIC ACIDS

### *A. C-10 Adipic Acids*

The longest known necic acid is senecic acid. In 1935, Orekhov and Tidebel [1] obtained the substance $C_{10}H_{14}O_4$ by hydrolysis of platyphylline and called it platynecic acid. Later on, the same substance was obtained by Barger and Blackie [2] from the hydrolysis of senecionine. He called it senecic acid and characterized it as an unsaturated acid with one lactone ring and three C–$CH_3$ groups. By oxidation of this acid, Manske [3] obtained acetic acid by which he demonstrated the presence of an ethylidene grouping in the molecule. Richardson and Warren [4] isolated senecic acid from rosmarinine and established that senecic acid had the empirical formula $C_{10}H_{16}O_5$ and that platynecic acid was its lactone. The structure of senecic acid was then inferred by Kropman and Warren [5]. Colour reaction with ferric chloride showed that senecic acid was an $\alpha$-hydroxy acid; its ozonolysis gave acetaldehyde (from the ethylidene grouping) and a dicarboxylic hydroxyketo acid, which was oxidized with lead tetraacetate to the keto acid $C_6H_{10}O_3$. This keto acid was further oxidized with hypobromite to afford (+)-methylsuccinic acid. On this basis, the structure of $\alpha$- or $\beta$-methyllevulinic acid was assigned to the keto acid $C_6H_{10}O_3$. To confirm this structure, the authors carried out hydrogenation of senecic acid to obtain the dihydro derivative which on oxidation with lead tetraacetate gave a keto acid. Further oxidation of this keto acid with hypobromite yielded $\alpha$-methyl-$\alpha'$-ethylglutaric acid. The following scheme shows the sequence of reactions from which the structure I of senecic acid has been deduced:

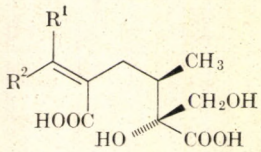



Senecic acid (I) is isomeric with integerrinecic acid obtained [3] by the hydrolysis of integerrimine. The elucidation of its structure (II) was based on the fact that it is formed by isomerization of the lactone of senecic acid on heating with sodium hydroxide [6]. These two acids belong to the group of C-10 adipic acids which are typical for the alkaloids of the plants of the family Asteraceae. They were, however, also found to occur in the plants of the genus *Crotalaria*. All the acids of this group (except for two) have a uniform skeleton which is formed by abnormal bonding of two isoprene units.

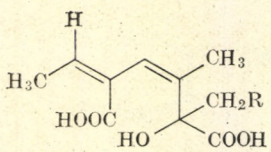
Two other acids of this group are isatinecic acid and retronecic acid [7, 8]. Both yield the same dihydro derivative on hydrogenation, but they differ owing to geometric isomerism on the double bond. Oxidative degradation, carried out as in the case of senecic acid (I), afforded α-methyl-α′-ethylglutaric and α-methyl-α′-ethylideneglutaric acids. A comparison of the UV spectra led to the assignment of structure III to isatinecic acid and structure IV to retronecic acid.

Isoseneciphyllic acid arises from the hydrolysis of the alkaloid seneciphylline [9]. Adams *et al.* [10] effected the oxidative degradation of tetrahydroisoseneciphyllic acid to α-methyl-α′-ethylglutaric acid which showed that isoseneciphyllic acid had the same skeleton as senecic acid (I). It

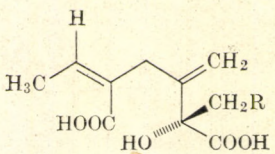
remained to determine the position of the two double bonds in the molecule. Since ozonolysis of isoseneciphyllic acid gave only acetaldehyde, it was ascribed structure V. On the basis of degradation reactions [11], riddelic acid was then assigned structure VI which bears analogy to the structure of isoseneciphyllic acid (V). In 1959, however, Masamune [12] obtained formaldehyde by the oxidation of isoseneciphyllic acid with permanganate–periodate. He also provided NMR evidence for the presence of a methylene group. Consequently, the correct structure of isoseneciphyllic acid is VII. Nair and Adams [13] then also revised the structure of riddelic acid, and on NMR spectroscopic evidence, they assigned it the correct structure VIII.




III $R^1 = H$, $R^2 = CH_3$
IV $R^1 = CH_3$, $R^2 = H$



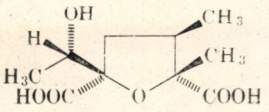

V R=H
VI R=OH



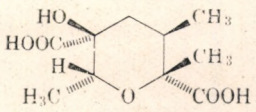

VII R=H
VIII R=OH

Alkaline hydrolysis of either jacobine or jaconine yielded jaconecic acid and isojaconecic acid. After some failures, Bradbury and Masamune [14] and Geissman [15] succeeded in elucidating the structure of these acids. The structure of jaconecic acid (IX) followed particularly from its oxidation with lead tetraacetate to acetaldehyde, from the oxidation with nitric acid to dimethylmalic acid and, furthermore, from the reactions of the alcohol, prepared by the reduction of dimethyljaconecate with lithium aluminium hydride. Isojaconecic acid was assigned the structure X on the basis of oxidative degradation of the acid and of the corresponding alcohol; the latter was obtained by the reduction of dimethyl isojaconecate with lithium aluminium hydride. These acids do not occur in nature; the tetrahydropyran or tetrahydrofuran rings arise only during the alkaline hydroysis of the alkaloid.

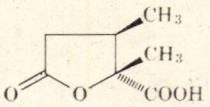
The absolute configuration of necic acids was determined by X-ray analysis of jacobine bromohydrin [16] and of the bromodilactone obtained from



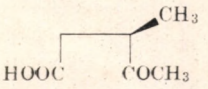

IX




X



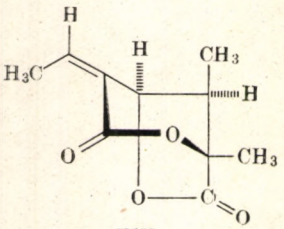

XI



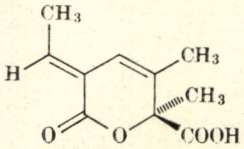

XII

jacobine [17]. This led to the absolute configurations IX and X for jaconecic and isojaconecic acid, respectively. In view of the fact that senecic and seneciphyllic acids were correlated with the acids obtained from jacobine [18, 19], the other mentioned necic acids have the absolute configurations I–VIII. The absolute configurations of necic acids from jacobine were chemically confirmed by Masamune [20]. Oxidation of jaconecic acid (IX) with lead tetraacetate gave rise to the lactone XI and to the keto acid XII. Masamune converted the keto acid by the Bayer–Villiger reaction to (—)-3-hydroxybutyric acid anhydride of known absolute configuration. He synthesized the lactone XI from the racemic keto acid XII which he subdivided into two stereoisomeric racemates and then to optically active substances; one of these substances he found to be identical with the lactone obtained from the oxidation. Other reactions showed that in the lactone XI the methyls at C-2 and C-3 are in *cis*-position. Reaction of the phenylglyoxylate ester of dimethyl jaconecate with methylmagnesium iodide gave (+)-atrolactic acid, which also led to the elucidation of the absolute configuration at C-5 and thus of the whole molecule of jaconecic acid (IX), because the stereochemistry at C-6 is determined by the opening of the epoxide ring of the native acid in jacobine.

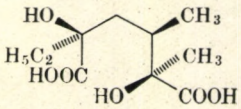
Hygrophyllinecic acid was obtained from the hydrolysis of hygrophylline [21] in the form of a monolactone $C_{10}H_{14}O_5$. Ozonolysis of this monolactone yielded acetaldehyde. Hydrogenolysis over Adams catalyst in the presence of perchloric acid converted the monolactone into dihydrosenecic lactone. Consequently, hygrophyllinecic acid is a hydroxy derivative of senecic acid; the hydroxyl group was found to be located at C-4 because the triol obtained on reduction of the alkaloid possesses only one glycol grouping (demonstrated by oxidation with periodic acid). Since hygrophyllinecic acid also forms the dilactone XIII, the absolute configuration at C-4 is thus also established. If the absolute configuration at C-4 were reversed, formation of the dilactone XIII would be impossible.



XIII



XIV

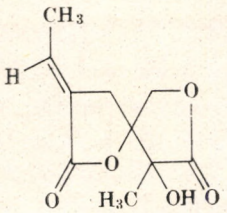


XV

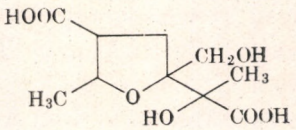
Clivonecic acid was obtained by the hydrolysis of the alkaloid clivorine. Hydrogenation afforded [22] the tetrahydro derivative which on oxidative degradation gave α-ethyl-α′-methylglutaric acid. It follows that this acid has the same skeleton as senecic acid (I). The position of the two double bonds was determined by ozonolysis to acetaldehyde (ethylidene grouping) and by interpretation of the NMR, mass and UV spectra (conjugated double bonds). From these data, structure XIV was inferred for clivonecic acid. It was assigned [23, 24] the absolute configuration 2*S* on the basis of a comparison of the ORD spectra of tetrahydroclivonecic acid with the spectra of dihydrosenecic, tetrahydroseneciphyllic and monocrotalic acids of known absolute configurations, and in view of the asymmetric reactions. This was the first case of a necic acid with 2*S* absolute configuration. Isolinecic acid was isolated from the alkaloid isoline [25] and, on the basis of spectral studies, structure XV appeared to be the most plausible. A comparison of the CD spectra of the dilactones of senecic and isolinecic acids and the degradation of isolinecic acid to 3-methylheptane-2,5-dione of known absolute configuration showed that the absolute configuration was 2*S*, 3*R*, 5*S*. At present, this is the second known necic acid with a 2*S* absolute configuration.

Alkaline hydrolysis of the alkaloid erucifoline gives rise [27] to erucifolinecic acid and by acid hydrolysis the dilactone is obtained. The structures of these two substances were elucidated on the basis of the interpretation of the NMR and mass spectra, which led to the most likely structure XVI for the dilactone and structure XVII for erucifolinecic acid. The absolute configurations were not determined; these compounds are artifacts which arise after opening of the epoxide ring of the native unsaturated epoxy acid.

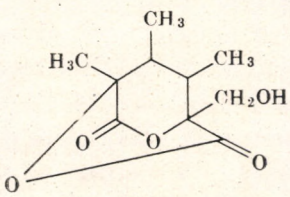
The last two C-10 adipic acids are sceleranecic acid and the acid from seazine. These two acids do not fall in with the so far common scheme of C-10 adipic acids. Sceleranecic acid was reduced [28] with lithium aluminium hydride to give a glycol which with periodic acid yielded formaldehyde



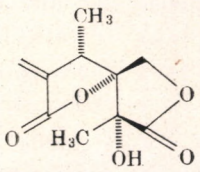
XVI



XVII



XVIII



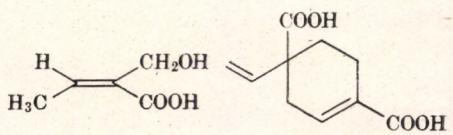
XIX

and 2,3-dimethyllevulinic acid. Oxidation of sceleranecic acid with lead tetraacetate gave rise to 2-hydroxy-2,3,4-trimethylglutaric acid lactone and to 2,3-dimethyllevulinic acid. On the basis of these reactions and the reinterpretation of the previously reported reactions, sceleranecic acid was assigned the structure XVIII.

Acid hydrolysis of swazine [29] afforded the dilactone whose structure XIX, inclusive of the absolute configuration 2*R*, 3*S*, 4*S*, was elucidated on the basis of X-ray analysis of its *p*-bromobenzoate. This dilactone is not a natural substance, either; it arises after opening of the epoxide ring in the native acid.

*B. C-5 Acids*

One of the most widely distributed C-5 acids is angelic acid. Hydrolysis of pyrrolizidine alkaloids also gave tiglic acid, α-methylbutyric acid and sarracinic acid. The latter arises on alkaline hydrolysis of sarracine [30] along with mikanecic acid. On the basis of the NMR spectrum, structure XX was ascribed to sarracinic acid. On hydrolysis sarracinic acid is partially dehydrated to a diene which undergoes Diels-Alder condensation giving rise to mikanecic acid (XXI).



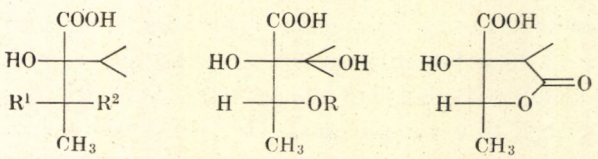
XX



XXI

*C. α-Isopropylbutyric Acids*

Hydroxy-substituted α-isopropylbutyric acids occur only in the alkaloids of the plant family Borraginaceae. The only exception are the alkaloids from *Eupatorium*. The most widely distributed acid is (+)-trachelanthic acid. (−)-Trachelanthic acid and the diastereoisomeric (−)-viridifloric acid were also found to occur in nature. The structure of these acids was elucidated [31, 32] on the basis of their degradation to 4-methylpentane-2,3-dione and 4-methylpentane-3-carboxylic acid. (+)-Trachelanthic acid was also obtained by demethylation of another natural necic acid, i.e. (−)-heliotric acid [33]. The absolute configurations of the above-mentioned acids were definitely established by Likhosherstov *et al.* [34] as 2*S*, 3*R* for (+)-trachelanthic (XXII) and (−)-heliotric (XXIII) acids, and 2*S*, 3*S* for (−)-viridifloric acid (XXIV), on the basis of a comparison of their degradation products with synthetic substances of known absolute configurations. Echimidinic, macrotomic and lasiocarpic acids possess an additional oxygen atom in the molecule. The structure of lasiocarpic acid (XXV) was determined [35] on the basis of its oxidation with periodic acid, colour reaction with ferric chloride and hydrolysis. Macrotomic acid is oxidized by periodic acid to acetaldehyde, acetone and oxalic acid; therefore, structure XXVI was ascribed to it [36]. The same oxidation products were also obtained [37] from echimidinic acid, which has the same structure (XXVI) as macrotomic acid; these two acids probably differ from each other in their stereochemistry. Crowley and Culvenor [38] published an interesting study dealing with the behaviour of lasiocarpic, echimidinic and macrotomic acids on acid hydrolysis. Later on, lasiocarpic acid (XXV) was ascribed [34] the same absolute configuration at C-3 as (+)-trachelanthic acid (XXII).



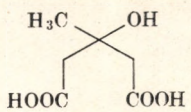

**XXII** $R^1=H$, $R^2=OH$ **XXV** $R=CH_3$ **XXVII**
**XXIII** $R^1=H$, $R^2=OCH_3$ **XXVI** $R=H$
**XXIV** $R^1=OH$, $R^2=H$

Latifolic acid (XXVII) can also be included into the group of α-isopropylbutyric acids. Its structure was determined [39] by NMR spectroscopy.

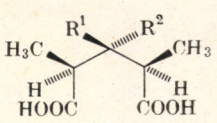
*D. C-6, C-8 and C-10 Glutaric Acids*

The acids of this group have been isolated from alkaloids of the plant family Leguminoseae, mainly from the genus *Crotalaria*; exceptionally, they are also found among the alkaloids of the plant family Borraginaceae.

The only C-6 glutaric acid known so far is dicrotalic acid. It is optically inactive and is readily acetylated to acetyldicrotalic acid anhydride. Thermal treatment of this compound splits off a molecule of acetic acid to give rise to an unsaturated substance which on hydrolysis yields *cis*-$\beta$-methylglutaconic acid [40]. Since dicrotalic acid does not form a lactone, the hydroxyl group must be in the $\beta$-position (XXVIII).

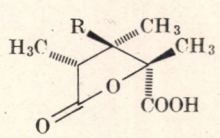



XXVIII




XXIX $R^1=OH$, $R^2=CH_3$

XXX $R^1=CH_3$, $R^2=OH$



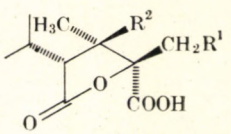

XXXI R=OH

XXXII R=H

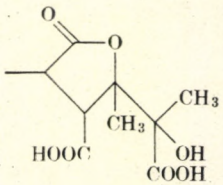
At present four C-8 acids are known. Fulvinic acid was isolated from the hydrolysis of fulvine [41]. Optically inactive fulvinic acid is a dicarboxylic acid which has no $\alpha$-hydroxyl group. Its NMR spectrum revealed the presence of a tertiary methyl and two equivalent $C-CH(CH_3)-C$ units, which led to structure XXIX. The optically inactive crispatic acid (XXX) from crispatine [42] is isomeric with fulvinic acid. Monocrotalic acid is formed by the hydrogenolysis of monocrotaline [43]. Its structure XXXI was definitely determined [44] on the basis of degradation reactions of the alcohol obtained by reduction of methyl monocrotalate with lithium aluminium hydride. The absolute configuration of monocrotalic acid, 2*R*, 3*R*, 4*R* was also established [24, 45, 46]. Hydrolysis of retusine gave [47] two isomeric acids; one of them, the so-called $\alpha$-acid, was identified as dihydroanhydromonocrotalic acid XXXII; it is a native acid. The $\beta$-acid arises by isomerization at C-4 during hydrolysis.

The longest known C-10 glutaric acids are trichodesmic acid and junceic acid. Trichodesmic acid was isolated from the hydrogenolysis of trichodesmine as early as 1935 [48]. Its hydrolysis gave (±)-lactic acid and methyl isobutyl ketone, which along with the interpretation of other reactions of the alkaloid led to structure XXXIII. The absolute configuration (2*R*, 3*R*, 4*R*) was elucidated together with the absolute configuration of monocrotalic acid [45, 46]. The hydrolysis of junceic acid ($C_{10}H_{16}O_6$) obtained by the

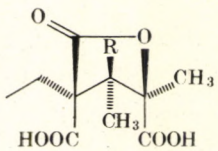
hydrogenolysis of junceine [49], also affords methyl isobutyl ketone. Oxidation of junceine with periodic acid yields formaldehyde with the consumption of 2 moles of periodic acid, which indicates that junceic acid has three vicinal hydroxyl groups; it was ascribed structure XXXIV. Incanic acid (XXXV) is formed, along with the isomeric isoincanic acid, in the hydrolysis of incanine [50]. Its oxidation with chromic acid yields acetone and acetic acid. Its methyl ester is reduced with lithium aluminium hydride to a triol whose treatment with periodic acid gives formaldehyde. The absolute configuration of the acids XXXIV and XXXV are not known.



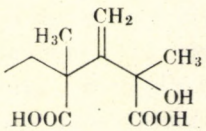
**XXXIII** $R^1=H$, $R^2=OH$
**XXXIV** $R^1=R^2=OH$
**XXXV** $R^1=OH$, $R^2=H$



**XXXVI**

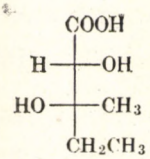


**XXXVII** R=OH
**XXXVIII** R=H

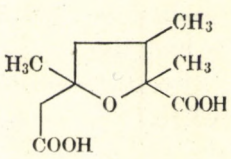


**XXXIX**

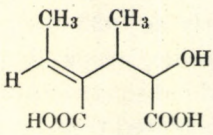
Grantianic acid from the alkaloid grantianine is a lactone acid [51]. Wunderlich [52] suggested structure XXVII instead of the originally proposed structure XXXVI, in view of the assumed similarity of grantianic acid with the acid XXXVIII contained in the alkaloid retusamine. Emilinic acid was obtained [53] from the hydrolysis of the alkaloid emiline. Its structure (XXXIX) was elucidated on the basis of mass and NMR spectral data and is similar to the two last mentioned acids.



**XL**



**XLI**



**XLII**

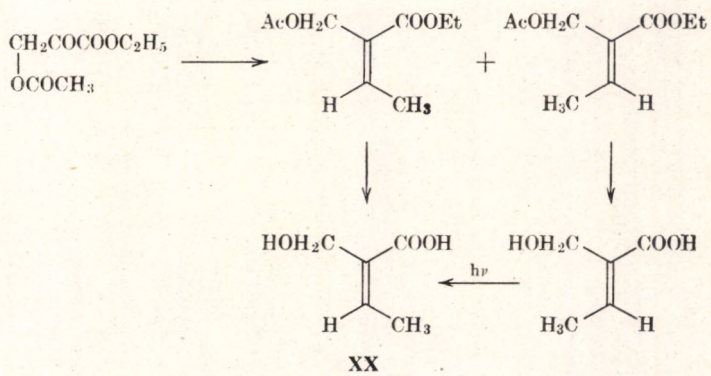
Three of the so far isolated necic acids cannot be allocated to any of the above-mentioned groups; these are *trans*-$\beta$-methylthioacrylic acid from planchonelline [54], nemorensic acid, and the acid from strigosine. The acid from strigosine has structure XL and on oxidation it yields butanone and carbon dioxide [55]. Nemorensic acid was obtained by hydrolysis of the alkaloid nemorensine and its structure XLI was proposed [56] on the evidence of NMR spectral data.

The only C-9 acid isolated so far is nilgiric acid. Its structure (XLII) was inferred [57] from an analysis of the NMR and mass spectra.

The structure of a series of pyrrolizidine alkaloids has been studied by physico-chemical methods and their corresponding acids are not known. Some of the necic acids are also very unstable and they readily undergo hydrolytic cleavage, so that they are not known in the native form. A great number of the necic acids reported in this chapter are artifacts which have arisen by isomerization, lactonization, etherification, opening of the epoxy ring, etc. of the native acids. The acids isolated from pyrrolizidine alkaloids in pure state are listed in Table IV.

## 2. SYNTHESIS OF NECIC ACIDS

Of the C-5 acids, sarracinic acid (XX) has been synthesized [58]. Reaction of ethyl acetoxypyruvate with ethylidene triphenylphosphorane gave a mixture of two isomeric esters which were separated by column chromatography. These two isomers were subjected to alkaline hydrolysis. The synthetic acid with a *trans*-arrangement of the methyl and the hydroxymethyl groups was then found to be identical with natural sarracinic acid (XX) and was also prepared by UV irradiation of the second isomer of the above synthesis:




XX

*Table IV*

Structure and Physical Constants of Necic Acids

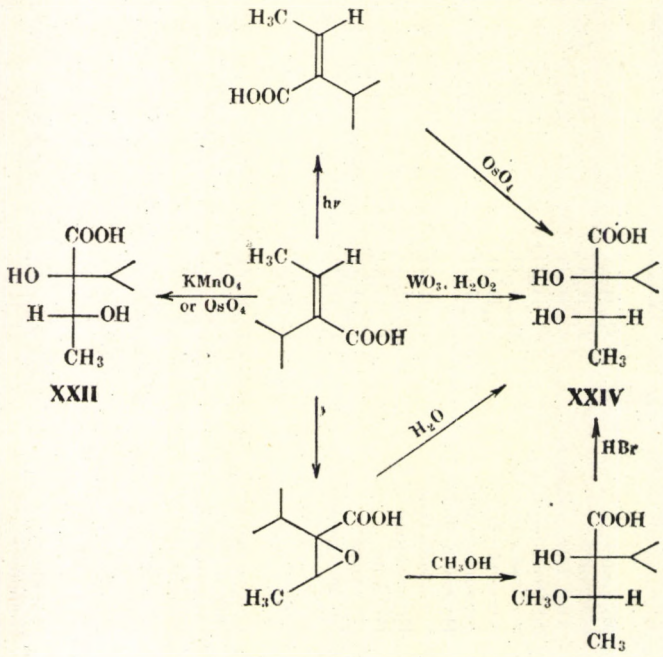
| Necic acid | Empirical formula | Structure | M.p., °C | $[\alpha]_D^{EtOH}$ | Parent Alkaloid |
|---|---|---|---|---|---|
| *trans*-β-Methylthio-acrylic | $C_4H_6O_2S$ | | — | — | Planchonelline |
| Angelic | $C_5H_8O_2$ | | 45–46 | — | Angelylheliotridine, Angelylheliotridine trachelanthate, Angelylheliotridine viridiflorate, Angelylretronecine, Brychyglottine, Cynoglossophine, Echiumine, Laburnine angelate, Lasiocarpine, Latifoline, Macrophylline, Sarracine, Turneforcine, Lindelofamine |
| Tiglic | $C_5H_8O_2$ | | 64 | — | Anadoline, Laburnine tiglate |
| Sarracinic | $C_5H_8O_3$ | XX | 57 | — | Sarracine |
| 2,3-Dihydroxy-3-methyl-valeric | $C_6H_{12}O_4$ | XL | — | −17.6 | Strigosine |
| Dicrotalic | $C_6H_{10}O_5$ | XXVIII | 109 | — | Dicrotaline |
| (+)-Trachelanthic | $C_7H_{14}O_4$ | XXII | 93–94 | +3.7 | Anadoline, Echiumine, Intermedine, Lindelofamine, Lindelofine, Rinderine, Supinine, Trachelanthamine |
| (−)-Trachelanthic | $C_7H_{14}O_4$ | | 94 | $-3.4^{H_2O}$ | Acetylindicine, Indicine |
| (−)-Viridifloric | $C_7H_{14}O_4$ | XXIV | 128–132 | $-2.0^{H_2O}$ | Amabiline, Cynaustine, Cynaustraline, Echinatine, Lycopsamine, Symphytine, Viridiflorine |
| Macrotomic | $C_7H_{14}O_5$ | XXVI | — | — | Macrotomine |
| Echimidinic | $C_7H_{14}O_5$ | XXVI | — | +16.4 | Echimidine, Heliosupine |
| Latifolic | $C_7H_{10}O_5$ | XXVII | 165–166 | +94 | Latifoline |
| α-Dihydroanhydromono-crotalic | $C_8H_{12}O_4$ | XXXII | 130–131 | +3.3 | Retusine |
| Monocrotalic | $C_8H_{12}O_5$ | XXXI | 182 | $-5.3^{H_2O}$ | Monocrotaline, Spectabiline |
| Crispatic | $C_8H_{14}O_5$ | XXX | 133–134 | 0 | Crispatine |
| Fulvinic | $C_8H_{14}O_5$ | XXIX | 113–114 | 0 | Fulvine |
| (−)-Heliotric | $C_8H_{16}O_4$ | XXIII | 93 | $-10.8^{H_2O}$ | Heleurine, Heliotrine |
| Lasiocarpic | $C_8H_{16}O_5$ | XXV | 97 | +10.6 | Europine, Lasiocarpine |
| Nilgiric | $C_9H_{14}O_5$ | XLII | 126–127 | — | Nilgirine |
| Hygrophyllinecic dilactone | $C_{10}H_{12}O_4$ | XIII | 103–105 | −97.6 | Hygrophylline |

*(Table IV—continued)*

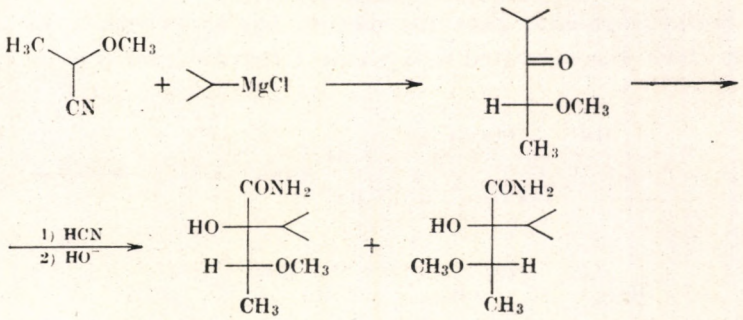
| Necic acid | Empirical formula | Structure | M.p., °C | $[\alpha]_D^{EtOH}$ | Parent Alkaloid |
|---|---|---|---|---|---|
| Mikanecic | $C_{10}H_{12}O_4$ | XXI | 234 | 0 | Sarracine |
| Clivonecic | $C_{10}H_{12}O_4$ | XIV | 142–144 | −208 | Clivorine |
| Acid from swazine | $C_{10}H_{12}O_5$ | XIX | 191 | −116 | Swazine |
| Sceleratinic dilactone | $C_{10}H_{13}O_4Cl$ | | 208 | — | Sceleratine, Chlorodesoxysceleratine |
| Sceleratinic | $C_{10}H_{14}O_5$ | XVIII | 156 | $-9.3^{H_2O}$ | Sceleratine |
| Seneciphyllic | $C_{10}H_{14}O_5$ | *trans*-VII | 145 | — | Angularine, Seneciphylline |
| Isoseneciphyllic | $C_{10}H_{14}O_5$ | VII | 105–108 | −8.6 | Seneciphylline |
| Hygrophyllinecic monolactone | $C_{10}H_{14}O_5$ | | 180–181 | −18.8 | Hygrophylline |
| Retusaminic | $C_{10}H_{14}O_6$ | XXXVIII | 164 | −45 | Retusamine |
| Riddellic | $C_{10}H_{14}O_6$ | VIII | 103 | +2.6 | Riddelline |
| Grantianic | $C_{10}H_{14}O_7$ | XXXVI? | — | — | Grantianine |
| Integerrinecic | $C_{10}H_{16}O_5$ | II | 151 | +15.9 | Hastacine, Integerrimine, Madurensine, Neoplatyphylline |
| Senecic | $C_{10}H_{16}O_5$ | I | 152 | +11.8 | Acetylsenkirkine, Anacrotine, Platyphylline, Rosmarinine, Senecionine, Senkirkine |
| Usaromoensic | $C_{10}H_{16}O_5$ | *iso*-I? | 170 | +6.6 | Usaramoensine |
| Incanic | $C_{10}H_{16}O_5$ | XXXV | 163–164 | +25 | Crosemperine, Incanine |
| Isoincanic | $C_{10}H_{16}O_5$ | | 122–123 | −25 | Incanine |
| Nemorensic | $C_{10}H_{16}O_5$ | XLI | 174–178 | +87 | Nemorensine |
| Emilinic | $C_{10}H_{16}O_5$ | XXXIX | 126–128 | — | Emiline |
| Trichodesmic | $C_{10}H_{16}O_5$ | XXXIII | 209 | — | Trichodesmine |
| Isatinecic | $C_{10}H_{16}O_6$ | III | 147–148 | — | Hydroxysenkirkine, Retrorsine N-oxide |
| Jaconecic | $C_{10}H_{16}O_6$ | IX | 182 | +31.7 | Florosenine, Jacobine, Jaconine, Othosenine |
| Isojaconecic | $C_{10}H_{16}O_6$ | X | 113–114 | +75 | Jacobine, Jaconine |
| Junceic | $C_{10}H_{16}O_6$ | XXXIV | 180–182 | — | Junceine |
| Retronecic | $C_{10}H_{16}O_6$ | IV | 181 | $+5.7^{H_2O}$ | Retrorsine, Usaramine |
| Erucifolinecic | $C_{10}H_{16}O_7$ | XVII | 180–182 | −9 | Erucifoline |
| Isolinecic | $C_{10}H_{18}O_6$ | XV | 140–142 | +66 | Isoline, Bisline |

The acid from strigosine (XL) had been synthesized [59] before it was found in natural material. Another C-6 necic acid — dicrotalic acid (XXVIII) — was prepared [40] by the hydrolysis of the diethyl ester obtained in the Reformatzky reaction of ethyl acetoacetate with ethyl chloroacetate.

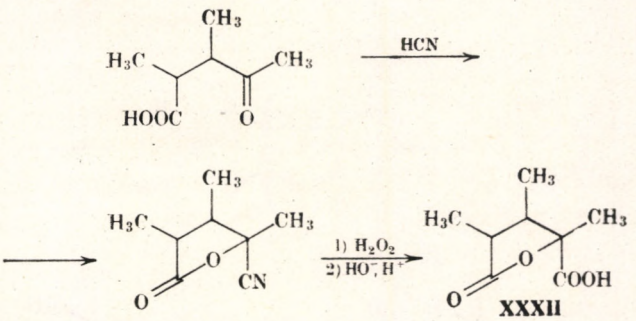
Of the C-7 acids, trachelanthic acid and viridifloric acid have been prepared. The starting substance for the synthesis [60] was *trans*-α-isopropylcrotonic acid. *Cis*-hydroxylation with potassium permanganate gave (±)-trachelanthic acid, which after resolution afforded natural (+)-trachelanthic acid (XXII). Epoxidation of the starting unsaturated acid gave an epoxide which, on the one hand, was hydrolyzed to (±)-viridifloric acid giving natural (−)-viridifloric acid (XXIV) after resolution and, on the other hand, it was methanolyzed [61] to the *erythro*-isomer of heliotric acid. The synthesis carried out by Kochetkov *et al.* [62] was also based on *trans*-α-isopropylcrotonic acid. By its *cis*-hydroxylation with osmium tetroxide, they prepared (±)-trachelanthic acid. *Trans*-hydroxylation with tungsten trioxide and hydrogen peroxide gave (±)-viridifloric acid. Furthermore, UV-irradiation of the starting unsaturated acid yielded its *cis*-isomer, which on *cis*-hydroxylation with osmium tetroxide afforded (±)-viridifloric acid. All the racemic acids were separated into the optical enantiomers by resolution with (−)-α-phenylethylamine:



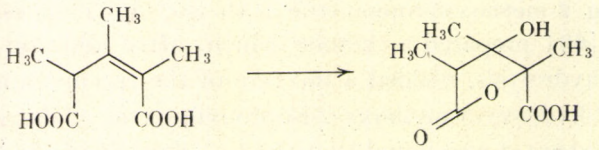
By reacting 2-methoxypropionitrile with isopropylmagnesium chloride, Adams *et al.* [63] prepared a ketone which, after addition of hydrogen cyanide and hydrolysis, yielded a mixture of the racemic amides of *threo*- and *erythro*-2-hydroxy-3-methoxy-2-isopropylbutyric acids. After separation of the mixture it was found that the (+)-*threo*-amide had an IR spectrum similar to that of (+)-heliotramide:



Dihydroanhydromonocrotalic acid (XXXII) was synthesized [64] from 3-methylpentane-4-one-2-carboxylic acid. The cyanohydrin obtained after addition of hydrogen cyanide is immediately lactonized to the lactone nitrile which, on oxidation with hydrogen peroxide and hydrolysis, yields a mixture of racemates of dihydroanhydromonocrotalic acid. After resolution of the mixture by means of the brucine salt, the (+)-isomer of the natural acid XXXII was isolated:

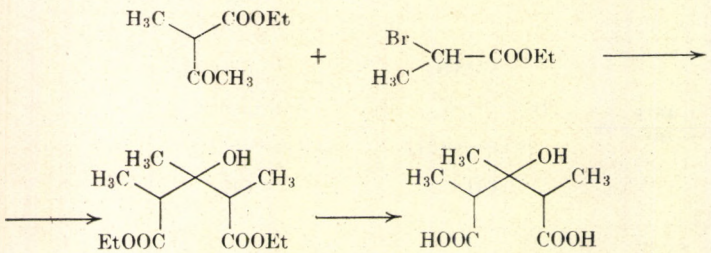


Racemic monocrotalic acid (XXXI) was prepared [65] by *trans*-hydroxylation of *cis*-trimethylglutaconic acid:

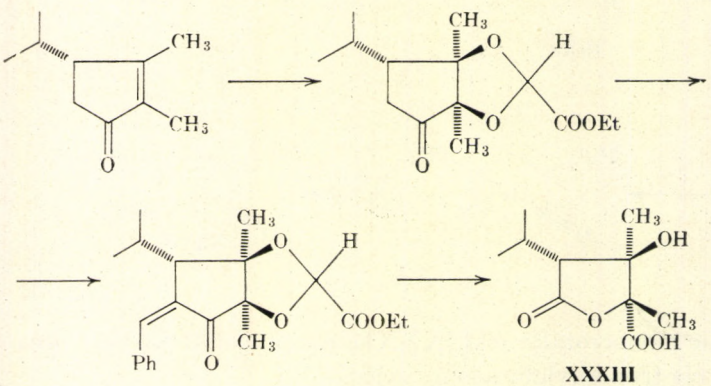


XXXI

Fulvinic acid (XXIX) and crispatic acid (XXX) have also been synthesized [66]. The Reformatsky reaction of ethyl methylacetoacetate with ethyl α-bromopropionate gave the diester. On hydrolysis it yielded the racemate which was separated into *S-meso* (crispatic) and *R-meso* (fulvinic) acids:

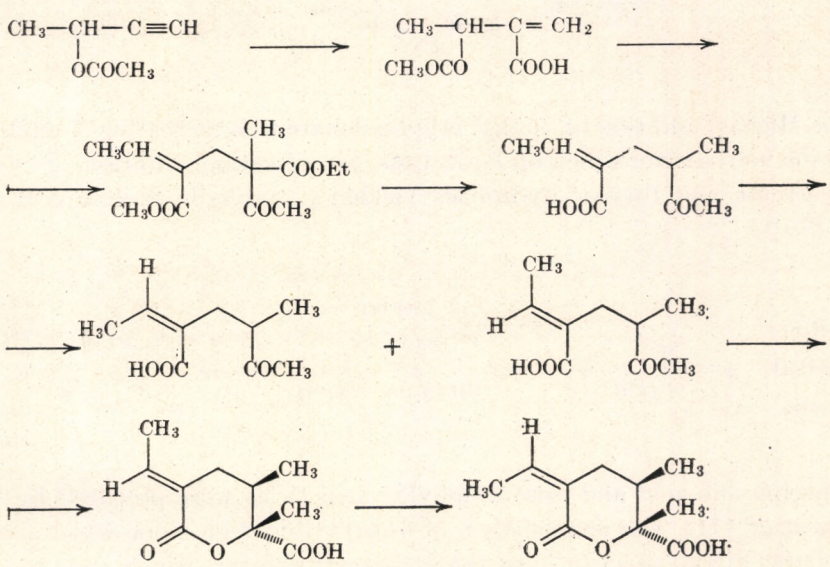


Trichodesmic acid (XXXIII) was synthesized by Edwards and Matsumoto [67]. Hydroxylation of (±)-2,3-dimethyl-4-isopropyl-2-cyclopentanone by osmium tetroxide gave the *cis*-glycol. After blocking the free hydroxyl groups with ethyl orthoformate, the product was condensed with benzaldehyde to the benzal derivative, which on ozonolysis and oxidative hydrolysis yielded (±)-trichodesmic acid. Natural (+)-trichodesmic acid (XXXIII) was prepared by resolution of the racemate by means of the cinchonidine salt:

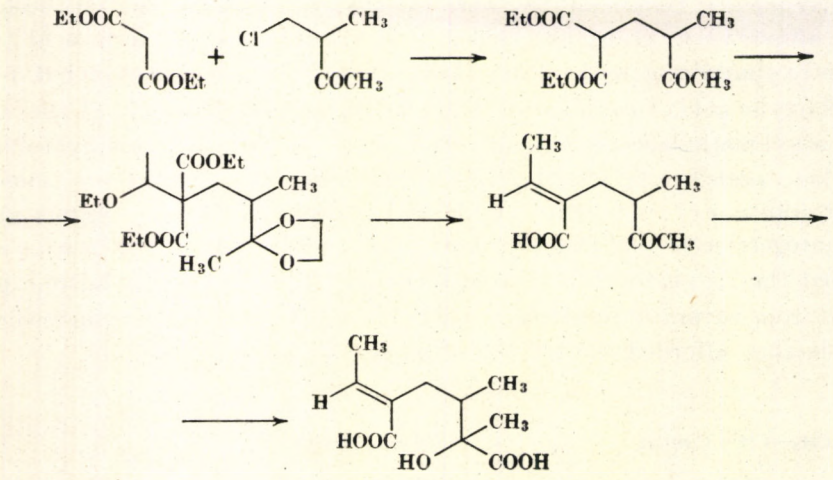


XXXIII

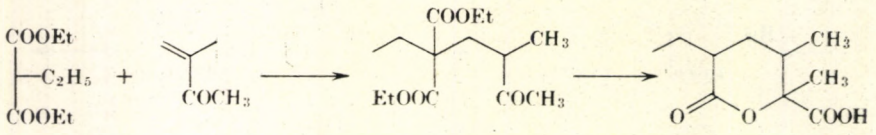
Culvenor and Geissman [68] were the first to carry out the synthesis of C-10 adipic necic acids, i.e. senecic acid (I) and integerrinecic acid (II). 3-Acetoxybut-1-yne with nickel tetracarbonyl yielded 3-acetoxybut-1-en-2-carboxylic acid. Esterification and the Michael condensation of this acid with ethyl methylacetoacetate afforded a keto-diester which was hydrolyzed to a keto acid. The keto acid was separated into the *cis*- and *trans*- isomers. After addition of hydrogen cyanide and hydrolysis, the *trans*-isomer gave (±)-integerrinecic acid lactone whose resolution by way of the brucine salt yielded the (+)-lactone, which was found identical with the lactone prepared from natural integerrinecic acid (II). UV irradiation of integerrinecic acid lactone afforded the lactone of senecic acid (I):



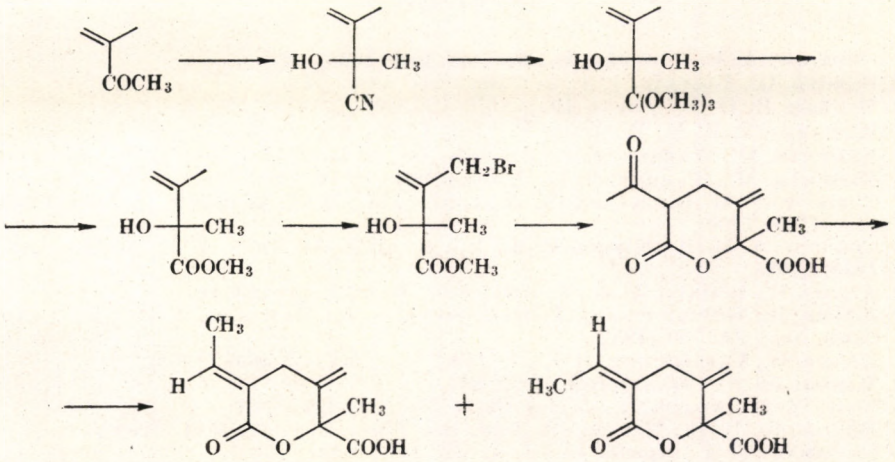
(±)-Integerrinecic acid (II) was also prepared by Kochetkov *et al.* [69]. Diethyl malonate was alkylated with 3-chloromethyl-2-butanone to the keto diester which after shielding of the keto group with ethylene glycol was alkylated with 1-chloro-1-ethoxyethane. The resulting ketal diester was hydrolyzed, decarboxylated and after splitting off ethanol, it yielded an unsaturated acid which after the addition of hydrogen cyanide and hydrolysis yielded (±)-integerrinecic acid:



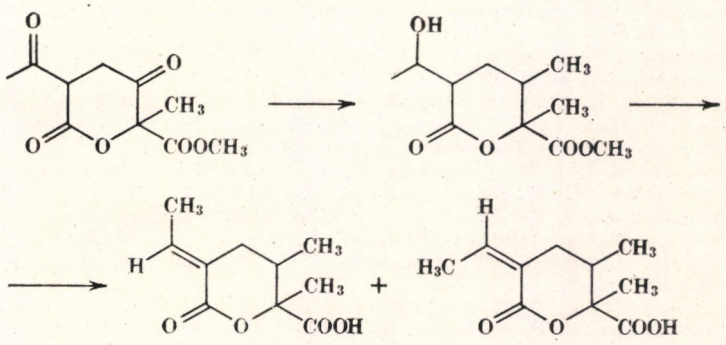
The Michael addition of diethyl ethylmalonate to 3-methylbut-3-en-2-one gave the keto-diester which on hydrolysis, esterification, addition of hydrogen cyanide and further hydrolysis yielded (±)-dihydrosenecic acid lactone [70]:



Seneciphyllic acid and isoseneciphyllic acid (VII) were prepared by Edwards *et al.* [71]. The cyanohydrin of 3-methylbut-3-en-2-one was converted by the Pinner reaction into the orthomethyl ester, which gave an unsaturated ester on hydrolysis. Treatment of this ester with N-bromosuccinimide yielded the corresponding allyl bromide. Alkylation of methyl acetoacetate with this halide and hydrolysis yielded the keto-lactone. Selective reduction of the β-oxo group with morpholine borane and dehydration with phosphoric acid afforded a mixture of (+)-*cis*- and (±)-*trans*-seneciphyllic acid lactones. Separation of this mixture, resolution of the racemates using the cinchodinine salts and hydrolysis gave all four possible isomeric forms of seneciphyllic acid:



For a new synthesis of senecic acid (I) and integerrinecic acid (II), Edwards *et al.* [72, 73] used the keto-lactone from the previous synthesis of isoseneciphyllic acid. Its reduction with morpholine borane and subsequent hydrogenation gave the saturated hydroxy-lactone which was dehydrated to a mixture of (±)-*cis*- and (±)-*trans*-lactones. Careful separation of the mixtures and resolution of the racemates *via* the brucine salts yielded all eight possible diastereo-isomeric lactones of senecic acid and also all eight possible diastereo-isomeric decanecic acids:



## REFERENCES


1. Orekhov, A. P., Tidebel, V., *Ber.*, *68*, 650 (1935).
2. Barger, G., Blackie, J. J., *J. Chem. Soc.*, *1936*, 743.
3. Manske, R. H. F., *Can. J. Res.*, *17B*, 1 (1939).
4. Richardson, M. F., Warren, F. L., *J. Chem. Soc.*, *1943*, 452.
5. Kropman, M., Warren, F. L., *J. Chem. Soc.*, *1949*, 2852.
6. Kropman, M., Warren, F. L., *J. Chem. Soc.*, *1950*, 700.
7. Christie, S. M. H., Kropman, M., Leisegang, E. C., Warren, F. L., *J. Chem. Soc.*, *1949*, 1700.
8. Christie, S. M. H., Kropman, M., Novellie, L., Warren, F. L., *J. Chem. Soc.*, *1949*, 1703.
9. Adams, R., Looker, J. H., *J. Am. Chem. Soc.*, *73*, 134 (1951).
10. Adams, R., Govindachari, T. R., Looker, J. H., Edwards, J. D., Jr., *J. Am. Chem. Soc.*, *74*, 700 (1952).
11. Adams, R., VanDuuren, B. L., *J. Am. Chem. Soc.*, *75*, 4638 (1953).
12. Masamune, S., *Chem. & Ind.*, *1959*, 21.
13. Nair, M. D., Adams, R., *J. Am. Chem. Soc.*, *83*, 922 (1961).
14. Bradbury, R. B., Masamune, S., *J. Am. Chem. Soc.*, *81*, 5201 (1959).
15. Geissman, T. A., *Austral. J. Chem.*, *12*, 247 (1959).
16. Fridrichsons, J., Mathieson, A. McL., Sutor, D. J., *Tetrahedron Letters*, *1960* (No. 23) 35; *Acta Cryst.*, *16*, 1075 (1963).
17. Mathieson, A. McL., Taylor, J. C., *Tetrahedron Letters*, *1961* (No. 17) 590; *Acta Cryst.*, *16*, 1075 (1963).
18. Koretskaya, N. I., Danilova, A. V., Utkin, L. M., *Zh. obsch. khim.*, *32*, 3823 (1962).
19. Koretskaya, N. I., Danilova, A. V., Utkin, L. M., *Zh. obsch. khim.*, *34*, 1348 (1964).
20. Masamune, S., *J. Am. Chem. Soc.*, *82*, 5253 (1960).
21. Schlosser, F. D., Warren, F. L., *J. Chem. Soc.*, *1965*, 5707.
22. Klásek, A., Neuner-Jehle, N., Šantavý, F., *Coll. Czechoslov. Chem. Commun.*, *34*, 1459 (1969).
23. Červinka, O., Hub, L., Klásek, A., Šantavý, F., *Chem. Commun.*, *1968*, 261.
24. Červinka, O., Hub, L., *Coll. Czechoslov. Chem. Commun.*, *33*, 2933 (1968).
25. Coucourakis, E. D., Gordon-Gray, C. G., *J. Chem. Soc. (C)*, *1970*, 2312.
26. Coucourakis, E. D., Gordon-Gray, C. G., Whiteley, C. G., *J. Chem. Soc. (C)*, *1972*, 2339.
27. Sedmera, P., Klásek, A., Duffield, A. M., Šantavý, F., *Coll. Czechoslov. Chem. Commun.*, *37*, 4112 (1972).
28. de Waal, H. L., Wiechers, A., Warren, F. L., *J. Chem. Soc.*, *1963*, 953.
29. Gordon-Gray, C. G., Wells, R. B., Hallak, N., Hursthouse, M. B., Neidle, S., Toube, T. P., *Tetrahedron Letters*, *1972*, 707.
30. Culvenor, C. C. J., Geissman, T. A., *J. Org. Chem.*, *26*, 3045 (1961).
31. Menshikov, G. P., *Zh. obsch. khim.*, *17*, 343 (1947).
32. Menshikov, G. P., *Zh. obsch. khim.*, *18*, 1736 (1948).
33. Adams, R., van Duuren, B. L., *J. Am. Chem. Soc.*, *75*, 4636 (1953).
34. Likhosherstov, A. M., Kulakov, V. N., Kochetkov, N. K., *Zh. obsch. khim.*, *39*, 1405 (1969).
35. Drummond, L. J., *Nature*, *167*, 41 (1951).
36. Menshikov, G. P., Petrova, M. F., *Zh. obsch. khim.*, *12*, 1457 (1942).
37. Culvenor, C. C. J., *Austral. J. Chem.*, *9*, 512 (1956).
38. Crowley, H. C., Culvenor, C. C. J., *Austral. J. Chem.*, *13*, 269 (1960).
39. Crowley, H. C., Culvenor, C. C. J., *Austral. J. Chem.*, *15*, 139 (1962).
40. Adams, R., van Duuren, B. L., *J. Am. Chem. Soc.*, *75*, 2377 (1953).
41. Schoental, R., *Austral. J. Chem.*, *16*, 233 (1963).
42. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem.*, *16*, 239 (1963).
43. Adams, R., Rogers, E. F., Sprules, F. J., *J. Am. Chem. Soc.*, *61*, 2819 (1939).
44. Adams, R., Govindachari, T. R., *J. Am. Chem. Soc.*, *72*, 158 (1950).
45. Robins, D. J., Crout, D. H. G., *J. Chem. Soc. (C)*, *1969*, 1386.

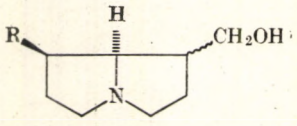
46. Robins, D. J., Crout, D. H. G., *J. Chem. Soc. (C), 1970*, 1334.
47. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem., 10*, 464 (1957).
48. Menshikov, G. P., Rubinstein, W., *Ber., 68*, 2039 (1935).
49. Adams, R., Gianturco, M., *J. Am. Chem. Soc., 78*, 1926 (1956).
50. Yunusov, S. Yu., Plekhanova, N. V., *Zh. obsch. khim., 29*, 677 (1959).
51. Adams, R., Gianturco, M., *J. Am. Chem. Soc., 78*, 4458 (1956).
52. Wunderlich, J. A., *Chem. & Ind., 1962*, 2089; *Acta Cryst., 23*, 846 (1967).
53. Kohlmünzer, S., Tomczyk, H., Saint-Firmin, A., *Dissert. Pharm. Pharmacol., 23*, 419 (1971).
54. Hart, N. K., Lamberton, J. A., *Austral. J. Chem., 19*, 1259 (1966).
55. Mattocks, A. R., *J. Chem. Soc., 1964*, 1974.
56. Klásek, A., Sedmera, P., Boeva, A., Šantavý, F., *Coll. Czechoslov. Chem. Commun., 38*, 2504 (1973).
57. Sawhney, R. S., Atal, C. K., *Planta Med., 21*, 435 (1972).
58. Edwards, J. D., Jr., Matsumoto, T., Hase, T., *J. Org. Chem., 32*, 244 (1967).
59. Sjolander, J. R., Folkers, K., Adelberg, E. A., Tatum, E. L., *J. Am. Chem. Soc., 76*, 1085 (1954).
60. Dry, L. J., Warren, F. L., *J. Chem. Soc., 1952*, 3445.
61. Dry, L. J., Warren, F. L., *J. Chem. Soc., 1955*, 65.
62. Kochetkov, N. K., Likhosherstov, A. M., Kulakov, C. N., *Tetrahedron, 25*, 2313 (1969).
63. Adams, R., Culvenor, C. C. J., Robinson, C. N., Stingl, H. A., *Austral. J. Chem., 12*, 706 (1959).
64. Adams, R., Hauserman, F. B., *J. Am. Chem. Soc., 74*, 694 (1952).
65. Adams, R., van Duuren, B. L., Braun, B. H.,*J. Am. Chem. Soc., 74*, 5608 (1952).
66. Warren, F. L., "Senecio Alkaloids", in "The Alkaloids" (Ed. R. H. F. Manske), Vol. XII, Academic Press, New York, 1970, Ref. No. 151.
67. Edwards, J. D., Jr., Matsumoto, T., *J. Org. Chem., 32*, 2561 (1967).
68. Culvenor, C. C. J., Geissman, T. A., *J. Am. Chem. Soc., 83*, 1647 (1961).
69. Kochetkov, N. K., Vasil'ev, A. E., Levchenko, S. N., *Zh. obsch. khim., 34*, 2202 (1964).
70. Kochetkov, N. K., Vasil'ev, A. E., Levchenko, S. N., *Izv. Akad. Nauk SSSR, 1962*, 2240.
71. Edwards, J. D., Jr., Hase, T., Ichikawa, N., *Chem. Commun., 1965*, 364; *J. Heterocycl. Chem., 4*, 487 (1967).
72. Edwards, J. D., Jr., Hase, T., Hignite, C., Matsumoto, T., *J. Org. Chem., 31*, 2282 (1966).
73. Edwards, J. D., Jr., Matsumoto, T., *J. Org. Chem., 32*, 1837 (1967).

# IV. ALKALOIDS

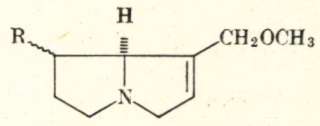
## 1. THE STRUCTURE AND ABSOLUTE CONFIGURATION OF PYRROLIZIDINE ALKALOIDS

### *A. Simple Pyrrolizidine Alkaloids*

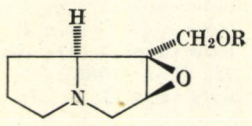
Along with ester alkaloids, free necines have also been found in plant material. Laburnine (I) was isolated [1,2] from *Cytisus laburnum*, trachelanthamidine (II) from *Eupatorium maculatum* [3] and *Heliotropium strigosum* [4], lindelofidine (III) from *Thesium minkwitzianum* [5], and retronecine (IV) from *Crotalaria retusa* [6]. From *Vanda cristata*, the acetate of laburnine (acetate of I) was obtained [7]. For the elucidation of the structure of these necines see Chapter II.




**I** 1$\beta$, 8$\beta$, R=H
**II** 1$\alpha$, 8$\alpha$, R=H
**III** 1$\alpha$, 8$\beta$, R=H
**IV** 8$\alpha$, $\triangle^{1,2}$, R=OH




**V** R=H
**VI** R=$\beta$-OH
**VIII** R=$\alpha$-OH
**IX** R=$\beta$-OAc

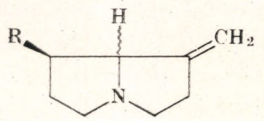



**VII** R=$CH_3$
**X** R=H

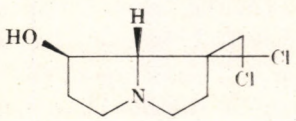
From *Crotalaria trifoliastrum* and *C. aridicola*, six new bases have been isolated [8–10]. On hydrogenation and subsequent degradation with hydrobromic acid, the base *A* ($C_9H_{15}NO$) yields isoretronecanol; it follows that it is the methyl ether of supinidine (V). The base *B* ($C_9H_{15}NO_2$) was hydrogenated to a substance which by reaction with hydrobromic acid and then with sodium hydroxide was converted to anhydroplatynecine. Therefore, base *B* must be a methyl ether of retronecine (VI). The structures of these two bases were confirmed by etherification of supinidine and retronecine with methyl iodide and potassium *t*-butoxide. The structure of the base *C* was elucidated as 1$\beta$, 2$\beta$-epoxy-1$\alpha$-methoxymethyl-8$\alpha$-pyrrolizidine (VII)

mainly on the evidence of spectral data. For the other three simple alkaloids isolated from the studied plants, structures VIII, IX and X were proposed on the basis of NMR and mass spectral data; these have been confirmed by their synthesis from the available necines. The presence of the methyl ethers of supinidine (V) and retronecine (VI) was also demonstrated [11] in *Crotalaria medicaginea*.

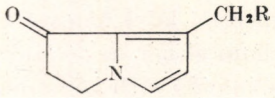
Another simple alkaloid was found [12] in *Crotalaria anagyroides*. On hydrogenation, this compound yielded a mixture of heliotridane and pseudoheliotridane; on ozonolysis it gave formaldehyde and 1-oxopyrrolizidine; it was, therefore, assigned structure XI. From *Crotalaria goreensis*, two diastereoisomeric simple alkaloids, $C_8H_{13}NO$ (bases *A* and *B*), were isolated. On hydrogenation, base *A* afforded the enantiomer of hydroxyheliotridane and on oxidation with osmium tetroxide and periodic acid, formaldehyde, which indicated structure XII. The base *B* differs from base *A* only by its absolute configuration at C-8, and has been ascribed structure XIII. These two substances have also been synthesized from the availabe necines. The base $C_9H_{13}NOCl_2$ (XIV) occurs in *C. goreensis* [14]. This spiro-compound is, however, an artifact formed by the reaction of base *A* (XII) with chloroform during isolation. Later on, base *A* (XII) and base *B* (XIII) were also found [15] in *Crotalaria maypurensis*.




**XI** 8α, R=H
**XII** 8β, R=OH
**XIII** 8α, R=OH




**XIV**

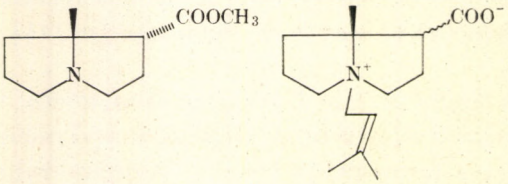



**XV** R=OH
**XVI** R=H

In 1970, a new simple alkaloid, $C_8H_9NO_2$, was isolated from *Urechites karwinsky;* it was called loroquine. Acetylation revealed the presence of one hydroxyl group; the presence of a keto group was demonstrated by reduction with sodium borohydride. Structure XV was suggested mainly on the basis of the interpretation of the NMR spectra and confirmed by the fact that the diol obtained on reduction of loroquine was identical with the previously prepared [17] synthetic product. The analogue of loroquine (XV) is an unsaturated ketone XVI, found [18] in the exocrine secretion of danaid butterflies.

The simple alkaloid $C_9H_{15}NO_2$, designated as chysine A, was isolated from *Chysis bractensis* [19]. Its correct structure XVII was established [20]

on the basis of its reduction to lindelofidine (III) and confirmed by synthesis.

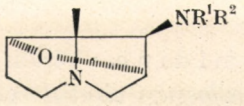



**XVII** **XVIII** 1$\beta$ **XIX** 1$\alpha$ **XX**

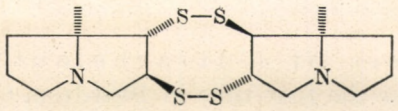
From *Anodendron affine*, two diastereoisomeric zwitterionic simple alkaloids — anodendrine and alloanodendrine — have been isolated [21]. The structural elucidation was mainly based on the finding that on hydrogenolysis over a Pd catalyst anodendrine (XVIII) yielded (+)-laburninic acid, whereas alloanodendrine (XIX) gave (+)-isoretronecanolic acid. The nature of the chain on the quaternary nitrogen was determined on the basis of spectral data and the two structures were confirmed by synthesis.

The definite structure of laburnamine, isolated [22] from *Cytisus laburnum*, is not known as yet. On the basis of the interpretation of the mass spectra, laburnamine was ascribed the partial structure XX, with the side-chain either in position 1 or 2. This is one of the few cases of pyrrolizidine alkaloids with an amino group in the side chain. Other alkaloids of this type have been isolated from plants of the family Gramineae. Th alkaloids loline ($C_8H_{14}N_2O$), norloline ($C_7H_{12}N_2O$) and lolinine ($C_{10}H_{16}N_2O_2$) occur in *Lolium cuneatum* [23]. These alkaloids have a common heterocyclic skeleton (they differ only in the substitution on the amino group) which is a pyrrolizidine ring with an ether bridge [24, 25]. On the basis of Hofmann exhaustive methylation and oxidative degradation, structure XXI was assigned to norloline, XXII to loline, and XXIII to lolinine. The alkaloid festucine was isolated [28] from *Festuca arundinacea*. Later on, it was found [29] to be identical with loline (XXII). Temuline, isolated [30] from *Lolium temulentum*, is probably identical with norloline (XXI).

Decorticasine, $C_{10}H_{16}N_2O_2$ was isolated from *Adenocarpus* species. On hydrolysis it yielded propionic acid and an amine which was identical [31] with norloline (XXI). Therefore, the structure of decorticasine is XXIV. On the basis of X-ray crystallography, these bases were assigned [32] the absolute configuration XXI–XXIV.




**XXI** $R^1=R^2=H$
**XXII** $R^1=H$, $R^2=CH_3$
**XXIII** $R^1=CH_3$, $R^2=COCH_3$
**XXIV** $R^1=H$, $R^2=COC_2H_5$

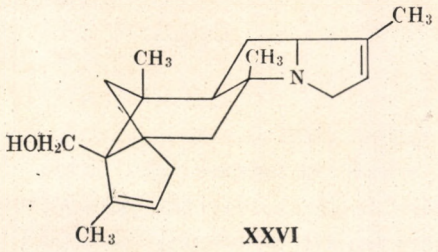



**XXV**

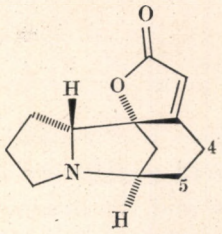
Cassipourine $C_{14}H_{22}N_2S_4$ was isolated [33] from *Cassipourea gummiflua*. On distillation with zinc dust it yielded [34] the pyrrolo [1,2-*a*] pyrrolidine and on hydrogenation pyrrolizidine. It was assigned [35] structure XXV, on the basis of X-ray crystallographic evidence.

Thelepogine $C_2H_{31}NO$ (XXVI) was isolated from *Thelepogon elegans* and its structure was established by X-ray crystallography [36, 37].

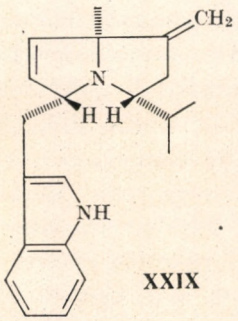
The alkaloids norsecurinine (XXVII) and dihydronorsecurinine (XXVIII) can also be considered as pyrrolizidine derivatives; they have been isolated [38] from *Securinega virosa*. The last, so far known alkaloid of this group is peduncularine, isolated from *Aristotelia peduncularis*. Its structure XXIX was determined mainly by NMR spectroscopy [39].




**XXVI**



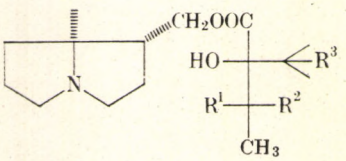

**XXVII** $\Delta^{4,5}$
**XXVIII**



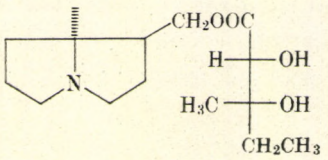

**XXIX**

*B. Monoester Pyrrolizidine Alkaloids*

(a) Esters of (−)-trachelanthamidine. Viridiflorine was isolated for the first time in 1948 from *Cynoglossum viridiflorum* [40]. On alkaline hydrolysis, it yielded trachelanthamidine and (−)-viridifloric acid. The structures of these two hydrolytic products have been elucidated (see Chapters II and III) and viridiflorine is assigned structure XXX. Its diastereoisomer is trachelanthamine (XXXI) which was isolated for the first time from *Trachelanthus korolkovii* [41]; on hydrolysis it yielded trachelanthamidine and (+)-trachelanthic acid. Macrotomine (XXXII) was isolated from *Macrotomia echioides* [42]; its alkaline hydrolysis gave trachelanthamidine and acetone, on oxidation with periodic acid it yielded acetone, oxalic acid and acetaldehyde. Strigosine (XXXIII) was isolated [4] from *Heliotropium strigosum*. On alkaline hydrolysis, it gave trachelanthamidine and the acid $C_6H_{12}O_4$, which was identified as (−)-α,β-dihydroxy-β-methylvaleric acid.



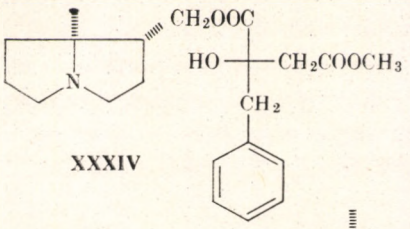

**XXX** $R^1$=OH, $R^2$=$R^3$=H
**XXXI** $R^1$=$R^3$=H, $R^2$=OH
**XXXII** $R^1$, $R^2$=H, OH, $R^3$=OH



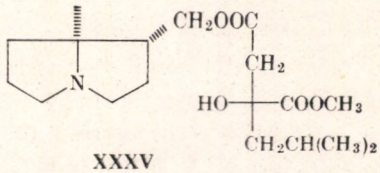

**XXXIII**

*Phalaenopsis amabilis* and *P. mannii* were the sources of isolation [43] of the alkaloid phalaenopsine T, which on methanolysis yielded trachelanthamidine and the dimethyl ester $C_{13}H_{16}O_5$. Spectral data showed that this ester was dimethyl (−)-2-benzylmalate, which was confirmed by synthesis. By reaction with peroxytrifluoroacetic acid, diethyl benzylidenesuccinate was converted to an epoxide which on hydrogenolysis and transesterification afforded racemic dimethyl 2-benzylmalate. The mass spectrum of phalaenopsine T exhibits a peak M-73 ($M-CH_2COOCH_3$) which indicates structure XXXIV for this compound. Cornucervine (XXXV) was isolated [44] from *Phalaenopsis cornu-cervi*. Methanolysis yielded trachelanthamidine and dimethyl (−)-2-isobutylmalate which was also synthesized by condensation of methyl isobutyl ketone with diethyl carbonate to ethyl 5-methyl-3-oxo-hexanoate and, thereafter, by preparation of the cyanohydrin followed by acid methanolysis. The intense peak M-59 ($M-COOCH_3$) and the absence of the peak M-73 in the mass spectrum of cornucervine demonstrates the nature of the methyl ester grouping in this compound.

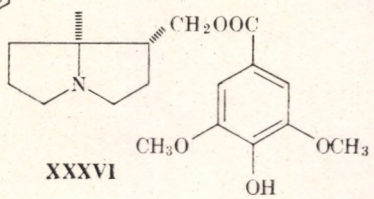
The absolute configuration of the acid components of strigosine, phalaenopsine T and cornucervine is not known as yet.



XXXIV



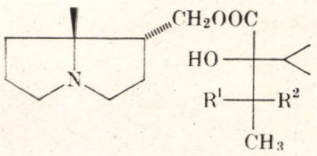
XXXV

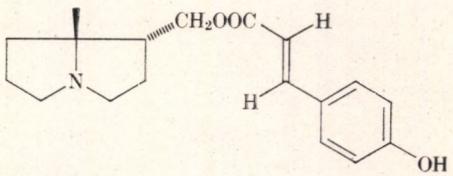


XXXVI

In 1971, a new alkaloid, alafine, was isolated from *Alafia multiflora* [45]. Its hydrolysis yielded (−)-trachelanthamidine and syringic acid; therefore it is assigned the structure XXXVI.

(b) Esters of lindelofidine. Lindelofine and lindelofamine were found in *Lindelofia macrostyla* [46, 47]. Hydrolysis of lindelofine (XXXVII) yielded lindelofidine and (+)-trachelanthic acid. Lindelofamine is an ester of lindelofine (XXXVII) with angelic acid or tiglic acid, and its probable structure is XXXVIII.





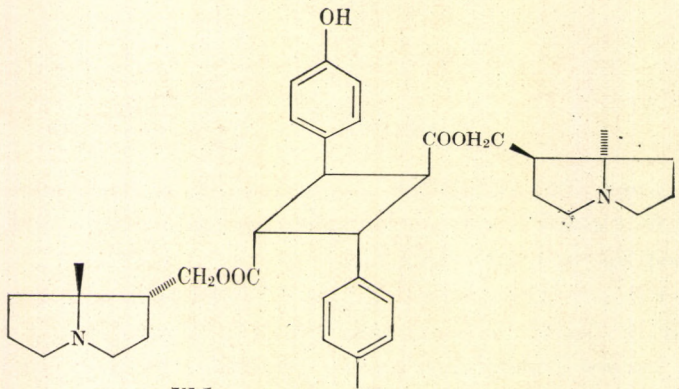
**XXXVII** $R^1$=H, $R^2$=OH
**XXXVIII** $R^1$=H, $R^2$=OOC(CH$_3$)=CHCH$_3$
**XXXIX** $R^1$=OH, $R^2$=H

XL

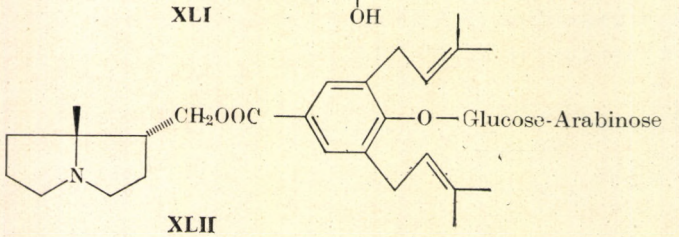
Cynaustraline (XXXIX) was isolated [48] from *Cynoglossum australe*. On hydrolysis it yielded lindelofidine and (−)-viridifloric acid. Thesinine and thesine were found [5] in *Thesium minkwitzianum*. Hydrolysis of these two alkaloids gave lindelofidine. The isolated acidic component was *p*-hydroxy-*trans*-cinnamic acid from thesinine whose structure is XL. On hydrolysis, thesine yielded the dimeric acid $C_{18}H_{16}O_6$. Oxidation of the dimethyl ester of thesinic acid gave [49] two moles of anisic acid; it follows that thesinic acid is a *p,p'*-dihydroxydiphenylcyclobutane dicarboxylic

acid. The structure of thesinic acid was confirmed [50] by its synthesis from α-truxillic acid, which indicates structure XLI for the alkaloid thesine.

The last so far known ester of lindelofidine is nervosine which was isolated [51, 52] from *Liparis* species. On alkaline hydrolysis, the obtained acidic component of nervosine was $C_{28}H_{40}O_{12}$ which on mild acid hydrolysis afforded the acid $C_{17}H_{22}O_3$; hydrogenation followed by acid hydrolysis yielded the acid $C_{17}H_{26}O_3$, D-glucose and L-arabinose. The structures of the acids were elucidated by NMR spectroscopy from which the structure XLII follows for nervosine.

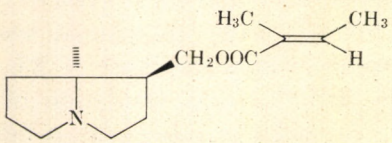



XLI

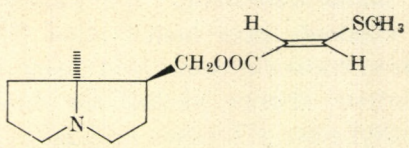



XLII

(c) Esters of (−)-isoretronecanol. Only two esters of (−)-isoretronecanol have been isolated so far. (−)-Isoretronecanyl tiglate (XLIII) and (−)-isoretronecanyl *trans*-3-methylthiopropenate (XLIV) occur in *Mimusops elengi* [53]. Unequivocal proof of the presence of (−)-isoretronecanol as a basic component could, however, not be provided.

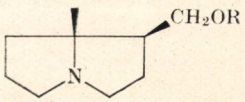



XLIII

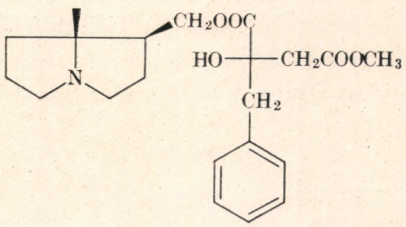



XLIV

(d) Esters of laburnine. From *Planchonella* species three esters of laburnine [54] were isolated. The major alkaloid is planchonelline whose structure (XLV) was suggested on the evidence of its NMR spectrum and that of the thio-acid $C_4H_6O_2S$ obtained from planchonelline by hydrolysis. The two other alkaloids were identified as the tigloyl (XLVI) and benzoyl (XLVII) esters of laburnine.

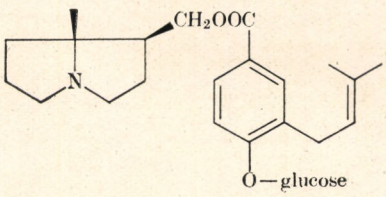



**XLV** R=*trans*-β-methylthioacryloyl
**XLVI** R=tigloyl
**XLVII** R=benzoyl

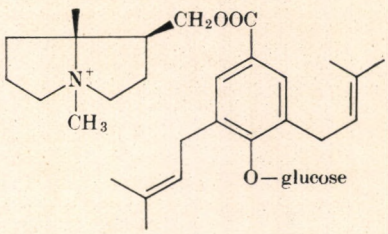



**XLVIII**

Phalaenopsine La was isolated [43, 55] from *Phalaenopsis mannii* and *Kingiella taenialis*. The structure XLVIII was elucidated in a similar manner as the structure of phalaenopsine T (XXXIV); these two alkaloids are enantiomers. Malaxine ($C_{26}H_{37}NO_8$) was found [56] in *Malaxis congesta*. Acid hydrolysis of malaxine gave laburnine and glucose; alkaline hydrolysis yielded 4-hydroxy-3-(3-methyl-2-butenyl)-benzoic acid. On the basis of these facts and the interpretation of the NMR, IR and mass spectra, malaxine was assigned structure XLIX. Very similar to malaxine is kumokirine (L) which was found in *Liparis* species [51, 52] together with nervosine (XLII). Hydrolysis of kumokirine yielded N-methyllaburnine, the corresponding unsaturated hydroxy acid and glucose.



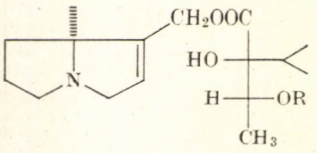

**XLIX**



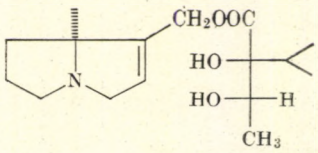

**L**

(e) Esters of (−)- and (+)-supinidine. Three esters of (−)-supinidine have been isolated. The first of them is supinine (LI) obtained from *Heliotropium supinum* [57, 58] and, later on, also from *H. europaeum*

[59], *Tournefortia sarmentosa* [60], *Trichodesma zeylanicum* [61] and *Eupatorium serotinum* [62]. It is hydrolyzed to (−)-supinidine and (+)-trachelanthic acid. Its methyl ether, heleurine (LII), was isolated from *Heliotropium europaeum* [59]; its hydrolysis yields (−)-heliotric acid. The diastereoisomer of supinine is amabiline (LIII) which was hydrolyzed to (−)-supinidine and (−)-viridifloric acid. It was isolated from *Cynoglossum amabile* [48]. Cynaustine (LIV) was obtained from *Cynoglossum australe* [48] along with cynaustraline (XXXIX). On hydrolysis it affords (+)-supinidine and (−)-viridifloric acid. This is the first ester of a (+)-enantiomer of an allylic amino alcohol which was found in plant material.

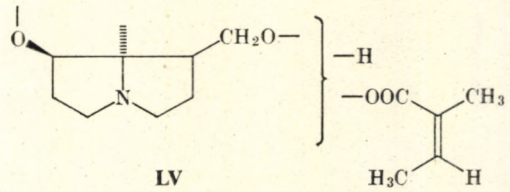


**LI** R=H
**LII** R=$CH_3$

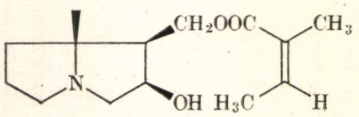


**LIII** 8α
**LIV** 8β

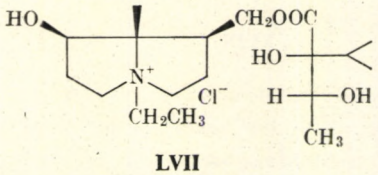
(f) Esters of saturated dihydroxynecines. Only three monoesters of saturated dihydroxynecines are known so far. Turneforcine was isolated [63] from *Turnefortia sibirica* and hydrolyzed to turneforcidine (see Chapter II) and angelic acid. Consequently, it is angelyl-turneforcidin (LV). It is, however, not known which hydroxyl group of turneforcidine is esterified. Macrophylline was isolated [64] from *Senecio macrophyllus* and identified as the ester of angelic acid with a primary alcoholic group of macronecine [65]. The correct structure of macronecine was established much later by synthesis (see Chapter II), and thus the structure of macro-



**LV**



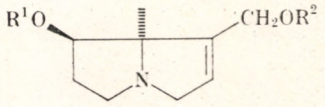
**LVI**



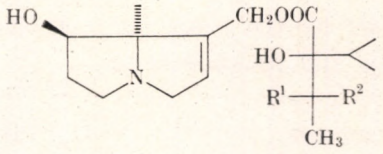
**LVII**

phylline is LVI. The last alkaloid of this group has been isolated from *Lindelofia macrostyla* [47] and is designated as the Lindelofia base $C_{17}H_{32}NO_5Cl$ (LVII). Its acid hydrolysis gave (+)-trachelanthic acid and a quaternary chloride, which on the basis of the IR spectrum and $R_F$ values was identified as hastanecine ethochloride.

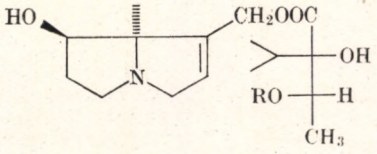
(g) Esters of retronecine. $O^7$-Angelylretronecine (LVIII) was isolated from *Cynoglossum latifolium* [66]. $O^9$-Angelylretronecine (LIX) was obtained in the form of its N-oxide from *Bhesa archboldiana* [67]; its hydrolysis afforded angelic acid and retronecine N-oxide; hydrogenolysis gave retronecanol and 2-methylbutyric acid. Consequently, it is an alkaloid of the allyl ester type, whose angeloyl rest is attached to C-9. Intermedine (LX), lycopsamine (LXI) and sincamidine (LXII) were isolated [68] from *Amsinckia intermedia*. Their structures were determined by identification of the products of hydrolysis and hydrogenolysis. The stereochemistry of sincamidine (LXII) is still obscure. Its acid is probably [68] the *erythro* isomer of heliotric acid.




**LVIII** $R^1$=Angeloyl, $R^2$=H
**LIX** $R^1$=H, $R^2$=Angeloyl




**LX** $R^1$=H, $R^2$=OH
**LXI** $R^1$=OH, $R^2$=H
**LXII** $R^1$, $R^2$=H, $OCH_3$

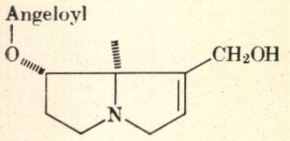



**LXIII** R=H
**LXIV** R=Acetyl

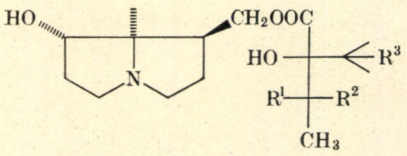
*Heliotropium indicum* generates [69, 70] the alkaloids indicine and acetylintricine. The structure of indicine (LXIII) was assigned on the basis of its hydrolysis to retronecine and (−)-trachelanthic acid. This is the first alkaloid which was found to contain (−)-trachelanthic acid. The hydrogenolysis of indicine (LXIII) gave retronecanol along with (−)-trachelanthic acid, which indicated an alkaloid of the allyl ester type. Gentle hydrolysis of acetylindicine (LXIV) afforded indicine (LXIII) and on acetylation both

substances were converted to the same diacetyl derivative. The position of the acetyl group in acetylindicine (LXIV) was determined by its hydrogenolysis; the products were retronecanol and acetyltrachelanthic acid, which gave a positive reaction with ferric chloride (α-hydroxy acids).

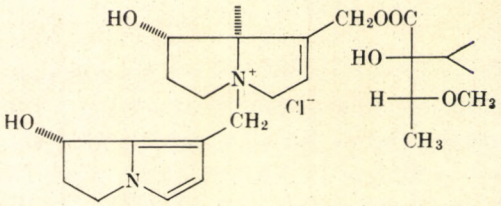
(h) Esters of heliotridine. $O^7$-Angelylheliotridine (LXV) was found for the first time [58] in *Heliotropium supinum* as one of the six alkaloids present therein. Later on, it was also isolated from *Senecio rivularis* [71] and *Cynoglossum officinalis* [72]. On hydrogenation it does not afford the hydrogenated acid and dihydrodeoxynecine, but $O^7$-heliotridine 2-methylbutyrate, which is evidence for the presence of an angeloyl residue at C-7. Rinderine (LXVI) was obtained from *Rindera baldschuanica* [73], *Solenanthus turkestanicus* [74] and *Eupatorium serotinum* [62]. Echinatine (LXVII) was found for the first time in *Rindera echinata* [75] and, later on, in *Cynoglossum amabile* [48], *C. officinale* [76], *Eupatorium maculatum* [3], *Heliotropium supinum* [58], *Lindelofia tschimganica* [77], some *Rindera* species [77] and *Solenanthus karateginus* [78]. Heliotrine (LXVIII) is one of the first known pyrrolizidine alkaloids which was found only in the *Heliotropium species* [78–85]. The structure of these three alkaloids was determined by the usual methods, i.e. identification of the products of hydrolysis and hydrogenolysis.




**LXV**




**LXVI** $R^1=R^3=H$, $R^2=OH$
**LXVII** $R^1=OH$, $R^2=R^3=H$
**LXVIII** $R^1=R^3=H$, $R^2=OCH_3$
**LXIX** $R^1$, $R^2=H$, $OCH_3$, $R^3=OH$



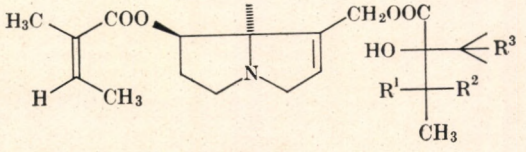

**LXX**

Europine (LXIX) was isolated [59] from *Heliotropium europaeum* and hydrolyzed to heliotridine and lasiocarpic acid, whose stereochemistry is not known. The last alkaloid of this group known so far is the base $C_{24}H_{37}N_2O_6Cl$ which was also isolated [59] from *Heliotropium europaeum*. Its structure (LXX) was elucidated [86] by zinc reduction which yielded heliotrine (LXVIII), and on the basis of the NMR and mass spectra. Confirmatory evidence was provided by its synthesis from heliotrine and dehydroheliotrine.

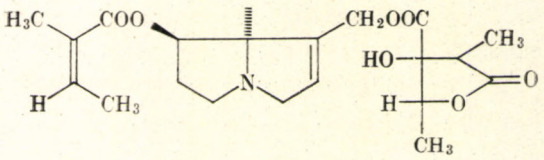
### *C. Acyclic Diester Alkaloids*

One of the acids present in all the diester acyclic alkaloids is angelic acid (in one case tiglic acid) which is bound to the hydroxyl group in the position 7 of the pyrrolizidine nucleus. The second hydroxyl at C-9 is usually esterified by hydroxylated α-isopropylbutyric acids.

(a) Diesters of retronecine. Echiumine (LXXI) and echimidine (LXXII) were found [87] in *Echium lycopsis*. Later on, echiumine was also isolated from *Amsinckia hispida*, *A. intermedia* and *A. lycopsoides* [68]. The hydrolysis of these two alkaloids gives retronecine and angelic acid which, on the basis of the results of hydrogenolysis, esterifies the hydroxyl at C-7. They differ, however, from each other by the presence of an additional acid. Since hydrolysis of echiumine affords (+)-trachelanthic acid, it has the structure LXXI. Echimidine (LXXII) contains echimidinic acid whose stereochemistry is not known. The diastereoisomer of echiumine (LXXI) is symphytine (LXXIII) which was found [88] in *Symphytum officinale*. Its structure was elucidated by application of the usual methods, i.e. hydrolysis and hydrogenolysis. The last alkaloid of this group is latifoline which was isolated [66] from *Cynoglossum latifolium* along with $O^7$-angelylretronecine (LVIII). The hydrolysis of latifoline yields retronecine, angelic acid and a mixture of some other acids. On hydrogenolysis, latifoline gives latifolic acid whose structure has been elucidated by NMR spectroscopy. Latifoline has been assigned the structure LXXIV; the stereochemistry of the acid moiety is not known yet.

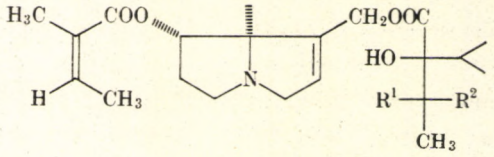



**LXXI** $R^1=R^3=H$, $R^2=OH$
**LXXII** $R^1$, $R^2=H$, OH, $R^3=OH$
**LXXIII** $R^1=OH$, $R^2=R^3=H$

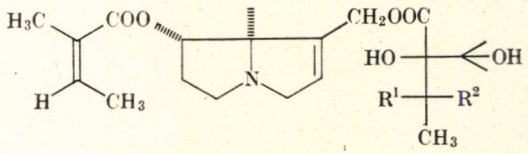


**LXXIV**

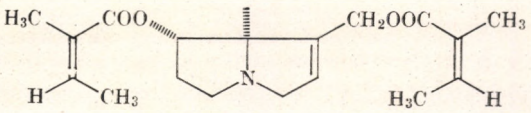
(b) Diesters of heliotridine. From *Heliotropium supinum* [58], three alkaloids have been isolated along with supinine (LI) and echinatine (LXVII); they are angelylheliotridine trachelanthate (LXXV), angelylheliotridine viridiflorate (LXXVI) and heliosupine (LXXVII). The structures of these three alkaloids were determined by the usual methods, i.e. identification of the products of hydrolysis and hydrogenolysis. The methyl ether of heliosupine (LXXVII) is lasiocarpine (LXXVIII), which was found for the first time in *Heliotropium lasiocarpum* [84, 85] and, later on, also in *H. europaeum* [82]. The hydrogenolysis of heliotridine gave angelic acid and lasiocarpic acid. *Cynoglossum officinale* was also found [72] to contain acetylheliosupine (LXXIX). Asperumine (LXXX), isolated from *Senecio asperum*, differs somewhat from the other alkaloids of this group, because on hydrolysis it yields 2 moles of angelic acid [89, 90] instead of the hydroxylated α-isopropylbutyric acid.



**LXXV** $R^1$=H, $R^2$=OH
**LXXVI** $R^1$=OH, $R^2$=H



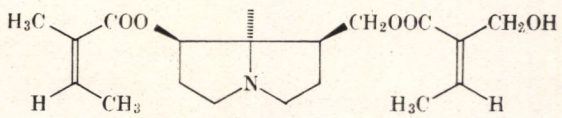
**LXXVII** $R^1$, $R^2$=H, OH
**LXXVIII** $R^1$, $R^2$=H, $OCH_3$
**LXXIX** $R^1$, $R^2$=H, OAc



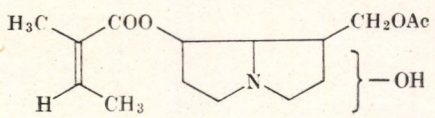
LXXX

(c) Diesters of other necines. Along with the diesters of retronecine and heliotridine, only three other alkaloids of the diester type have been found so far. The first of them was sarracine, isolated from *Senecio sarracenicus* [91] and *S. mikanioides* [92]. On alkaline hydrolysis, it yields mikanecic acid which is a dimer arising from two native C-5 acids (see Chapter III). Mild alkaline hydrolysis of sarracine gives sarracinic acid and $O^7$-angelylplatynecine, which indicates that sarracinic acid is bound to the primary hydroxyl group of platynecine. The structure LXXXI was assigned to sarracine on the basis of its NMR spectrum and that of sarracinic acid. The structure of sarracinic acid was also confirmed by synthesis. Noticeable is the striking conformity of the structures of sarracine (LXXXI) and asperumine (LXXX). Contrary to other acyclic diesters, these two alkaloids occur in the plants of the genus *Senecio*, for which the presence of macrocyclic diester alkaloids is typical. It is likely that these two alkaloids are intermediates in the biosynthesis of macrocyclic alkaloids.

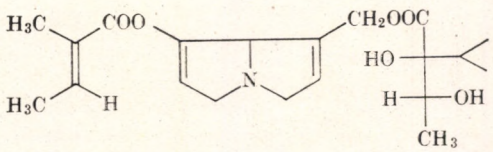
Brachyglottine was isolated from *Brachyglottis repanda* [93] and hydrolyzed to angelic acid and acetic acid. The isolated basic moiety was a saturated trihydroxynecine which was not, however, identical with rosmarinecine. Thus, the partial structure of brachyglottine is given by the formula LXXXII.



LXXXI



LXXXII

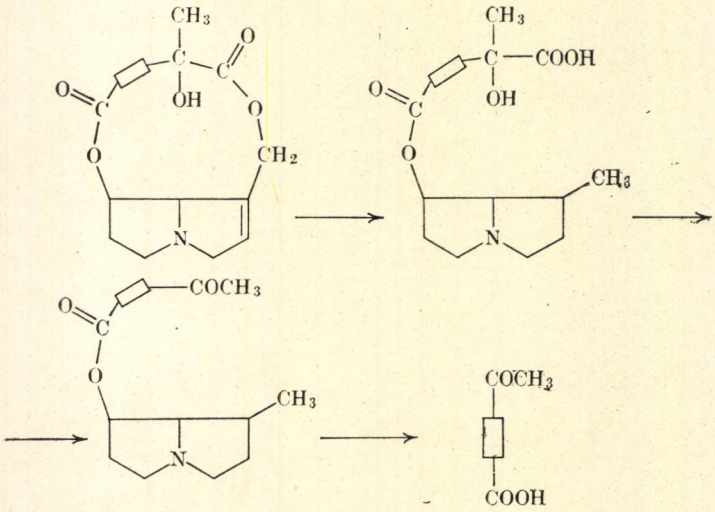


LXXXIII

Anadoline was isolated from *Symphytum orientale* [94]. Alkaline hydrolysis yielded tiglic acid, (+)-trachelanthic acid, and a doubly-unsaturated necine. Hydrogenolysis gave 7-(2′-methylbutyroyl)-retronecanol. The NMR spectra showed that the double bonds in the basic moiety of the molecule were located in the positions 1,2 and 6,7, which led to the assignment of structure LXXXIII to anadoline.

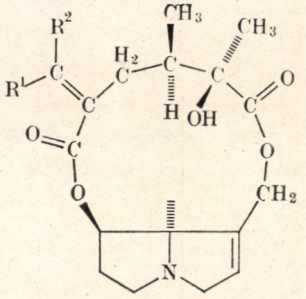
### *D. Macrocyclic Diester Alkaloids with 12-Membered Ring*

(a) Diesters of retronecine. The first alkaloid of this group is senecionine which has been found in many plants of the genera *Senecio* and *Crotalaria*, as well as in some other plants. For details of the determination of the structure of senecionine and some other long known alkaloids of this group, see the pertaining summarizing reports (References to Introduction). Since the alkaloids of this group are present in a great number of plants, the enumeration of all the papers dealing with their occurrence in plant material would be beyond the scope of this paper. The structures of the macrocyclic diesters and their absolute configurations have usually been elucidated separately by studying the structure of the acid and the basic moiety after hydrolysis (see Chapters II and III). The only problem was to determine how the dicarboxylic acid was bound to dihydroxynecine. This problem was resolved by hydrogenolysis of the alkaloid to the half-ester whose oxidation and subsequent hydrolysis gave the corresponding keto acid:

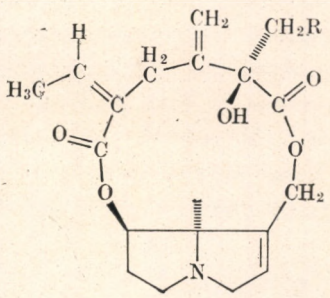


Thus, the primary hydroxyl group of the necine is bound to that side of the dicarboxylic acid which has a hydroxyl at the α-carbon atom. This fact was confirmed in numerous alkaloids by mass spectrometry and X-ray crystallography, and no exception has been encountered in that respect as yet. This rule results from the qualitative difference between the hydroxyl groups of the necine and the considerably different dissociation constants of the carboxyl groups of the corresponding acid.

By application of the above method, the structures of senecionine (LXXXIV), its *trans* isomer integerrimine (LXXXV), seneciphylline (LXXXVI) and riddelline (LXXXVII) have been elucidated.

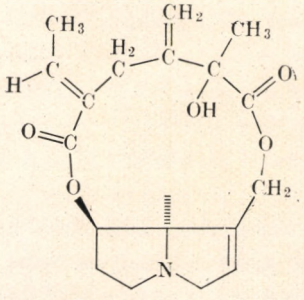


**LXXXIV** $R^1=CH_3$, $R^2=H$
**LXXXV** $R^1=H$, $R^2=CH_3$

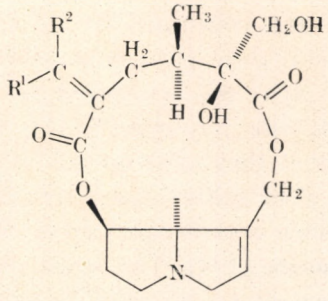


**LXXXVI** R=H
**LXXXVII** R=OH

The alkaloid spartioidine has been isolated from *Senecio spartioides*. Adams and Gianturco [95] assume that this alkaloid is an ester of retronecine with some of the diastereoisomers of seneciphyllic acid. Since all the possible diastereoisomers of seneciphyllic and isoseneciphyllic acids have already been synthesized (see Chapter III) and because the physical constants of none of them are consistent with those of spartioidic acid, the structure LXXXVIII of spartioidine is still questionable.



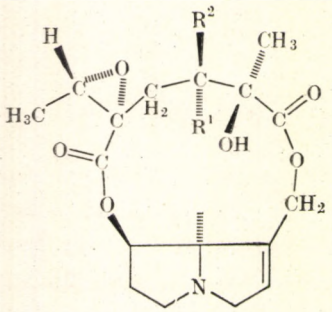
**LXXXVIII**



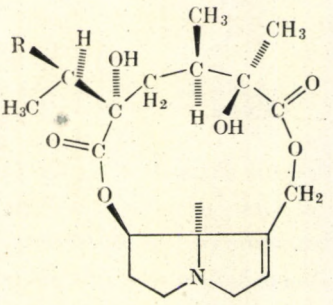
**LXXXIX** $R^1=CH_3$, $R^2=H$
**XC** $R^1=H$, $R^2=CH_3$

Identification of the acid and of the basic component led to the assignment of the structure LXXXIX to retrorsine. In 1966, the alkaloid usaramine was isolated [96] from *Crotalaria usaramoensis* and was identified as the *trans* isomer of retrorsine. The structure of usaramine (XC) was also confirmed by its conversion into retrorsine (LXXXIX) by ultraviolet irradiation. Isomerization of retrorsine (LXXXIX) to usaramine (XV) was also carried out by the addition of bromine followed by debromination with zinc dust in acetic acid [97]. In 1968, mucronatinine was isolated from *Crotalaria mucronata* [98], and the NMR and mass spectral data showed that it was a diastereoisomer of *trans*-retrorsine. Later on, proof could, however, be provided [99] that mucronatinine was identical with usaramine (XC) and, therefore, the denomination mucronatinine should be discarded from the literature.

*Senecio jacobaea* has four alkaloids, the main of which is jacobine. The definite structure XCI of jacobine was proposed by Geissman [100] and Bradbury and Masamune [101]. The absolute configuration was determined by X-ray crystallography of jacobine bromohydrin [102] and, independently, by chemical data [103].



**XCI** $R^1$=H, $R^2$=$CH_3$
**XCII** $R^1$+$R^2$=$CH_2$

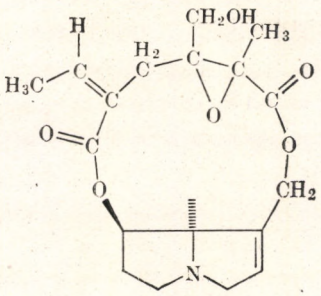


**XCIII** R=Cl
**XCIV** R=OH

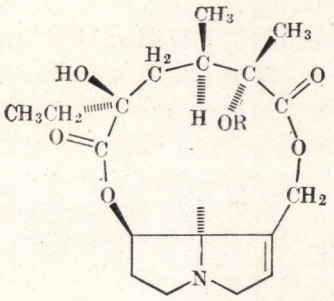
The structure of jacozine (XCII) was determined on the basis of the NMR spectrum and confirmed by its conversion to seneciphylline (LXXXVI) by reaction with potassium selenocyanate [103]. In the same manner, jacobine (XCI) was converted to senecionine (LXXXIV). The structures of the alkaloids jaconine (XCIII) and jacoline (XCIV) were confirmed by the fact that they arise on mild hydrolysis of jacobine (XCI) with hydrochloric acid or sulfuric acid [100, 104].

Erucifoline was isolated from *Senecio erucifolius* and then hydrolyzed to retronecine [105]. The acids obtained after hydrolysis were artifacts which arose after opening of the epoxide ring of the native acid (see Chapter III). The total structure of erucifoline (XCV) resulted from the interpreta-

tion of the NMR and mass spectra of the alkaloid, its acetyl derivative and the hydrolytic products [106]. The presence of an ethylidene grouping in erucifoline was also demonstrated by oxidation with osmium tetroxide and periodic acid to acetaldehyde; the *cis* arrangement was shown by UV spectroscopy. The absolute configuration of the acid moiety of erucifoline is not known as yet.

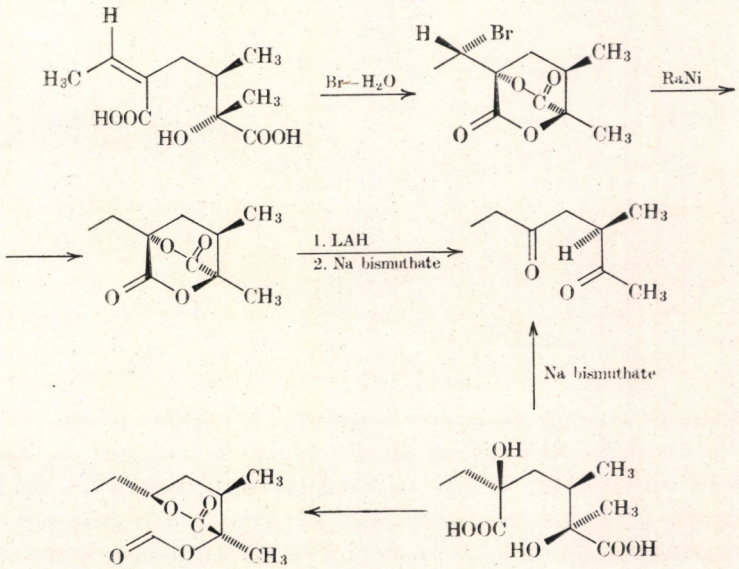


**XCV**



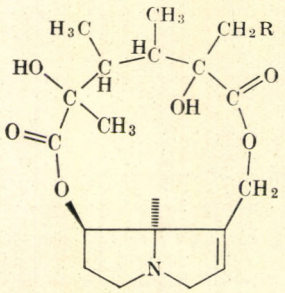
**XCVI** R=Ac
**XCVII** R=H

From *Senecio othonniformis*, the alkaloids bisline and its acetyl derivative isoline were isolated [107]. Hydrolysis of isoline gave retronecine and isolinecic acid $C_{10}H_{18}O_6$. It was found [108] that isolinecic acid was a diastereoisomer of the dilactone of senecic acid. These two substances were degraded to *R*-(+)-3-methylheptane-2,5-dione:

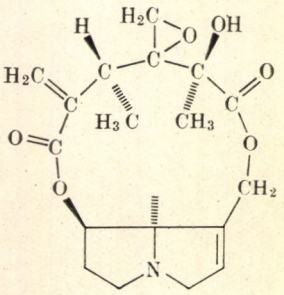


On the basis of these reactions, the CD spectra, and the fact that isolinecic acid also forms a dilactone, the absolute configuration 2*S*, 3*R*, 5*S* has been ascribed to isolinecic acid. Isoline has the structure XCVI and bisline XCVII.

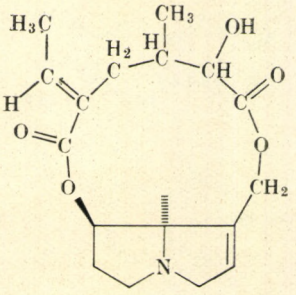
Sceleratine and chlorodeoxysceleratine have been isolated from *Senecio sceleratus*. Hydrolysis of sceleratine affords retronecine and an acid whose structure is given in Chapter III. Thus, the structure of sceleratine is XCVIII. Hydrolysis of chlorodeoxysceleratine yields the same products as sceleratine, therefore, the structure XCIX has been assigned [109] to it. An attempt to carry out the partial hydrolysis of chlorodesoxysceleratine (XCIX) to sceleratine (XCVIII) failed, since it resulted in closure of the six-membered ether ring; the absolute configuration of these two alkaloids has not been elucidated yet.



**XCVIII** R=OH
**XCIX** R=Cl



**C**



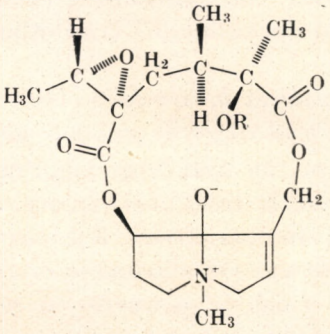
**CI**

The alkaloid swazine has been isolated [110] from *Senecio swaziensis*. On the basis of X-ray analysis of the dilactone obtained by hydrolysis (along with retronecine), it was ascribed the structure of an unsaturated epoxide with a twelve-membered ring. In view of this structure, the α-hydroxycarboxylic group must be bound to the secondary hydroxyl group

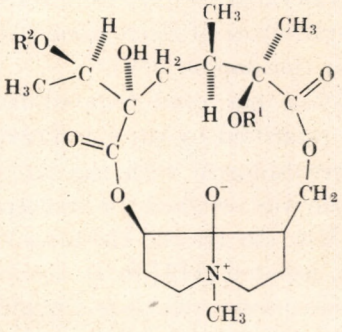
of retronecine, which has not been observed in any of the alkaloids studied so far. However, X-ray analysis of swazine methiodide has revealed [111] that the interorientation of the dicarboxylic acid and necine is the same as in the other macrocyclic alkaloids. Thus, the structure of swazine, including its absolute configuration, is C.

The last of the retronecine esters is nilgirine (CI), isolated from *Crotalaria mucronata* [112]. It differs from the other alkaloids of this group by the presence of an acid with 9 carbon atoms. Its structure was determined on the basis of the interpretation of the NMR spectrum.

(b) Diesters of othonecine. The structures of the alkaloids belonging to this group remained unknown for a long time, owing to the fact that it was difficult to isolate and identify the basic component, othonecine. The elucidation of the structure of othonecine and its conversion to the stable, identifiable dihydrodesoxy derivative (see Chapter II) led to the determination of the structure of its diesters. These diesters have characteristic IR spectra which exhibit a broad absorption band at about 1600 $cm^{-1}$. This band is attributable to the keto group which is in transannular interaction with the tertiary nitrogen atom. When the IR spectra of the salts of the esters of othonecine are measured, the above-mentioned band disappears from the spectrum. Wunderlich [113] determined the structure of the alkaloid retusamine by X-ray analysis (see this Chapter, Section F(b)). On the basis of its analogy with the alkaloids othosenine, onetine and renardine, he assigned to them the structures CII, CIV and CVII. In view of the identity of the acids from othosenine (CII) and onetine (CIV) with those of jacobine (XCI) and jacoline (XCIV), the absolute configuration of the former two alkaloids could also be inferred.

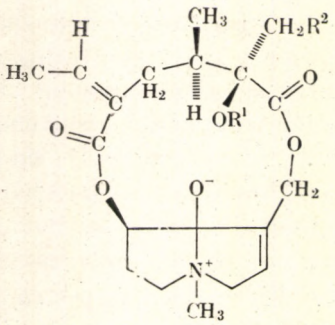


**CII** R=H
**CIII** R=Ac

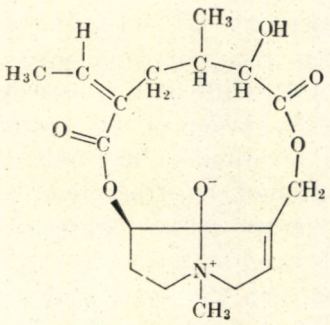


**CIV** $R^1$=$R^2$=H
**CV** $R^1$=Ac, $R^2$=H
**CVI** $R^1$=$R^2$=Ac

Renardine (CVII) was found to be identical with senkirkine which was isolated [114] from *Senecio kirkii*. For the substance CVII, the designation senkirkine is still in use. Later on, the structure of senkirkine (CVII) was confirmed by a series of chemical reactions and by the interpretation of the NMR spectra. *Senecio kirkii* also gave O-acetylsenkirkine (CVIII) [115]. In 1972, hydroxysenkirkine (CIX), another derivative of senkirkine, was isolated [116] from *Crotalaria laburnifolia*. Its structure resulted from the identification of isatinecic acid as its acidic component.



**CVII** $R^1=R^2=H$
**CVIII** $R^1=Ac$, $R^2=H$
**CVIX** $R^1=H$, $R^2=OH$



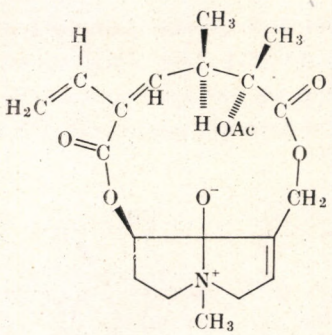
**CX**

In addition of othosenine (CII), three of its analogues were isolated [117] from *Cacalia floridana*. Florosenine (CIII) is an acetylothosenine, floridanine (CV) a monoacetylonetine, and floricaline (CVI) is a diacetylonetine. The structures of the alkaloids have been elucidated on the basis of the NMR and mass spectra, and confirmed by the acetylation of othosenine (CII) and floridanine (CV) to florosenine (CIII) and floricaline (CVI) and by partial hydrolysis of florosenine (CIII) and floricaline (CVI) to othosenine (CII) and floridanine (CV).

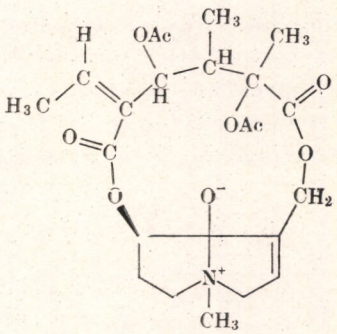
Crotafoline has been isolated from *Crotalaria laburnifolia* [116]. Othonecine is esterified by the C-9 dicarboxylic acid which is probably identical or diastereoisomeric with the *cis* isomer of the acid from nilgirine (CI). Crotafoline was assigned the structure CX on the basis of the interpretation of the NMR, the IR and the mass spectra. Another diester of othonecine is clivorine, isolated [118] from *Ligularia clivorum*. On alkaline hydrolysis, it yielded clivonecic acid (see Chapter III) which is, however, an artifact. Along with clivonecic acid acetic acid was isolated. Later on, the originally proposed [119] structure with a $\beta$-ketoester arrangement was revised [120], because it was not consistent with the UV and CD spectra. The newly

assigned structure CXI is in good accord in all respects and was confirmed by X-ray crystallographic studies [120, 121], which also led to the absolute configuration of clivorine. The *Ligularia* species were also found to contain the alkaloids ligularine and ligudentine. As far as the structure of ligudentine is concerned, only some of its fragments are known. On the basis of an analysis of the NMR spectrum, ligularine was assigned [122] the structure CXII. The absolute configuration of ligularine (CXII) which formally arises by addition of acetic acid to the diene system of clivorine (CXI) is not known.

(c) Diesters of other necines. In addition to a great number of 12-membered diesters of retronecine and othonecine, there are known 7 alkaloids whose base is formed by another necine.

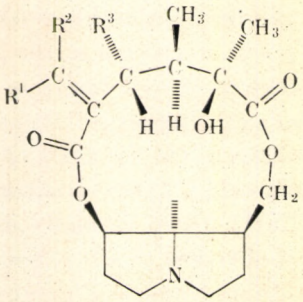


**CXI**

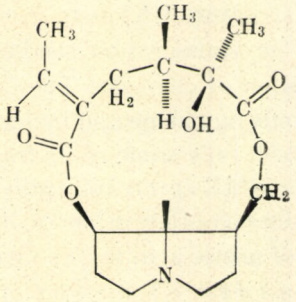


**CXII**

Platyphylline was isolated from a series of plants of the genus *Senecio* and also from *Petasites laevigatus;* along with platyphylline, the minor alkaloid neoplatyphylline was isolated from *Senecio rhombifolius.* It was found [123] that hydrolysis of these two alkaloids gave rise to the same products, i.e. platynecine and integerrinecic acid. Acidic hydrolysis of platyphylline yielded senecic acid and the integerrinecic acid lactone, which shows that these two alkaloids are isomeric; platyphylline (CXIII) is a *cis* and neoplatyphylline (CXIV) a *trans* isomer. The structure of neoplatyphylline (CXIV) was also elucidated [124] in connection with the structure of hastacine which was isolated from *Cacalia hastata.* It was established that hastacine is diastereoisomeric with neoplatyphylline (CXIV); these two substances have the same acid, and they differ from each other by the necine. The structure first proposed [124] (basic moiety–hastanecine with a 1$\alpha$, 7$\beta$, 8$\alpha$ configuration) was, however, incorrect. Later on, it was revised (see Chapter II) and the correct structure CXVI was assigned to hastacine.

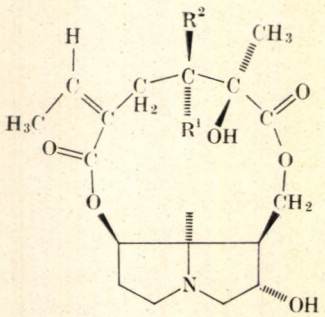


**CXIII** $R^1=CH_3$, $R^2=R^3=H$
**CXIV** $R^1=R^3=H$, $R^2=CH_3$
**CXV** $R^1=CH_3$, $R^2=H$, $R^3=OH$

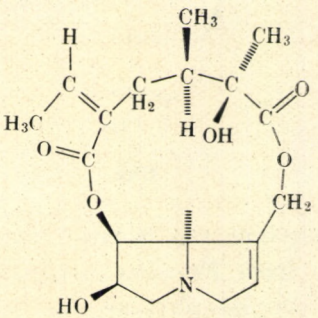


**CXVI**

Hygrophylline was isolated from *Senecio hygrophyllus;* on hydrolysis it gave platynecine and hygrophyllinecic acid [125]. The NMR spectrum of hygrophyllinecic acid dilactone revealed that its methyl is in *trans* arrangement to the carboxyl, whereas that of the native alkaloid is *cis* (CXV); isomerization takes place on hydrolysis.



**CXVII** $R^1=H$, $R^2=CH_3$
**CXVIII** $R^1+R^2=CH_2$



**CXIX**

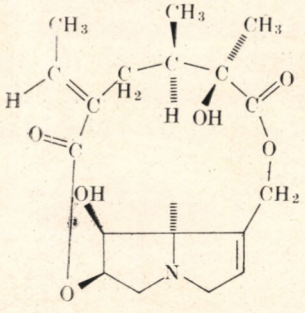
Rosmarinine and angularine occur in *Senecio angulatus* [126]. Rosmarinine had been found earlier in various *Senecio* species. Hydrolysis of the mixture of these two alkaloids yields [126] rosmarinecine and a mixture of senecic and isoseneciphyllic acids. Rosmarinine (CXVII) is an ester of rosmarinecine with senecic acid, which was also confirmed [127] by its conversion to senecionine (LXXXIV). The conversion was accomplished by refluxing rosmarinine toluene-*p*-sulfonate with pyridine. Consequently, isoseneciphyllic acid, which arises on hydrolysis of the above-mentioned mixture, is attributable to angularine. Therefore it has the structure CXVIII. This assignment is also consistent with the NMR spectral data.

Anacrotine was isolated from *Crotalaria anagyroides* [128]. Alkaline hydrolysis gave senecic acid and crotanecine (see Chapter II). On the basis of NMR and mass spectral data, anacrotine was assigned the structure CXIX. The alkaloid crotalaburnine from *Crotalaria laburnifolia* was found to be identical [129] with anacrotine (CXIX). The latter was also obtained from *Crotalaria incana* [130].

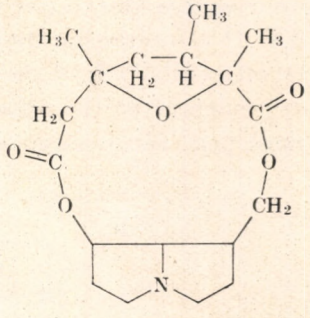
*E. Macrocyclic Diesters with 13-Membered Ring*

Only two alkaloids with 13-membered ring have been isolated so far. Madurensine was found together with anacrotine (CXIX) in *Crotalaria madurensis* and *C. agatiflora* [128] and, later on, also in *C. laburnifolia* [116]. The statement that madurensine was *trans*-anacrotine [128] was then refuted. On the evidence of NMR spectroscopy, madurensine was assigned [131] the structure CXX.

Nemorensine was isolated [132] from *Senecio nemorensis*. It was assigned the structure CXXI on the basis of the NMR and mass spectral properties and those of the acid obtained on its hydrolysis. The absolute configuration of the acid and of the basic component is unknown.



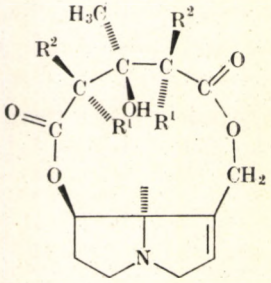
**CXX**



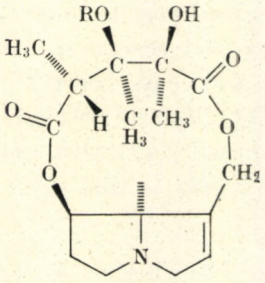
**CXXI**

*F. Macrocyclic Diesters with 11-Membered Ring*

(a) Diesters of retronecine. The simplest alkaloid of this group is dicrotaline, isolated [133] from *Crotalaria dura* and *C. globifera*. On hydrolysis, it affords dicrotalic acid and retronecine. Adams and van Duuren [134] determined the structure of dicrotalic acid and confirmed it by synthesis; thus, structure CXXII is attributable to dicrotaline.

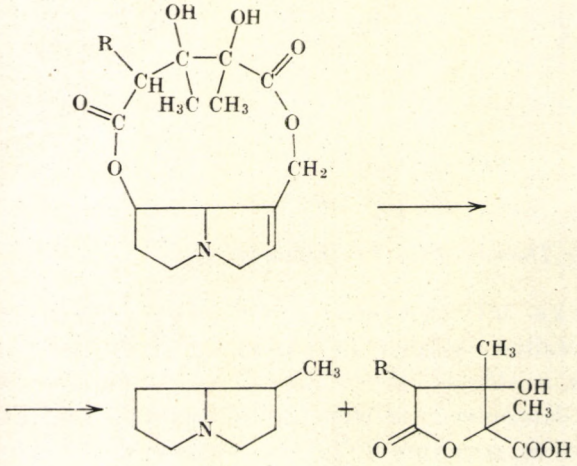


**CXXII** $R^1=R^2=H$
**CXXIII** $R^1=H$, $R^2=CH_3$
**CXXIV** $R^1=CH_3$, $R^2=H$



**CXXV** R=H
**CXXVI** R=Ac
**CXXVII** R=—COCH($CH_3$)$C_2H_5$

The dimethyl derivatives of dicrotaline (CXXII) are crispatine and fulvine which have been isolated from *Crotalaria fulva* and *C. crispata* [135, 136]. Fulvinic and crispatic acids are optically inactive enantiomers which have already been synthesized (see Chapter III); thus structures CXXIII and CXXIV are assignable to fulvine and crispatine. Monocrotaline is the first known and widely distributed alkaloid of this group. It is a diester of retronecine and monocrotalic acid. The correct structure of monocrotaline has been proposed by Adams *et al.* [137]. Since the absolute configuration of monocrotalic acid is known (see Chapter III), monocrotaline has the structure CXXV. It is interesting that hydrogenolysis of monocrotaline (CXXV) and of the other alkaloids of the monocrotaline type usually does not yield half-esters, contrary to the alkaloids of the senecionine type (LXXXIV). For the most part, retronecanol and the lactone acid are directly formed:



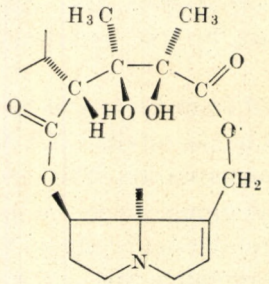
The orientation of the dicarboxylic acid to dihydroxynecine can, however, be successfully determined by the interpretation of the mass spectra [138].

Spectabiline was isolated from *Crotalaria spectabilis* and identified [139] as an acetyl derivative of monocrotaline (CXXV); this was also confirmed by the acetylation of monocrotaline. The acetyl group was found to be localized at the $\beta$-hydroxyl group, since the hydrogenolysis of spectabiline also gave anhydromonocrotalic acid (elimination of acetic acid), whereas on hydrogenolysis of monocrotaline this acid was not formed. Consequently, spectabiline has the structure CXXVI. Another ester of monocrotaline (CXXV) is grahamine. This alkaloid was isolated from *Crotalaria grahamiana* [140]. On hydrolysis it yields (−)-2-methylbutyric acid, monocrotalic acid and retronecine, and on partial hydrolysis monocrotaline (CXXV). On the basis of these findings and the interpretation of the NMR spectra, grahamine was assigned the structure CXXVII.

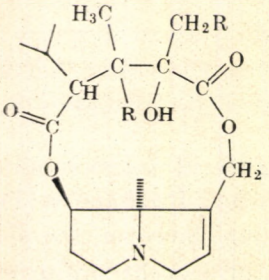
Trichodesmine and junceine are analogues of monocrotaline (CXXV) having an isopropyl group at C-4. Yunusov and Plekhanova [141] assigned the structure CXXVIII to trichodesmine (not, however, the absolute configuration). On the basis of the formation of a cyclic sulfite with thionyl chloride, they established that both hydroxyl groups must be in *cis* configuration. Since monocrotalic and trichodesmic acids were correlated (see Chapter III) and it was shown [142] that they had the same absolute configuration, trichodesmine has the structure CXXVIII. On alkaline hydrolysis, junceine yields a methyl isobutyl ketone and retronecine; with 2 moles of periodate it gives formaldehyde. On the basis of these reactions and the reaction of junceic acid (obtained by hydrogenolysis of junceine), Adams and Gianturco [143] proposed the structure CXXIX for junceine. Later on, this structure was confirmed [138] by interpretation of the NMR spectra of junceine.

Incanine was isolated from *Trichodesma incanum*. Alkaline hydrolysis of incanine yielded incanic and isoincanic acids, and its hydrogenolysis retronecanol and incanic acid; it was assigned [141] the structure CXXX. The absolute configuration of junceine and that of incanine are not known.

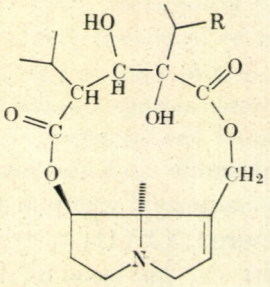
Axillarine has been isolated [144] from *Crotalaria axillaris*, and it is isomeric with junceine (CXXX). It has a hydroxyethyl group instead of a methyl and hydroxymethyl group. Axillarine was assigned the structure CXXXI on the basis of the NMR and mass spectral data and the oxidation with periodate [144, 145]. Axillaridine, a minor alkaloid obtained from *Crotalaria axillaris*, has structure CXXXII [145]. The absolute configurations of these two alkaloids are not known as yet.



**CXXVIII**



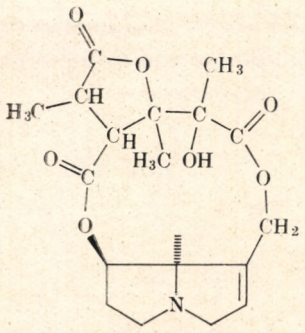
**CXXIX** R=OH
**CXXX** R=H



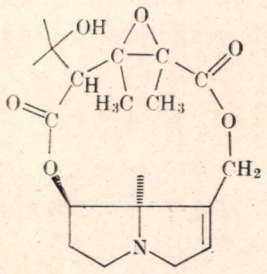
**CXXXI** R=OH
**CXXXII** R=H

The structures of the last two alkaloids of this group are still obscure. Grantianine was isolated from *Crotalaria grantiana*. Adams and Gianturco [146] assigned to it the structure CXXXIII because, on the basis of the IR spectrum, grantianine contains a $\gamma$-lactone grouping. The arrangement of the skeleton is analogous to that of trichodesmine (CXXVIII). Wunderlich [113] is, however, of the opinion that grantianine should have the structure of a dialkyl ethylmalonate bearing analogy to retusamine (see below), which also possesses a $\gamma$-lactone grouping and whose structure was determined by X-ray crystallography. Culvenor *et al.* [147] separated a sample of the alkaloid obtained from *Crotalaria grantiana* and obtained a $\gamma$-lactone which he found identical with grantianine; in addition he obtained still another alkaloid, grantaline. By measuring the NMR spectra of grantianine, he excluded the structure suggested by Wunderlich. The interpretation of the complex spectrum did not, however, provide unequivocal evidence for the assignment of the structure CXXXIII.

Grantaline was hydrolyzed [147] to retronecine and the dicarboxylic grantalic acid. On the basis of the interpretation of the NMR spectra of the alkaloid and the acid, Culvenor proposed structure CXXXIV for grantaline, but he did not exclude other possibilities.

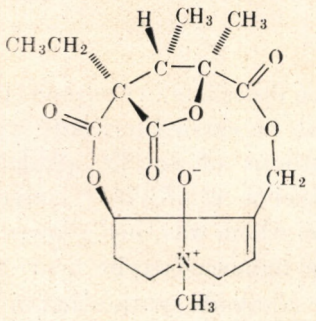


**CXXXIII**

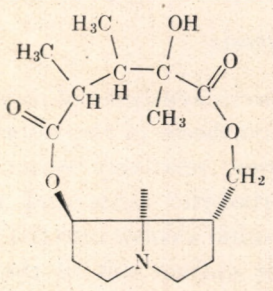


**CXXXIV**

(b) Diesters of other necines. So far, only 4 alkaloids of this group are known, three of which are diesters of othonecine. Retusamine and retusine have been isolated from *Crotalaria retusa* [6]. The structure of retusamine (CXXXV) was determined by X-ray crystallography [113]. This was the first diester of othonecine whose structure could be definitely resolved. Retusine was hydrolyzed [6] to a base which was identical with turneforcidine. The acidic moiety was identified as α-dihydroanhydromonocrotalic acid. The stereochemistry of turneforcidine has been resolved and confirmed by partial synthesis only recently (see Chapter II), which led to the assignment of the correct structure CXXXVI to retusine.



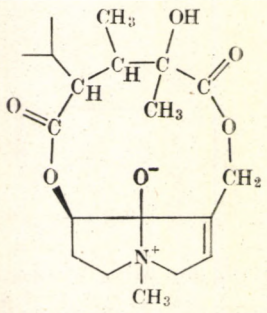
**CXXXV**



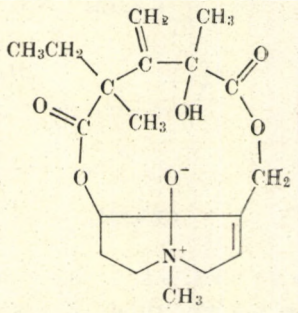
**CXXXVI**

Crosemperine has been isolated from *Crotalaria semperflorens* [148]. On hydrolysis it yields incanic acid and on hydrogenolysis dihydrodesoxyothonecine. Consequently it is a diester of othonecine and incanic acid. Thus, the assignment of the structure CXXXVII to crosemperine is in good agreement with the interpretation of its NMR spectrum. The last of the 11-membered diesters is emiline which was isolated [149] from *Emilia flammea*. Its hydro-

lysis gave emilic acid. The structure CXXXVIII for emiline was determined [150] on the basis of its NMR, IR and mass spectra and those of emilic acid. The basic component is, in all probability, othonecine.



**CXXXVII**



**CXXXVIII**

The physical constants of all the alkaloids isolated so far, whose structures have been elucidated, are listed in Table V. Table VI gives the synonyma and the designation of the alkaloid mixtures, and Table VII lists the alkaloids with yet unknown structures.

## 2. SYNTHESIS AND BIOSYNTHESIS OF PYRROLIZIDINE ALKALOIDS

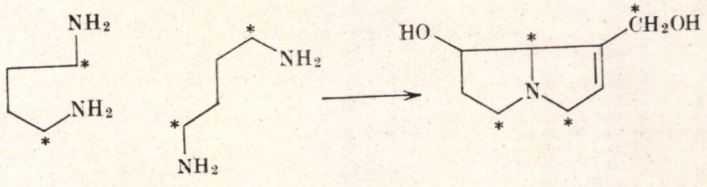
### *A. Synthesis*

Numerous syntheses of necines have been described in Chapter II, Section 3. The syntheses of the ester alkaloids can be carried out only with great difficulties; relatively easier is the synthesis of acyclic alkaloids from monocarboxylic acids and necines. Culvenor *et al.* [151] converted an allylic amino alcohol into the allyl chloride, which was then allowed to react with the sodium salt of the appropriate monocarboxylic acid; yields of about 50% were obtained. The first of the alkaloids synthesized in this way were lycopsamine (LXI) and intermedine (LX) which were prepared [68] by the combination of the allyl chloride derived from retronecine with the sodium salt of (−)-viridifloric acid and (+)-trachelanthic acid, respectively. The other two synthetic acyclic alkaloids were heliotrine (LXVIII) and supinine (LI) which were made [151] by the combination of the allyl chlorides derived from heliotridine and supinidine with the sodium salt of (−)-heliotric acid and (+)-trachelanthic acid. The formation of the esters is complicated by the tendency of the allyl chloride to form quaternary

ammonium derivatives. A combination of the components gave [54] laburnine benzoate (XLVII). The total syntheses of trachelanthamine (XXXI), viridiflorine (XXX) and lindelofine (XXXVII) have also been achieved. Russian authors [152] converted (+)-trachelanthic acid to its methyl ester by reaction with diazomethane. With benzyl chloride in dimethylformamide in the presence of sodium hydride, the methyl ester yielded methyl O,O′-dibenzyl-(+)-trachelanthate. On refluxing this substance with (−)-trachelanthamidine in n-heptane in the presence of sodium methoxide, it was transesterified to O,O′-dibenzyltrachelanthamine. Trachelanthamine (XXXI) was then obtained by hydrogenolysis of the dibenzyl derivative. In a similar manner, the combination of methyl O,O′-dibenzyl-(−)-viridiflorate with (−)-trachelanthamidine gave viridiflorine (XXX) and the combination of methyl O,O′-dibenzyl-(+)-trachelanthate with lindelofidine yielded lindelofine (XXXVII). The synthesis of the macrocyclic diesters has not been achieved yet.

*B. Biosynthesis*

(a) Biosynthesis of necines. Robinson [153] suggested that ornithine was a precursor of the necines. 2-[$^{14}$C]-Ornithine was incorporated by Nowacki and Byerrum [154] into the molecule of monocrotaline in *Crotalaria spectabilis*, and by Hughes *et al.* [155] into the molecule of retrorsine in *Senecio isatideus*. In this way, confirmatory evidence was provided to show that ornithine was, indeed, a precursor in the biosynthesis of necines. Bottomley and Geissman [156] fed 1,4-[$^{14}$C]-putrescine, 2-[$^{14}$C]- and 5-[$^{14}$C]-ornithine to *Senecio douglasii* which contains the alkaloids retrorsine, riddelline, senecionine and seneciphylline. All these alkaloids are diesters of retronecine. Since 25% of the activity was found to be present in the hydroxymethyl group of retronecine, these authors assume that the utilization of 2- and 5-labelled ornithine proceeded *via* a symmetrical intermediate:




The results obtained by Hughes *et al.* [155] are, however, contradictory. Though 26% of the activity was found in the hydroxymethyl group (C-9), there was no activity at C-3 and C-5; at C-7 + C-8, the activity amounted

*Table V*

Structure and Physical Constants of Pyrrolizidine Alkaloids

| Alkaloid | Formula | Structure | M.p., °C | $[\alpha]_D$ | Plant genera |
|---|---|---|---|---|---|
| 7$\beta$-Acetoxy-1-methoxymethyl-1,2-dehydro-8$\alpha$-pyrrolizidine | $C_{11}H_{17}NO_3$ | IX | — | +15.2[a] | *Crotalaria* |
| Acetylheliosupine | $C_{22}H_{33}NO_8$ | LXXIX | | | *Cynoglossum* |
| Acetylindicine | $C_{17}H_{27}NO_6$ | LXIV | — | — | *Heliotropium* |
| O-Acetylsenkirkine | $C_{21}H_{29}NO_7$ | CVIII | 195–196 | −34[c] | *Senecio* |
| Alafine | $C_{17}H_{23}NO_5$ | XXXVI | | | *Alafina* |
| Alloanodendrine | $C_{13}H_{21}NO_2$ | XIX | — | +18[a] | *Anodendron* |
| Amabiline | $C_{15}H_{25}NO_4$ | LIII | — | −7.1[a] | *Cynoglossum* |
| Anacrotine | $C_{18}H_{25}NO_6$ | CXIX | 195 | +30[a] | *Crotalaria* |
| Anadoline | $C_{20}H_{29}NO_6$ | LXXXIII | 186 | +9.2[b] | *Symphytum* |
| $O^7$-Angelylheliotridine | $C_{13}H_{19}NO_3$ | LXV | 116–117 | +10.8[a] | *Heliiotropium, Senecio* |
| $O^7$-Angelylheliotridine trachelanthate | $C_{20}H_{31}NO_6$ | LXXV | — | — | *Heliotropium* |
| $O^7$-Angelylheliotridine viridiflorate | $C_{20}H_{31}NO_6$ | LXXVI | — | — | *Heliotropium* |
| $O^7$-Angelylretronecine | $C_{13}H_{19}NO_3$ | LVIII | 76–77 | +49[a] | *Cynoglossum* |
| $O^9$-Angelylretronecine-N-oxide | $C_{13}H_{19}NO_4$ | LIX | 153–154 | +30[b] | *Bhesa* |
| Angularine | $C_{18}H_{25}NO_6$ | CXVIII | 200–201 | −98[a] | *Senecio* |
| Asperumine | $C_{18}H_{25}NO_4$ | LXXX | | | *Senecio* |
| Anodendrine | $C_{13}H_{21}NO_2$ | XVIII | — | +9.5[a] | *Anodendron* |
| Axillaridine | $C_{18}H_{27}NO_6$ | CXXXII | 148–152 | +241[c] | *Crotalaria* |
| Axillarine | $C_{18}H_{27}NO_7$ | CXXXI | 205 | +65.1[d] | *Crotalaria* |
| Bisline | $C_{18}H_{27}NO_6$ | XCVII | 169 | — | *Senecio* |
| Brachyglottine | $C_{15}H_{23}NO_5$ | LXXXII | 98–99 | +88[a] | *Brachyglottis* |
| Cassipourine | $C_{14}H_{22}N_2S_4$ | XXV | 212 | −11.8[b] | *Cassipourea* |
| Chlorodeoxysceleratine | $C_{18}H_{26}NO_6Cl$ | XCIX | 196 | +32.4[a] | *Senecio* |
| Chysine A | $C_9H_{15}NO_2$ | XVII | — | +64[b] | *Chysis* |
| Clivorine | $C_{21}H_{27}NO_7$ | CXI | 147–149 | +79[b] | *Ligularia* |
| Cornucervine | $C_{17}H_{29}NO_5$ | XXXV | — | −4.3[a] | *Phalaenopsis* |
| Crispatine | $C_{16}H_{23}NO_5$ | CXXIV | 137–138 | +40.7[a] | *Crotalaria* |
| Crosemperine | $C_{19}H_{29}NO_6$ | CXXXVII | 117–118 | +45[b] | *Crotalaria* |
| Crotafoline | $C_{18}H_{25}NO_6$ | CX | 176–182 | — | *Crotalaria* |
| Cynaustine | $C_{15}H_{25}NO_4$ | LIV | — | +13.2[a] | *Cynoglossum* |
| Cynaustraline | $C_{15}H_{27}NO_4$ | XXXIX | — | +48[a] | *Cynoglossum* |
| Decorticasine | $C_{10}H_{16}N_2O_2$ | XXIV | — | +26.1[a] | *Adenocarpus* |
| Dicrotaline | $C_{14}H_{19}NO_5$ | CXXII | 170 | +25.7[f] | *Crotalaria* |

*(Table V — continued)*

| Alkaloid | Formula | Structure | M.p., °C | $[\alpha]_D$ | Plant genera |
|---|---|---|---|---|---|
| Dihydronorsecurinine | $C_{12}H_{15}NO_2$ | XXVIII | 135–136 | — | *Securinega* |
| Echimidine | $C_{20}H_{31}NO_7$ | LXXII | — | +13.4[a] | *Echium* |
| Echinatine | $C_{15}H_{25}NO_5$ | LXVII | — | +15[a] | *Cynoglossum, Eupatorium, Heliotropium, Rindera, Lindelofia, Solenanthus* |
| Echiumine | $C_{20}H_{31}NO_6$ | LXXI | 99–100 | +14.4[a] | *Echium, Amsinckia* |
| Emiline | $C_{19}H_{27}NO_6$ | CXXXVIII | 105–107 | −13.1[b] | *Emilia* |
| Erucifoline | $C_{18}H_{23}NO_6$ | XCV | 195–197 | −134[b] | *Senecio* |
| Europine | $C_{16}H_{27}NO_6$ | LXIX | — | +10.9[a] | *Heliotropium* |
| Floricaline | $C_{23}H_{33}NO_{10}$ | CVI | 177–178 | +74.3[b] | *Cacalia* |
| Floridanine | $C_{21}H_{31}NO_9$ | CV | 195–196 | +66.5[b] | *Cacalia* |
| Florosenine | $C_{21}H_{29}NO_8$ | CIII | 100–103 | +31.9[b] | *Cacalia* |
| Fulvine | $C_{16}H_{23}NO_5$ | CXXIII | 213–214 | −1.5[a] | *Crotalaria* |
| Grahamine | $C_{21}H_{31}NO_7$ | CXXVII | 163 | +100[a] | *Crotalaria* |
| Grantaline | $C_{18}H_{25}NO_6$ | CXXXIV ? | 219–220 | +101[a] | *Crotalaria* |
| Grantianine | $C_{18}H_{23}NO_7$ | CXXXIII ? | 209 | +64[a] | *Crotalaria* |
| Hastacine | $C_{18}H_{27}NO_5$ | CXVI | 171–172 | −79.5[b] | *Cacalia* |
| Heleurine | $C_{16}H_{27}NO_4$ | LII | 67–68 | −12[a] | *Heliotropium* |
| Heliosupine | $C_{20}H_{31}NO_7$ | LXXVII | — | −4.3[a] | *Cynoglossum, Echium, Heliotropium* |
| Heliotrine | $C_{16}H_{27}NO_5$ | LXVII | 128 | +17.6[a] | *Heliotropium* |
| Heliotropium base | $C_{24}H_{37}N_2O_6Cl$ | LXX | 158–159 | — | *Heliotropium* |
| 7α-Hydroxy-1-methoxymethyl-1,2-dehydro-8α-pyrrolizidine | $C_9H_{15}NO_2$ | VIII | 54 | +24.8[a] | *Crotalaria* |
| 7β-Hydroxy-1-methoxymethyl-1,2-dehydro-8α-pyrrolizidine | $C_9H_{15}NO_2$ | VI | 36–38 | +38[a] | *Crotalaria* |
| 7β-Hydroxy-1-methylene-8α-pyrrolizidine | $C_8H_{13}NO$ | XIII | 35–36 | −150[a] | *Crotalaria* |
| 7β-Hydroxy-1-methylene-8β-pyrrolizidine | $C_8H_{13}NO$ | XII | 34–36 | +36.1[a] | *Crotalaria* |
| 1α-Hydroxymethyl-1β,2β-epoxy-8β-pyrrolizidine | $C_8H_{13}NO_2$ | X | 65–66 | −49.5[a] | *Crotalaria* |
| Hydroxysenkirkine | $C_{19}H_{27}NO_7$ | CIX | 124–125 | +5.3[a] | *Crotalaria* |
| Hygrophylline | $C_{18}H_{27}NO_6$ | CXV | 173–174 | −67.3[a] | *Senecio* |
| Incanine | $C_{18}H_{27}NO_5$ | CXXX | 97 | −38.8[a] | *Trichodesma* |
| Indicine | $C_{15}H_{25}NO_5$ | LXIII | 97–98 | +22.3[a] | *Heliotropium* |
| Integerrimine | $C_{18}H_{25}NO_5$ | LXXXV | 172 | +4.3[b] | *Cacalia, Crotalaria, Senecio* |
| Intermedine | $C_{15}H_{25}NO_5$ | LX | — | +4.8[a] | *Amsinckia* |

*(Table V — continued)*

| Alkaloid | Formula | Structure | M.p., °C | $[\alpha]_D$ | Plant genera |
|---|---|---|---|---|---|
| Isoline | $C_{20}H_{29}NO_7$ | XCVI | 173 | $-4.8^a$ | *Senecio* |
| (+)-Isoretronecanol | $C_8H_{15}NO$ | III | 39–40 | $+76^a$ | *Thesium* |
| (−)-Isoretronecanyl-*trans*-3-methylthiopropenate | $C_{12}H_{19}NO_2S$ | XLIV | — | — | *Mimusops* |
| (−)-Isoretronecanyl tiglate | $C_{13}H_{21}NO_2$ | XLIII | 111–113 | $-57^a$ | *Planchonella* |
| Jacobine | $C_{18}H_{25}NO_6$ | XCI | 228 | $-46.3^b$ | *Senecio* |
| Jacoline | $C_{18}H_{27}NO_7$ | XCIV | 221 | $+48^b$ | *Senecio* |
| Jaconine | $C_{18}H_{26}NO_6Cl$ | XCIII | 147 | $+27^b$ | *Senecio* |
| Jacozine | $C_{18}H_{23}NO_6$ | XCII | 228 | $-140^b$ | *Senecio* |
| Junceine | $C_{18}H_{27}NO_7$ | CXXIX | 191–192 | $-3^d$ | *Crotalaria* |
| Kumokirine | $C_{32}H_{48}NO_8^+$ | L | — | — | *Liparis* |
| Laburnamine | $C_{12}H_{22}N_2O$ | XX | 125–127 | — | *Cytisus* |
| Laburnine | $C_8H_{15}NO$ | I | — | $+15.5^a$ | *Cytisus* |
| Laburnine acetate | $C_{10}H_{17}NO_2$ | acetate of I | — | $+13^a$ | *Vanda* |
| Laburnine benzoate | $C_{15}H_{19}NO_2$ | XLVII | — | — | *Planchonella* |
| Laburnine tiglate | $C_{13}H_{21}NO_2$ | XLVI | — | — | *Planchonella* |
| Lasiocarpine | $C_{21}H_{33}NO_7$ | LXXVIII | 96–97 | $-3.5^a$ | *Cynoglossum, Heliotropium* |
| Latifoline | $C_{20}H_{27}NO_7$ | LXXIV | 102–103 | $+57^a$ | *Cynoglossum* |
| Ligularine | $C_{23}H_{31}NO_9$ | CXII | — | $-34^b$ | *Ligularia* |
| Lindelofamine | $C_{20}H_{33}NO_5$ | XXXVIII | 88 | — | *Lindelofia* |
| Lindelofia base | $C_{17}H_{32}NO_5Cl$ | LVII | 149–151 | $+20.3^c$ | *Lindelofia* |
| Lindelofine | $C_{15}H_{27}NO_4$ | XXXVII | 106–107 | $+50^a$ | *Lindelofia* |
| Loline | $C_8H_{14}N_2O$ | XXII | — | $+4.6^a$ | *Lolium, Festuca* |
| Lolinine | $C_{10}H_{16}N_2O_2$ | XXIII | 73 | $+51^e$ | *Lolium* |
| Loroquine | $C_8H_9NO_2$ | XV | 77–78 | — | *Urechites* |
| Lycopsamine | $C_{15}H_{25}NO_5$ | LXI | — | $+3.1^a$ | *Amsinckia* |
| Macrophylline | $C_{13}H_{21}NO_3$ | LVI | 42–44 | $+34.5^a$ | *Senecio* |
| Macrotomine | $C_{15}H_{27}NO_5$ | XXXII | 95–97 | $-6.9^a$ | *Macrotonia* |
| Madurensine | $C_{18}H_{25}NO_6$ | CXX | 175–176 | $+55.7^a$ | *Crotalaria* |
| Malaxine | $C_{26}H_{37}NO_8$ | XLIX | 151–159 | $-31^a$ | *Malaxis* |
| 1-Methoxymethyl-1,2-dehydro-8α-pyrrolizidine | $C_9H_{15}NO$ | V | — | $-24^a$ | *Crotalaria* |
| 1α-Methoxymethyl-1β,2β-epoxy-8α-pyrrolizidine | $C_9H_{15}NO_2$ | VII | — | $-63^a$ | *Crotalaria* |
| 1-Methylene-8α-pyrrolizidine | $C_8H_{13}N$ | XI | — | $-100^a$ | *Crotalaria* |
| Monocrotaline | $C_{16}H_{23}NO_6$ | CXXV | 202–203 | $-55^a$ | *Crotalaria* |
| Nemorensine | $C_{18}H_{27}NO_5$ | CXXI | 132–134 | $-58^b$ | *Senecio* |

*(Table V — continued)*

| Alkaloid | Formula | Structure | M.p., °C | $[\alpha]_D$ | Plant genera |
|---|---|---|---|---|---|
| Neoplatyphylline | $C_{18}H_{27}NO_5$ | CXIV | 131–133 | $+1.9^b$ | *Senecio* |
| Nervosine | $C_{36}H_{53}NO_{12}$ | XLII | — | — | *Liparis* |
| Nilgirine | $C_{17}H_{23}NO_5$ | CI | 127–128 | $+31.5^a$ | *Crotalaria* |
| Norloline | $C_7H_{12}N_2O$ | XXI | — | $+15.1^c$ | *Lolium* |
| Norsecurinine | $C_{12}H_{13}NO_2$ | XXVII | 81–82 | $-19.5^a$ | *Securinega* |
| Onetine | $C_{19}H_{29}NO_8$ | CIV | 192–193 | $+73^b$ | *Senecio* |
| Othosenine | $C_{19}H_{27}NO_7$ | CII | 221–222 | $+20.8^b$ | *Senecio* |
| Peduncularine | $C_{20}H_{24}N_2$ | XXIX | 155–157 | $-24^c$ | *Aristotelia* |
| Phalaenopsine La | $C_{20}H_{27}NO_5$ | XLVIII | 125–135 | $+10^a$ | *Kingiella, Phalaenopsis* |
| Phalaenopsine T | $C_{20}H_{27}NO_5$ | XXXIV | 104–105 | $-15^a$ | *Phalaenopsis* |
| Planchonelline | $C_{12}H_{19}NO_2S$ | XLV | — | $+9^b$ | *Planchonella* |
| Platyphylline | $C_{18}H_{27}NO_5$ | CXIII | 129 | $-59^a$ | *Cynoglossum, Petasites, Senecio* |
| Retronecine | $C_8H_{13}NO_2$ | IV | 117–118 | $+50^a$ | *Crotalaria* |
| Retrorsine | $C_{18}H_{25}NO_6$ | LXXXIX | 219–220 | $-61.4^b$ | *Crotalaria, Senecio* |
| Retusamine | $C_{19}H_{25}NO_7$ | CXXXV | 174.5 | $+13^a$ | *Crotalaria* |
| Retusine | $C_{16}H_{25}NO_5$ | CXXXVI | 174–175 | $+16.2^a$ | *Crotalaria* |
| Riddelline | $C_{18}H_{23}NO_6$ | LXXXVII | 198 | $-108.9^b$ | *Crotalaria, Senecio* |
| Rinderine | $C_{15}H_{25}NO_5$ | LXVI | 98–100 | $+20.3^a$ | *Eupatorium, Rindera, Solenanthus* |
| Rosmarinine | $C_{18}H_{27}NO_6$ | CXVII | 209 | $-90.2^a$ | *Senecio* |
| Sarracine | $C_{18}H_{27}NO_5$ | LXXXI | 51–52 | $-129.7^b$ | *Senecio* |
| Sceleratine | $C_{18}H_{27}NO_7$ | XCVIII | 178 | $+54^a$ | *Senecio* |
| Senecionine | $C_{18}H_{25}NO_5$ | LXXXIV | 244–245 | $-55.8^b$ | *Crotalaria, Emilia, Erechtites, Petasites, Senecio, Tussilago* |
| Seneciphylline | $C_{18}H_{23}NO_5$ | LXXVI | 217 | $-139^b$ | *Crotalaria, Erechtites, Senecio* |
| Senkirkine | $C_{19}H_{27}NO_6$ | CVII | 196–197 | $-16^c$ | *Brachyglottis, Crotalaria, Nardosmia. Petasites, Senecio* |
| Sincamidine | $C_{16}H_{27}NO_5$ | LXII | — | — | *Amsinckia* |
| Spartioidine | $C_{18}H_{23}NO_5$ | LXXXVIII ? | 178 | $-83.7^a$ | *Senecio* |
| Spectabiline | $C_{18}H_{25}NO_7$ | CXXVI | 185–186 | $+143^a$ | *Crotalaria* |
| Strigosine | $C_{14}H_{25}NO_4$ | XXXIII | — | $-19.3^a$ | *Heliotropium* |
| Supinine | $C_{15}H_{25}NO_4$ | LI | 148–149 | $-12^a$ | *Eupatorium, Heliotropium, Trichodesma, Tournefortia* |
| Swazine | $C_{18}H_{23}NO_6$ | C | 165 | $-103.5^a$ | *Senecio* |
| Symphytine | $C_{20}H_{31}NO_6$ | LXXIII | — | $+3.6^a$ | *Symphytum* |
| Thelepogine | $C_{20}H_{31}NO$ | XXVI | 184–185 | — | *Thelepogon* |
| Thesine | $C_{34}H_{42}N_2O_6$ | XLI | 254–256 | — | *Thesium* |
| Thesinine | $C_{17}H_{21}NO_3$ | XL | 38–40 | — | *Thesium* |

*(Table V — continued)*

| Alkaloid | Formula | Structure | M.p., °C | $[\alpha]_D$ | Plant genera |
|---|---|---|---|---|---|
| Turneforcine | $C_{13}H_{21}NO_3$ | LV | — | $-58.6^f$ | *Tournefortia* |
| Trachelanthamidine | $C_8H_{15}NO$ | II | — | $-13.8^a$ | *Eupatorium, Heliotropium* |
| Trachelanthamine | $C_{15}H_{27}NO_4$ | XXXI | 91–92 | $-18.1^f$ | *Rindera, Trachelanthus* |
| Trichodesmine | $C_{18}H_{27}NO_6$ | CXXVIII | 160–162 | $+38.2^a$ | *Crotalaria, Heliotropium, Trichodesma* |
| Usaramine | $C_{18}H_{25}NO_6$ | XC | 182–183 | $+7.1^a$ | *Crotalaria* |
| Viridiflorine | $C_{15}H_{27}NO_4$ | XXX | 102–103 | $-11.7^a$ | *Cynoglossum, Lindelofia, Paracaryum* |

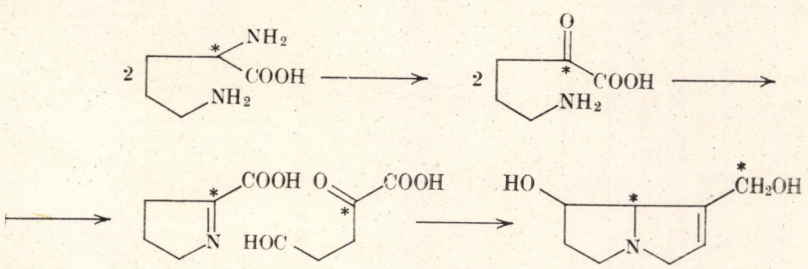
[a] Ethanol; [b] Chloroform; [c] Methanol; [d] Pyridine; [e] Acetone; [f] Water.

*Table VI*

Older Synonyma and Mixtures of Pyrrolizidine Alkaloids

| Older denomination | Present denomination |
|---|---|
| Aureine | Senecionine |
| Brasilinecine | Mixture of senecionine, seneciphylline and jacobine |
| Base S-C | Erucifoline |
| Base from *Senecio coronopifolius* | Senecionine |
| Crotalaburnine | Anacrotine |
| Damarensine | 1-Methylene-8α-pyrrolizidine |
| Douglassine | Mixture of senecionine, seneciphylline, retrorsine, and riddelline |
| Eremophylline | Mixture of senecionine, seneciphylline, retrorsine |
| Festucine | Loline |
| Hieracifoline | Mixture of senecionine and seneciphylline |
| Isatidine | Retrorsine N-oxide |
| Jacodine | Seneciphylline |
| α-Longilobine | Seneciphylline |
| β-Longilobine | Retrorsine |
| 1-α-Methoxycarbonyl-8β-pyrrolizidine | Chysine A |
| Mikanoidine | Mixture of sarracine and non-identified alkaloid |
| Mucronatinine | Usaramine |
| Pterophine | Mixture of senecionine and seneciphylline |
| Renardine | Senkirkine |
| Rivularine | $O^7$-Angelylheliotridine |
| Senecifolidine | Non-specified mixture |
| Senecifoline | Non-specified mixture |
| Squalidine | Integerrimine |
| Tomentosine | Othosenine |

to 71%. It follows that the biosynthesis of retronecine proceeds through two different intermediates:



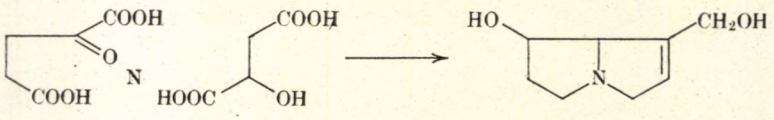

Degradation studies of retronecanol obtained by hydrogenolysis of the alkaloids from *Senecio sceleratus* after the incorporation of 2-[$^{14}$C]-acetate

*Table VII*

Pyrrolizidine Alkaloids with Unknown Structure

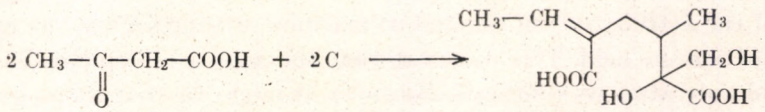
| Denomination | Formulae | Source |
|---|---|---|
| | $C_5H_9NO$ | *Senecio sarracenicus* |
| | $C_7H_{13}NO_2$ | *Senecio doria* |
| | $C_8H_{13}NO$ | *Senecio sarracenicus* |
| | $C_8H_{13}NO$ | *Crotalaria goreensis* |
| | $C_9H_{15}NO_2$ | *Senecio fuchsii* |
| | $C_{12}H_{17-19}NO_4$ | *Heliotropium europaeum* |
| | $C_{13}H_{21}NO_3$ | *Senecio sarracenicus* |
| | $C_{18}H_{27}NO_5$ | *Senecio palustris* |
| Alkaloid S-F | $C_{15}H_{21}NO_3$ | *Senecio viscosus* |
| Campestrine | $C_{13}H_{19}NO_3$ | *Senecio campestris* |
| Carategine | ? | *Lindelofia tschimganica, Rindera oblongifolia, Solenthus karateginus, S. circinatus* |
| Cruentine A | $C_{18}H_{25}NO_5$ | *Senecio cruentus* |
| Cruentine B | $C_{18}H_{25}NO_6$ | *Senecio cruentus* |
| Franchetine | ? | *Senecio francheti* |
| Fuchsisenecionine | $C_{12}H_{21}NO_3$ | *Senecio fuchsii* |
| Indicinine | ? | *Heliotropium indicum* |
| Ligudentine | ? | *Ligularia clivorum, L. dentata, L. elegans* |
| Lolinidine | ? | *Lolium cuneatum* |
| Mucronatine | ? | *Crotalaria mucronata* |
| Paucicaline | $C_{18}H_{27}NO_8$ | *Senecio paucicalyculatus* |
| Pictumine | $C_{14}H_{23}NO_4$ | *Cynoglossum pictum* |
| Ruwenine | $C_{18}H_{27}NO_6$ | *Senecio ruwenzoriensis* |
| Ruzorine | $C_{18}H_{27}NO_8$ | *Senecio ruwenzoriensis* |
| Silvasenecine | $C_{12}H_{21}NO_4$ | *Senecio sylvaticus* |
| Thelepogidine | $C_{18}H_{29}NO_2$ | *Thelepogon elegans* |
| Thesinicine | $C_{10}H_{11}NO_2$ | *Thesium minkwitzianum* |
| Turkestanine | ? | *Rindera baldschuanica, Solenanthus turkestanicus* |

show [155] that ring A is probably formed from ketoglutaric acid and ring B from malic acid:



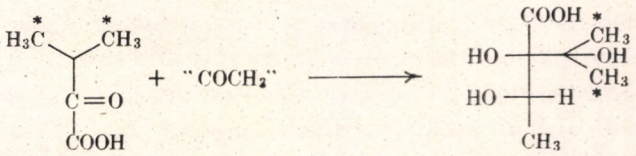

(b) Biosynthesis of necic acids. Hughes and Warren [157] incorporated sodium 1-[$^{14}$C]- and 2-[$^{14}$C]-acetate into *Senecio isatideus*. Thus they obtained labelled retrorsine, which was then hydrolyzed to the 4-[$^{14}$C]-retronecic acids A and B. These acids were degraded and the results showed

that retronecic acid was, in all probability, formed of two acetoacetate units and two other single-carbon sources:

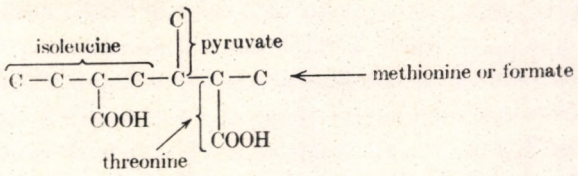


Crout *et al.* [158] fed labelled L-threonine and L-isoleucine to *Senecio douglasii* and they isolated labelled seneciphyllic acid. On the basis of the known metabolic pathway of threonine through isoleucine to acetyl CoA and the fact that angelic acid is common in pyrrolizidine alkoloids, isoleucine was examined as a precursor. Uniformly labelled isoleucine was incorporated at a high level and specifically into seneciphyllic acid; 1-[$^{14}$C]-isoleucine, however, gave low and non-specific incorporation. It was concluded that threonine and isoleucine provided only the left-hand half of the seneciphyllic acid (C-4, C-5, C-6, C-7, C-10). Threonine may supply the C-1, C-2 units, and C-9 comes from methionine formate.

Crout [159a] incorporated 4-[$^{14}$C]-D,L-valine to echimidinic acid, the main esterifying acid of heliosupine from *Cynoglossum officinale*. The activity (90%) of incorporated valine was found in the isopropyl group, indicating that valine reacted in the form of 3-methyl-2-ketobutyric acid:



The following scheme illustrates Crout's opinion regarding the origin of the main group of C-10 acids:




Incorporation of U-[$^{14}$C]-L-isoleucine and sodium 2-[$^{14}$C]-acetate into *Cynoglossum officinale* showed [159b] that 98% of the activity was present in the angeloyl residue of heliosupine. In their most recent paper, Crout *et al.* [160] reported that all the C-10 dicarboxylic acids of senecic acid type

are biosynthesized by the coupling of metabolites derived from isoleucine. (c) Biosynthesis of the alkaloids. Hughes *et al.* [161] incorporated labelled integerrinecic acid into *Senecio adnatus* and found that 89% of the activity of the isolated rosmarinine was present in the carboxyl group of senecic acid. This indicates that the acid is incorporated into the alkaloid only after its synthesis. However, it might be that the macrocyclic diesters are formed by intramolecular coupling of two C-5 units esterifically bound to the necine molecule [157]. This assumption is also supported by the occurrence of acyclic alkaloids with C-5 acids along with macrocyclic alkaloids, particularly by the occurrence of sarracine with two C-5 acids along with macrocyclic diesters.

## 3. PHYSICAL AND SPECTROSCOPIC PROPERTIES OF PYRROLIZIDINE ALKALOIDS

Since the physical and spectroscopic properties of the pyrrolizidine alkaloids have been discussed in detail in the monograph of Bull *et al.* [162] mention will be made here only of the papers published after the year 1967.

The ultraviolet spectroscopy of pyrrolizidine alkaloids has been studied by Šimánek *et al.* [163]. As predicted, the contribution of the acid and the base to the resulting absorption maximum of the corresponding alkaloid is additive. On the basis of the measurements of the data taken from older literature, these alkaloids have been subdivided into several groups according to the nature of their UV spectra. The first group comprises the macrocyclic alkaloids with an unsaturated necine and an unsaturated acid, which exhibit an absorption maximum at about 214 nm ($\log\varepsilon$ about 3.97 for the *cis* isomers and $\log\varepsilon$ about 4.02 for the *trans* isomers). The second group includes the macrocyclic alkaloids with an unsaturated base and a saturated epoxy-acid, whose absorption maximum is at about 218 nm ($\log\varepsilon$ about 3.30). The acyclic alkaloids with an unsaturated base and acid also absorb at about 218 nm ($\log\varepsilon$ about 4.07). Finally, the acyclic alkaloids with an unsaturated base and a saturated acid absorb at about 213 nm ($\log\varepsilon$ about 3.28), which are values similar to those of non-esterified unsaturated necines. Such a classification on the basis of the UV spectra is useful for a rapid orientative relegation of an unknown alkaloid to one of the group types.

Mattocks [164] reported a new method of detection of pyrrolizidine alkaloids after thin-layer chromatography. The detection is based on the conversion of unsaturated pyrrolizidine alkaloids by the action of hydrogen peroxide and acetic anhydride to dihydropyrrolizine derivatives (the so-

called 'pyrrols') which give a characteristic colour reaction with the Ehrlich reagent. The spectrophotometric determination of unsaturated pyrrolizidine alkaloids [165] is based on the same principle. This method is very useful for the determination of the content of pyrrolizidine alkaloids and metabolites in biological materials; it was, later on, also studied by Bingley [166].

Abdullaev *et al.* [167] studied the mass spectra of acyclic pyrrolizidine alkaloids of the heliotridane series. The mass and NMR spectra of pyrrolizidine alkaloids have also been dealt with in many other publications, the enumeration of which would surpass the scope of this paper. Practically all the alkaloids isolated after 1966 have been studied by physico-chemical methods and are discussed in the corresponding paper cited in this Chapter, Section 1. It is rather difficult to make a summarizing evaluation of all the NMR data, since the measurements have not been carried out under comparable conditions. Recently, the X-ray analysis of pyrrolizidine alkaloids has also been dealt with in several papers, particularly the determination of the absolute configuration of the alkaloids of the loline group [32], and the elucidation of the structure of the alkaloids cassipourine [35] and clivorine [120, 121]. Two groups of workers have been concerned with the circular dichroism of pyrrolizidine alkaloids. Culvenor *et al.* [168] measured 17 alkaloids and discussed their CD spectra in relation to the nature and the relative position of the chromophores of the free acids and bases and of alkaloids. Hrbek *et al.* [169] measured the CD spectra of 10 acyclic and 20 macrocyclic pyrrolizidine alkaloids along with the CD spectra of the free acids and bases. It has been found that the Cotton effects of the free acids and bases are not additive, which indicates that the conformations of both moieties in the free and the esterified state differ from each other. CD was also suggested as a very sensitive probe for the detection of the othonecine esters.

Frahn [170] measured the electrophoretic mobilities of 27 pyrrolizidine alkaloids in seven electrolytes. The results show that mixtures of pyrrolizidine alkaloids can be separated by electrophoresis at controlled pH, according to the difference in molecular weight and the strength of the bases; furthermore, the tendency of the alkaloids with vicinal glycol groups to form anionic complexes with sodium borate also makes their electrophoretic separation possible.

## REFERENCES


1. Galinovsky, F., Vogl, O., Nesvadba, H., *Sci. Pharm.*, *21*, 256 (1953).
2. Galinovsky, F., Vogl, O., Nesvadba, H., *Monatsh.*, *85*, 913 (1954).
3. Tsuda, Y., Marion, L., *Can. J. Chem.*, *41*, 1919 (1963).
4. Mattocks, A. R., *J. Chem. Soc.*, *1964*, 1974.
5. Arendaruk, A. P., Proskurnina, N. F., Konovalova, R. A., *Zh. obsch. khim.*, *30*, 670 (1960).
6. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem.*, *10*, 464 (1957).
7. Lindström, B., Lüning, B., *Acta Chem. Scand.*, *23*, 3352 (1969).
8. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem.*, *15*, 121 (1962).
9. Culvenor, C. C. J., Morrison, J. D., Nicholson, A. J. C., Smith, L. W., *Austral. J. Chem.*, *16*, 131 (1963).
10. Culvenor, C. C. J., O'Donovan, G. M., Smith, L. W., *Austral. J. Chem.*, *20*, 757 (1967).
11. Sawhney, R. S., Atal, C. K., *J. Indian Chem. Soc.*, *47*, 741 (1970).
12. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem.*, *12*, 255 (1959).
13. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem.*, *14*, 284 (1961).
14. Culvenor, C. C. J., Smith, L. W., Woods, W. G., *Tetrahedron Letters*, *1965*, 2025.
15. Culvenor, C. C. J., Sawhney, R. S., Smith, L. W., *Austral. J. Chem.*, *21*, 2135 (1968).
16. Borges del Castillo, J., España de Aguirre, A. G., *Tetrahedron Letters*, *1970*, 1219.
17. Culvenor, C. C. J., Edgar, J. A., Smith, L. W., Tweedale, H. J., *Tetrahedron Letters*, *1969*, 3599.
18. Meinwald, J., Meinrald, Y. C., 4th IUPAC Symp. Chem. Nat. Prod., Stockholm, 1966, Abstr. p. 127.
19. Lüning, B., Tränkner, H., *Tetrahedron Letters*, *1965*, 921.
20. Lüning, B., Tränkner, H., *Acta Chem. Scand.*, *22*, 2324 (1968).
21. Sasaki, K., Hirata, Y., *Tetrahedron Letters*, *1969*, 4065; *Tetrahedron*, *26*, 2119 (1970).
22. Neuer-Jehle, N., Nesvadba, H., Spiteller, G., *Monatsh.*, *96*, 321 (1965).
23. Yunusov, S. Yu., Akramov, S. T., *Zh. obsch. khim.*, *25*, 1813 (1955).
24. Yunusov, S. Yu., Akramov, S. T., *Zh. obsch. khim.*, *30*, 677 (1960).
25. Yunusov, S. Yu., Akramov, S. T., *Zh. obsch. khim.*, *30*, 683 (1960).
26. Yunusov, S. Yu., Akramov, S. T., *Zh. obsch. khim.*, *30*, 3132 (1960).
27. Akramov, S. T., Yunusov, S. Yu., *Khim. prirod. soed.*, *1965*, 262.
28. Yates, S. G., Tookey, H. L., *Austral. J. Chem.*, *18*, 53 (1965).
29. Aasen, A. J., Culvenor, C. C. J., *Austral. J. Chem.*, *22*, 2021 (1969).
30. Hofmeister, F., *Arch. Exptl. Pathol. Pharmakol.*, *30*, 203 (1892).
31. Ribas-Barceló, M., Ribas-Marqués, I., *Anales Real Soc. Españ. de Fís. y Quim., Ser. B*, *64*, 637 (1968).
32. Bates, R. B., Morehead, S. R., *Tetrahedron Letters*, *1972*, 1629.
33. Wright, W. G., Warren, F. L., *J. Chem. Soc. (C)*, *1967*, 283.
34. Cooks, R. G., Warren, F. L., Williams, D. H., *J. Chem. Soc. (C)*, *1967*, 286.
35. Gafner, G., Admiraal, L. J., *Acta Cryst.*, *25B*, 2114 (1969).
36. Crow, W. D., *Austral. J. Chem.*, *15*, 159 (1962).
37. Fridrichsons, J., Mathieson, A. McL., *Tetrahedron Letters*, *1960* (No. 26) 18; *Acta Cryst.*, *16*, 206 (1963).
38. Saito, S., Tanaka, T., Kotera, K., Nakai, H., Sugimoto, N., Horii, Z., Ikeda, M., Tamura, Y., *Chem. Pharm. Bull.*, *13*, 786 (1965).
39. Bick, I. R. C., Bremmer, J. B., Preston, N. W., *Chem. Commun.*, *1971*, 1155.
40. Menshikov, G. P., *Zh. obsch. khim.*, *18*, 1736 (1948).
41. Menshikov, G. P., Borodina, G. M., *Zh. obsch. khim.*, *15*, 225 (1945).
42. Menshikov, G. P., Petrova, M. F., *Zh. obsch. khim.*, *22*, 1457 (1952).
43. Brandäge, S., Lüning, B., *Acta Chem. Scand.*, *23*, 1151 (1969).
44. Brandäge, S., Lüning, B., Moberg, Ch., Sjöstrand, E., *Acta Chem. Scand.*, *25*, 349 (1971).

45. Pais, M., Jarreau, F. X., Fouché, P., Goutarel, R., *Ann. pharm. franc.*, *29*, 57 (1971).
46. Labenskii, A. S., Menshikov, G. P., *Zh. obsch. khim.*, *18*, 1836 (1948).
47. Tsyrul'nikova, L. G., Labenskii, A. S., Utkin, L. M., *Zh. obsch. khim.*, *32*, 2705 (1962).
48. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem.*, *20*, 2499 (1967).
49. Arendaruk, A. P., Skoldinov, A. P., *Zh. obsch. khim.*, *30*, 484 (1960).
50. Arandaruk, A. P., Skoldinov, A. P., *Zh. obsch. khim.*, *30*, 489 (1960).
51. Nishikawa, K., Hirata, Y., *Tetrahedron Letters*, *1967*, 2591.
52. Nishikawa, K., Miyamura, M., Hirata, Y., *Tetrahedron Letters*, *1967*, 2597.
53. Hart, N. K., Johns, S. R., Lamberton, J. A., *Austral. J. Chem.*, *21*, 1393 (1968).
54. Hart, N. K., Lamberton, J. A., *Austral. J. Chem.*, *19*, 1259 (1966).
55. Brandäge, S., Gravelli, I., Lüning, B., *Acta Chem. Scand.*, *24*, 354 (1970).
56. Leander, K., Lüning, B., *Tetrahedron Letters*, *1967*, 3477.
57. Menshikov, G. P., Gurevich, E. I., *Zh. obsch. khim.*, *19*, 1382 (1949).
58. Crowley, H. C., Culvenor, C. C. J., *Austral. J. Chem.*, *12*, 694 (1959).
59. Culvenor, C. C. J., *Austral. J. Chem.*, *7*, 287 (1954).
60. Crowley, H. C., Culvenor, C. C. J., *Austral. J. Chem.*, *8*, 464 (1955).
61. O'Kelly, J., Sargeant, K., *J. Chem. Soc.*, *1961*, 484.
62. Locock, R. A., Beal, J. L., Doskotch, R. W., *Lloydia*, *29*, 201 (1966).
63. Menshikov, G. P., Denisova, S. O., Massagetov, P. S., *Zh. obsch. khim.*, *22*, 1465 (1952).
64. Danilova, A. V., Utkin, L. M., Massagetov, P. S., *Zh. obsch. khim.*, *25*, 831 (1955).
65. Danilova, A. V., Utkin, L. M., *Zh. obsch. khim.*, *30*, 345 (1960).
66. Crowley, H. C., Culvenor, C. C. J., *Austral. J. Chem.*, *15*, 139 (1962).
67. Culvenor, C. C. J., Johns, S. R., Lamberton, J. A., Smith, L. W., *Austral. J. Chem.*, *23*, 1279 (1970).
68. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem.*, *19*, 1955 (1966).
69. Mattocks, A. R., Schoental, R., Crowley, H. C., Culvenor, C. C. J., *J. Chem. Soc.*, *1961*, 5400.
70. Mattocks, A. R., *J. Chem. Soc.*, *1967*, 329.
71. Klásek, A., Vrublovský, P., Šantavý, F., *Coll. Czechoslov. Chem. Commun.*, *32*, 2512 (1967).
72. Pedersen, E., *Dansk Tidsskr. Farm.*, *44*, 287 (1970); *Chem. Abstr.*, *74*, 72 780 (1971).
73. Akramov, S. T., Kiyamitdinova, F., Yunusov, S. Yu., *Uzbeksk. khim. Zh.*, *1961*, 36; *Chem. Abstr.*, *57*, 16 676 (1962).
74. Akramov, S. T., Kiyamitdinova, F., Yunusov, S. Yu., *Dokl. Akad. Nauk Uz SSR*, *19*, 29 (1962); *Chem Abstr.*, *61*, 11 005 (1964).
75. Menshikov, G. P., Denisova, S. O., *Sb. Statei Obsch. Khim. Akad. Nauk SSSR*, *2*, 1458 (1953).
76. Jerzmanowska, Z., Sykulska, Z., *Dissertationes Pharm.*, *16*, 71 (1964); *Chem. Abstr.*, *61*, 16 438 (1964).
77. Akramov, S. T., Kiyamitdinova, R., Yunusov, S. Yu., *Dokl. Akad. Nauk UzSSR*, *22*, 35 (1965); *Chem. Abstr.*, *63*, 16 770 (1965).
78. Akramov, S. T., Samatov, A. S., Yunusov, S. Yu., *Dokl. Akad. Nauk UzSSR*, *21*, 28 (1964).
79. Zolotavina, Z. M., *Tr. Inst. Botan. Akad. Nauk KazSSR*, *17*, 170 (1963); *Chem. Abstr.*, *61*, 11 013 (1964).
80. Akramov, S. T., Kiyamitdinova, F., Yunusov, S. Yu., *Dokl. Akad. Nauk UzSSR*, *18*, 30 (1961); *Chem. Abstr.*, *60*, 16 209 (1964).
81. Gandhi, R. N., Rajagopalan, T. R., Seshadri, T. R., *Current Sci. (India)*, *35*, 121 (1966).
82. Culvenor, C. C. J., Drummond, L. J., Price, J. R., *Austral. J. Chem.*, *7*, 277 (1954).
83. Crowley, H. C., Culvenor, C. C. J., *Austral. J. Appl. Sci.*, *7*, 359 (1956).
84. Menshikov, G. P., *Ber.*, *65*, 974 (1932).
85. Menshikov, G. P., *Ber.*, *66*, 875 (1933).
86. Culvenor, C. C. J., Smith, L. W., *Tetrahedron Letters*, *1969*, 3603.

87. Culvenor, C. C. J., *Austral. J. Chem.*, *9*, 512 (1956).
88. Furuya, T., Araki, K., *Chem. Pharm. Bull. (Japan)*, *16*, 2512 (1968).
89. Man'ko, I. V., Korotkova, M. P., Shevtsova, N. M., *Rast. Resur.*, *5*, 508 (1969); *Chem. Abstr.*, *72*, 87 175 (1970).
90. Man'ko, I. V., Kotovskii, B. K., Denisov, Y. G., *Rast. Resur.*, *6*, 582 (1970); *Chem. Abstr.*, *74*, 84 023 (1971).
91. Danilova, A. R., Konovalova, R. A., Massagetov, P., Garina, M., *Dokl. Akad. Nauk. SSSR*, *89*, 865 (1953).
92. Culvenor, C. C. J., Geissman, T. A., *J. Org. Chem.*, *26*, 3045 (1961).
93. Mortimer, P. H., White, E. P., *Nature*, *214*, 1255 (1967).
94. Ulubelen, A., Doganca, S., *Tetrahedron Letters*, *1970*, 2583.
95. Adams, R., Gianturco, M., *J. Am. Chem. Soc.*, *79*, 174 (1957).
96. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem.*, *19*, 2127 (1966).
97. Mattocks, A. R., *J. Chem. Soc.*, *1968*, 225.
98. Bhacca, N. S., Sharma, R. K., *Tetrahedron*, *24*, 6319 (1968).
99. Sawhney, R. S., Atal, C. K., *J. Indian Chem. Soc.*, *47*, 667 (1970).
100. Geissman, T. A., *Austral. J. Chem.*, *12*, 247 (1959).
101. Bradbury, R. B., Masamune, S., *J. Am. Chem. Soc.*, *81*, 5201 (1959).
102. Fridrichsons, J., Mathieson, A. McL., Sutor, D. J., *Acta Cryst.*, *16*, 1075 (1963).
103. Culvenor, C. C. J., *Austral. J. Chem.*, *17*, 233 (1964).
104. Bradbury, R. B., *Chem. & Ind.*, *1954*, 1022.
105. Kompiš, I., Šantavý, F., *Coll. Czechoslov. Chem. Commun.*, *27*, 1413 (1962).
106. Sedmera, P., Klásek, A., Duffield, A. M., Šantavý, F., *Coll. Czechoslov. Chem. Commun.*, *37*, 4112 (1972).
107. Coucourakis, E. D., Gordon-Gray, C. G., *J. Chem. Soc. (C)*, *1970*, 2312.
108. Coucourakis, E. D., Gordon-Gray, C. G., Whiteley, C. G., *J. Chem. Soc.*, *1972*, 2339.
109. Gordon-Gray, C. G., *J. Chem. Soc. (C)*, *1967*, 781.
110. Gordon-Gray, C. G., Wells, R. B., Hallak, N., Hursthouse, M. B., Neidle, S., Toube, T. P., *Tetrahedron Letters*, *1972*, 707.
111. Laing, M., Sommerville, P., *Tetrahedron Letters*, *1972*, 5183.
112. Atal, C. K., Sawhney, R. S., Culvenor, C. C. J., Smith, L. W., *Tetrahedron Letters*, *1968*, 5605.
113. Wunderlich, J. A., *Chem. & Ind. (London)*, *1962*, 2089; *Acta Cryst.*, *23*, 846 (1967).
114. Briggs, L. H., Mangan, J. L., Russell, W. E., *J. Chem. Soc.*, *1948*, 1891.
115. Briggs, L. H., Cambie, R. C., Candy, B. J., O'Donovan, G. M., Russell, R. H., Seelye, R. N., *J. Chem. Soc.*, *1965*, 2492.
116. Crout, D. H. G., *J. Chem. Soc. (Perkin I)*, *1972*, 1602.
117. Cava, M. P., Rao, K. V., Weisbach, J. A., Raffauf, R. F., Douglas, B., *J. Org. Chem.*, *33*, 3570 (1968).
118. Klásek, A., Vrublovský, P., Šantavý, F., *Coll. Czechoslov. Chem. Commun.*, *32*, 2512 (1967).
119. Klásek, A., Sedmera, P., Šantavý, F., *Coll. Czechoslov. Chem. Commun.*, *35*, 956 (1970).
120. Birnbaum, K. B., Klásek, A., Sedmera, P., Snatzke, G., Johnson, L. F., Šantavý, F., *Tetrahedron Letters*, *1971*, 3421.
121. Birnbaum, K. B., *Acta Cryst.*, *B28*, 2825 (1972).
122. Klásek, A., Sedmera, P., Šantavý, F., *Coll. Czechoslov. Chem. Commun.*, *36*, 2205 (1971).
123. Danilova, A. V., Utkin, L. M., Kozyreva, C. V., Syrneva, Yu. I., *Zh. obsch. khim.*, *29*, 2432 (1959).
124. Culvenor, C. C. J., Koretskaya, N. L., Smith, L. W., Utkin, L. M., *Austral. J. Chem.*, *21*, 1671 (1968).
125. Schlosser, F. D., Warren, F. L., *J. Chem. Soc.*, *1965*, 5707.
126. Porter, L. A., Geissman, T. A., *J. Org. Chem.*, *27*, 4132 (1962).
127. Koekemoer, M. J., Warren, F. L., *J. Chem. Soc.*, *1955*, 63.
128. Atal, C. K., Kapur, K. K., Culvenor, C. C. J., Smith, L. W., *Tetrahedron Letters*, *1966*, 537.
129. Sawhney, R. S., Atal, C. K., *J. Indian Chem. Soc.*, *48*, 887 (1971).

130. Mattocks, A. R., *J. Chem. Soc. (C), 1968*, 235.
131. Culvenor, C. C. J., Smith, L. W., Willing, R. I., *Chem. Commun., 1970*, 65.
132. Klásek, A., Sedmera, P., Boeva, A., Šantavý, F., *Coll. Czechoslov. Chem. Commun., 38*, 2504 (1973).
133. Marais, J. S. C., *Onderstepoort M. Vet. Sci. Animal Ind., 20*, 61 (1944).
134. Adams, R., van Duuren, B. L., *J. Am. Chem. Soc., 75*, 2377 (1953).
135. Schoental, R., *Austral. J. Chem., 16*, 233 (1963).
136. Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem., 16*, 239 (1963).
137. Adams, R., Shafer, P. R., Braun, B. H., *J. Am. Chem. Soc., 74*, 5612 (1952).
138. Atal, C. K., Sharma, R. K., Culvenor, C. C. J., Smith, L. W., *Austral. J. Chem. 19*, 2189 (1966).
139. Colvenor, C. C. J., Smith, L. W., *Austral. J. Chem., 10*, 474 (1957).
140. Atal, C. K., Culvenor, C. C. J., Sawhney, R. S., Smith, L. W., *Austral. J. Chem., 22*, 1773 (1969).
141. Yunusov, S. Yu., Plekhanova, N. N., *Zh. obsch. khim., 29*, 677 (1959).
142. Robins, D. J., Crout, D. H. G., *J. Chem. Soc. (C), 1969*, 1386.
143. Adams, R., Gianturco, M., *J. Am. Chem. Soc., 78*, 1926 (1956).
144. Crout, D. H. G., *Chem. Commun., 1968*, 429.
145. Crout, D. H. G., *J. Chem. Soc., 1969*, 1379.
146. Adams, R., Gianturco, M., *J. Am. Chem. Soc., 78*, 4458 (1956).
147. Bull, L. B., Culvenor, C. C. J., Dick, A. T., "The Pyrrolizidine Alkaloids", North-Holland Publ. Co., Amsterdam, 1968, p. 85.
148. Atal, C. K., Culvenor, C. C. J., Sawhney, R. S., Smith, L. W., *Austral. J. Chem., 20*, 805 (1967).
149. Tomczyk, H., Kohlmünzer, S., *Herba Polon., 17*, 226 (1971).
150. Kohlmünzer, S., Tomczyk, H., Saint-Firmin, A., *Dissert. Pharm. Pharmacol., 23*, 419 (1971).
151. Culvenor, C. C. J., Dann, A. T., Smith, L. W., *Chem. & Ind. (London), 1959*, 20.
152. Kochetkov, N. K., Likhosherstov, A. M., Kulakov, V. N., *Tetrahedron, 25*, 2313 (1969).
153. Robinson, R., "The Structural Relations of Natural Products", Oxford Univ. Press, London, 1955, p. 72.
154. Nowacki, E., Byerrum, R. U., *Life Sci., 5*, 157 (1962).
155. Hughes, C. A., Letcher, R., Warren, F. L., *J. Chem. Soc., 1964*, 4974.
156. Bottomley, W., Geissman, T. A., *Phytochemistry, 3*, 357 (1964).
157. Hughes, C. A., Warren, F. L., *J. Chem. Soc., 1962*, 34.
158. Crout, D. H. G., Benn, M. H., Imaseki, H., Geissman, T. A., *Phytochemistry, 5*, 1 (1966).
159a. Crout, D. H. G., *J. Chem. Soc. (C), 1966*, 1968.
159b. Crout, D. H. G., *J. Chem. Soc. (C), 1967*, 1233.
160. Crout, D. H. G., Davies, N. M., Smith, E. H., Whitehouse, D., *Chem. Commun., 1970*, 635; *J. Chem. Soc. (Perkin I), 1972*, 671.
161. Hughes, C. A., Gordon-Gray, C. G., Schlosser, F. D., Warren, F. L., *J. Chem. Soc., 1965*, 2370.
162. Bull, L. B., Culvenor, C. C. J., Dick, A. T., "The Pyrrolizidine Alkaloids", North-Holland Publ. Co., Amsterdam, 1968, pp. 33–58.
163. Šimánek, V., Klásek, A., Šantavý, F., *Coll. Czechoslov. Chem. Commun., 34*, 1832 (1969).
164. Mattocks, A. R., *J. Chromatogr., 27*, 505 (1967).
165. Mattocks, A. R., *Anal. Chem., 39*, 443 (1967).
166. Bingley, J. B., *Anal. Chem.*, 40, 1166 (1968).
167. Abdullaev, U. A., Rashkes, J. V., Shakhidoatov, C., Yunusov, S. Yu., *Khim. Prirod. Soed., 1972*, 634.
168. Culvenor, C. C. J., Crout, D. H. G., Klyne, W., Mose, W. P., Renwick, J. D., Scopes, P. M., *J. Chem. Soc. (C), 1971*, 36.
169. Hrbek, J., Jr., Hruban, L., Klásek, A., Kochetkov, N. K., Likhosherstov, A. M., Šantavý, F., Snatzke, G., *Coll. Czechoslov. Chem. Commun., 37*, 3918 (1972).
170. Frahn, J. L., *Austral. J. Chem., 22*, 1655 (1969).

## V. BIOLOGICAL PROPERTIES OF PYRROLIZIDINE ALKALOIDS

The pharmacological effect of pyrrolizidine alkaloids has been known since ancient times. Much information on the pharmacological properties of *Senecio* plants is contained for instance in the work by Dioscorides (69 B. C.). From that we get to know that *Senecio* leaves and flowers have a cooling effect, and when applied to inflammations, inflamed wounds and ulcers, they act curatively. Drinking of the fresh extract from flowers produces a stifling effect, whereas the boiled extract from the whole plant has a beneficial action upon gastric pains. Later on, many new findings were made in connection with the discovery and investigation of new botanical species. Plinius (23 to 79 A. D.) already drew attention to the beneficial effect of an extract of *Senecio* as a means against worms, epileptic seizures, jaundice, liver- and heart diseases. The findings made in ancient times and in the middle ages have been summarized to a large extent by A. Müller [1]. It was, however, only by the end of the nineteenth century that the alkaloids contained in the plants were recognized as the biologically active principles. This finding was a landmark in the investigation of the pharmacological properties of this plant. The isolation of toxic pyrrolizidine alkaloids from *Senecio* plants has led to the clarification of the etiology of a disease of domestic animals which was caused by fodder from meadows infested with such plants, thus leading to enormous loss of cattle and horses and consequently to considerable economical waste. The disease has been known under different names, e.g., as the Pictou disease in Canada [2], the Winton disease in New Zealand [2], the Molteno disease in South Africa [3], and the Žďar disease of horses in the region of Southern Bohemia [4–6]. In this century, not only veterinarians, but also physicians were concerned with the effects of *Senecio*. Acute intoxication in man (with clinical manifestations of an acute intoxication of the liver) due to the consumption of bread made of flour contaminated with *Senecio* is an almost endemic disease in Western India, but frequently it is also found to occur in South Africa. In those regions, but sometimes also in other areas, a chronic intoxication

(with clinical manifestation of liver cirrhosis) is observed which is caused by bread contaminated with *Senecio* and also by drinking a "curative tea", the so-called bush tea (used in cases of gastrointestinal disorders and in children against cold). For details concerning the biological effects of pyrrolizidine alkaloids see the monograph by Bull, Culvenor and Dick [7] and the survey by McLean [8].

At present, the biological effects of over 100 pure preparations of pyrrolizidine alkaloids are being studied. Much attention is paid to the toxic effect of these alkaloids on the individual organs in the animal organism, to the mutagenic effect, and to their ability to affect chromosomes. Another sphere of intense studies is the carcinogenic effect of some pyrrolizidine alkaloids and, on the other hand, the antitumorous activity of some of them.

## 1. TOXIC EFFECTS OF PYRROLIZIDINE ALKALOIDS

### *A. Acute Hepatic Lesions*

The hepatotoxic effect of pyrrolizidine alkaloids was experimentally determined for the first time by Cushny in 1911 [9]. In the following years many papers [5, 10–32] were published on this subject. The experiments were mostly carried out by using the alkaloids lasiocarpine [14, 16, 24–26, 28, 31, 93], heliotrine [11, 14, 15, 18, 29], retrorsine [19, 23, 27, 29, 32], senecionine [20, 33, 34], and monocrotaline [21, 29]. In some isolated cases, retronecine [21], integerrimine [20], jacobine [20], longilobine [20], spartioidine [20], riddelline [29], and fulvine [94] were also examined. While studying the hepatotoxic effect of pyrrolizidine alkaloids, much attention was paid particularly to the observation of the histological changes which took place after the administration of these alkaloids. Thus at present, we already possess a very accurate picture of the histological lesions encountered in the liver and, recently, numerous findings made by using the electron microscope [31] have been added.

The interpretation of the results obtained from the histological examination of the livers of animals after intoxication with pyrrolizidine alkaloids is still controversial. The main question is whether the pyrrolizidine alkaloids are primarily toxic for the liver cell or for the vascular system of the liver. Davidson [19] is of the opinion that primarily the vascular system of the liver is disturbed, whereas the other lesions in the liver can be considered secondary. Bras and Hill [12], Berry and Bras [10] and Hill and Martin [22] concluded on the basis of the observations made in man after intoxication with pyrrolizidine alkaloids that they primarily affect the vascular component of the liver which is reminiscent of a veno-occlusive disease. Many

authors [13–17, 20, 21, 24, 28–30] emphasize, however, that the liver cells are involved in the first place from which follows the assumed primary effect upon its metabolism.

We have analysed in great detail the morphological picture of the liver during acute intoxication with senecionine in correlation with some biochemical changes [33–35]. This alkaloid has a toxic effect particularly on the intermediary and, later on, on the central portion of the lobe, whereas the periportal region remains practically unchanged. The degree of the injury differs in dependence on sex, and proceeds from dystrophy of the liver cells up to monocellular, focal, and zonal necroses. After the administration of pyrrolizidine alkaloids, the liver parenchyma of female rats is found to be markedly congested and the formation of extensive blood pools is observed in the necrotic area. This finding is consistent with the conclusions made by Bull *et al.* [16] who described the histological lesions in the liver after administration of heliotrine and lasiocarpine; they did not, however, study the sex-dependence.

### *B. Chronic Hepatic Lesions*

Chronic lesions in the liver after administration of pyrrolizidine alkaloids were observed for the first time by Cushny [9] and Davidson [19]. Bull [13] carried out the first systematic experimental study on sheep. He called the produced disease "pyrrolizidine alkaloidosis". McLean [8] prefers the designation "pyrrolizidine intoxication". The principal and constant feature of the thus arisen hepatic lesion is megalocytosis of the liver cells in connection with disorders of the liver function [13]. Along with the enormously enlarged hepatocytes, other histological changes were also observed, such as proliferation of the bile-ducts [36], fatty degeneration and fibrosis [7], cirrhosis [37], and vascular changes [29]. Megalocytes in the liver were found to be present after administration of a single dose of some alkaloids (lasiocarpine and heliotrine) to young rats [28, 29, 36], after repeated doses to rats [38] and mice [39], or after repeated feeding of sheep [13, 40], cattle [41–44], horses [22, 45, 46] and pigs [47] with *Senecio* plants. Similar cells were also found in cultures of human foetal livers after previous expositon of the tissue culture to pyrrolizidine alkaloids [48]. Chronic damage to the liver due to pyrrolizidine alkaloids offers two interesting problems, i.e. that the disease arises only with a certain period of latency after application of a small single dose to young rats, or after repeated small doses to adult rats, without apparent signs of acute intoxication [24, 36]. Furthermore, the produced disease is irreversible and is characterized by permanent

progression. Thus, this type of injury of the liver parenchyma differs from that produced by other hepatotoxic substances, the administration of which is followed by the appearance of megalocytes in the liver. Such substances are for instance thioacetamide [49], bentonite [50], dimethylnitrosoamine [51], and aflatoxine [52]. Schoental [53] studied the biochemical function of the thus damaged liver and found an increase in the bilirubin level and a decrease in the level of total proteins after administration of isatidine. In calves fed with *Senecio jacobaea*, Ford *et al.* [54] observed a decrease in the albumin–globulin ratio of serum proteins, an increase in the activity of glutamic oxalic transaminase (GOT), the glutamate dehydrogenase and the ornithine carbamyl transferase, and an unaltered activity of glutamic pyruvic transaminase (GPT). A high blood ammonia level was found in horses [46] and in rats [7]. We have studied the changes in the level of liver indicator enzymes (GOT, GPT and lactic dehydrogenase (LDH)), the reduced glutathione in the liver and erythrocytes, and the level of total proteins in the serum inclusive of their sex-linked electrophoretic representation in rats after administration of senecionine [33].

### *C. Theories Concerning the Development of Megalocytes*

The mechanism of the development of megalocytes is still obscure. According to Bull and Dick [14], the megalocytes might be polyploid cells which are usually seen in advanced age, consequently cells which have grown old before their time. They assume that the development of such cells is due to a disorder in the cellular division of hepatocytes without any effect upon the DNA synthesis in the nucleus, so that the progressing cellular growth might lead to megalocytosis. In their opinion, the sensitivity to the effect of alkaloids is dependent on the capacity of cell division and on the actual phase of cell growth during the period of application of the alkaloid. This would mean that at the time of intense growth the sensitivity to the effect of the alkaloid would be much higher than in older individuals. A more recent report by Peterson [25] that lasiocarpine N-oxide inhibits mitoses without acting upon the synthesis of DNA stands in support of the hypothesis postulated by Bull and Dick. The observation that megalocytes contain an increased amount of succinyl dehydrogenase, $\beta$-glucuronidase and unspecific esterases in the histochemical picture led Scheuer [55] to the hypothesis that the megalocytes are atypical, in all probability, preneoplastic forms of liver cells. This assumption was again supported by Schoental and Bensted [56] who observed the development of a hepatoma a year or two after the application of a single dose of retrorsine. These theories do

not exclude each other, their confirmation will, however, require further studies.

*D. Toxic Injury to Other Organs*

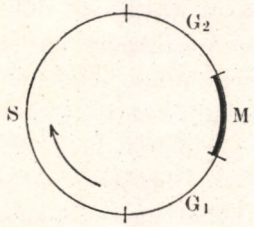
The lungs are another organ in which lesions due to intoxication have been studied. The involvement of the lungs after administration of pyrrolizidine alkaloids was described in rats [19, 53, 57–60], mice [20], frogs [9], turkeys [37, 61], pigs [47], rabbits [7], and horses [62]. Post-mortem examination usually showed pulmonal oedema and congestion and, in the pleural cavity, the presence of a fluid. Microscopy revealed epithelial hyperplasia in the alveoles [63], an intimal hyperplasia of the lung vessels [63], and capillary thrombosis [64]. Turner and Lalich [63] suggest that mast cell hyperplasia is associated with the release of 5-hydroxytryptamine which is causing pulmonary hypertension thus giving rise to cor pulmonale. For details of the role played by the mast cells and 5-hydroxytryptamine see Kay *et al.* [65, 66]. Contrary to Turner and Lalich, they report that the mast cells are present in larger quantities only preterminally, and their occurrence is put into relationship with the exudative pulmonary lesions. They are of the opinion that the invasion of these cells is connected with failure of the right heart ventricle, rather than with the genesis of primary pulmonary hypertension. Measuring of the quantity of free-plasma and platelet bound 5-hydroxytryptamine in rats after a 5-week course of feeding showed no rise as compared with the controls. They assume that the alveolar lesions and pulmonary hypertension are due to an increased permeability of the capillaries or constriction of the pulmonary veins.

Other organs have also been studied in this connection. After administration of pyrrolizidine alkaloids, lesions were found to be present in the brain [19, 39, 67–69], kidneys [7, 39, 58, 68, 70–73], muscle [37, 61], myocardium [74, 75], coronary arteries [76], and lymphatic tissues [29, 57, 77].

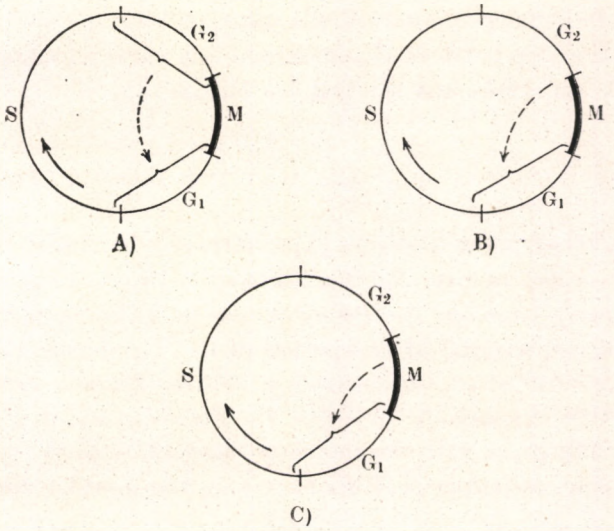
## 2. ANTIMITOTIC EFFECT OF PYRROLIZIDINE ALKALOIDS

In connection with the interpretation of the causative agent of megalocytosis in the liver, it has been mentioned that according to Bull and Dick [14] pyrrolizidine alkaloids have an inhibitory effect upon mitoses. Schoental [78] also assumes the presence of a specific inhibitory effect. Peterson [25] and Downing and Peterson [79] demonstrated a decrease (up to 50%) in the mitotic activity of the liver cells in partially hepatectomized rats after the administration of heliotrine, lasiocarpine or lasiocarpine N-oxide. Similar conclusions were also drawn by Rogers [80] who studied the antimitotic effect of lasiocarpine. We have found [35] that administration of 0.30 $LD_{50}$

of senecionine is followed by a decrease in the mitotic activity in regenerating rat liver. This decrease is independent of the age of the rats when senecionine is applied from the third to the seventh week of age. Furthermore, a decrease in the mitotic activity in the regenerating liver was also observed five weeks after the application of senecionine (decrease in the mitotic index in 80%) of the animals, 8 weeks after application (in 50%), and after 16 to 18 weeks (in 75%), showing a long lasting effect upon the liver tissue. The direct effect of pyrrolizidine alkaloids on the cellular cycle was dealt with by McLean [8]. Similarly to Howard and Pelc [81], McLean is of the opinion that the phase of cellular division and the preparatory phase of the DNA synthesis fall in under the concept of a 'cell cycle'.




It consists of four phases. The phase of the mitosis ($M$) is followed by the interval $G_1$ which precedes the phase of the DNA synthesis ($S$). It is separated by another interval $G_2$ from the proper onset of mitosis. McLean is of the opinion that the effect of the pyrrolizidine alkaloids manifests itself by the formation of a "by-pass" in this cycle which can proceed along three pathways:



A) passage from an unknown site in $G_2$ to an unknown point in $G_1$; the phase $S$ would remain without alteration, and the phase $M$ would completely be left out, B) transfer from the early phase of mitosis (prophase) to an unknown site in $G_1$, C) passage from the metaphase of the mitosis to $G_1$, which might explain the presence of abnormal mitoses.

## 3. MUTAGENIC ACTION AND CHROMOSOME DAMAGE

As early as 1959 Clark [82] drew attention to the mutagenic effect of heliotrine. He made a detailed study of the mutagenic effect of pyrrolizidine alkaloids [83, 84]. Nine pyrrolizidine alkaloids were tested in adult male *Drosophila melanogaster* and it was found that, in a concentration of 0.02 M, monocrotaline, lasiocarpine and heliotridine were strongly mutagenic. Less effective were echinatine, echimidine, senecionine and supinine; jacobine and platyphylline had only negligible activity. Bick and Jackson [85] observed in short-term cultures of peripheral leukocytes of the macupial, *Potorous tridactylus*, a linear relationship between the number of chromosomal breaks and the concentration of heliotrine. Chromosomal breaks can be detected as soon as 72 hours after the administration of a concentration of $10^{-6}$M of heliotrine. The ability of pyrrolizidine alkaloids to produce chromosomal breaks in growing cells of the root tip of the onion *(Allium)* and in wild peas *(Vicia faba)* was studied by Avanzi [86–89]. He also carried out experiments on leukocyte cultures obtained from a Tasmanian kangaroo *(Potorous tridactylus)*. For testing he used the alkaloids heliotrine, lasiocarpine, monocrotaline, jacobine, supinine and seneciphylline. All the tested alkaloids affected the chromosomes, the most active was, however, lasiocarpine. The relationship between chromosomal lesions, the mutagenic activity and the DNA synthesis was studied by McLean [8].

## 4. CARCINOGENIC AND ANTITUMOROUS EFFECTS

Cook *et al.* [90] were the first who experimentally demonstrated the hepatocarcinogenic effect of pyrrolizidine alkaloids. Rats were given per os an extract of *Senecio jacobaea*. According to the quantity of the alkaloid administered, the rats expired after a period of 1 to 11 months. Rats surviving less than one month after administration of the alkaloid, showed necroses and degenerative changes in the liver. In rats expiring at a later period, autopsy revealed, along with degenerative changes, sites of rapid regeneration and bile-duct hyperplasia. Rats surviving ten months showed nodular

regeneration and the development of a hepatoma or formation of bile-duct cystadenoma. Schoental and Head [91] administered pyrrolizidine alkaloids to rats over a period of 11–17 months and found a hepatoma arising from a colony of regenerating hepatocytes. Therefore it is assumed that liver carcinoma arises along with the regeneration of the liver parenchyma. Continuous administration of alkaloids [14] resulted in severe degenerative changes in the liver (high level of megalocytes, low regeneration and an atrophic liver) not, however, in a hepatoma. Intermittent administration or administration which was discontinued after a certain period (allowing the liver to regenerate) brought about the formation of carcinoma. The causative agent of the carcinogenic effect has not been explained satisfactorily; however, most frequently it is brought into relationship with the mutagenic properties of pyrrolizidine alkaloids.

The studies of the antimitotic effect of pyrrolizidine alkaloids also led to the investigation of their antitumorous effect. The antitumorous activity of many pyrrolizidine alkaloids could experimentally be confirmed. The testing of alkaloids on experimentally produced standard tumours was carried out by the Cancer Chemotherapy National Service Centre, Bethesda, and by the Abbot laboratories, North Chicago. The results were published by Culvenor [92]. So far, the antitumorous activity of pyrrolizidine alkaloids has not found wider use because of the numerous side effects, particularly the hepatotoxic activity.

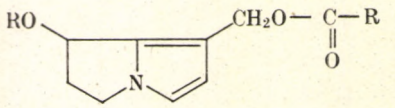
## 5. THE MECHANISM OF THE EFFECTS OF PYRROLIZIDINE ALKALOIDS

### *A. Alkylation Hypothesis*

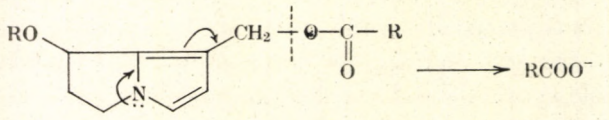
The hypothesis of the alkylating effect of pyrrolizidine alkaloids was postulated for the first time by Culvenor *et al.* in 1962 [95]. The postulation of this hypothesis was preceded, on the one hand, by a series of experimental–pathological studies and, on the other, by a series of chemical papers dealing with the possible synthesis and the degradation of these alkaloids *in vitro*. The experiments carried out with pyrrolizidine alkaloids (applied per os, subcutaneously or intraperitoneally) showed that they were toxic substances with a particular affinity to the liver tissue. It has also been found that the alkaloid is probably interfering with the mitoses in other tissues, though lesions of such a depth and extent as those observed in the liver are only rarely produced. Such an interference with cell division was observed, e.g., in the cells of the epithelium of the rat duodenum [7], in the germinal cells

of mice [39], *Drosophila* germ cells [84], in cultures of human foetal liver [48, 64], and in various standard tumours [92, 105, 106]. Nuclear abnormalities were found in Kupfer cells [7] and in reticuloendothelial cells of the spleen [107]. Except in liver cell cultures, these changes did not, however, lead to necrosis or megalocytosis. In view of these facts it has been concluded that, in all probability, the toxic substance is not the alkaloid itself, but some metabolite which is formed in the liver perhaps in connection with its degradation or detoxication.

Culvenor *et al.* [95] undertook chemically to prepare *in vitro* the dehydropyrrolizidine analogues (in abbreviation called pyrrols) of the corresponding alkaloids. In contrast to the corresponding primary alkaloids, they are very reactive and have a high alkylating ability. Their alkylating capacity is comparable with that of, e.g., nitrogen mustard or mitomycine C, which were found to inhibit mitoses by bonding to both DNA chains [8]. Culvenor assumes that similarly to the chemical preparation of these 'chemical pyrrols', the organism is able to metabolize the pyrrolizidine alkaloids to the so-called 'metabolic pyrrols'. The pyrrols (I) arise on dehydrogenation of the primary alkaloid [95]. Mattocks is of the opinion that the assumed site of this process in the cell are the microsomes of the liver cells [23]. An analysis of the individual cellular fractions has shown that the pyrrols are mainly contained in the microsomes [23]. Furthermore, experimental evidence has been provided that the formation of the pyrrols takes place *in vitro* after the addition of rat liver slices or of microsomal fractions obtained from rat liver [23].



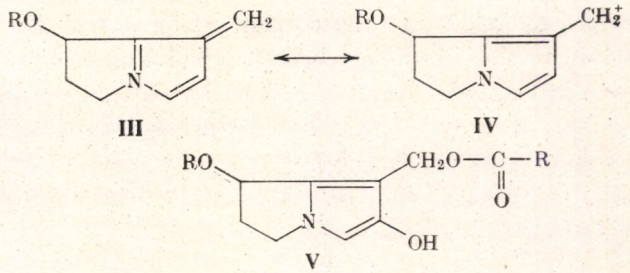

I




II

The resulting pyrrols are very reactive; their alkylating capacity depends on the capacity and possibility of a transfer of electrons from the nitrogen atom to the first carbon atom of the side chain (C-9) (II), and on the facility of the cleavage of the ester linkage. Thus an intermediate is formed having the structure III which resonates with structure IV. This structure is the

proper alkylating agent. It may be bound with a covalent bond to some cellular moiety, in all probability to DNA, RNA or to a protein [96–98]. By alkylation, the normal metabolism of these compounds is affected which might manifest itself by a disturbance of growth (after small doses) or by destruction of the cells (after larger doses). The transfer of the electrons to C-9 is rendered difficult by the incorporation of the hydroxyl group into the pyrrol nucleus (formation of a new electrophilic centre) (V).




III IV V

Hydroxylation, which can be carried out by microsomal hydroxylating enzymes, thus prevents the formation of the active pyrrol intermediate [8]. Principally, it might represent the detoxicating mechanism of pyrrol substances. Thus, the toxic effect of the alkaloids would result from the difference in the quantity of formed and the quantity of detoxicated pyrrol derivatives.

### *B. The Determination of Pyrrols*

Some of the steps of the assumed mechanism of the alkylating effect of the alkaloids have already been demonstrated *in vitro*. An increase in the pyrrol content (Ehrlich-positive substances) can be demonstrated in the urine after application of pyrrolizidine alkaloids. A certain quantity of the administered alkaloid is always eliminated from the organism without any alterations [7, 99–101], more precisely, it is metabolically not converted into pyrrols; by comparing the eliminated alkaloid and the quantity of eliminated pyrrols with the amount of the applied alkaloid, the retained quantity can be estimated. The retained portion of the alkaloid is then the amount which might have been converted into alkylating pyrrols. It must, however, be admitted that some of the administered alkaloids may be metabolized or detoxicated by another, so far unknown, mechanism.

The determination of the pyrrol metabolites of pyrrolizidine alkaloids is based on the colour reaction with 4-dimethylamino azobenzaldehyde

(Ehrlich reagent) measured spectrophotometrically. The reaction is brought about by making use of the modified Ehrlich reagent. 4-Dimethylamino-azobenzaldehyde is dissolved in a Lewis acid (boron trifluoride) in the form of a methanolic complex. This reagent has an absorption maximum at 562 nm and produces a comparatively strong and permanent color. For details of the methods of determining pyrrol contents, see Mattocks [102–104].

The determination of metabolically unaltered pyrrolizidine alkaloids is based on their conversion into the corresponding pyrrol derivatives along chemical pathways. The first stage includes oxidation to the corresponding N-oxide, which in the following step is dehydrated to a pyrrol with boiling acetic anhydride. The pyrrol reacts with the modified Ehrlich reagent to give rise to a blue-violet color. The metabolically altered alkaloid, which is eliminated directly as a pyrrol, reacts with the Ehrlich reagent without previous oxidation and dehydration.

## REFERENCES


1. Müller, A., *Heil- und Gewürz-Pflanzen*, *7*, 1 (1924).
2. Gilruth, J. A., *11th Ann. Rept. Dept. Agr., N. Z., Div. Vet. Sci.*, 228 (1903).
3. Chase, W. H., *Agr. J. Cape Good Hope*, *25*, 675 (1904).
4. Bobek, K., Vaněk, J., *Lékařské Listy*, *9*, 361 (1954).
5. Vaněk, J., *Sborník ČAZV*, *29*, 705 (1956).
6. Vaněk, J., *Acta Morphol. Acad. Sci. Hung.*, *8*, 323 (1958).
7. Bull, L. B., Culvenor, C. C. J., Dick, A. T., "The Pyrrolizidine Alkaloids". North-Holland Publ. Co., Amsterdam, 1968.
8. McLean, E. K., *Pharmacol. Rev.*, *22*, 429 (1970).
9. Cushny, A. R., *J. Pharmacol. exp. Ther.*, *2*, 531 (1911).
10. Berry, D. M., Bras, C., *N. Amer. Vet.*, *38*, 323 (1957).
11. Bick, A. A. E., Jackson, W. D., *Austr. J. biol. Sci.*, *21*, 469 (1968).
12. Bras, G., Hill, K. R., *Lancet*, *2*, 161 (1956).
13. Bull, L. B., *Aust. Vet. J.*, *31*, 33 (1955).
14. Bull, L. B., Dick, A. T., *J. Pathol. Bacteriol.*, *78*, 483 (1959).
15. Bull, L. B., Dick, A. T., *Aust. J. exp. biol. Med. Sci.*, *38*, 515 (1960).
16. Bull, L. B., Dick, A. T., McKenzie, J. S., *J. Pathol. Bacteriol.*, *75*, 17 (1958)
17. Bull, L. B., Rogers, E. S., Keast, J. C., Dick, A. T., *Aust. Vet. J.*, *37*, 37 (1961).
18. Dann, A. T., *Nature*, *186*, 1051 (1960).
19. Davidson, J., *J. Pathol. Bacteriol.*, *40*, 285 (1935).
20. Harris, P. N., Anderson, R. C., Chen, K. K., *J. Pharmacol. exp. Ther.*, *75*, 69a (1942).
21. Harris, P. N., Anderson, R. C., Chen, K. K., *J. Pharmacol. exp. Ther.*, *75*, 78b (1942).
22. Hill, K. R., Martin, H. M., *Brit. Vet. J.*, *114*, 345 (1958).
23. Mattocks, A. R., *Nature*, *217*, 723 (1968).
24. Nolan, J. P., Scheic, R. L., Klatskin, G., *Amer. J. Pathol.*, *49*, 129 (1966).
25. Peterson, J. E., *J. Pathol. Bacteriol.*, *89*, 153 (1965).
26. Reddy, J., Harris, C., Svoboda, D., *Nature*, *217*, 659 (1968).
27. Selzer, G., Parker, R. G. F., Sapeika, N., *Brit. J. exp. Pathol.*, *32*, 14 (1951).
28. Schoental, R., Magee, P. N., *J. Pathol. Bacteriol.*, *74*, 305 (1957).
29. Schoental, R., Magee, P. N., *J. Pathol. Bacteriol.*, *78*, 471 (1959).

30. Schoental, R., Mattocks, A. R., *Nature, 185*, 842 (1962).
31. Svoboda, D., Soga, J., *Amer. J. Pathol., 48*, 347 (1966).
32. Villa-Trevino, S., Leaver, D. D., *Biochem. J., 109*, 87 (1968).
33. Weinbergová, O., Černoch, M., Jezdinská, V., Pelikán, V., Seidlová, V., *Acta hepato-splenol., 18*, 373 (1971).
34. Weinbergová, O., Vrublovský, P., Slezáček, B., Jezdinská, V., Pelikán, V., Seidlová, V., *Acta hepato-splenol.* (In the press.)
35. Weinbergová, O., Unpublished results.
36. Schoental, R., Magee, P. N., *Acta Unio Int. Contra Cancer, 15*, 212 (1959).
37. Allen, J. R., Lalich, J. J., Schmittle, *Lab. Invest., 12*, 512 (1963).
38. Levin, G. S., *Farmakol. Toxicol., 32*, 170 (1969).
39. Rosenfeld, I., Beath, O. A., *Amer. J. Clin. Pathol., 15*, 407 (1945).
40. Bull, L. B., Dick, A. T., Keast, J. C., Edgar, G., *Aust. J. Agr. Res., 7*, 281 (1956).
41. Bull, L. B., Scott-Rogers, E., Keast, J. C., Dick, A. T., *Aust. Vet. J., 37*, 37 (1961).
42. Flowler, M. E., *J. Amer. Vet. Med. Ass., 152*, 1131 (1968).
43. Markson, L. M., *Proc. Roy. Soc. Med., 53*, 283 (1960).
44. Thorpe, E., Ford, E. J. H., *J. Comp. Pathol., 78*, 195 (1968).
45. Rose, A. L., Gardner, C. A., McConnell, J. D., Bull, L. B., *Aust. Vet. J., 33*, 25 (1957).
46. Rose, A. L., Gardner, C. A., McConnell, J. D., Bull, L. B., *Aust. Vet. J., 33*, 49 (1957).
47. Harding, J. D. J., Lewis, G., Done, J. T., Allcroft, R., *Pathol. Vet., 1*, 204 (1964).
48. Sullman, S. F., Zuckerman, A. J., *Brit. J. exp. Pathol., 50*, 361 (1969).
49. Rather, L. J., *Bull. Johns Hopkins Hosp., 88*, 38 (1958).
50. Wilson, J. W., Leduc, E. H., *J. Pathol. Bacteriol., 76*, 441 (1968).
51. Christie, G. S., LePage, R. N., *Lab. Invest., 10*, 729 (1961).
52. Butler, W. H., *J. Cancer, 18*, 756 (1964).
53. Schoental, R., *Voeding, 16*, 268 (1955).
54. Ford, E. J. H., Ritchie, H. E., Thorpe, E., *J. Comp. Pathol., 78*, 207 (1968).
55. Scheuer, P. J., *J. Pathol. Bacteriol., 85*, 507 (1963).
56. Schoental, R., Bensted, J. P. M., *Brit. J. Cancer, 17*, 242 (1963).
57. Harris, P. N., Rose, C. L., Chen, K. K., *Arch. Pathol., 64*, 152 (1957).
58. Ratnoff, O. D., Mirick, G. S., *Bull. Johns Hopkins Hosp., 84*, 507 (1949).
59. Schoental, R., Head, M. A., *Brit. J. Cancer, 9*, 229 (1955).
60. Selzer, G., Parker, R. G. F., Sapeika, N., *Brit. J. Exp. Pathol., 32*, 14 (1951).
61. Allen, J. R., *Avian Dis., 7*, 318 (1963).
62. Theiler, A., "Seventh and Eighth Reports of the Director of Veterinary Research", Cape Times, Ltd., Gov't Printers, Capetown, Union of South Africa, 1920.
63. Turner, J. H., Lalich, J., *Arch. Pathol., 79*, 409 (1965).
64. Hirchinson, V., Hill, K. R., *Brit. J. Cancer, 14*, 637 (1960).
65. Kay, J. M., Crawford, N., Heath, D., *Experientia, 24*, 1149 (1968).
66. Kay, J. M., Gillund, T. D., Heath, D., *Amer. J. Pathol.*, 51, 1031 (1967).
67. Markson, L. M., Terlecki, S., *Brit. Vet. J., 124*, 309 (1968).
68. Masugi, Y., Oami, H., Aihara, K., Hashimoto, K., Hakozaki, T., *Acta Pathol. Jap., 15*, 407 (1965).
69. Shtenberg, A. I., Orlova, N. V., *Vop. Pitan., 14*, 27 (1955).
70. Allen, J. R., Carstens, L. A., Katagigi, G., *Arch. Pathol., 87*, 279 (1969).
71. Carstens, L. A., Allen, J. R., *Amer. J. Pathol., 60*, 75 (1970).
72. Chen, K. K., *Ann., Rev. Physiol., 7*, 695 (1945).
73. Hayashi, Y., Lalich, J. J., *Proc. Soc. Exp. Biol. Med., 124*, 392 (1967).
74. Lalich, J. J., Merkow, M. S., *Lab. Invest., 10*, 744 (1961).
75. Schoental, R., *Bull. World Health Organ.*, 29, 823 (1963).
76. Blaustein, R. L., Blaustein, A., Rosenblat, *Arch. Pathol., 79*, 335 (1965).
77. Carstens, L. A., Allen, J. R., *Fed. Proc., 27*, 250 (1968).
78. Schoental, R., *J. Exptl. Med. Sci., 4*, 126 (1961).
79. Downing, D. T., Peterson, J. E., *Aust. J. Exp. Biol. Med. Sci., 46*, 493 (1968).
80. Rogers, A. E., *Fed. Proc., 29*, 756 (1970).
81. Howard, A., Pelc, S. R., *Heredity* (Suppl.), *6*, 261 (1953).

82. Clark, A. M., *Nature, 183* 731 (1959).
83. Clark, A. M., *Z. Vererbungsl., 91*, 74 (1960).
84. Clark, A. M., *Z. Vererbungsl., 94*, 115 (1963).
85. Bick, Y. A. E., Jackson, W. D., *Aust. J. biol. Sci., 21*, 469 (1968).
86. Avanzi, S., *Caryologia, 14*, 251 (1961).
87. Avanzi, S., *Caryologia, 15*, 351 (1962).
88. Avanzi, S., *Caryologia, 16*, 493 (1963).
89. Avanzi, S., Mariani, A., *Caryologia, 16*, 479 (1963).
90. Cook, J. W., Duffy, E., Schoental, R., *Brit. J. Cancer*, 4, 405 (1950).
91. Schoental, R., Head, M. A., *Brit. J. Cancer, 8*, 458 (1954).
92. Culvenor, C. C. J., *J. Pharm. Sci., 57*, 1112 (1968).
93. Rogers, A. E., Newborne, P. M., *Toxicol. Appl. Pharmacol., 18*, 356 (1971).
94. Persaud, T. V. N., Putzke, H. P., Tessmann, D., Bienengraber, A., *Acta Histochem., 37*, 369 (1970).
95. Culvenor, C. C. J., Dann, A. T., Dick, A. T., *Nature, 195*, 570 (1962).
96. Culvenor, C. C. J., Downing, D. T., Edgar, J. A., Jago, M. V., *Ann. N. Y. Acad. Sci., 168*, 837 (1969).
97. Mattocks, A. R., *J. Chem. Soc., 1969*, 1155.
98. Mattocks, A. R., White, I. N. H., *Nature New Biol., 231*, 114 (1971).
99. Schoental, R., *Bull. World Health Organ., 29*, 823 (1963).
100. Hayashi, Y., *Fed. Proc., 25*, 688 (1966).
101. Jago, M. V., Bingley, J. B., Piercy, D. W. T., Whitem, J. H., Lanigan, G. W., Titchen, D. A., *J. Pathol. Bacteriol., 98*, 115 (1969).
102. Mattocks, A. R., *Anal. Chem., 39*, 443 (1967).
103. Mattocks, A. R., *Anal. Chem., 40*, 1749 (1968).
104. Mattocks, A. R., *Nature, 219*, 5153 (1968).
105. Kupchan, S. M., Doskotsch, R. W., Venevhoven, P. W., *J. Pharm. Sci., 53*, 343 (1964).
106. Pukhalskaya, A. Ch., Petrova, M. F., Man'ko, I. V., *Bull. Exp. Biol. Med., 48*, 91 (1959).
107. Svoboda, D., Racela, A., Higginson, J., *Biochem. Pharmacol., 16*, 651 (1967).

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# ADVANCES IN THE SYNTHETIC CHEMISTRY OF GLYCOSAMINIDES

# I. INTRODUCTION

Amino sugars and, first of all, 2-amino-2-deoxyhexoses, being important structural components of polysaccharides and complex biopolymers, play an essential role in life processes. The nature of the chemical bond linking the monomeric units of the macromolecules is the central problem in the chemistry of biopolymers containing amino sugar residues. Since in polysaccharides and other carbohydrate-containing polymers amino sugar residues are, as a rule, linked by means of a glycosaminidic bond, it is of prime importance for the chemistry of amino sugars that the methods for the formation of this bond and investigating its properties should be elaborated.

The present review deals only with the methods of glycosaminide synthesis involving the formation of the glycosaminidic bond; no mention is made of the procedures involving chemical transformations of glycosides and oligosaccharides resulting in amino sugar derivatives produced without the formation of new glycosidic bonds. The methods for synthesizing glycosaminides available at present are analyzed from the standpoint of the general requirements for glycoside synthesis, i.e.:

(1) The reaction should have structural and steric selectivity;

(2) Sufficiently mild conditions should be provided for the reaction, as the protections of the "aglycon" component are rather labile;

(3) The groups protecting the glycosylating agent should be sufficiently stable under the conditions of the glycosylation reaction and they should be readily and selectively removable from the final products without change of the structure.

From the chemical point of view, the synthesis of 2-amino-2-deoxy sugar glycosides is of special interest as it is different in a number of points from the glycoside synthesis in the series of neutral and other amino sugars, this being due to the presence of the amino group localized in the immediate proximity to the glycosidic centre.

In this review we confined ourselves to analyzing only those works in which the fundamentals of glycosaminide synthesis have been elaborated,

as well as those in which the reaction patterns have been studied or the existing methods substantially modified. The main attention is paid to the synthesis of N-acetylglycosaminides including oligosaccharides containing N-acetylglycosaminyl residues, as in most cases natural amino sugars and their derivatives are N-acetylated.

The problems of glycosaminide synthesis have been dealt with in several reviews [13, 40, 59]. However, the rapid progress in this field of carbohydrate chemistry and especially in the synthetic chemistry of amino sugar oligosaccharides has necessitated the present review covering the literature up to the first half of 1973.

## II. SYNTHESIS OF ALKYL AND ARYL GLYCOSAMINIDES

### 1. ALCOHOLYSIS OF MONOSACCHARIDES

The Fischer method widely used in the synthesis of neutral sugar alkyl glycosides is sometimes employed for obtaining mixtures of anomeric glycosides of N-substituted 2-amino-2-deoxy sugars. For example, treatment of N-acetyl-D-glucosamine (or its acetate) with alcohols in the presence of hydrogen chloride, *p*-toluenesulfonic acid, ion-exchange resins (in $H^+$ form) or Lewis acids leads to mixtures of alkyl 2-acetamido-2-deoxy-α- and β-D-glucopyranosides [19, 36, 41, 88, 89, 140, 142, 160, 219, 224] in which the α-anomers prevail. Glycosylation of benzyl alcohol with N-acetyl-D-glucosamine also gives a mixture of anomeric glycosides from which benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside is isolated [49, 84, 171], which is used in the synthesis of numerous derivatives of 2-amino-2-deoxy sugars. Treatment of N-acetyl-D-galactosamine (or its acetate) with lower alcohols gives similar results [89, 115, 173, 180]. Galactosaminides are generally formed under the same conditions as glucosaminides, whatever the nature of the aglycon; therefore, their preparation is not specially described below.

2-Amino-2-deoxy sugars themselves do not form glycosides in the course of the Fischer's procedure because of the electrostatic shielding produced by the $NH_3^+$ group which prevents the cationoid groups from approaching the glycosidic centre [140].

### 2. THE HELFERICH SYNTHESIS OF ARYL GLYCOSAMINIDES

The Helferich method (see [27]) consisting in the fusion of peracetylated amino sugars with phenols in the presence of a catalytic amount of *p*-toluenesulfonic acid or zinc chloride, was used to synthesize aryl glycosami-

nides [27, 35, 42, 198, 199, 201]. *p*-Toluenesulfonic acid preferentially catalyzes the formation of 1,2-*trans*-glycosaminides, whereas in the presence of zinc chloride a mixture of anomeric aryl glycosaminides is formed. This method is hardly applicable to the synthesis of glycosaminides with a complex aglycon and, in particular, to the glycosylation of labile compounds.

### 3. THE ACTION OF ALKYLATING AGENTS

Methyl iodide in dimethylformamide in the presence of barium oxide and barium hydroxide converts N-acetyl-D-glucosamine (and its acetate) into methyl 2-acetamido-2-deoxy-3,4,6-tri-O-methyl-β-D-glucopyranoside [87]. Alkylation of N-acetyl-D-glucosamine derivatives by dimethyl sulfate yields both α- and β-D-glycosides depending on the reaction conditions [116, 161]. Kuhn and Baer [83] suggested a very mild procedure for glycosylation, in which N-acetyl-D-glucosamine and its N-formyl and N-benzyloxycarbonyl analogues are treated with diazomethane in aqueous methanol to give methyl β-D-glycosides. The above procedures are too specific to have a sufficient preparative value.

## III. THE KOENIGS–KNORR REACTION AND ITS MODIFICATIONS IN GLYCOSAMINIDE SYNTHESIS

### 1. THE BEHAVIOUR OF AMINO SUGAR GLYCOSYL HALIDES IN THE KOENIGS–KNORR REACTION

The Koenigs–Knorr reaction (see reviews [27, 34, 53]) based on the interaction of acylated glycosyl halides with alcohols and phenols in the presence of hydrogen halide acceptors is widely used in the synthesis of glycosaminides. (The available data on glycosyl halides of 2-amino-2-deoxy sugars are listed in Table I.) It was demonstrated as early as 1911 [69] that 3,4,5-tri-O-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide (I), reacting with methanol, is converted into the glycoside hydrobromide II. However, bromides of completely acetylated 2-amino-2-deoxy sugars essentially differ from those of the respective neutral analogues [53]. That is why the structure and properties of acylated bromides and chlorides of 2-amino-2-deoxy sugars should be dealt with in greater detail.

Bromide I and the respective chloride are sufficiently stable compounds and resemble the glycosyl halides of neutral hexopyranoses. The bromide of acetylated D-glucosamine (III) possesses a much higher reactivity as

*Table*

Glycosyl Halides of

| Compound | Yield[a] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent[b] | References |
|---|---|---|---|---|
| Glycosyl chlorides | | | | |
| 3,4-Di-O-acetyl-2-deoxy-2-nitroso-α-D-xylopyranosyl chloride, dimer | 90 | 102–106 | +164 C | [104] |
| 2-Amino-2-deoxy-α-D-glucopyranosyl chloride | | | | |
| 3,4,6-tri-O-acetylhydrochloride | — | 161–163 d. | +146 C | [62] |
| N-acetyl- | 86 | 125–126 d. | | [15] |
| | — | 133–134 d. | +120 C | [141] |
| | 69 | 133–134 d. | +118 C | [92] |
| | 86 | 126–128 d. | +119 C | [228] |
| β-anomer | 60 | syrup | +20 C | [146] |
| N-benzoyl- | 53 | 124–127 | +36 P-W | [135] |
| N-bis-(4-nitrobenzyl)-phosphoryl- | 80 | 148–149 | +39 C | [223] |
| N-chloroacetyl- | 61 | 143 d. | +116 C | [146] |
| N-(3,5-dinitrobenzoyl)- | 70 | 144 | +133 C | [8] |
| N-diphenylphosphoryl- | 90 | 125–126 | +97 C | [223] |
| N-(4-methoxybenzoyl)- | 11 | 137 d. | +153 C | [146] |
| N-(4-nitrobenzoyl)- | 86 | 105–107 | +152 C | [146] |
| 2-nitroso analogue, dimer | 80 | 129–130 | +149 C | [101,104] |
| N,N-phthaloyl-, β-anomer | 66 | 149 | +62 C | [6, 146] |
| | 86 | 154 | +28 B | [17] |
| N-propionyl- | 64 | 122–124 | +132 A | [48] |
| N,N-succinyl-, β-anomer | 61 | 132–134 | +21 C | [7, 146] |
| N-(*p*-toluenesulfonyl)- | 67 | 139–141 | +107 C | [136] |
| | 80 | 138 | +107 C | [6] |
| N-methyl- | | 139–140 | +103 C | [136] |
| 3,4,6-tri-O-benzoyl-N-acetyl- | 45 | 128–130 | | [114] |
| 3,4,6-Tri-O-acetyl-2-amino-2-deoxy-α-D-galactopyranosyl chloride | | | | |
| N-acetyl- | 43 | 131–136 d. | +134 C | [180] |
| | | 130 | +138 C | [57] |
| 2-nitroso analogue, dimer | 84 | 128–131 | +128 C | [104] |
| 2-Acetamido-3,4-di-O-acetyl-2,6-dideoxy-α-D-glucopyranosyl chloride | 71 | 145 d. | +153 C | [148] |
| 2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride | 66 | 208–209 d. | +41 C | [147] |
| | 60 | 202–204 d. | +34 C | [228] |

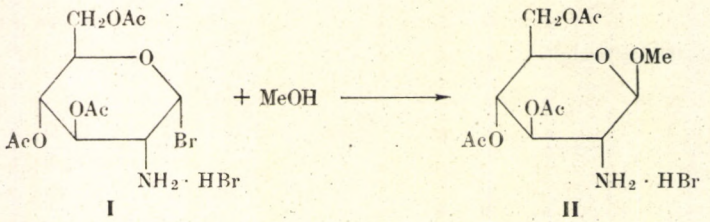
[a] The yields are given only for the halogenation step, excluding the step of N-substituent introduction.

[b] In this and the following tables the following abbreviations are used: A — acetone, B — benzene, C — chloroform, D — dimethylformamide, M — methanol, N — nitromethane, P — pyridine, T — tetrahydrofuran, W — water.

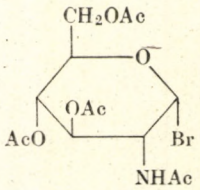
*I*

2-Amino-2-deoxy Sugars

| Compound | Yield[a] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent[b] | References |
|---|---|---|---|---|
| O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride | 34 | 169 d. | +33 C | [149] |
| Glycosyl bromides | | | | |
| 2-Amino-2-deoxy-α-D-glucopyranosyl bromide | | | | |
| 3,4,6-tri-O-acetyl- | | 82–83 | +75 C | [38] |
| hydrobromide | — | 149–150 d. | +148 A | [69] |
| | 85 | 149–150 | +151 A | [212, 223] |
| N-acetyl- | 10 | 116–118 | +86 C | [38] |
| | 30 | 90 d. | +148 C | [137] |
| N-benzyloxycarbonyl- | 33 | 97–98 | +146 C | [223] |
| N-benzylsulfonyl- | 72 | 65–75 | +89 C | [145] |
| N-diphenylphosphoryl- | 91 | 126–127 | +125 C | [223] |
| N-(4-methoxybenzylidene)- | 64 | 112–114 | +217 C | [223] |
| N-phenylcarbamoyl- | 70 | 105 d. | +137 C | [127] |
| N,N-phthaloyl-, β-anomer | 48 | 136–137 | +51 C | [5, 6] |
| | 50 | 130 | +18 B | [17] |
| N-(*p*-toluenesulfonyl)- | 83 | 148 | +64 C | [143] |
| | 83 | 148 d. | +134 C | [138] |
| N-trifluoroacetyl- | 74 | 95–97 | | [176] |
| | 92 | 96 | +126 C | [204] |
| N-(2,4-dinitrophenyl)- | | | | |
| 3,4,6-tri-O-acetyl- | 97 | 162–164 d. | +46 C | [111, 112, 191] |
| 3,4-di-O-acetyl-6-O-methyl- | 78 | 135 | +28 C | [107] |
| 3,6-di-O-acetyl-4-O-methyl- | 70 | 150–152 | +68 C | [107] |
| 4,6-di-O-acetyl-3-O-methyl- | — | amorph. | −5 C | [107] |
| 3,4,6-tri-O-benzoyl- | 70 | 150 d. | +68 C | [194] |
| | 94 | 143 d. | +119 C | [196] |
| hydrobromide | 81 | 163 d. | +71 T | [196] |
| N-benzoyl- | 86 | 126 d. | +127 C | [136] |
| | 88 | 128 | +125 C | [195, 196] |
| N-benzyloxycarbonyl- | 47 | 102–104 | +105 C | [196] |
| N-dichloroacetyl- | 85 | 125–127 | +89 C | [167] |
| 2-Amino-2-deoxy-α-D-galactopyranosyl bromide | | | | |
| 3,4,6-tri-O-acetyl-, hydrobromide | 76 | 144–148 d. | +160 C | [208] |
| 3,4,6-tri-O-benzoyl-N-dichloroacetyl- | — | amorph. | | [3] |



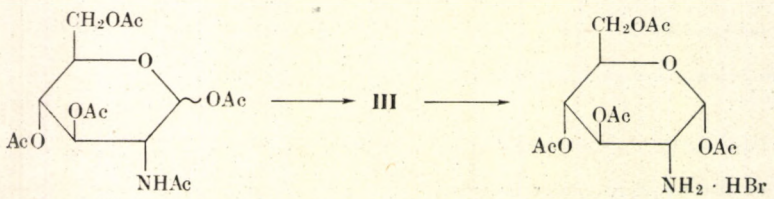

I II




III

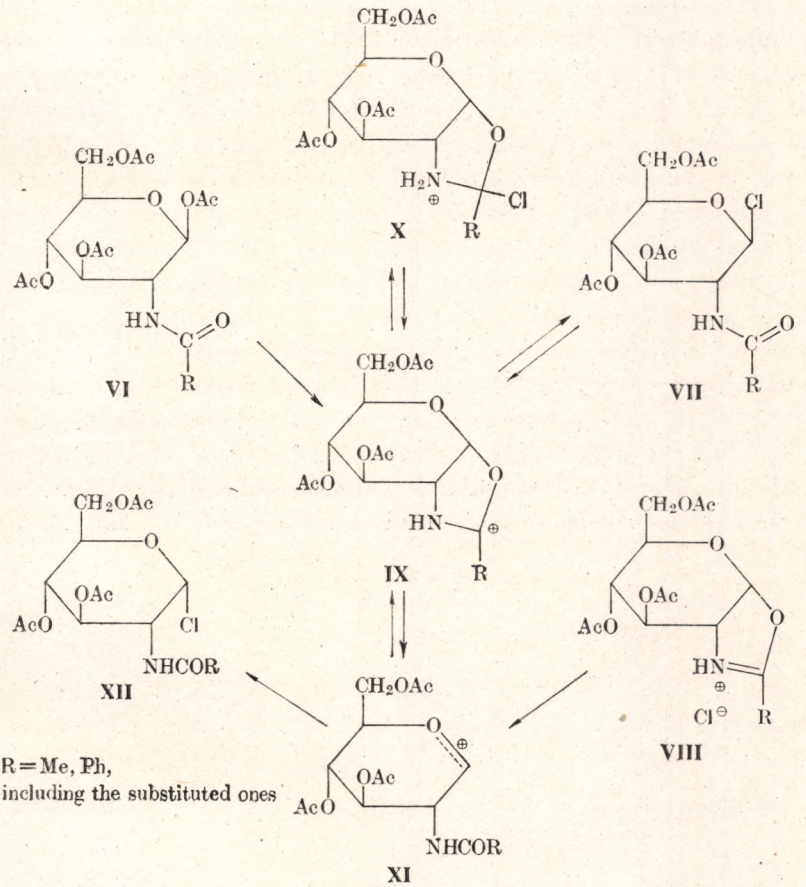
compared with tetra-O-acetyl-α-D-glucopyranosyl bromide. This is due to the fact that after heterolysis of the C-1-Br bond the acetamido group at C-2 becomes involved in the formation of the stable intramolecular cyclic derivative between the N-substituent and the C-1 atom to a greater extent than the acetoxy group at C-2 in the D-glucose analogue. The reactivity may be decreased (and the stability of glycosyl halides increased) by increasing the electronegativity of the substituents in the acylamido group.

The extreme sensitivity of the bromide III to moisture has for a long time prevented its isolation as an individual compound. Under the action of hydrogen bromide on the acetate IV in acetic acid, instead of the expected bromide III, the hydrobromide V was isolated [140], as was demonstrated later [65, 92, 131]. Nevertheless, the unpurified bromide III formed in this reaction, as well as a bromide of the D-*galacto* series [180] prepared under similar conditions, were used, as will be shown below, in glycosaminide synthesis. It was much later that the individual bromide III was isolated from the reaction of a limited amount of hydrogen bromide with the acetate IV in acetic acid. Of course, measures were taken to prevent the product from coming into contact with moisture [137, *cf.* 38].




IV V

Osawa [146] has described in detail the processes occurring under the action of hydrogen chloride, titanium tetrachloride or aluminium chloride on 1,2-*trans*-acetylated derivatives of 2-amino-2-deoxy sugars (VI). First a rapid elimination of the acetoxy group at C-1 takes place with participation of the neighbouring acylamido group, leading to an unstable β-D-glycosyl halide (VII). At this step the N-aroyl derivatives rapidly stabilize by rearrangement into an oxazoline derivative salt (VIII) in which the C=N



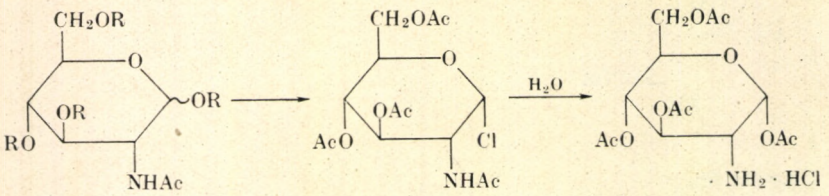

bond is conjugated with the aromatic ring. If R is an alkyl group, the resulting intermediate cation (IX) may react with hydrogen halide to give an oxazolidine cation (X). Strongly electronegative N-substituents, such as *p*-nitrobenzoyl and chloroacetyl, favour the rapid formation of an oxonium ion (XI) which is an immediate precursor of α-D-glycosyl halide (XII).

A mixture of anomeric acetates reacts with hydrogen bromide in acetic acid in a more complicated fashion [137].

The stability of acylated 2-amino-2-deoxy sugar bromides may be increased not only by electronegative substituents, but also by aryl or arylalkylidene group insertion into the amino group of amino sugar glycosyl halides; thereby the group at C-2 is rendered incapable of the participation effect. For example, O-acylated α-D-glycosyl bromides have been obtained with the following N-substituents: benzoyl [136, 195], *p*-toluenesulfonyl [138, 143], benzylsulfonyl [145], 2,4-dinitrophenyl [111, 112, 191], diphenylphosphoryl [223], anisylidene [223], benzyloxycarbonyl [223], trifluoroacetyl [176, 204], dichloroacetyl [2, 3, 167], and some others [5, 6, 15, 17, 68, 127, 204, 223] which were further used in glycosaminide synthesis.

The stability of the glycosyl halides of the 2-amino-2-deoxy sugars also depends on the nature of the halogen atom and increases in the following order: bromides, chlorides, and fluorides. No iodides have been synthesized so far and the fluorides possess such a low reactivity that they may be deacetylated in the presence of sodium methoxide, without the C—F bond being broken [138].

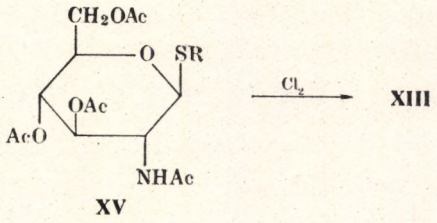
Of the amino sugar chlorides, greatest interest is attached to peracetylated 2-amino-2-deoxy-α-D-glycosyl chlorides, formed when 2-acetamido-2-deoxy sugars (or their acetates) are treated with hydrogen chloride [4, 15, 91, 92, 141, 220], acetyl chloride [61, 130] or both [228], or with titanium tetrachloride [15, 65, 180]. These methods have been used to prepare chlorides of di- and trisaccharides of acetylated amino sugars [149, 220, 228].




R = H or Ac **XIII** **XIV**

Chloride XIII is a sufficiently stable compound; it is slowly converted into the hydrochloride XIV, which allows its isolation with moisture-containing solvents [65, 92, 146].

Another method of preparing acetylated glycosaminyl chlorides [62], including furanosyl chlorides [215], is the chlorination of S-substituted 1-thio analogues (XV, R = Et, CSOEt, Ac) (*cf.* [23]), for example:



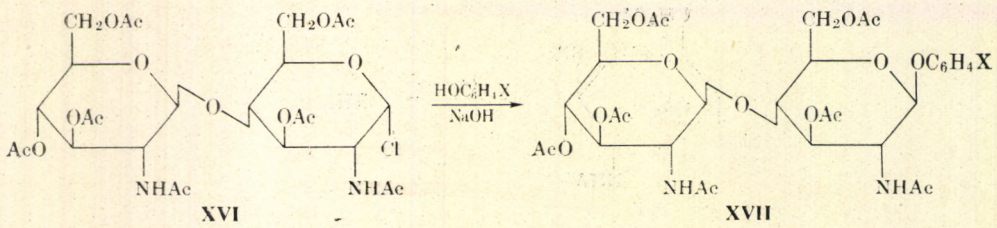

XV

From the preparative point of view, however, this method can hardly compete with that described above.

The stable bromide I has only been used in glycosaminide syntheses for obtaining the glycosides of lower alcohols [69] and some phenols [120]; this is due to its poor solubility in the solvents used in the Koenigs–Knorr reaction. Unstable bromides of acetylated amino sugars (which were not, as a rule, isolated individually) or stable but insufficiently reactive chloride have been used to prepare 2-acetamido-2-deoxy-α-D-glucopyranosides and α-D-galactopyranosides of lower alcohols [85, 91, 92, 130, 180, 181], phenols [18, 20, 47, 48, 57, 91, 92, 228], monoterpenic [81, 82], steroid [58], and some other alcohols [30, 93, 141]. Various monofunctional hydroxyl-containing compounds were glycosylated by bromides with other N-substituents [5–17, 52, 67, 107, 109, 111, 112, 126, 127, 136, 138, 143, 145, 146, 176–178, 191, 194–196, 223]. Though the yields of the end-products were higher in these cases than with N-acetylated bromides, the use of bromides in which the substituents at C-2 are incapable of participation due to structural reasons, often leads to a mixture of anomeric glycosides [107, 109 191].

In glycoside synthesis the key problem is, apparently, the steric selectivity. The fact that a given anomer may be isolated from a reaction mixture (sometimes multicomponent as it is the case, e.g., with glycosides with a complex aglycon) does not signify the stereospecificity of the reaction. This question may be answered unequivocally only after an accurate analysis of all the reaction products has been made. Hence, the data on the stereospecificity of glycosylation should be interpreted with care.

The Koenigs–Knorr method [181, 220] and its modifications [16, 113, 147, 149, 184, 228] have been used for preparing alkyl or aryl glycosides of oligosaccharides with 2-acetamido-2-deoxy-β-D-glucopyranosyl residues at the reducing end. The disaccharide chloride XVI reacted with phenols to give aryl β-chitobioside acetates (XVII, X = H, Me, Et, MeO, Cl, $NO_2$).




XVI XVII

## 2. SYNTHETIC OLIGOSACCHARIDES*

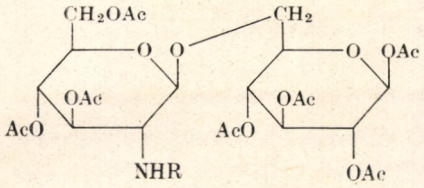
Of all the known methods of di- and oligosaccharide synthesis, those based on the Koenigs–Knorr reaction or its modifications have been the only ones used to synthesize oligosaccharides with 2-acetamido-2-deoxy-D-glycopyranosyl residues. A number of derivatives of disaccharides (XXIII–XXXI) with $\beta$-(1 → 6)-glycosaminidic bonds between the monosaccharide units have been obtained in the reaction of a freshly prepared solution of the bromide III [65] or its N-benzoyl analogue (XVIII) and partially protected derivatives of D-glucose (XIX) [86, 174], D-galactose (XX) [86], N-acetyl-D-glucosamine (XXI; R = Ac or *p*-nitrophenyl) [147, 192], and N-acetylmuramic acid (XXII) [36, 37, 150, 218], under the standard conditions of the Koenigs-Knorr reaction.

The bromide III was also used for obtaining the acetate of the non-reducing disaccharide, 2,2′-diamino-2,2′-dideoxy-$\beta$,$\beta$-trehalose [65] in a low yield.

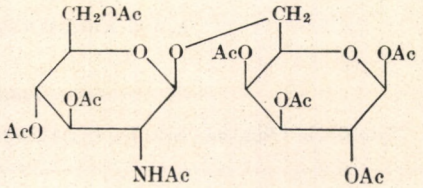
It should be noted that in all cases the yields of the required products were much lower than in the glycosylation of the same (or similar) 'aglycons' by glycosyl halides of neutral sugars by the Koenigs–Knorr procedure.

Because of the great instability of the bromide III which leads to low yields of disaccharide derivatives, it was later replaced by more stable glycosyl halides. Zervas and Konstas [223] were the first to apply a stable bromide XXXII to the synthesis of disaccharides. The reaction was carried out in the presence of mercuric cyanide and gave a protected disaccharide (XXXIV) in 60% yield. A similar disaccharide derivative (XXXV) was synthesized later [71] in the same yield, *via* glycosylation of compound XXXIII.

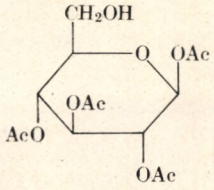
* See Table II summarizing the data of synthetic amino sugar oligosaccharides and their derivatives.



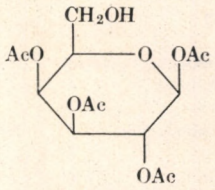
**XXIII** (R=Ac), 15 %
**XXIV** (R=Bz), 33 %



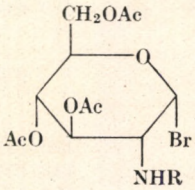
**XXV**, 20 %



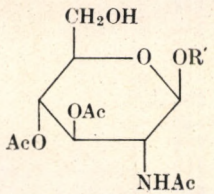
**XIX**



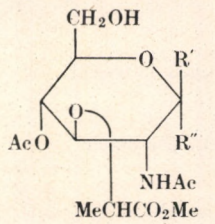
**XX**



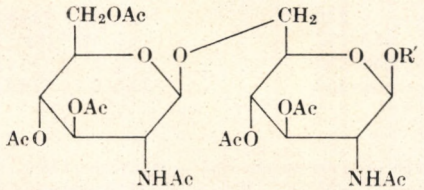
**III** (R=Ac)
**XVIII** (R=Bz)



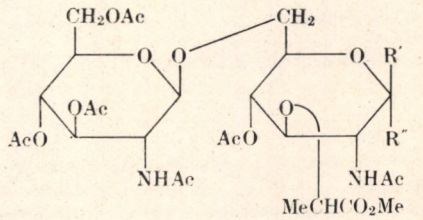
**XXI**



**XXII**



**XXVI** (R′=Ac), 5%
**XXVII** (R′=$OC_6H_4NO_2$), 2.4%



**XXVIII** (R′=H, R″=OMe), 12%
**XXIX** (R′=H, R″=$OCH_2Ph$), 3.5%
**XXX** (R′=OPh, R″=H) 1.3%
**XXXI** (R′=H, R″=OAc), 6%

*Table II*

Synthetic Oligosaccharides Containing 2-Amino-2-deoxyglycosyl Residues

| Oligosaccharides[a] and their derivatives | Method of synthesis and yield,[b] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| Glc*p*N-α-(1→3)-Glc | | | | |
| N-acetyl- | | 212–215 | +182→+158 W | [99] |
| 1,2 : 5,6-di-O-isopropylidene-3′,4′,6′-tri-O-acetyl-, α-furanose | 75[c] | 168–170 | +32 C | [99] |
| 2′-oximino analogue | NC 59 | 160–161 | +66 C | [96] |
| Glc*p*N-β-(1→3)-Glc | | | | |
| N-acetyl- | | amorph. | +59→+39 W | [226] |
| 1,2 : 5,6-di-O-isopropylidene-3′,4′,6′-tri-O-acetyl-, α-furanose | Z 49 | 219–220 | +68 C | [226] |
| N-benzylsulfonyl-1,2 : 5,6-di-O-isopropylidene-3′,4′,6′-tri-O-acetyl-, α-furanose | K 6 | 219 | −36 C | [145] |
| Glc*p*N-β-(1→4)-Glc | | | | |
| N-acetyl- | | 190–195 | +30 W | [168] |
| hepta-O-acetyl-, α-anomer | | 148–151 | +24 C | [168] |
| penta-O-acetyl-1,6-anhydro- | | 194–195 | −29 C | [168] |
| *p*-nitrophenyl-β-pyranoside | E 49 | 256–258 | | [159] |
| N-dichloroacetyl-2,3-di-O-acetyl-1,6-anhydro-3′,4′,6′-tri-O-benzoyl- | K 10 | 112 | −38 C | [168] |
| Glc*p*N-β-(1→6)-Glc | | | | |
| hydrochloride, hepta-O-acetyl-, β-anomer | | 233 d. | +32 D | [223] |
| N-acetyl- | | amorph. | +3 W | [11, 86] |
| hepta-O-acetyl-, β-anomer | | 217–218 | −9 C | [223] |
| | K 16 | 218–219 | −10 C | [86] |
| | Z 46 | 222–223 | −7 C | [11] |
| benzyl-β-pyranoside (monohydrate) | | 240–241 | −50 M | [71] |
| hexa-O-acetyl- | Z 46 | 210–211 | −26 C | [71] |
| N-benzylsufonyl-hepta-O-acetyl-, β-anomer | K 34 | 246 | −18 C | [145] |
| N-benzoyl- | | 175–177 | −6 W | [128, 129] |
| hepta-O-acetyl-, β-anomer | K 33 | 252–253 | −2 C | [174] |
| | Z 51 | 251–253 | −2 C | [128] |

[a] The following abbreviations not shown in [70] are used: Xyl — D-xylose, GlcN — D-glucosamine, GlcNAc — N-acetyl-D-glucosamine, GalNAc — N-acetyl-D-galactosamine, ManN — D-mannosamine, TalN — D-talosamine, MurNAc — N-acetylmuramic acid, 3-$NH_2$-3deGlc — 3-amino-3-deoxy-D-glucose.

[b] E — enzymic synthesis, K — the Koenigs–Knorr reaction and its modifications, NC — nitrosochloride method [96, 102], Z — oxazoline method. The yields are given only for glycosylation steps.

[c] At the step of reduction of the respective oximino derivative.

*(Table II — continued)*

| Oligosaccharides[a] and their derivatives | Method of synthesis and yield,[b] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| N-benzoyl- (cont.) | | | | |
| methyl-α-pyranoside | | 260–265 d. | +35 W | [128] |
| hexa-O-acetyl- | Z 55 | 214–216 | +69 C | [128] |
| hexa-O-methyl- | | 196–198 | +84 C | [129] |
| N-benzol-methyl-β-pyranoside, hexa-O-methyl- | | 200–202 | +14 C | [129] |
| N-diphenylphosphoryl- | | | | |
| hepta-O-acetyl-, β-anomer | K 60 | 221–223 | +3 C | [223] |
| benzyl-β-pyranoside, hexa-O-acetyl- | K 61 | 202–203 | −20 C | [71] |
| Glc*p*N-β-(1→3)-Gal | | | | |
| N-acetyl- | | | | |
| hepta-O-acetyl-, α-anomer | | 212–213 | +80 C | [167] |
| penta-O-acetyl-1,6-anhydro- | | 202–204 | −78 C | [167] |
| N-dichloroacetyl-2-O-acetyl-1,6-anhydro-3′,4′,6′-tri-O-benzoyl- | K 19 | 235–236 | −41 C | [167] |
| 4-O-acetyl- | | syrup | | [167] |
| Glc*p*N-β-(1→4)-Gal | | | | |
| N-acetyl- | | 162–165 | +8 W | [167] |
| hepta-O-acetyl-, α-anomer | | 178–179 | +56 C | [167] |
| penta-O-acetyl-1,6-anhydro- | | 238–239 | −29 C | [167] |
| N-dichloroacetyl-2-O-acetyl-1,6-anhydro-3′,4′,6′-tri-O-benzoyl- | K 26 | 146–148 | −40 C | [167] |
| 3-O-acetyl- | K 9 | 235–236 | −33 C | [167] |
| Glc*p*N-α-(1→6)-Gal | | | | |
| hydrochloride (dihydrate) | | 50–60[d] | +112 W | [110] |
| N-acetyl- | | amorph. | +126 W | [99] |
| (dihydrate) | | 60[d] | +126 W | [110] |
| 1,2 : 3,4-di-O-isopropylidene-3′,4′,6′-tri-O-acetyl- | 77[c] | 128–129 | +23 C | [99] |
| 2′-oximino analogue | NC 67 | 137–138 | +9 C | [96] |
| N-(2,4-dinitrophenyl)- | | 170[d] | +103 M | [110] |
| 1,2 : 3,4-di-O-isopropylidene | K 30 | 80[d] | −40 C | [110] |
| 3′,4′,6′-tri-O-acetyl- | | 201–202 | −28 C | [99] |
| Glc*p*N-β-(1→6)-Gal | | | | |
| hydrochloride | | 197 d. | +15 W | [110] |
| 1,2 : 3,4-di-O-isopropylidene-3′,4′,6′-tri-O-acetyl- | | 245 d. | −21 M | [145] |
| N-acetyl- | | amorph. | +10 W | [11, 110] |
| | | amorph. | +9 W | [139] |
| hepta-O-acetyl- | K 10 | 197–198 | +6 C | [86] |
| β-anomer | | 196–197 | +8 C | [139] |
| 1,2 : 3,4-di-O-isopropylidene-3′,4′,6′-tri-O-acetyl- | Z 48 | 198 d. | +85 M | [11] |
| benzyl-β-pyranoside, 2,3,6-tri-O-benzyl- | | 213–215 | +23 P | [139] |
| 3′,4′,6′-tri-O-acetyl- | K 12 | 195–195.5 | −28 C | [139] |

[d] The temperature of softening.

*(Table II — continued)*

| Oligosaccharides[a] and their derivatives | Method of synthesis and yield,[b] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| Glc*p*N-β-(1→6)-Gal (cont.) | | | | |
| N-benzylsufonyl-1,2 : 3,4-di-O-isopropylidene- | K 50 | 90–100 | −80 C | [145] |
| N-(2,4-dinitrophenyl)- | | 215–216 | +33 C | [110] |
| 1,2 : 3,4-di-O-isopropylidene- | K 29 | 198–199 | −82 C | [110] |
| Glc*p*N-β-(1→6)-Man | | | | |
| N-acetyl- | | 128–130 d. | −9 W | [118] |
| hepta-O-acetyl-, β-anomer | Z 26 | 187–190 | −26 C | [118] |
| Glc*p*N-α-(Y→)-GlcN | | | | |
| N-acetyl-4,6-O-benzylidene-, benzyl-α-pyranoside | | | | |
| N′-dibenzylphosphoryl- | | 214–217 | +75 P | [54, 55] |
| N′-diphenylphosphoryl-3′,4′,6′-tri-O-acetyl- | K 5 | 147–149 | +81 C | [54, 55] |
| Glc*p*N-β-(1→3)-GlcN | | | | |
| di-N-acetyl- | | 198–199 | +14→+6 W | [54, 55] |
| | | 180 d. | +40→+4 W | [10, 226] |
| | | 194–195 d. | | [10, 226] |
| benzyl-β-pyranoside | | 219–220 | −8 AcOH | [10, 226] |
| 3′,4′,6′-tri-O-acetyl- | | 297–298 | −43 P | [10, 226] |
| 4,6-O-benzylidene | Z 81 | 208–209 d. | +65 W | [54] |
| N-acetyl- | | 268–269 d. | +76 P | [54] |
| 4,6-O-benzylidene- | | 191–192 | +72 M | [54] |
| N′-dibenzylphosphoryl- | | 179–180 | +38 C | [54] |
| 4,6-O-benzylidene- | | | | |
| N′-diphenylphosphoryl- | | | | |
| 3′,4′,6′-tri-O-acetyl-4,6-O-benzylidene- | K 26 | 102.5–103.5 | +66 C | [54, 55] |
| Glc*p*N-α-(1→4)-GlcN | | | | |
| di-N-acetyl-(ethanolate) | | 150 d. | +96 M | [55, 56] |
| 5,6-O-isopropylidene-, ethylacetal | | syrup | | |
| | | 177–179 | +106 W | [56] |
| di-N-acetyl- | | syrup | +62 C | [56] |
| tetra-O-acetyl- | | 100–102 | +27 M | [56] |
| 2-N,3-O-carbonate | | 112–115 | +18 C | [56] |
| N′-dibenzylphosphoryl- | | | | [55, 56] |
| N′-diphenylphosphoryl- | | 116–118 | +30 C | |
| 3′,4′,6′-tri-O-acetyl- | K 35 | | | [55, 56] |
| Glc*p*N-β-(1→4)-GlcN | | | | |
| di-N-acetyl-, *p*-nitrophenyl-β-pyranoside | E 62 | 253–256 | | [31] |
| 5,6-O-isopropylidene-2-N,3-O-carbonate, diethyl acetal | | syrup | −49 M | |
| | | 173–176 | −40 C | [56] |
| N′-dibenzylphosphoryl- | | | | [56] |
| N′-diphenylphosphoryl- | | 93–95 | −23 C | |
| 3′,4′,6′-tri-O-acetyl- | K 10 | | | [55, 56] |

*(Table II — continued)*

| Oligosaccharides[a] and their derivatives | Method of synthesis and yield,[b] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| Glc*p*N-α-(1→6)-GlcN | | | | |
| di-N-acetyl- — | 8[e] | 215 | +140→+125 W | [39] |
| Glc*p*N-β-(1→6)-GlcN | | | | |
| di-N-acetyl- | 3.2[e] | 200 d. | +12→+6 W | [39] |
| | | amorph. | +4 W | [192] |
| | | 199–201 | +10 W | [25] |
| | | 200 | +8 W | [33] |
| (monohydrate) | | 190–192 | +22→−55 W | [171] |
| hexa-O-acetyl-, α-anomer | K 5.3 | 236–238 | +12 C | [192] |
| *p*-nitrophenyl-β-pyranoside | | 218–219 | −43 M-W | [229] |
| (monohydrate) | | 220–221 | −55 M-W | [147] |
| penta-O-acetyl- | Z 49 | 258.5–260 | −27 C | [229] |
| (monohydrate) | K 2.4 | 257–258 | −43 C | [147] |
| 3,3′,4′,6′-tetra-O-acetyl-benzyl-α-pyranoside | Z 46 | 245–246 | −25 M | [229] |
| (monohydrate) | | 272–273 | +97 M | [171] |
| penta-O-acetyl- | Z 50 | 254–255 | +67 C | [171] |
| N-acetyl- | K,Z 58 | 242 | +65 C | [33] |
| N′-dibenzylphosphoryl-, benzyl-α-pyranoside | | 230–231 | −15 M | [25] |
| N′-diphenylphosphoryl-hexa-O-acetyl-, β-anomer | K 41 | 206–207 | −5 C | [25] |
| benzyl-α-pyranoside, penta-O-acetyl- | K 65 | 179–181 | +68 C | [25] |
| Glc*p*N-β-(1→4)-2-deGlc | | | | |
| N-acetyl-, *p*-nitrophenyl-β-pyranoside | E 12 | | | [159] |
| Glc*p*N-α-(1→6)-3-$NH_2$-3-deGlc | | | | |
| dihydrochloride | | amorph. | +76 W | [125] |
| N′-(2,4-dinitrophenyl)-3′,4′,6′-tri-O-acetyl-1,2-O-isopropylidene-, 3-azido analogue, α-furanose | K 37 | 203–205 | +58 C-M | [125] |
| Glc*p*N-β-(1→6)-3-$NH_2$-3-deGlc | | | | |
| dihydrochloride | | amorph. | +15 W | [125] |
| 1,2-O-isopropylidene-, 3-azido analogue, α-furanose | | | | |
| N′-(2,4-dinitrophenyl)-3′,4′,6′-tri-O-acetyl- | K 12 | 90–95 | +15 M | [125] |
| N′-trifluoroacetyl- | | amorph. | −16 M | [125] |
| 3′,4′,6′-tri-O-acetyl- | K 65 | syrup | −18 M | [125] |

[e] Obtained by acid reversion of N-acetyl-D-glucosamine.

*(Table II — continued)*

| Oligosaccharides[a] and their derivatives | Method of synthesis and yield,[b] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| Glc*p*N-β-(1→4)-MurNAc | | | | |
| methyl ester | | | | |
| N-acetyl-tetra-O-acetyl-, α-anomer | | 237–238 | +38 C | [121] |
| N-diphenylphosphoryl-3′,4′,6′-tri-O-acetyl-1,6-anhydro- | K 15 | 203–204 | −29 C | [121] |
| Glc*p*N-β-(1→6)-MurNAc | | | | |
| N-acetyl- (monohydrate) | | amorph. | +16→+14 W | [74] |
| penta-O-acetyl-, methyl ester | K 6 | 240–241 | +40 C | [150] |
| benzyl-α-pyranoside | | 227–228 | +87 M | [37] |
| tetra-O-acetyl-, methyl ester | K 3.5 | 252–253, 260 | +58 C | [37] |
| methyl-α-pyranoside, tetra-O-acetyl-, methyl ester | K 12 | 288–289 | +54 C | [36] |
| phenyl-β-pyranoside | | 235–237 | −32 W | [218] |
| 3′,4′,6′-tri-O-acetyl-, methyl ester | K 1.3 | 261–262 d. | −7 C | [218] |
| Gal*p*N-α-(1→3)-Glc | | | | |
| N-acetyl- | | 212–215 | +177→+187 W | [99] |
| 1,2 : 5,6-di-O-isopropylidene-, α-furanose | 35[c] | 223–224 | +95 D | [99] |
| 3′,4′,6′-tri-O-acetyl-,2′-oximino analogue | NC 53 | 149–150 | +62 C | [96] |
| Gal*p*N-β-(1→3)-Glc | | | | |
| N-acetyl- | | 208–210 | +78→+50 W | [73] |
| 3′,4′,6′-tri-O-acetyl-1,2 : 5,6-di-O-isopropylidene-, α-furanose | Z 52 | amorph. | −23 C | [72, 73] |
| Gal*p*N-β-(1→6)-Glc | | | | |
| N-acetyl- | | 186–187 d. | +36→+18 W | [73] |
| hepta-O-acetyl-, β-anomer | Z 74 | 193–194 | −12 C | [72, 73] |
| Gal*p*N-β-(1→3)-Gal | | | | |
| N-acetyl- | | 163–165 | +56 W | [165] |
| hepta-O-acetyl-, α-anomer | | 96–97 | +59 C | [165] |
| penta-O-acetyl-1,6-anhydro- | | 208 | −66 C | [165] |
| N-dichloroacetyl-2-O-acetyl-1,6-anhydro-3′,4′,6′-tri-O-benzoyl- | K 15 | 139–140 | +5 C | [165] |
| Gal*p*N-β-(1→4)-Gal | | | | |
| N-acetyl- | | 148–150 | +56 W | [165] |
| hepta-O-acetyl-, α-anomer | | 104–106 | +43 C | [165] |
| penta-O-acetyl-1,6-anhydro- | | 122–123 | −27 C | [165] |

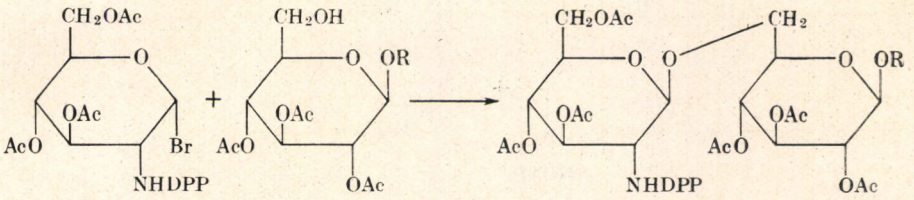
[f] Including the steps of removal of the protective groups.

*(Table II — continued)*

| Oligosaccharides[a] and their derivatives | Method of synthesis and yield,[b] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| N-dichloroacetyl-2-O-acetyl-1,6-1,6-anhydro-3′,4′,6′-tri-O-benzoyl- | K 30 | 194–195 | −2 C | [165] |
| Gal*p*N-α-(1→6)-Gal | | | | |
| N-acetyl- | | | +137 W | [99] |
| 1,2 : 3,4-di-O-isopropylidene- | 29[c] | 151–153 | +69 D | [99] |
| 3′,4′,6′-tri-O-acetyl-,2′-oximino analogue | NC 80 | 171–172 | +14 C | [99] |
| Gal*p*N-β-(1→6)-Gal | | | | |
| N-acetyl- | | 204–205 | +38 W | [3] |
| | | 182–184 | +38 W | [119] |
| | | 204–206 | +44 W | [73] |
| 3′,4′,6′-tri-O-acetyl-1,2,3-tri-O-benzoyl-, α-anomer | Z 44 | 209–211 | +114 C | [119] |
| 1,2 : 3,4-di-O-isopropylidene- | | 142–144 | −62 C | [3] |
| | Z 81 | 141–142 | −63 C | [72, 73] |
| 2′-oximino analogue | NC 9 | 90–92 | −94 C | [96] |
| N-dichloroacetyl-1,2,3,4-tetra-O-acetyl-3′,4′,6′-tri-O-benzoyl- | K 76 | 156–157 | +4 C | [3] |
| 1,2 : 3,4-di-O-isopropylidene-3′,4′,6′-tri-O-benzoyl- | K 90 | 125 | −4 C | [3] |
| Gal*p*N-β-(1→6)-GlcN | | | | |
| di-N-acetyl-, *p*-nitrophenyl-β-pyranoside | | 208–210 | −41 P | [73] |
| penta-O-acetyl- | Z 71 | 226–227 | −28 M | [72, 73] |
| Man*p*N-(1→6)-Glc | | | | |
| N-acetyl-(dihydrate) | Z 55[f] | amorph. | +36 W | [11] |
| Tal*p*N-α-(1→3)-Glc | | | | |
| N-acetyl- | | 190–195 | +82→ +70 W | [99] |
| 1,2 : 5,6-di-O-isopropylidene-, α-furanose | 47[c] | 163–165 | +27 D | [99] |
| Tal*p*N-α-(1→6)-Gal | | | | |
| N-acetyl- | | | +65 W | [99] |
| 1,2 : 3,4-di-O-isopropylidene- | 59[c] | 119–121 | −10 D | [99] |
| Glc*p*N-α-(1→1)-α-Glc*p* | K 18 | 197 d. | +146 W | [188] |
| octa-acetyl- | | 99 | +152 C | [188] |
| Glc*p*N-β-(1→1)-β-Glc*p*N | | | | |
| octa-acetyl- | K 2.3 | 324—326 d. | +2 C | [65] |
| Glc*p*N-α-(1→1)-α-Man*f* | | | | |
| 2,3 : 5,6-di-O-isopropylidene-3′,4′,6′-tri-O-acetyl-, 2′-oximino analogue | NC 41 | 86–87 | +88 C | [96] |

*(Table II — continued)*

| Oligosaccharides[a] and their derivatives | Method of synthesis and yield,[b] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| Glc*p*N-$\beta$-(1→1)-1-S-$\beta$-Glc*p*N | | | | |
| di-N-acetyl- | | 219–220 | −37 W | [4] |
| hexa-O-acetyl- | K 50 | 314–316 d. | −146 C | [4] |
| Glc*p*N-$\beta$-(1→3)-Gal*p*-$\beta$-(1→4)-Glc | | | | |
| N-acetyl- | | 205–209 | +40 W | [1] |
| N-dichloroacetyl-, benzyl-$\beta$-pyranoside | | 194–195 | −23 M | [1] |
| 2,3,6,2′,6′-penta-O-acetyl-3″,4″,6″-tri-O-benzoyl- | K 19 | 125–127 | −50 C | [1] |
| Glc*p*NAc-$\beta$-(1→4)-Glc*p*NAc-$\beta$-(1→6)-Glc | Z 29[f] | amorph. | +1 W | [229] |
| Glc*p*NAc-$\beta$-(1→3)-Glc*p*NA-$\beta$-(1→6)-GlcNAc | | | | |
| *p*-nitrophenyl-$\beta$-pyranoside | | 209–210 | +8 M-W | [226] |
| hepta-O-acetyl- | Z 78 | 280–281 | +75 C-M | [226] |
| Glc*p*NAc-$\beta$-(1→4)-Glc*p*NAc-$\beta$-(1→6)-GlcNAc | | | | |
| *p*-nitrophenyl-$\beta$-pyranoside (dihydrate) | | 239–241 d. | −48 W | [229] |
| hepta-O-acetyl- | Z 30 | 289–290 d. | −31M-N | [229] |
| Glc*p*NAc-$\beta$-(1→6)-Glc*p*NAc-$\beta$-(1→4)-GlcNAc | | | | |
| *p*-nitrophenyl-$\beta$-pyranoside | | 257.5–259 | −2 W | [227] |
| 2,6,3′,3″,4″,6″-hexa-O-acetyl- | Z 14 | 273.5–275 | −44 N | [227] |
| Glc*p*NAc-$\beta$-(1→4)-MurNAc-$\beta$-(1→4)-GlcNAc | E 15 | | −20 W | [153] |
| Glc*p*NAc-$\beta$-(1→4)-MurNAc-$\beta$-(1→4)-Xyl | E 9 | | −38 W | [153] |
| Glc*p*NAc-$\beta$-(1→4)-MurNAc-$\beta$-(1→2)-Gal | E 13 | | −14 W | [153] |
| Glc*p*NAc-$\beta$-(1→4)-Glc*p*NAc-$\beta$-(1→6)-Glc*p*NAc-$\beta$-(1→4)-GlcNAc | | | | |
| *p*-nitrophenyl-$\beta$-pyranoside | Z 13[f] | 248–249 | −28 W | [227] |

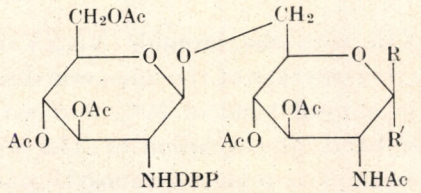


**XXXII** DPP = P(O)(OPh)$_2$

**XIX** (R = Ac)
**XXXIII** (R = $CH_2Ph$)

**XXXIV** (R = Ac)
**XXXV** (R = $CH_2Ph$)

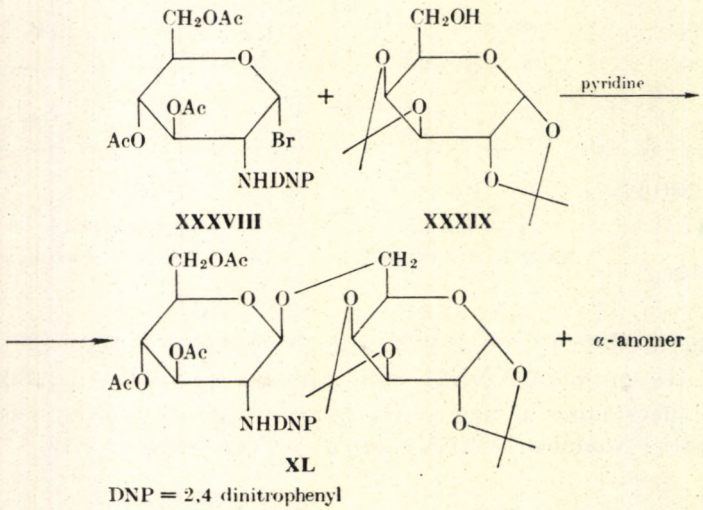
Satisfactory results in the glycosylation step were obtained [25] in the reaction of the bromide XXXII with partially protected derivatives of N-acetyl-D-glucosamine having a free hydroxyl group at C-6. The yields of glycosylation products (XXXVI and XXXVII) were 41–65%.



**XXXVI** (R = OAc, R′ = H)
**XXXVII** (R = H, R′ = $OCH_2Ph$)

The diphenylphosphoryl group is removed either by catalytic hydrogenation in the presence of platinum [223] or (less effectively) by alkaline hydrolysis [71].

In the reaction of 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide (XXXVIII) with 1,2 : 3,4-di-O-isopropylidene-α-D-galactopyranose (XXXIX) glycosylation is not stereospecific and depends on the nature of the solvent and the hydrogen bromide acceptor [108, 110]. Like in the case of alkyl glycosaminides synthesized from the bromide XXXVIII [109], in the presence of pyridine the α-anomer is predominantly (30%) formed, the quantity of the β-linked disaccharide (XL) amounting to only 15%. When the reaction was performed in the presence of silver carbonate in nitromethane, only the β-anomer XL was isolated in a yield of 29%. The dinitrophenyl group was removed by treatment with anionites in the base form [112].

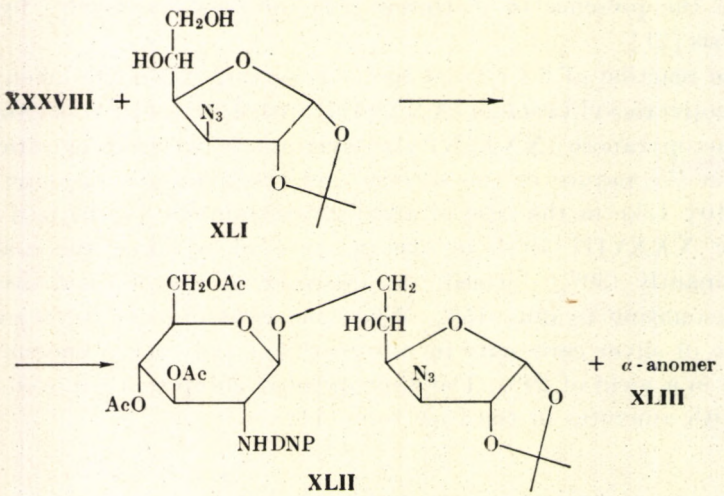



XXXVIII XXXIX

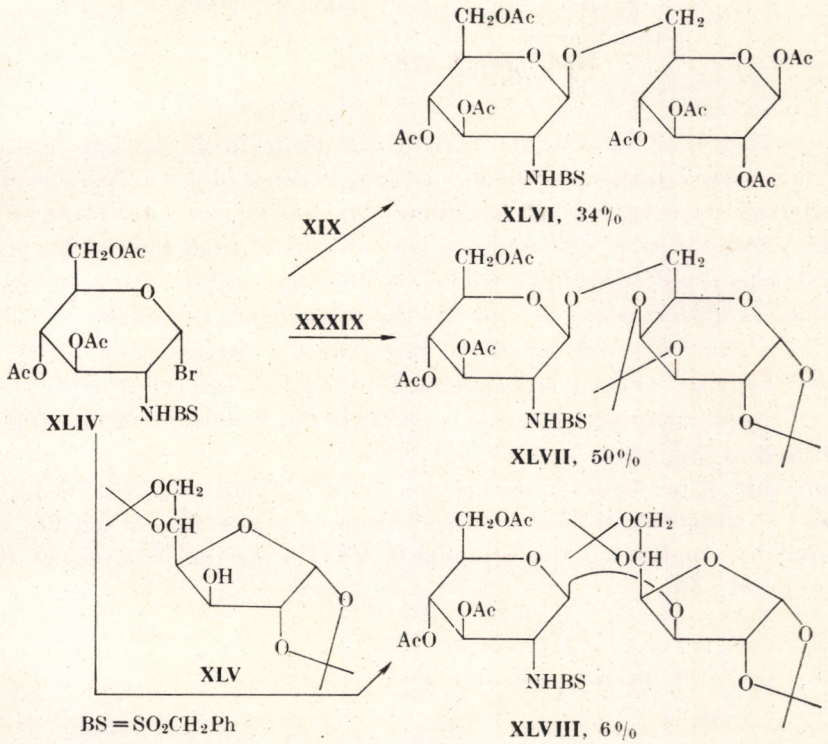
XL

DNP = 2,4 dinitrophenyl

Glycosylation of compound XLI with the bromide XXXVIII in the presence of mercuric salts also gave a mixture of the anomeric disaccharide derivatives (XLII and XLIII) in a total yield of 50% and with a 1 : 3 anomer ratio [125]. On the other hand, glycosylation of XLI with 3,4,6-tri-O-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide in the presence of mercuric cyanide yielded a single disaccharide similar to XLII [125].



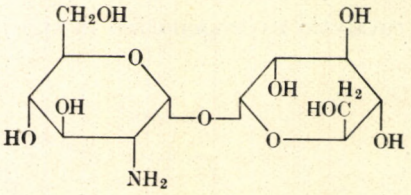
The bromide XLIV was reported to have been used as a glycosylating agent in the Koenigs–Knorr reaction. This resulted in the formation of the disaccharide derivatives XLVI–XLVIII, from which the benzylsulfonyl group was removed by catalytic hydrogenation in the presence of Raney nickel.




Thus the synthesis of (1 → 6)-linked disaccharides exemplifies the advantage of the use of stable glycosyl bromides with electronegative or other "non-participating" substituents in the amino group, giving the glycosylation products in markedly improved yields. The disadvantages of this procedure, for preparative purposes, are that it is a multi-step synthesis, especially when N-acetylated analogues of disaccharides are required, and sometimes the stereospecificity of glycosylation is lost.

Of the small number of synthetic non-reducing disaccharides containing 2-amino-2-deoxy sugars, mention should be made of trehalosamine ( (XLIX) prepared by the reaction of 3,4,6-tri-O-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)α-D-glucopyranosyl bromide with 2,3,4,6-tetra-O-benzyl-

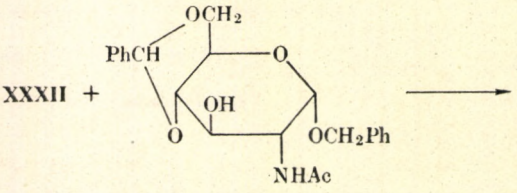
D-glucopyranose in a yield of 18% after the removal of the protective groups [188].



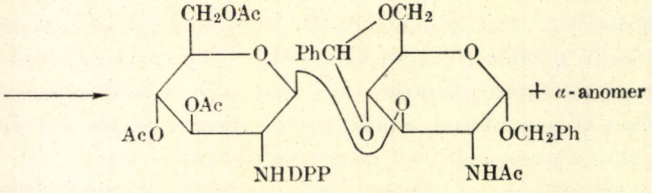

XLIX

Glycosylation of the secondary hydroxyl groups in saccharides has for a long time been a difficult problem, not only because of the absence of effective glycosylating agents in the amino sugar series, but also owing to the limited choice of such 'aglycon' components which could be readily glycosylated. This problem has been solved, on the one hand, by the use of stable bromides as glycosylating agents. On the other hand, pyranose derivatives [121, 156, 166–169] and acyclic monosaccharide derivatives [28, 55, 56] in which hydroxyl groups, especially at C-4, display a higher reactivity than in the *C*1 conformation of the pyranose form, have recently been proposed to be used as "aglycons".

A mixture of protected disaccharides (in 31% yield) with $\beta$-(1 → 3)- (LI) and $\alpha$-(1 → 3)-glucosaminidic bonds, containing about 85% of LI, has been prepared by condensing the bromide XXXII with the benzylidene derivative L [54, 55].



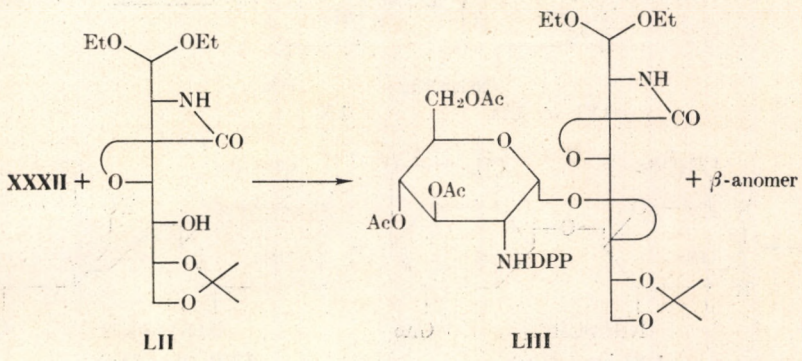

L



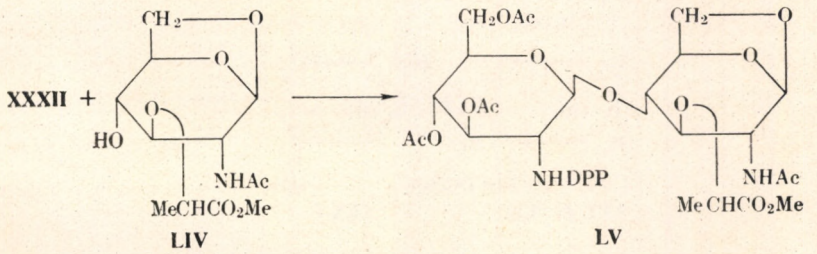

LI

Similarly, from the bromide XXXII and the acetal LII a mixture of anomeric disaccharide derivatives with $\alpha$-(1 → 4)- (LIII) and $\beta$-(1 → 4)-

glycosidic bonds have been synthesized, the yields being 35 and 10%, respectively [55, 56].

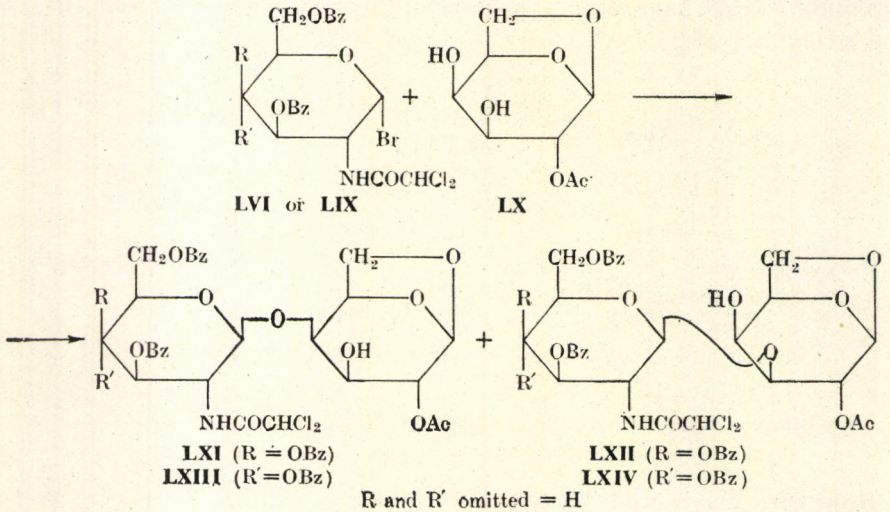



However, Merser and Sinaÿ [121] (*cf.* [25, 71] did not observe the formation of any β-linked disaccharide after glycosylation of the anhydro derivative LIV with the bromide XXXII; they reported the isolation of a protected disaccharide LV in a yield of 15%.

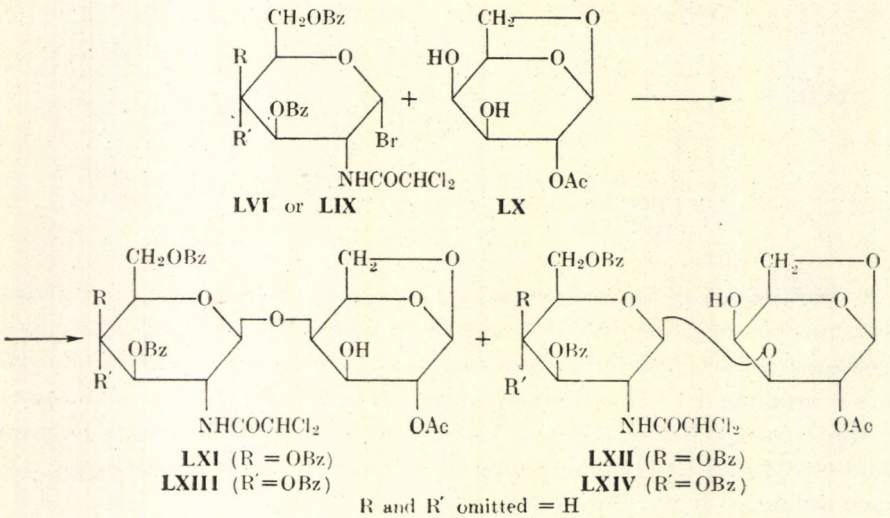



A synthesis of di- and trisaccharides which are fragments of carbohydrate determinants of glycoproteins and gangliosides and contain 2-acetamido-2-deoxy-β-D-galactopyranosyl residues, involving a reactive and relatively stable bromide LVI, has recently been reported [2, 3]. This modification of the Koenigs–Knorr reaction leads to the derivatives with β-glycosidic bond (LVII and LVIII) containing, as evidenced by IR spectroscopy, only trace amounts of α-anomers [3].

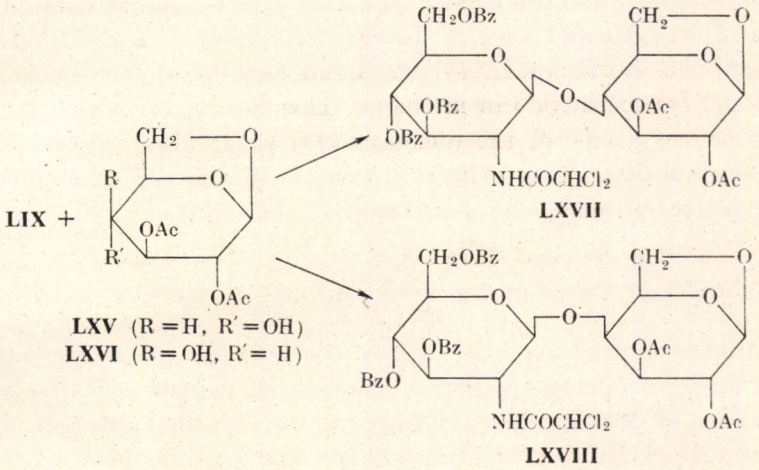
The bromide LVI and its D-*gluco* analogue (LIX) react with the 1,6-anhydro sugar LX to give mixtures of isomeric disaccharide derivatives with β-(1→4)-(LXI and LXIII) and β-(1 → 3)-glycosidic bonds (LXII and LXIV) in total yields of 45 and 47%, respectively [165, 167]. In both cases the ratios of isomers LXI : LXII and LXIII : LXIV were about 3 : 2. The glycosy-




lation products (**LXI** and **LXII**) were separated chromatographically. In another case the product **LXIII** was isolated after the (1 → 3)-isomer (**LXIV**) had been decomposed under mild alkaline conditions.

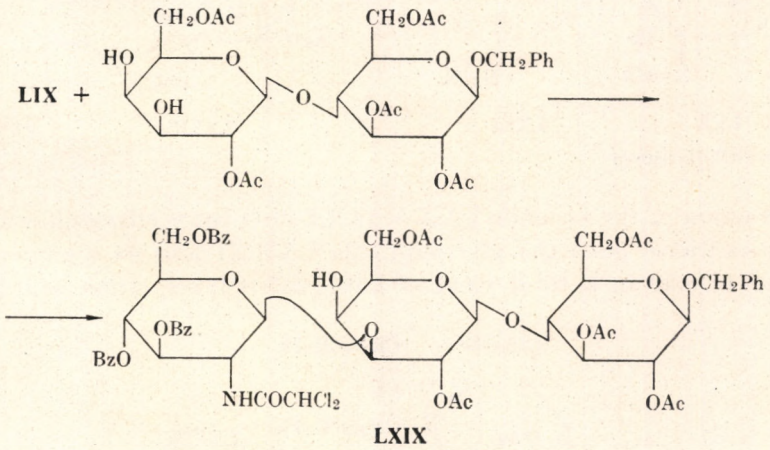



It is of interest that glycosylation of 2,3-di-O-acetates of 1,6-anhydro sugars (**LXV** and **LXVI**) gives, under the same conditions [167, 168], disaccharide derivatives (**LXVII** and **LXVIII**) in yields as low as 9 – 10%. However, glycosylation of the 1,6-anhydride **LXV** (or its analogue, i.e.




2-acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose) with neutral sugar glycosyl halides is much more effective, giving 50—80% yields of derivatives of lactose, N-acetyllactosamine, and N-acetylcellobiosamine [156, 169].

The same authors [1] reported to have achieved a synthesis of the protected trisaccharide **LXIX** in 19% yield.

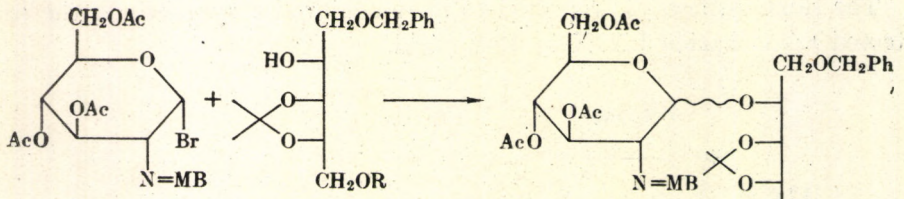



The most convenient method of conversion of the N-dichloroacetyl group into N-acetyl group turns out to be catalytic hydrogenolysis in the presence of palladium. Another route, involving hydrolysis of the N-dichloroacetyl group to the amino group with barium hydroxide followed by N-acetylation,

is applicable only for the derivatives with β-(1 → 4)-glycosaminidic bond (for example, LXI and LXIII). In the derivatives with β-(1 → 3)-glycosaminidic bond (LXII and LXIV), the bond is ruptured even under the action of sodium methoxide in methanol. This fact is accounted for by the high electronegativity of the dichloroacetyl group drawing the electrons from the glycosidic centre, which, in turn, facilitates degradation of the β-(1 → 3)-isomer through the β-elimination mechanism.

## 3. GLYCOSYLATION OF POLYALCOHOLS AND HYDROXY AMINO ACIDS

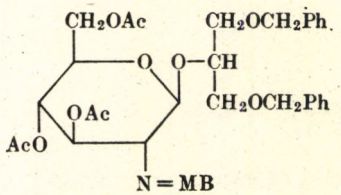
The products of glycosylation of polyalcohols and hydroxy amino acids may be used as model compounds in studies of glycolipids and glycoproteins. Derivatives of 2-amino-2-deoxy-D-glucopyranosyl-D-ribitol and -glycerol have been prepared by the Koenigs–Knorr reaction from glycosyl bromides and partially protected polyalcohols. Glycosylation of D-ribitol derivatives (LXXI, R = $CPh_3$ or COPh) by the bromide LXX in the presence of silver carbonate gives the anomeric glycosaminide mixtures (LXXII, R = $CPh_3$ or COPh) in 15% yield, in which the α-anomers prevail [52].




LXX LXXI LXXII

MB = $CHC_6H_4OMe$-p

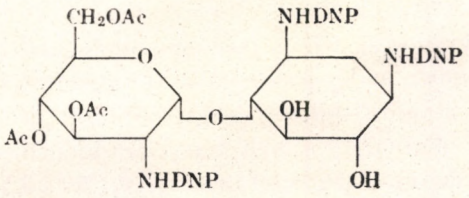
Interaction of the bromide LXX with 1,3-di-O-benzylglycerol, under the same conditions, gave the glycosaminide LXXIII and its α-anomer in a ratio of 1 : 0.39 in a total yield of 25% [51]. Glycosylation of 1,3-di-O-



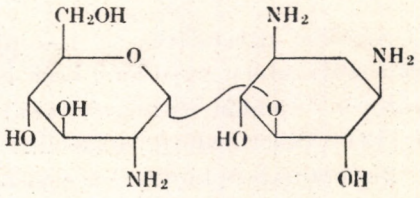

LXXIII

benzylglycerol by the bromide XXXII in the presence of mercuric cyanide yielded only the β-anomer, similar to LXXIII, in a yield of 55% [51].

The use of the bromide XXXVIII in the glycosylation of di-N-substituted 2-deoxystreptamine resulted in a mixture of glycosides, from which isomer LXXIV was isolated [185–187], which is a derivative of the antibiotic paromamine. A related antibiotic, LXXV, has also been synthesized [189].

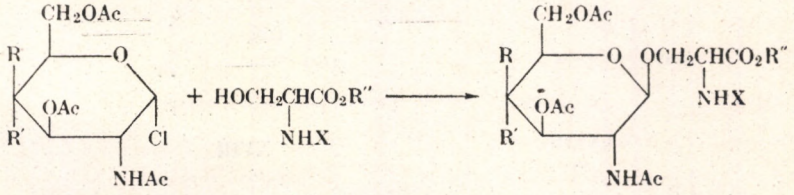



LXXIV




LXXV

The Koenigs–Knorr reaction has long been the major method of synthesizing 2-acetamido-2-deoxyglycosides of hydroxy amino acids. Condensation of the chloride XIII or its D-*galacto* analogue (LXXVI) with esters of N-benzyloxycarbonyl- or N-(2,4-dinitrophenyl)-L-serine in the presence of silver carbonate or mercuric cyanide gave the corresponding $\beta$-glycosaminides (LXXVII, R and R′ = H or OAc, R″ = Me or $CH_2Ph$, X = $CO_2CH_2Ph$ or 2,4-$(NO_2)_2C_6H_3$) in yields of up to 49% [24, 75, 190, 200].




XIII or LXXVI LXXVII

Under the same conditions N-benzyloxycarbonyl-DL-threonine amide was glycosylated with the chloride XIII [170]. It should be noted that the isolation of the glycosylation products is often a labour-consuming process.

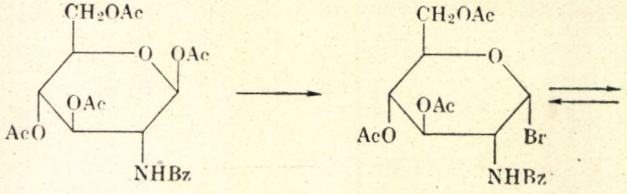
*

Thus, in spite of the fact that many investigations have dealt with the classical Koenigs–Knorr reaction and its modifications, very few systematic studies of glycosylation of partially protected saccharides and other "agly-

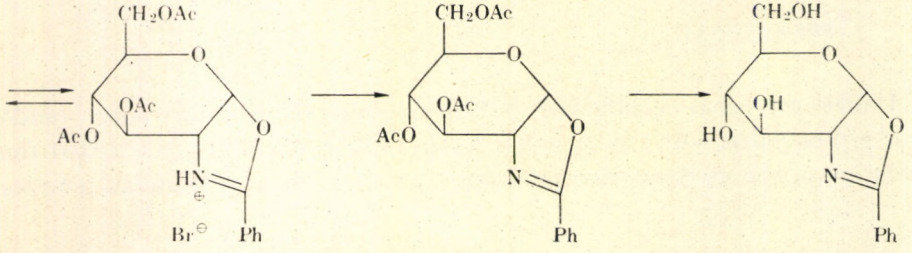
con" components of complex structures have been done. Therefore, though the method may be considered well-explored, the reaction conditions should be carefully chosen in every particular case.

## IV. 2-SUBSTITUTED GLYCO[2′,1′:4,5]-2-OXAZOLINES

Oxazoline derivatives of hexopyranoses, which are aza-analogues of sugar orthoesters, have long been known as by-products in the reaction of hydrogen bromide with acetylated 2-amino-2-deoxy sugars. White [202], (*cf.* [131]) described an "oxazoline" derivative which was later identified as 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-glucopyranose [76, 90, 92]. Micheel *et al.* [130, 132] were the first to isolate 2-phenyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano) [2′, 1′ : 4,5]-2-oxazoline hydrobromide (LXXIX) formed under the action of hydrogen bromide in acetic acid on the N-benzoate LXXVIII. The bromide XVIII which is the first to be formed, is readily converted into an oxazoline derivative (LXXIX). This process was later established to be an equilibrium one [193], the equilibrium being largely dependent on the nature of the solvent (as in the case of the O-benzoylated analogue of the bromide XVIII).




LXXVIII XVIII



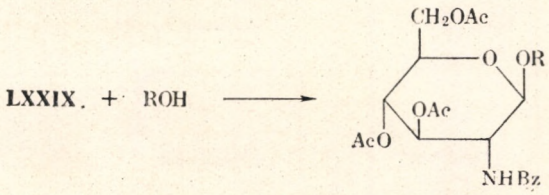

LXXIX LXXX LXXXI

Under similar conditions, the α-chloride XIII does not form an oxazoline derivative [135]. It follows from the data of Osawa [146] (see the scheme, page 11) that oxazoline derivatives are the intermediates in the synthesis

of O-acetylated 2-aroylamido-2-deoxy-α-D-glucopyranosyl chlorides from the corresponding β-acetates. When treated for a short period of time with aluminium chloride or titanium tetrachloride in chloroform, as well as with hydrogen chloride in acetic anhydride, these acetates were converted into O-acetylated 2-aryl-gluco[2′, 1′ : 4,5]-2-oxazolines in yields of up to 75%. If the reaction was continued, the yield of oxazoline derivatives decreased, and that of α-chlorides increased.

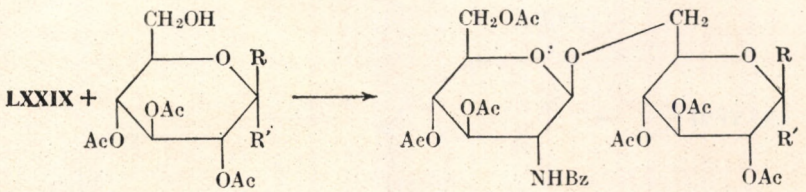
The hydrobromide LXXIX is converted by pyridine into a free base LXXX which may be deacetylated to the oxazoline LXXXI without cleavage of the heterocyclic ring [135].

The oxazoline derivatives LXXIX and LXXX proved to be effective glycosylating agents in the synthesis of 1,2-*trans*-2-benzamido-2-deoxy-D-glucopyranosides. The reaction of LXXIX with primary, secondary and tertiary alcohols, as well as with N-benzyloxycarbonyl-L(D or DL)-serine methyl ester in the presence of silver carbonate, gave acetates of 2-benzamido-2-deoxy-β-D-glucopyranosides (LXXXII) in 37–73% yields [132, 133, 135].




**LXXXII** (R=Me, Et, iso-Pr, *t*-Bu, $CH_2Ph$, $CHPh_2$·$CH_2CH(NHCO_2CH_2Ph)CO_2Me$)

Interaction of the oxazoline LXXIX with glucose tetraacetate (XIX) and with glycoside triacetate (LXXXIII) was the first example of sugar oxazoline derivatives being used in the synthesis of disaccharides with 2-benzamido-2-deoxy-β-D-glucopyranosyl residues, for instance, LXXXIV and LXXXV [128].



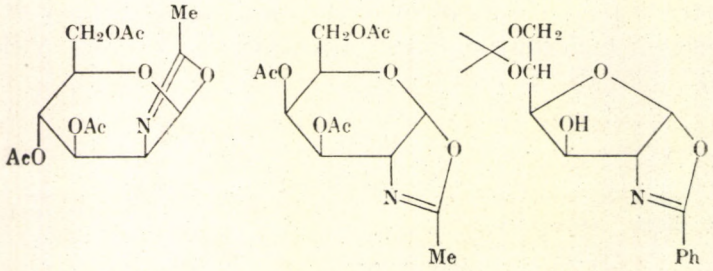

**XIX** (R=OAc)
**LXXXIII** (R′=OMe)

**LXXXIV** (R=OAc), 55%
**LXXXV** (R′=OMe), 51%

R and R′ omitted=H

For a long time no further progress was made in using sugar oxazoline derivatives in the synthesis of glycosaminides. No glycosylation method could be suggested without reliable general methods for producing the glycosylating agents themselves. This refers, first of all, to 2-methyl-glyco-[2′, 1′ : 4,5]-2-oxazolines which would have led to glycosides and oligosaccharides containing 2-acetamido-2-deoxyhexopyranosyl residues. For a long time general procedures for synthesizing sugar oxazoline derivatives were not available.

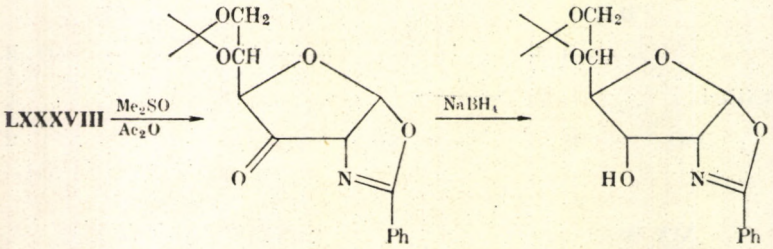
A 2-methyl substituted oxazoline of the D-*manno* series (LXXXVI) was prepared by treating acetylated D-mannosamine with hydrogen bromide in acetic acid [132]. Fletcher *et al.* [155] reported the formation of the oxazoline LXXXVI and its D-*galacto* analogue (LXXXVII) in yields of 11 and 26%, respectively, as by-products in the acetylation of N-acetyl-D-




LXXXVI LXXXVII LXXXVIII

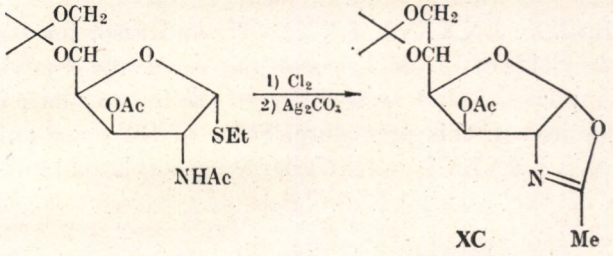
mannosamine and N-acetyl-D-galactosamine with acetic anhydride in the presence of a large amount of zinc chloride.

An oxazoline derivative of D-glucofuranose (LXXXVIII) was prepared by the treatment of N-benzoyl-D-glucosamine with acetone in the presence of hydrogen chloride [80, 106, 123]. Epimerization of the oxazoline LXXXVIII at C-3 gave the corresponding analogue of the D-*allo* series (LXXXIX) [124].



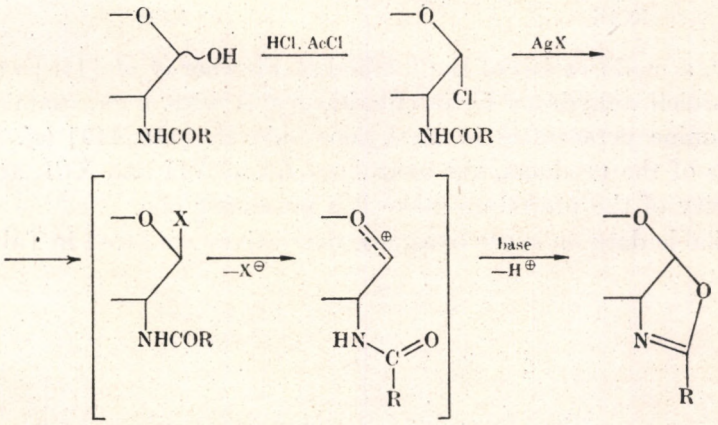

LXXXIX

The 2-methyl analogue of the oxazoline LXXXVIII, 2-methyl(3-O-acetyl-5,6-O-isopropylidene-1,2-dideoxy-α-D-glucofurano)-[2′,1′ : 4,5]-2-oxazoline (XC), was first synthesized by Wolfrom and Winkley [213] in 56% yield.




Until recently, the examples of sugar oxazoline derivatives being used in glycoside synthesis have been scanty and confined to the synthesis of glycosaminides (including furanosides) of lower alcohols and phenol [45, 122–124, 213]. The oxazoline group was mostly used for protecting the substituents at the C-1 and C-2 atoms in the synthesis of muramic acid and its derivatives [43, 44, 36, 106].

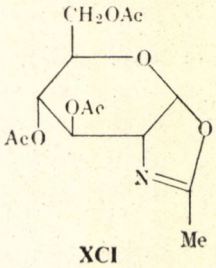
In 1968 in the authors' laboratory a general method of synthesizing 2-substituted glyco[2′,1′ : 4,5]-2-oxazolines was developed [77, 78]. The starting compounds were O-acetylated 2-acylamido-2-deoxy-α-D-glycosyl chlorides formed in high yields when N-acylated 2-amino-2-deoxy sugars were treated with hydrogen chloride in acetyl chloride [228]. The treatment of the chlorides with silver salts (nitrate, perchlorate, tosylate) in anhydrous acetone or acetonitrile [73] in the presence of organic bases (pyridine or



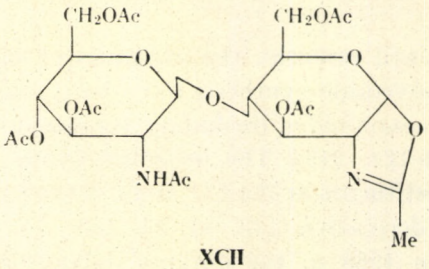

R=Me, Ph; X=$NO_3$, $ClO_4$, $OSO_2C_6H_4Me$-*p*

*sym.*-collidine) gave the corresponding oxazoline derivatives. The reaction seems to proceed *via* the formation of a carbonium cation which then becomes stabilized by conversion into the oxazoline. The best results were obtained with the use of silver nitrate and *sym.*-collidine.

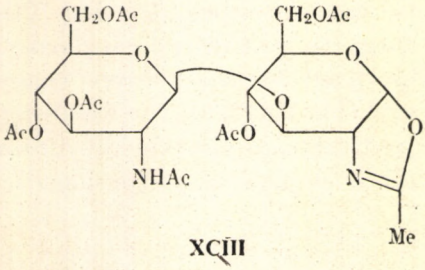
This synthesis was used to obtain oxazoline derivatives of mono-and disaccharides (LXXX, LXXXVI, LXXXVII, and also, for the first time, XCI—XCIV) in yields of 50—80% as calculated per free N-acylamido sugar. It should be emphasized that no isolation of the intermediate glycosaminyl chlorides is required in this procedure. Some of the oxazoline derivatives obtained (LXXX, LXXXVI, and XCI) were deacetylated in methanol using triethylamine as the catalyst [78].



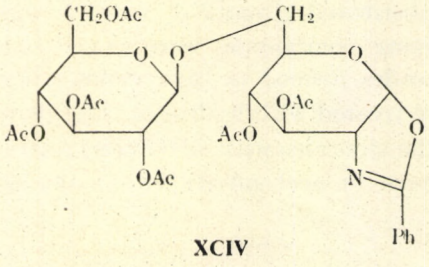

XCI




XCII




XCIII




XCIV

Recently, a modification of the method of Fletcher *et al.* [155] was suggested in which anhydrous ferric chloride reacts with D-glucosamine and D-galactosamine peracetates in methylene chloride [118, 119] (*cf.* [162]). High yields of the products, the oxazolines LXXXVII and XCI, and also the simplicity of the procedure make it a promising one.

The available data on sugar oxazoline derivatives are listed in Table III.

*Table III*

Glyco[2′,1′ : 4,5]-2-oxazolines

| Compound | Yield,[a] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| (1,2-Dideoxy-α-D-glucofurano)-2-oxazoline | | | | |
| 2-methyl-(3-O-acetyl-5,6-O-isopropylidene)- | 56 | 96–97 | +25 C | [213] |
| 2-phenyl-(5,6-O-isopropylidene)- | 61 | 159–160 | +4 C | [80] |
| | 100 | 160–161.5 | | [106] |
| hydrochloride | | 125–128 d. | | [80] |
| (3-O-acetyl)- | | 135–136 | −16 C | [80] |
| (3-O-D-1-carbamylethyl)- | | 192–193 | +73 C | [106] |
| (3-O-L-1-carbamylethyl)- | | 164.5–166 | +6 C | [106] |
| (3-O-carbamylmethyl)- | | 97–98.5 | +0.2 C | [106] |
| (3-O-D-1-carboxyethyl)- | | 163–164 | +63 C | [106] |
| | | 163–164 | +65 C | [43, 44] |
| (2-O-L-1-carboxyethyl)- | | 207–208 | −38 C | [106] |
| | | 208–210 | −34 C | [43, 44] |
| (3-O-carboxymethyl)- | | 185–186 | −22 C | [43] |
| | | 182–184 | −23 C | [106] |
| (3-O-L-1-carboxypropyl)- | | 217–217.5 | −34 C | [106] |
| [3-O-L-1-(methoxycarbonyl)-ethyl]- | | 88–90 | −31 C | [44] |
| (3-O-methyl)- | | 117–119 | −15 C | [43] |
| (3-O-methylsulfonyl)- | | 141–142 | +8 C | [123] |
| 2-phenyl-(5,6-O-benzylidene)- | | 201–202 | +64 C | [172] |
| (3-O-acetyl)- | | 88–89 | +34 C | [172] |
| (1,2-Dideoxy-α-D-glucopyrano)-2-oxazoline | | | | |
| 2-methyl- | | syrup | +13 C | [78] |
| (3,4,6-tri-O-acetyl)- | 51 | syrup | +10 C | [77, 78] |
| | 65 | syrup | +7 C | [162] |
| (4,6-O-benzylidene)- | | syrup | +3 C | [172] |
| 2-*p*-methoxyphenyl-(3,4,6-tri-O-acetyl)- | 74 | 106–108 | +67 C | [146] |
| 2-*p*-nitrophenyl-(3,4,6-tri-O-acetyl)- | 76 | 142–143 | +72 C | [146] |
| 2-phenyl- | | 142 | +48 C | [135] |
| | | 140–141 | +48 C | [78] |
| (3,4,6-tri-O-acetyl)- | 85 | 57 | +45 P-W | [132, 135] |
| | 69 | 56 | +52 C | [146] |
| | 82 | syrup | | [155] |
| | 98 | syrup | +44 C | [77, 78] |
| hydrobromide | 85 | 110 d. | +37 P-W | [130, 135] |
| hydrochloride | | 135 d. | +39 P-W | [146] |
| 2-trifluoromethyl-(3,4,6-tri-O-acetyl)- | — | syrup | +5 C | [125] |

[a] The yields are given only for those derivatives in which the oxazoline ring is formed.

*(Table III — continued)*

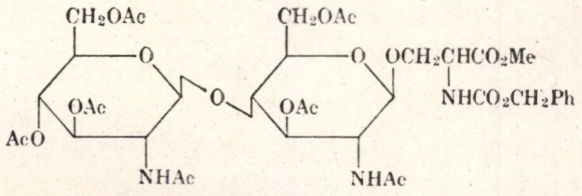
| Compound | Yield,[a] % | Melting point, °C | $[\alpha]_D$ (°) and rotation solvent | References |
|---|---|---|---|---|
| 2-Methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-galactopyrano)-2-oxazoline | 26 | syrup | +26 C | [155] |
| | 66 | syrup | +26 C | [119] |
| | 59 | syrup | +82 C | [72, 73] |
| 2-Methyl-(1,2-dideoxy-β-D-manno-pyrano)-2-oxazoline | | 166.5–167.5 | +9 C | [78] |
| (3,4,6-tri-O-acetyl)- | 11 | 133–134 | −31 C | [155] |
| | 82 | 132–133 | −31 C | [77, 78] |
| (4,6-O-benzylidene)- | | 172.5–174.5 | −23 C | [172] |
| 2-Phenyl-(1,2-dideoxy-α-D-allo-furano)-2-oxazoline | | | | |
| (5,6-O-isopropylidene)- | | 129–130 | +75 C | [124] |
| (3-keto) analogue | | 99–101 | +380 C | [124] |
| 2-Methyl-[4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-acetyl-1,2-dideoxy-α-D-glucopyrano]-2-oxazoline | 59 | 189–190 | −8 C | [78] |
| 2-Methyl-[3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4,6-di-O-acetyl-1,2-dideoxy-α-D-glucopyrano]-2-oxazoline | 53 | 181–182 | +8 C | [226] |
| 2-Phenyl-[6-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-3,4-di-O-acetyl-1,2-dideoxy-α-D-glucopyrano]-2-oxazoline | 60 | | +60 C | [77] |

## V. OXAZOLINE SYNTHESIS OF 1,2-*TRANS*-2-ACETAMIDO-2-DEOXYGLYCOSIDES

2-Methyl-glyco[2′,1′ : 4,5]-2-oxazolines, now available, are being studied, first of all, as glycosylating agents in the reactions with "aglycons" of complex structure. A detailed study has been made of the behaviour of sugar oxazoline derivatives in the synthesis of N-acetylglycosaminides, including amino sugar oligosaccharides, in the presence of acid catalysts, mainly *p*-toluenesulfonic and other acids [9, 79].

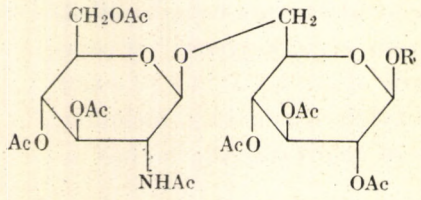
Alkyl [9, 72, 73, 79, 119, 135] and aryl 2-acetamido-2-deoxy-β-D-hexopyranosides [182, 225] are formed in high yields when the oxazolines LXXXVII and XCI react with alcohols and phenols in the presence of the above catalysts. Similarly, glycosylation of some monoterpenic alcohols with the oxazoline XCI gave *l*-bornyl and *l*-menthyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosides in 40–43% yield [182].

The effectiveness of the oxazoline method in the synthesis of the compounds serving as models of glycopeptide fragments of biopolymers was exemplified by the glycosylation of N-benzyl-oxycarbonyl-L-serine esters with oxazoline derivatives of mono- [9, 73] and disaccharide [105]. As a result, protected L-serine O-glycosides (LXXVII, R and R′ = H or Ac, R″ = Me or $CH_2Ph$, X = $CO_2CH_2Ph$; and XCV) were obtained in high yields (55–75% for the mono- and 40% for the disaccharide).

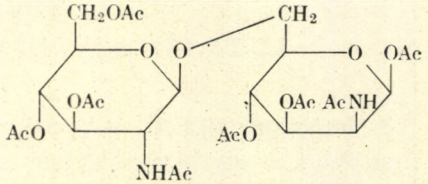



XCV

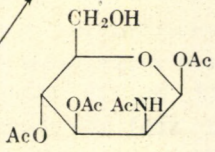
The oxazoline method has proved to be most successful in oligosaccharide synthesis (see Table II). Glycosylation by the oxazoline XCI of the primary hydroxyl group of derivatives of D-glucose (XIX and XXXIII) [11, 71], D-galactose (XXXIX) [11], D-mannose (XCVI) [118], and N-acetyl-D-glucosamine (XCVIII, R = H or Ac; and XCVIII) [77, 171, 229] gave the corresponding disaccharide derivatives (XXIII, XXVII, XCIX–CIII) in high yields, mostly amounting to 50%.



**XXIII** (R=Ac), 46%
**XCIX** (R=$CH_2Ph$), 46%

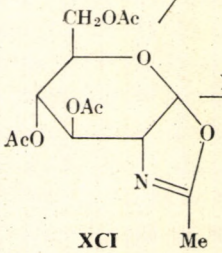


**CI**, 26%

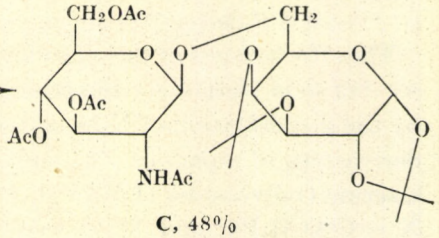


**XCVI**

**XIX** or **XXXIII**

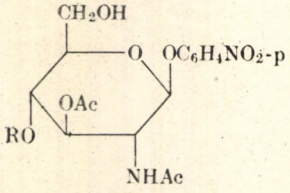


**XXXIX**

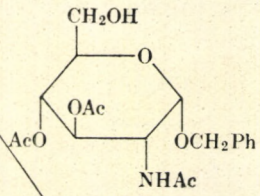


**C**, 48%

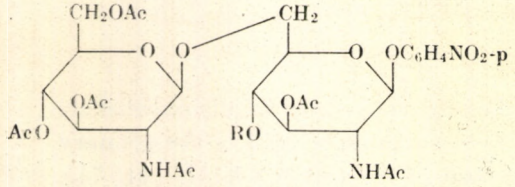
**XCI** Me



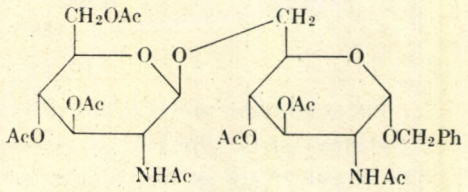
**XCVII**



**XCVIII**

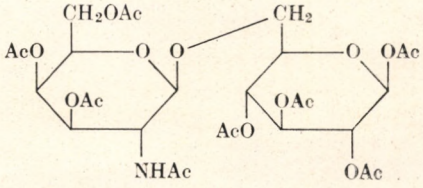


**XXVII** (R=Ac), 49%
**CII** (R=H), 46%

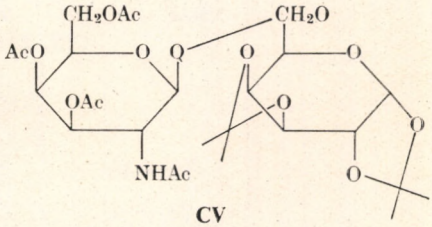


**CIII**, 50%

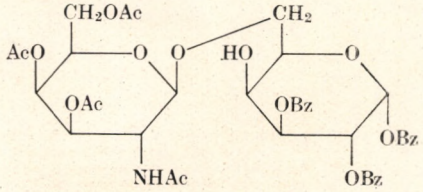
In the synthesis of (1 → 6)-linked disaccharides, oxazoline derivatives of the D-*galacto* [72, 73, 119] and D-*manno* series [11] proved just as effective, from which protected disaccharides CIV–CVIII were obtained in yields of 44–81%.



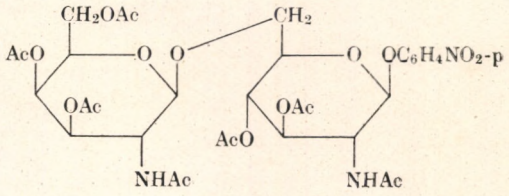

CIV



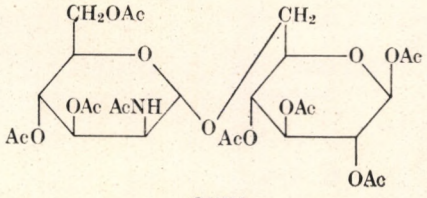

CV




CVI



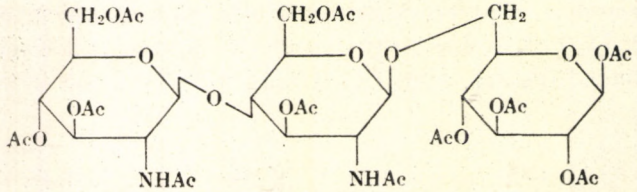

CVII




CVIII

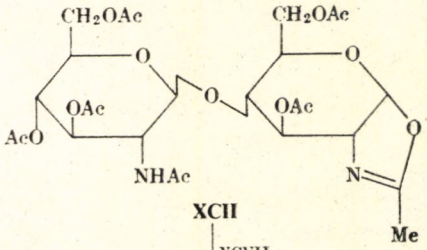
Glycosylation of partially protected monosaccharides with the oxazoline derivative of disaccharide (XCII) allowed the addition of two 2-acetamido-2-deoxy-β-D-glucopyranosyl residues to the oligosaccharide chain within a single step, which was demonstrated by the synthesis of the trisaccharide derivatives CIX [11] and CX [229]. This could not have been realized by other glycosylation procedures.

Oxazoline derivatives, like other glycosylating agents, possess a high selectivity with respect to the primary hydroxyl groups of saccharides, as compared with the secondary ones. As a result, branched oligosaccharides, when their formation was not excluded, were only detected in trace amounts, if at all [33, 229]. This allowed oligosaccharide derivatives having non-protected hydroxyl groups at C-4 and C-6 atoms in the terminal monosaccha-



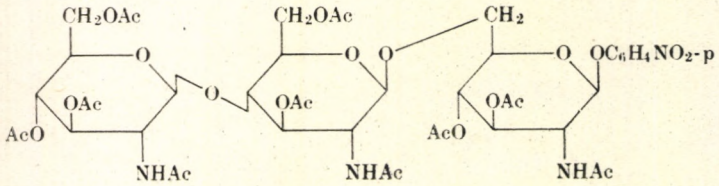
**CIX**, 29 %

XIX



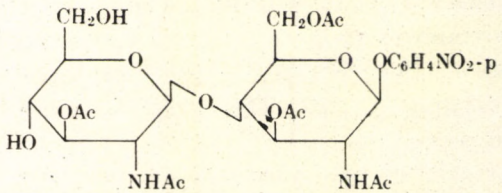
**XCII**

XCVII
(R = Ac)



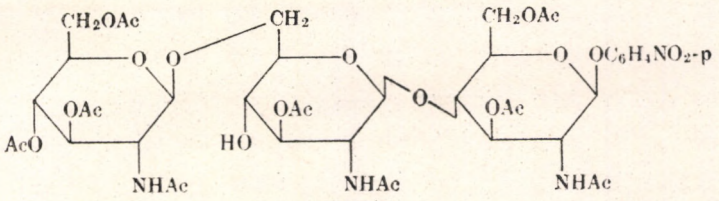
**CX**, 30 %

ride residue (for example, CXI), which are readily available from the corresponding 4,6-O-benzylidene derivatives, to be used as the component to

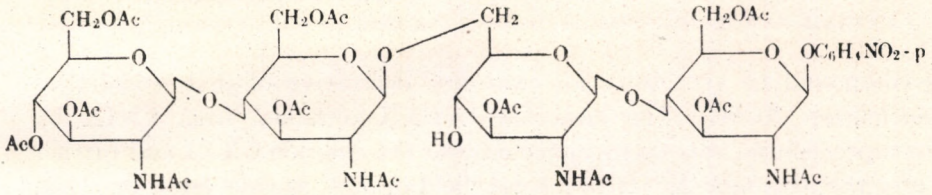


**CXI**

undergo glycosylation. In the reaction between the compound CXI and the oxazoline derivatives of mono- (XCI) and disaccharide (XCII) [227], tri- and tetrasaccharide glycosides (CXII and CXIII) were obtained. The low yields of these products (not exceeding 15%) are due to an extremely low



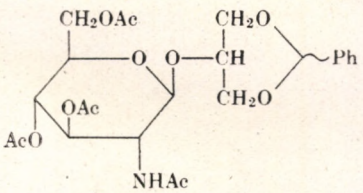
CXII



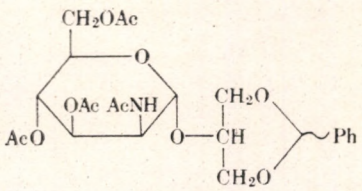
CXIII

solubility of the glycosylated component (CXI) even in such a polar solvent as nitromethane. In strongly polar solvents, *p*-toluenesulfonic acid catalyzes the isomerization of 2-methyl-glyco[2′, 1′ : 4,5]-2-oxazolines into 2-acetamidoglycals [162] which is, apparently, a competitive process in the cases considered.

The efficiency of the oxazoline method was especially high in the glycosylation of the secondary hydroxyl groups of polyalcohols and monosaccharides. Condensation of the oxazolines LXXXVI and XCI with 1,3-O-benzylideneglycerol gave glycerol 1,2-*trans*-glycosaminides (CXIV and CXV) in yields 34 and 31%, respectively (including the removal of the O-acetyl and benzylidene groups) [10, 226].



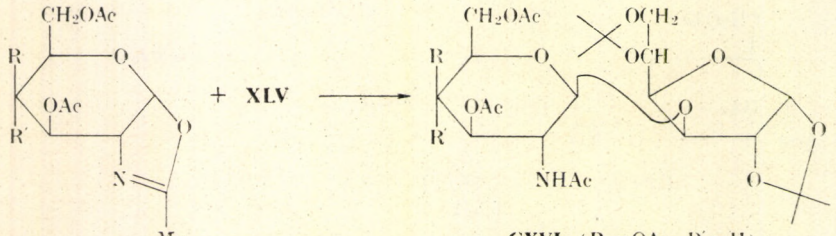
CXIV



CXV

The interaction of the oxazoline derivatives LXXXVII and XCI with 'diacetoneglucose' (XLV) yielded derivatives of $\beta$-(1 → 3)-linked disaccharides (CXVI and CXVII) in yields of about 50% [72, 73, 226].

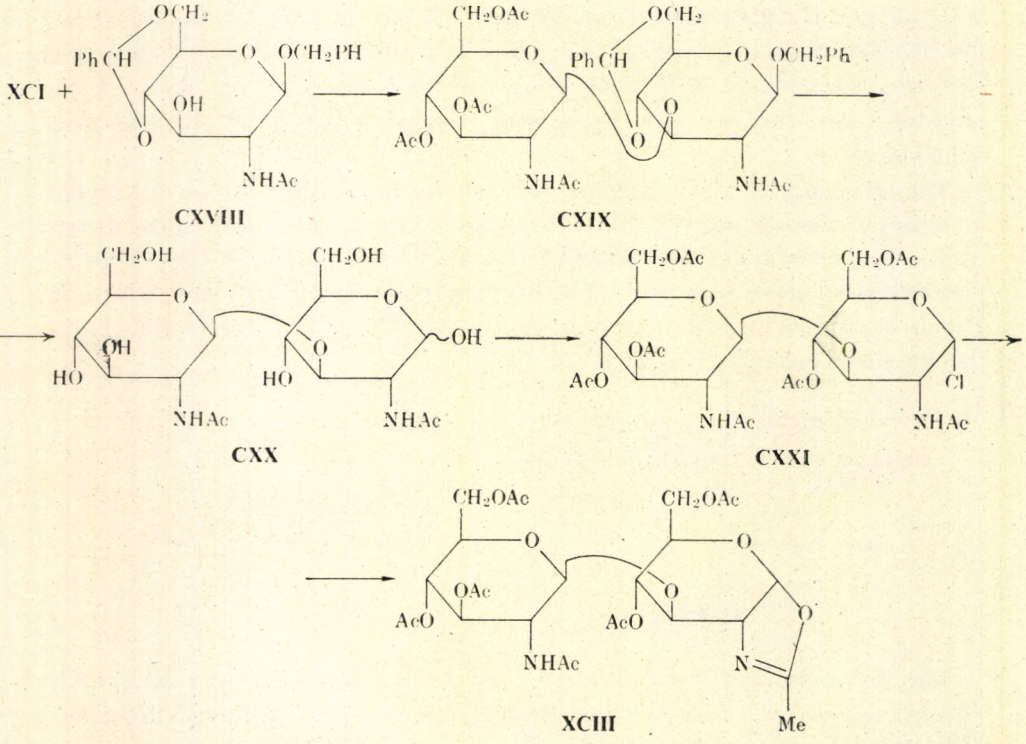
Glycosylation of the sterically hindered hydroxyl group at C-3 in the benzylidene derivative CXVIII with the oxazoline XCI gave a substituted disaccharide CXIX in a yield of 81% [226]. The latter was used as the start-




LXXXVII or XCI

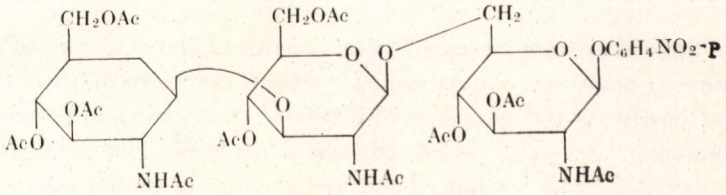
CXVI (R = OAc, R' = H)
CXVII (R = H, R' = OAc)

ing compound to synthesize an oxazoline derivative of $\beta$-(1 → 3)-linked disaccharide (XCIII). The disaccharide CXX obtained from CXIX by known procedures, was then converted into the chloride CXXI and further on into the oxazoline XCIII in a total yield of 53% as calculated for CXX [226].




CXVIII CXIX CXX CXXI XCIII

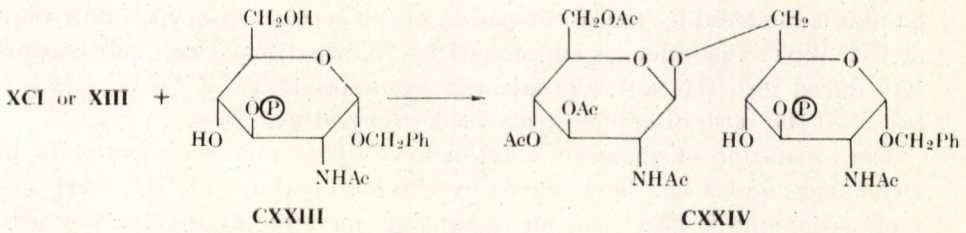
The oxazoline XCIII was used for the glycosylation of *p*-nitrophenyl-2-acetamido-3,4-di-O-acetyl-2-deoxy-$\beta$-D-glucopyranoside to obtain the trisaccharide derivative CXXII in 78% yield [226].



CXXII

This sequence of conversions was proved to be applicable to the synthesis of oligosaccharides having various combinations of 1,2-*trans*-glycosidic linkages between the amino sugar residues.

The recent work of Vignon *et al.* [33] on the synthesis of a disaccharide on a polymer support is actually a new word not only in the oxazoline synthesis of glycosaminides, but also in the synthesis of oligosaccharides in general. Glycosylation of the N-acetyl-D-glucosamine derivative attached to the polymer support with an ester bond (CXXIII), with the oxazoline XCI (or the chloride XIII under the conditions of the Koenigs–Knorr reaction) gave a high yield of a disaccharide derivative on the support (CXXIV) which, when treated with sodium methoxide in methanol, yielded



CXXIII CXXIV

Ⓟ - polymer support

the disaccharide glycoside. In addition to the well-known advantages of solid-phase syntheses (for example, that of peptides), this kind of the oxazoline synthesis of glycosaminides will allow, as it seems, the preparation of higher oligosaccharides, which is as yet difficult because of the low solubility of the glycosylated components [227].

By way of conclusion, it should be said about the oxazoline method of the synthesis of 1,2-*trans*-2-acetamido-2-deoxy-glycosides that sugar oxazoline derivatives allow the glycosylation of different types of hydroxyl-containing compounds, such as alcohols, phenols, hydroxy amino acids, polyalcohols, and partially substituted mono- and oligosaccharides (as well as thiols, which will be shown below). Several important classes of natural

'aglycons' have been demonstrated to be capable of the reactions of common types. Some conclusions could be made about the stereospecificity of the reaction after glycosylating agents of different structures, and various alcohol components have been tested. On the other hand, the same data show the stability of all the protective groups employed under the conditions required for glycosylation by sugar oxazoline derivatives. The groups tested were acyl, alkylidene, benzyl glycoside, and N-benzyloxycarbonyl (used in peptide synthesis).

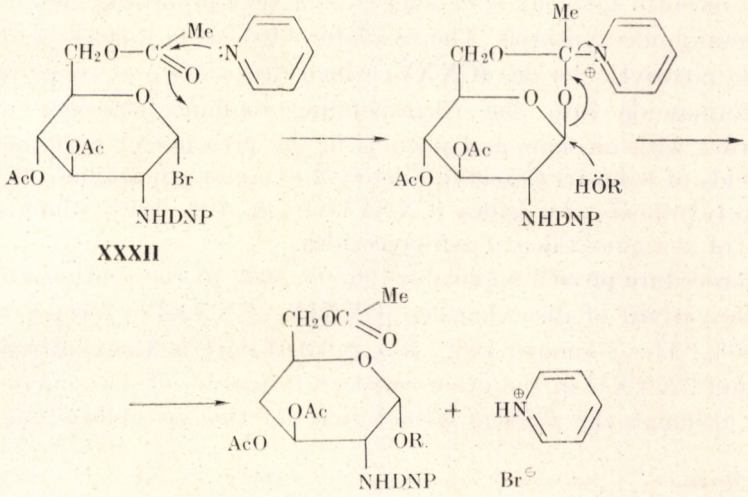
A comprehensive analysis of all the results obtained permits the conclusion that the oxazoline method is a general one for the formation of the 1,2-*trans*-glycosaminidic bond.

## VI. STEREOSPECIFIC SYNTHESIS OF 1,2-*CIS*-2-AMINO-2-DEOXYGLYCOSIDES

The methods of stereospecific synthesis of 1,2-*cis*-glycosaminides are in the stage of development, just as in the case of neutral sugars. It has been pointed out above that the formation of 1,2-*cis*-glycosides from glycosyl halides is hindered by the participation of the acetoxy or acylamido groups at C-2. When this effect is suppressed by "non-participating" substituents introduced into the amino group, the stereospecificity of glycosylation is lost, and the ratio of anomeric glycosides formed will greatly vary.

The separation of anomeric alkyl or aryl glycosaminides is not difficult any longer, and it can be achieved by chromatography [12, 117, 144]. The same technique is also used for separating anomeric glycosaminides with aglycons of complex structure (including disaccharide derivatives) formed in the Koenigs–Knorr syntheses. However, with larger oligosaccharide chains, even with trisaccharide derivatives, the possibilities of chromatography are not unlimited. That is why only stereospecific glycosylation may solve the problem of the synthesis of higher oligosaccharides having 1,2-*cis*-glycosaminidic bond.

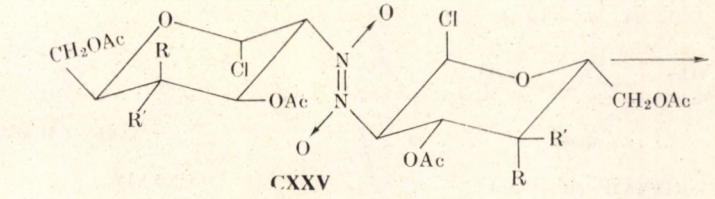
Preferential formation of 1,2-*cis*-glycosaminides has been observed only in some instances, as, for example, in the glycosylation by the bromide XXXII, in the presence of pyridine [107, 109]; the result is ascribed by the authors to the participation of the acetoxyl group at C-6. This was confirmed when 3,4-di-O-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-6-O-methyl-α-D-glucopyranosyl bromide was used as the glycosylating agent and the 1,2-*trans*-glycoside was predominantly formed [107]. Besides, in this case the



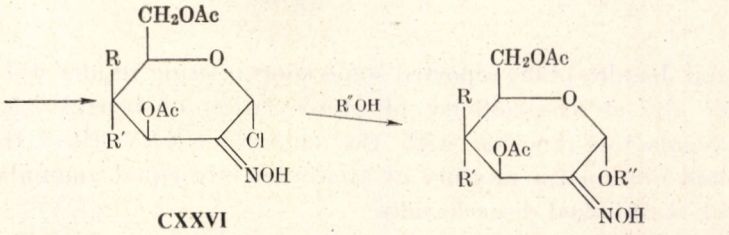

XXXII

reaction was markedly slower. The stereospecificity and the rate of the reaction do not change if the acetoxyl groups at C-3 and C-4 in the bromide XXXII are substituted by methyl groups.

A similar effect of participation of an acyloxyl substituent at the C-4 atom has recently been observed in the neutral sugar series [29]. Glycosylation with 2-O-benzyl-3,4-di-O-(*p*-nitrobenzoyl)-α-L-fucopyranosyl bromide gave α-L-fucosides with a high degree of stereospecificity.




CXXV

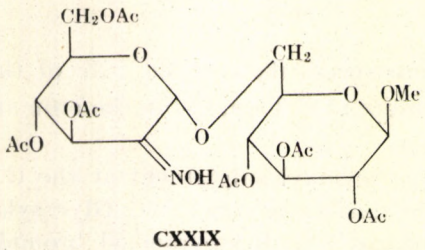



CXXVI

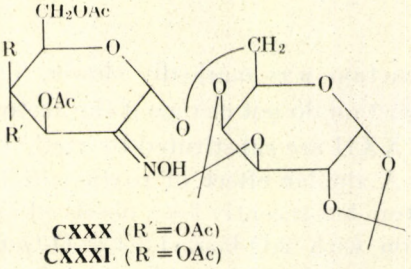
CXXVII (R=H, R′=OAc)
CXXVIII (R=OAc, R′=H)
R″ = alkyl, aryl

Quite recently Lemieux *et al.* suggested a very promising method of 1,2-*cis*-glycosaminide synthesis. The available [101, 104] dimeric D-*gluco* and D-*galacto* nitrosyl chlorides (CXXV) which dissociate and isomerize in dimethylformamide into the corresponding oximino chlorides (CXXVI) [103] react with alcohols and phenols in the presence of pyridine to give high yields of 3,4,6-tri-O-acetyl-2-deoxy-2-oximino-α-D-arabino- (CXXVII) and α-D-lyxo-hexopyranosides (CXXVIII) [96, 102, 139], which are precursors of 2-amino-2-deoxy-α-D-glycosides.

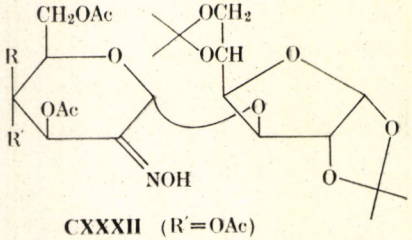
This procedure proved applicable [96, 97, 102] to the synthesis of 2′-oximino derivatives of disaccharides (CXXIX–CXXXIV) formed in yields of 50–80%. The β-anomer (9%) was isolated only in the synthesis of the compound CXXXI; in the other cases no formation of β-anomers was observed, although the authors do not rule out this possibility [96].



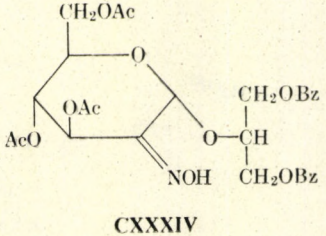

**CXXIX**




**CXXX** (R′ = OAc)
**CXXXI** (R = OAc)




**CXXXII** (R′ = OAc)
**CXXXIII** (R = OAc)




**CXXXIV**

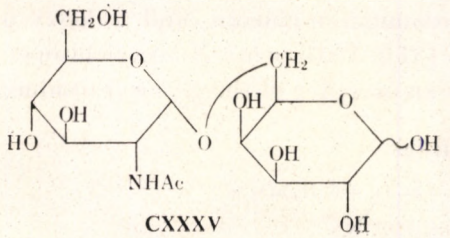
R and R′ omitted = H

Miyai and Jeanloz [139] reported some contradicting results; they failed to observe the stereospecificity of glycosylation of benzyl 2,3,4-tri-O-benzyl-β-D-galactopyranoside with the chloride CXXV (R = H, R′ = = OAc) and obtained a mixture of approximately equal amounts of the α- and β-(1 → 6)-linked disaccharides.

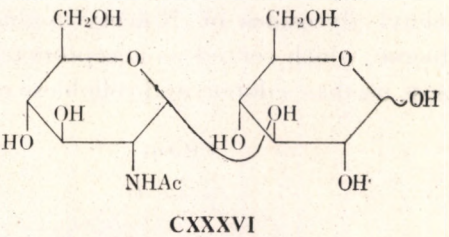
Preparatively, the method considered is not very good, since the stereospecificity of the reduction of the oximino derivatives into the corresponding 2-amino-2-deoxy sugars is unsatisfactory. Depending on the conditions

of reducing compounds CXXVII and CXXVIII, the ratio of the produced α-D-glycosaminides epimeric at C-2, greatly varied [95, 97, 139]. A high stereospecificity of the reaction was only observed when compound CXXVII was hydrogenated on palladium in the presence of hydrazine, or when the N-acetate of CXXVII was reduced with diborane in tetrahydrofuran. The resulting product contained 92–95% of α-D-glucosaminide and 5–8% of α-D-mannosaminide. Reduction of the oximino derivatives CXXVII or CXXVIII (and also of the N-acetate of the latter) with diborane or lithium aluminium hydride in tetrahydrofuran gave a mixture of the D-*gluco* and D-*manno* epimers (from CXXVII) and D-*galacto* and D-*talo* epimers (from CXXVIII) in comparable quantities.

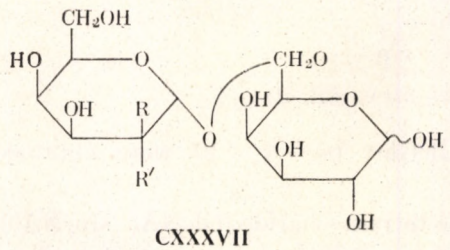
The regularities were generally the same when oximino derivatives of disaccharides (CXXX–CXXXIV) were reduced with diborane in tetrahydrofuran [99]. Individual disaccharides (CXXXV and CXXXVI) were obtained in high yields only from compounds CXXX and CXXXII after removal of the protective groups and N-acetylation. Reduction of compounds CXXXI and CXXXIII under the same conditions gave mixtures of the disaccharides CXXXVII and CXXXVIII (R + R′ = H, NHAc), respectively, with the predominance of the products having the D-*talo* configuration of the amino sugar component. The products were separated chromatographically.



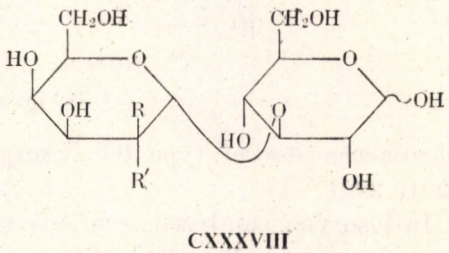
CXXXV



CXXXVI



CXXXVII



CXXXVIII

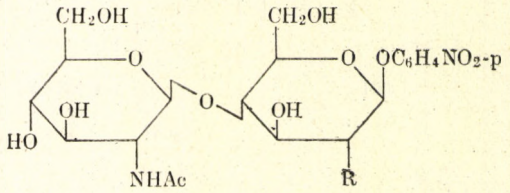
Reduction of the compound CXXIX also yields an approximately 1 : 1 mixture of disaccharide derivatives of the D-*gluco* and D-*manno* configuration of the non-reducing monosaccharide [95].

In spite of the occasionally ambiguous results of the reduction of sugar 2-oximino derivatives, the method of Lemieux *et al.* seems to be the only one available for the synthesis of 1,2-*cis*-glycosaminides with a structurally complex aglycon. It should be noted that 2-oximino-2-deoxy-α-D-glycosides are the precursors of not only α-D-glycosaminides, but also of α-D-glycopyranosides [97, 98], α-D-hexopyranosulosides [100], and of 2-deoxy-α-D-glycosides [94], including disaccharide derivatives.

## VII. ENZYMIC SYNTHESIS OF OLIGOSACCHARIDES CONTAINING AMINO SUGARS

A detailed analysis of the enzymic syntheses of oligosaccharides containing amino sugars is beyond the scope of the present review. We should only like to mention the enzymic syntheses of those compounds which may be interesting preparatively. They are based on the well-explored [25, 157] ability of hen egg-white lysozyme (E. C. 3.2.1.17) to display a transglycosylating activity.

Lysozyme and di-N-acetylchitobiose in the presence of methanol gave methyl 2-acetamido-2-deoxy-β-D-glucopyranoside [158]. Later, the formation of disaccharide glycosides (CXXXIX) was reported [159] under the action of lysozyme on tetra-N-acetyl-chitotetraose in the presence of *p*-nitrophenyl glycosides of N-acetyl-D-glucosamine, D-glucose, and 2-deoxy-D-glucose, which served as acceptors (*cf.* [31]). With penta-N-acetylchitopentaose used as a donor, and cellobiose as an acceptor of the glycosyl residues,




**CXXXIX** (R=NHAc, OH, H)

oligosaccharides of type $(GlcNAc)_n$-GlcGlc* ($n = 1 - 3$) were obtained [221, 222].

In lysozyme-catalyzed reactions, the tetrasaccharide $(GlcNAc–MurNAc)_2$ from bacterial cell walls (*Micrococcus lysodeikticus*) has proved to be a potent donor of glycosyl residues. When it was made to interact with mono-

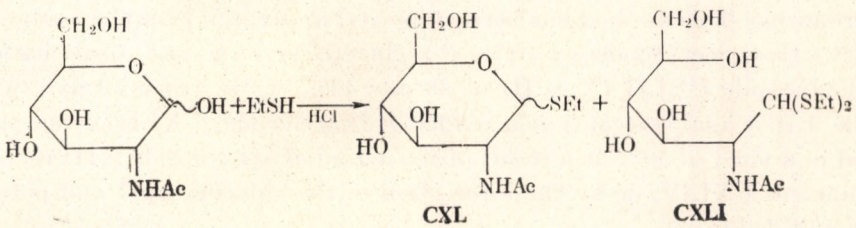
* For abbreviations, see Table II. The omitted positions of the glycosidic bonds correspond to β-(1→4) bonds.

saccharide acceptors (N-acetyl-D-glucosamine, D-glucose, D-galactose, and D-xylose), the following trisaccharides were obtained [152–154]; GlcNAc–MurNAc–GlcNAc, GlcNAc–MurNAc–Glc, GlcNAc–MurNAc–$\beta$–(1 → 2)-Glc, GlcNAc–MurNAc–$\beta$–(1 → 2)-Gal, GlcNAc–MurNAc–Xyl, GlcNAc–MurNAc–$\beta$-(1 → 2)-Xyl, and GlcNAc–MurNAc–$\beta$-(1 → 3)-Xyl. The yields of transglycosylation products were up to 15% (calculated for the donor). With oligosaccharides, di-N-acetylchitobiose and cellotetraose, the reaction proceeds in a similar way to give GlcNAc–MurNAc–$(GlcNAc)_2$ and GlcNAc–MurNAc–$(Glc)_4$ [154, 221].

The transglycosylating activity is a characteristic property of glycosidases and *endo*-glycanases, i.e. O-glycoside-hydrolases, catalyzing the hydrolytic cleavage of the O-glycosidic bond, the configuration of the anomeric glycosidic centre of the substrate thereby being retained. Enzymes of this type display a higher affinity in their active centres for the glycon moiety of the substrate; this fact is responsible for the adsorption of the activated glycosyl residue at the active centre, to be further transferred to the acceptor. In addition to lysozyme, other enzymes displaying transglycosylating activity may be used, for example $\alpha$- and $\beta$-hexosaminidases. But this has been hardly touched upon in the literature.

## VIII. SYNTHESIS OF S- AND N-GLYCOSAMINIDES

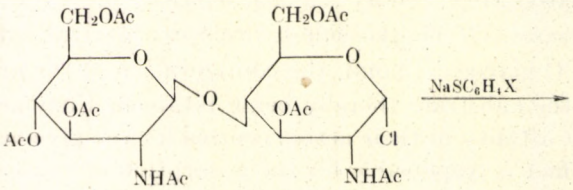
Unlike neutral monosaccharides, N-acetyl-D-glucosamine gives 1-thioglycosides under the conditions of the Fischer synthesis. For example, anomeric ethyl 2-acetamido-2-deoxy-1-thio-D-glucopyranosides (CXL) were obtained in a low yield under carefully controlled reaction conditions [53]; the main product of the reaction, however, was the mercaptal CXLI.



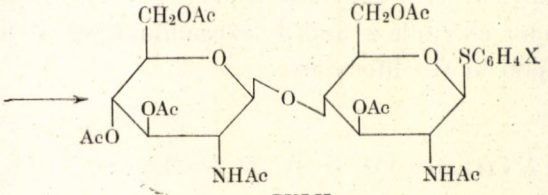

Principally, the methods for synthesizing amino sugar S-glycosides are the same as those employed for the neutral analogues [60]. Hydrolysis of the mercaptal CXLI in the presence of mercuric chloride and mercuric

oxide gave a mixture of anomeric ethyl 2-acetamido-2-deoxy-1-thio-D-glucofuranosides, the α-anomer being prevalent [211].

A variety of alkyl and aryl 2-amino-3,4,6-tri-O-benzoyl-2-deoxy-1-thio-β-D-glucopyranosides (as hydrobromides) [197] and also their N-benzoyl analogues [195] were synthesized from the respective glycosyl halides and thiols. In a similar way, using the chloride XVI, disaccharide phenyl 1-thioglycosides (CXLII, X = H, Me, $NO_2$) and their 1-seleno analogues have been synthesized [151, 183].




XVI



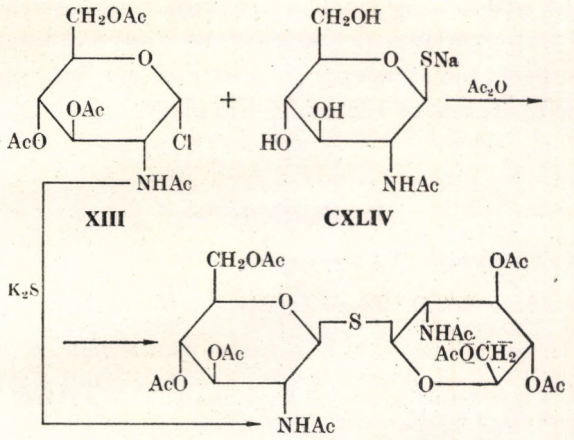

CXLII

Ethyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside results from the mercaptolysis of α-D-glucosamine peracetate in the presence of zinc chloride [63]. In this case, the stereospecificity is caused by the participation effect.

In the synthesis of 1-thioglycosaminides, the oxazoline method seems to be promising. This method has been employed to prepare phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside and disaccharide 1-thioglycoside CXLII (X = H) in 68 and 43% yields, respectively [225].

The 1-thio analogue of a non-reducing disaccharide (CXLIII) was prepared in a yield of 50% as a result of the action of the chloride XIII on the sodium salt CXLIV, or by the interaction of the chloride XIII and potassium sulfide [4].

In the synthesis of 2-amino-2-deoxy sugar N-glycosides, like in the case of neutral sugar N-glycosides [32], the methods used are the direct condensation of monosaccharides with aromatic amines [21, 22, 64, 66] or amino acids [22, 50], or else, the modified Koenigs–Knorr reaction. The difficulties



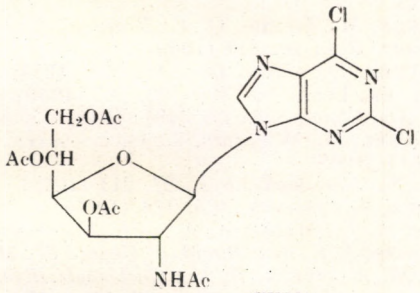

XIII CXLIV

CXLIII

with glycosyl halides as glycosylating agents are essentially the same as in the synthesis of 2-amino-2-deoxy sugar O-glycosides.

In the preparation of amino sugar nucleosides use has been made of O-acetylated glycosyl chlorides and bromides with various substituents at the nitrogen atom [14, 114, 163, 175, 179, 203, 205–207, 209, 210, 214, 217]. It should be noted that bromides having "non-participating" substituents at C-2 give, as a rule, mixtures of anomeric N-glycosaminides.

The synthesis of the nucleoside derivative CXLV is, so far, the only example of the oxazoline method used for obtaining N-glycosaminides [213] (*cf.* [135]).




CXLV

*

The data presented above allow the following conclusions. The synthesis of 1,2-*trans*-glycosaminides, including 2-amino-2-deoxy sugar oligosaccharides, is a well-explored field with preparative procedures being available. The oxazoline method is the most convenient, offering a wide range of

possibilities. The situation is much worse with 1,2-*cis*-glycosaminides, and it is the worst with synthetic polysaccharides built from amino sugar residues. In this respect, the synthetic chemistry of glycosaminides is yet lagging behind the synthesis of the neutral analogues [101, 164].

## REFERENCES


1. Acher, A. J., Rabinsohn, Y., Rachaman, E. S., Shapiro, D.: *J. Org. Chem.*, *35*, 2436 (1970).
2. Acher, A. J., Shapiro, D.: *Israel J. Chem.*, *5*, 61 (1967).
3. Acher, A. J., Shapiro, D.: *J. Org. Chem.*, *34*, 2652 (1969)
4. Akagi, M., Tejima, S., Haga, M.: *Chem. and Pharm. Bull.*, *9*, 360 (1961).
5. Akiya, S., Osawa, T.: *Yakugaku Zasshi*, *77*, 726 (1957); *C. A.*, *51*, 17 763 (1957)
6. Akiya, S., Osawa, T.: *Chem. and Pharm. Bull.*, *8*, 583 (1960).
7. Akiya, S., Osawa, T.: *Chem. and Pharm. Bull.*, *8*, 588 (1960).
8. Akiya, S., Osawa, T.: *Chem. and Pharm. Bull.*, *8*, 592 (1960).
9. Antonenko, T. S., Zaslavsky, B. Yu., Zurabyan, S. E., Shulman, M. L., Khorlin, A. Ya.: *Izv. Akad. Nauk SSSR, Ser. Khim.*, *1969*, 2622.
10. Antonenko, T. S., Zurabyan, S. E., Khorlin, A. Ya.: *Izv. Akad. Nauk SSSR, Ser. Khim.*, *1970*, 1153.
11. Antonenko, T. S., Zurabyan, S. E., Khorlin, A. Ya.: *Izv. Akad. Nauk SSSR, Ser. Khim.*, *1970*, 2766.
12. Austin, P. W., Hardy, F. E., Buchanan, J. G., Baddiley, J.: *J. Chem. Soc.*, *1963*, 5350.
13. Baer, H. H.: in "The Amino Sugars" (Edited by R. W. Jeanloz and E. A. Balazs), Vol. IA, Academic Press, New York, London, 1969, p. 268.
14. Baker, B. R., Joseph, J. P., Schaub, R. E.: *U. S. Pat.* 2 852 505 (1958); *C. A.*, *53*, 8175 (1959).
15. Baker, B. R., Joseph, J. P., Schaub, R. E., Williams, J. H.: *J. Org. Chem.*, *19*, 1786 (1954).
16. Ballardie, F. W., Capon, B.: *J. Chem. Soc. (Chem. Commun.)*, *1972*, 828.
17. Baluja, G., Chase, B. H., Kenner, G. W., Todd, A.: *J. Chem. Soc.*, *1960*, 4678.
18. Barker, S. A., Plevey, R. G., Simmons, R. G., Stacey, M.: *Tetrahedron*, Suppl. 8, Part II, 611 (1966).
19. Barker, S. A., Stacey, M., Tipper, D. J.: *Nature*, *184*, 1718 (1955).
20. Begbie, R.: *Carbohydr. Res.*, *10*, 311 (1969).
21. Bertho, A., Koziollek, D.: *Chem. Ber.*, *87*, 934 (1954).
22. Bertho, A., Koziollek, D.: *Chem. Ber.*, *92*, 627 (1959).
23. Bonner, W. A.: *J. Am. Chem. Soc.*, *70*, 3491 (1948).
24. Buddecke, E., Schauer, H., Werries, E., Gottschalk, A.: *Biochem. Biophys. Res. Commun.*, *34*, 517 (1969).
25. Bundle, D., Shaw, N.: *Carbohydr. Res.*, *21*, 211 (1972).
26. Chipman, D., Sharon, N.: *Science*, *165*, 454 (1969).
27. Conchie, J., Levvy, G. A., Marsh, C. A.: *Adv. Carbohydr. Chem. 12*, 157 (1957).
28. Curtis, E. J. C., Jones, J. K. N.: *Canad. J. Chem.*, *37*, 358 (1959).
29. Deiter-Juszynski, M., Flowers, H. M.: *Carbohydr. Res.*, *23*, 41 (1972).
30. Dunstan, D., Hough, L.: *Carbohydr. Res.*, *23*, 425 (1972).
31. van Eikeren, P., Chipman, D. M.: *J. Am. Chem. Soc.*, *94*, 4788 (1972).
32. Ellis, G. P., Honeyman, J.: *Advances in Carbohydrate Chemistry*, *10*, 95 (1955).
33. Excoffier, G., Gagnaire, D., Utille, J. P., Vignon, M.: *Tetrahedron Letters*, *1972*, 5065.
34. Evans, W. L., Reynolds, D. D., Talley, E. A.: *Adv. Carbohydr. Chem. 6*, 27 (1951).
35. Findlay, J., Levvy, G. A., Marsh, C. A.: *Biochem. J.*, *69*, 467 (1958).
36. Flowers, H. M., Jeanloz, R. W.: *J. Org. Chem.*, *28*, 1564 (1963).
37. Flowers, H. M., Jeanloz, R. W.: *J. Org. Chem.*, *28*, 2983 (1963).

38. Fodor, G., Ötvös, L.: *Ann., 604*, 29 (1957).
39. Foster, A. B., Horton, D.: *J. Chem. Soc., 1958*, 1890.
40. Foster, A. B., Horton, D.: *Adv. Carbohydr. Chem. 14*, 213 (1959).
41. Freudenberg, K., Eich, H., Knoevenagel, C., Westphal, W.: *Ber., 73*, 441 (1940).
42. Fujise, S., Yokoyama, K.: *Nippon Kagaku Zasshi, 77*, 728 (1951); *C. A., 46*, 11 116 (1952).
43. Gigg, R., Carroll, P. M.: *Nature, 191*, 495 (1961).
44. Gigg, R., Carroll, P. M., Warren, C. D.: *J. Chem. Soc., 1965*, 2975.
45. Gigg, R., Warren, C. D.: *J. Chem. Soc., 1965*, 1351.
46. Gigg, R., Warren, C. D.: *J. Chem. Soc. (C), 1969*, 295.
47. Greig, C. G., Leaback, D. H.: *Chem. and Ind., 1960*, 376.
48. Greig, C. G., Leaback, D. H., Walker, P. G.: *J. Chem. Soc., 1961*, 879.
49. Gross, P. H., Jeanloz, R. W.: *J. Org. Chem., 32*, 2759 (1967).
50. Hackman, R. H.: *Austral. J. Biol. Sci., 8*, 83 (1955).
51. Hardy, F. E.: *J. Chem. Soc., 1965*, 375.
52. Hardy, F. E., Buchanan, J. G., Baddiley, J.: *J. Chem. Soc., 1963*, 3360.
53. Haynes, L. J., Newth, F. H.: *Adv. Carbohydr. Chem. 10*, 207 (1955).
54. Heyns, K., Harrison, R., Paulsen, H.: *Chem. Ber., 100*, 271 (1967).
55. Heyns, K., Harrison, R., Propp, K., Paulsen, H.: *Chem. Commun., 1966*, 671.
56. Heyns, K., Propp, K., Harrison, R., Paulsen, H.: *Chem. Ber., 100*, 2655 (1967).
57. Heyworth, R., Leaback, D. H., Walker, P. G., *J. Chem. Soc., 1959*, 4121.
58. Hirschmann, R., Strachan, R. G., Buchschacher, P., Sarett, L. H., Steelman, S. L., Silber, R.: *J. Am. Chem. Soc., 86*, 3903 (1964).
59. Horton, D.: in "The Amino Sugars" (Edited by R. W. Jeanloz and E. A. Balazs), Vol. IA, Academic Press, New York, London, 1969, p. 3.
60. Horton, D., Hutson, D. H.: *Adv. Carbohydr. Chem. 18*, 123 (1963).
61. Horton, D., Wolfrom, M. L.: *J. Org. Chem., 27*, 1794 (1962).
62. Horton, D., Wolfrom, M. L., Garg, H. G.: *J. Org. Chem., 28*, 2922 (1963).
63. Hough, L., Taha, M. I.: *J. Chem. Soc., 1956*, 2042.
64. Inouye, Y., Onodera, K., Kitaoka, S.: *Nippon Nogeikagaku Kaisshi, 29*, 143 (1955); *C. A., 50*, 826 (1956).
65. Inouye, Y., Onodera, K., Kitaoka, S., Ochiai, H.: *J. Am. Chem. Soc., 79*, 421 (1957).
66. Inouye, Y., Onodera, K., Nakatami, J.: *Nippon Nogeikagaku Kaisshi, 25*, 550 (1951–52); *C. A., 48*, 2002 (1964).
67. Irvine, J. C., Earl, J. C.: *J. Chem. Soc., 121*, 2376 (1922).
68. Irvine, J. C., Hynd, A.: *J. Chem. Soc., 103*, 41 (1913).
69. Irvine, J. C., McNicoll, D., Hynd, A.: *J. Chem. Soc., 99*, 250 (1911).
70. IUPAC-IUB Combined Comission on Biochemical Nomenclature, *Biochemistry, 5*, 1445 (1966).
71. Iwamoto, R., Imanaga, Y.: *Carbohydr. Res., 24*, 133 (1972).
72. Jacquinet, J. C., Zurabyan, S. E., Khorlin, A. Ya.: *Izv. Akad. Nauk SSSR, Ser. Khim., 1973*, No. 8.
73. Jacquinet, J. C., Zurabyan, S. E., Khorlin, A. Ya.: *Carbohydr. Res.*, in the press.
74. Jeanloz, R. W., Sharon, N., Flowers, H. M.: *Biochem. Biophys. Res. Commun., 13*, 20 (1963).
75. Jones, J. K. N., Perry, M. B., Shelton, B., Walton, D.: *Canad. J. Chem., 39*, 1005 (1961).
76. van de Kamp, F.-P., Micheel, F.: *Chem. Ber., 90*, 2054 (1957).
77. Khorlin, A. Ya., Shulman, M. L., Zurabyan, S. E., Privalova, I. M., Kopaevich, Yu. L.: *Izv. Akad. Nauk SSSR, Ser. Khim., 1968*, 227.
78. Khorlin, A. Ya., Shulman, M. L., Zurabyan, S. E., Privalova, I. M., Kopaevich, Yu. L.: *Izv. Akad. Nauk SSSR, Ser. Khim., 1968*, 2094.
79. Khorlin, A. Ya., Zurabyan, S. E., Dubrovina, N. I., Bystrov, V. F., Vikha, G. V., Kaverzneva, E. D.: *Carbohydr. Res., 21*, 316 (1972).
80. Konstas, S., Photaki, I., Zervas, L.: *Chem. Ber., 92*, 1288 (1959).
81. Kozhin, S. A., Titkova, E. G.: *Zh. Obshch. Khim., 40*, 248 (1970).
82. Kozhin, S. A., Titkova, E. G.: *Zh. Obshch. Khim., 41*, 700 (1971).

83. KUHN, R., BAER, H. H.: *Chem. Ber., 86*, 724 (1953).
84. KUHN, R., BAER, H. H., SEELINGER, A.: *Ann., 611*, 236 (1958).
85. KUHN, R., KIRSCHENLOHR, W.: *Chem. Ber., 86*, 1331 (1953).
86. KUHN, R., KIRSCHENLOHR, W.: *Chem. Ber., 87*, 384 (1954).
87. KUHN, R., TRISCHMANN, H.: *Chem. Ber., 96*, 284 (1963).
88. KUHN, R., ZILLIKEN, F., GAUHE, A.: *Chem. Ber., 86*, 466 (1953).
89. KUSHIDA, H., HAYASHI, I.: *Japan. Pat.* 15 780 (1960); *C. A., 55*, 10 338 (1961).
90. LEABACK, D. H., WALKER, P. G.: *Chem. and Ind., 1957*, 1012.
91. LEABACK, D. H., WALKER, P. G.: *Chem. and Ind., 1957*, 1017.
92. LEABACK, D. H., WALKER, P. G.: *J. Chem. Soc., 1957*, 4754.
93. LEABACK, D. H., WALKER, P. G.: *Biochem. J., 78*, 151 (1961).
94. LEMIEUX, R. U., EARL, R. A., JAMES, K., NAGABHUSHAN, T. L.: *Canad. J. Chem., 51*, 19 (1973).
95. LEMIEUX, R. U., GUNNER, S. W.: *Canad. J. Chem., 46*, 397 (1968).
96. LEMIEUX, R. U., ITO, Y., JAMES, K., NAGABHUSHAN, T. L.: *Canad. J. Chem., 51*, 7 (1973).
97. LEMIEUX, R. U., JAMES, K., NAGABHUSHAN, T. L.: *Canad. J. Chem., 51*, 27 (1973).
98. LEMIEUX, R. U., JAMES, K., NAGABHUSHAN, T. L.: *Canad. J. Chem., 51*, 42 (1973).
99. LEMIEUX, R. U., JAMES, K., NAGABHUSHAN, T. L.: *Canad. J. Chem., 51*, 48 (1973).
100. LEMIEUX, R. U., JAMES, K., NAGABHUSHAN, T. L., ITO, Y.: *Canad. J. Chem., 51*, 33 (1973).
101. LEMIEUX, R. U., NAGABHUSHAN, T. L.: *Methods in Carbohydrate Chemistry, 6*, 487 (1972).
102. LEMIEUX, R. U., NAGABHUSHAN, T. L., GUNNER, S. W.: *Canad. J. Chem., 46*, 405 (1968).
103. LEMIEUX, R. U., NAGABHUSHAN, T. L., JAMES, K.: *Canad. J. Chem., 51*, 1 (1973).
104. LEMIEUX, R. U., NAGABHUSHAN, T. L., O'NEIL, I. K.: *Canad. J. Chem., 46*, 413 (1968).
105. LIKHOSHERSTOV, L. M., NOVIKOVA, O. S., DEREVITSKAYA, V. A.: *Izv. Akad. Nauk SSSR, Ser. Khim., 1971*, 652.
106. LINDBERG, B., AGBACK, H.: *Acta Chem. Scand., 18*, 185 (1964).
107. LLOYD, P. F., EVANS, B., FIELDER, R. J.: *Carbohydr. Res., 22*, 111 (1972).
108. LLOYD, P. F., ROBERTS, G. P.: *Proc. Chem. Soc., 1960*, 250.
109. LLOYD, P. F., ROBERTS, G. P.: *J. Chem. Soc., 1963*, 2962.
110. LLOYD, P. F., ROBERTS, G. P.: *J. Chem. Soc., 1965*, 6910.
111. LLOYD, P. F., STACEY, M.: *Chem. and Ind., 1955*, 917.
112. LLOYD, P. F., STACEY, M.: *Tetrahedron, 9*, 116 (1960).
113. LOWE, G., SHEPPARD, D., SINNOTT, M. L., WILLIAMS, A.: *Biochem. J., 104*, 893 (1967).
114. MCEVOY, F. J., WEISS, M. J., BAKER, B. R.: *J. Am. Chem. Soc., 82*, 205 (1960).
115. MASAMUNE, H., MAKI, M., HIYAMA, N.: *Tôhoku J. Exptl. Med., 54*, 313 (1951).
116. MASAMUNE, H., OKUYAMA, T., SINOHARA, H.: *Tôhoku J. Exptl. Med., 68*, 181 (1958).
117. MATSUSHIMA, Y., MIYAZAKI, T., PARK, J. T.: *J. Biochem., 54*, 109 (1963).
118. MATTA, K. L., BAHL, O. P.: *Carbohydr. Res., 21*, 460 (1972).
119. MATTA, K. L., JOHNSON, E. A., BARLOW, J. J.: *Carbohydr. Res., 26*, 215 (1973)
120. MAY, E. L., MOSETTIG, E.: *J. Org. Chem., 15*, 890 (1950).
121. MERSER, C., SINAŸ, P.: *Tetrahedron Letters, 1973*, 1029.
122. MEYER ZU RECKENDORF, W.: *Tetrahedron, 19*, 2033 (1963).
123. MEYER ZU RECKENDORF, W., BONNER, W. A.: *Chem. Ber., 95*, 996 (1962).
124. MEYER ZU RECKENDORF, W., FELDKAMP, J.: *Chem. Ber., 101*, 2289 (1968).
125. MEYER ZU RECKENDORF, W., WASSILIADOU-MICHELI, N.: *Chem. Ber., 103*, 1792 (1970).
126. MEYER ZU RECKENDORF, W., WASSILIADOU-MICHELI, N., MACHLEIDT, H.: *Arch. Pharm., 303*, 17 (1970).
127. MICHEEL, F., DRESCHER, E.: *Chem. Ber., 91*, 668 (1958).
128. MICHEEL, F., DRESCHER, E.: *Chem. Ber., 91*, 670 (1958).
129. MICHEEL, F., DRESCHER, E.: *Chem. Ber., 95*, 1020 (1962).
130. MICHEEL, F., VAN DE KAMP, F.-P., PETERSEN, H.: *Chem. Ber., 90*, 521 (1957).
131. MICHEEL, F., VAN DE KAMP, F.-P., WULFF, H.: *Chem. Ber., 88*, 2011 (1955).
132. MICHEEL, F., KÖCHLING, H.: *Chem. Ber., 90*, 1597 (1957).

133. MICHEEL, F., KÖCHLING, H.: *Chem. Ber., 91,* 673 (1958).
134. MICHEEL, F., KÖCHLING, H.: *Chem. Ber., 92,* 2832 (1959).
135. MICHEEL, F., KÖCHLING, H.: *Chem. Ber., 93,* 2372 (1960).
136. MICHEEL, F., MICHAELIS, E.: *Chem. Ber., 96,* 1959 (1963).
137. MICHEEL, F., PETERSEN, H.: *Chem. Ber., 92,* 298 (1958).
138. MICHEEL, F., WULFF, H.: *Chem. Ber., 89,* 1521 (1956).
139. MIYAI, K., JEANLOZ, R. W.: *Carbohydr. Res., 21,* 45 (1972).
140. MOGGRIDGE, R. C. G., NEUBERGER, A.: *J. Chem. Soc., 1938,* 745.
141. MOREL, C. J.: *Experientia, 12,* 419 (1956).
142. NEUBERGER, A., PITT RIVERS, P. V.: *J. Chem. Soc., 1939,* 122.
143. NEUBERGER, A., PITT RIVERS, P. V.: *Biochem. J., 33,* 1580 (1939).
144. NEUBERGER, A., WILSON, B. M.: *Carbohydr. Res., 17,* 89 (1971).
145. ONODERA, K., KITAOKA, S., OCHIAI, H.: *J. Org. Chem., 27,* 156 (1962).
146. OSAWA, T.: *Chem. and Pharm. Bull., 8,* 597 (1960).
147. OSAWA, T.: *Carbohydr. Res., 1,* 435 (1966).
148. OSAWA, T.: *Carbohydr. Res., 7,* 217 (1968).
149. OSAWA, T., NAKAZAWA, Y.: *Biochim. Biophys. Acta, 130,* 56 (1966).
150. OSAWA, T., JEANLOZ, R. W.: *Carbohydr. Res., 1,* 181 (1965).
151. OTSON, R., REYES-ZAMORA, C., TANG, J. Y.: *Canad. J. Biochem., 51,* 1 (1973).
152. POLLOCK, J. J., SHARON, N.: *Biochem. Biophys. Res. Commun., 34,* 673 (1969).
153. POLLOCK, J. J., SHARON, N.: *Carbohydr. Res., 13,* 211 (1970).
154. POLLOCK, J. J., SHARON, N.: *Biochemistry, 9,* 3913 (1970).
155. PRAVDIĆ, N., INCH, T. D., FLETCHER, H. G., JR.: *J. Org. Chem., 32,* 1815 (1967).
156. RABINSOHN, Y., ACHER, A. J., SHAPIRO, D.: *J. Org. Chem., 38,* 202 (1973(.
157. RAFTERY, M. A., DAHLQUIST, F. W.: *Fortschritte der Chemie organischer Naturstoffe, 27,* 340 (1969).
158. RAFTERY, M. A., RAND-MEIR, T.: *Biochemistry, 7,* 3281 (1968).
159. RAND-MEIR, T., DAHLQUIST, F. W., RAFTERY, M. A.: *Biochemistry, 8,* 4206 (1969).
160. ROSEMAN, S., LUDOWIEG, J.: *J. Am. Chem. Soc., 76,* 301 (1954).
161. ROTH, W., PIGMAN, W.: *J. Am. Chem. Soc., 82,* 4608 (1960).
162. SALO, W. L., FLETCHER, H. G., JR.: *J. Org. Chem., 34,* 3189 (1969).
163. SATO, T., ISHIDO, Y.: *Japan. Pat.* 6695 (1964); *C. A., 61,* 14 772 (1964).
164. SCHUERCH, C., ZACHOVAL, J., VERUOVIČ, B.: *Chemické listy, 66,* 1124 (1972).
165. SHAPIRO, D., ACHER, A. J.: *J. Org. Chem., 35,* 229 (1970).
166. SHAPIRO, D., ACHER, A. J., RABINSOHN, Y., DIVER-HABER, A.: *J. Org. Chem., 36,* 832 (1971).
167. SHAPIRO, D., ACHER, A. J., RACHAMAN, E. S.: *J. Org. Chem., 32,* 3767 (1967).
168. SHAPIRO, D., RABINSOHN, Y., ACHER, A. J., DIVER-HABER, A.: *J. Org. Chem., 35,* 1464 (1970).
169. SHAPIRO, D., RABINSOHN, Y., DIVER-HABER, A.: *Biochem. Biophys. Res. Commun., 37,* 28 (1969).
170. SHEN, T. Y., LI, J. P., DORN, C. P., EBEL, D., BUGIANESE, R., FECHER, R.: *Carbohydr., Res., 23,* 87 (1972).
171. SHULMAN, M. L., ABRAMOVA, G. V., PISKAEVA, V. N., KHORLIN, A. YA.: *Izv. Akad. Nauk SSSR, Ser. Khim., 1971,* 630.
172. SHULMAN, M. L., PRIVALOVA, I. M., KHORLIN, A. YA.: *Izv. Akad. Nauk SSSR, Ser. Khim., 1968,* 2655.
173. STACEY, M.: *J. Chem. Soc., 1944,* 272.
174. STEVENS, C. L., BLUMBERGS, P.: *J. Org. Chem., 30,* 2723 (1965).
175. STEVENS, C. L., NAGARAJAN, K.: *J. Med. Pharm. Chem., 5,* 1124 (1962).
176. STRACHAN, R. G., RUYLE, W. V., SHEN, T. Y., HIRSCHMANN, R.: *J. Org. Chem., 31,* 507 (1966).
177. SUAMI, T., OGAWA, S., UMEZAWA, S.: *Bull. Chem. Soc. Japan, 35,* 474 (1962).
178. SUAMI, T., OGAWA, S., UMEZAWA, S.: *Bull. Chem. Soc. Japan, 36,* 459 (1963).
179. SUGAWA, T., KUWADA, Y., IMAI, K., MORINAGA, M., KAJIWARA, K., TANAKA, M.: *Takeda Kenkyusho Nempo, 20,* 7 (1961); *C. A., 58,* 3497 (1963).
180. TARASIEJSKA, Z., JEANLOZ, R. W.: *J. Am. Chem. Soc., 80,* 6325 (1958).
181. THOMAS, E. W.: *Carbohydr. Res., 13,* 225 (1970).
182. TITKOVA, E. G., KOZHIN, S. A., ZURABYAN, S. E., KHORLIN, A. YA.: *Zh. Obshch. Khim., 41,* 1174 (1971).

183. Tsai, C. S., Reyes-Zamora, C., Otson, R.: *Biochim. Biophys. Acta, 250*, 172 (1971).
184. Tsai, C. S., Tang, J. Y., Subbarao, S. C.: *Biochem. J., 114*, 529 (1969).
185. Umezawa, S., Koto, S.: *J. Antibiotics, Ser. A, 17*, 186 (1964).
186. Umezawa, S., Koto, S.: *Bull. Chem. Soc. Japan, 39*, 2014 (1966).
187. Umezawa, S., Koto, S.: *J. Antibiotics, Ser. A., 19*, 88 (1966).
188. Umezawa, S., Tatsuta, K., Muto, R.: *J. Antibiotics, Ser. A., 20*, 388 (1967).
189. Umezawa, S., Tsuchiya, T., Fujita, H.: *J. Antibiotics, Ser. A., 19*, 222 (1966).
190. Vercellotti, J. R., Luetzow, A. E.: *J. Org. Chem., 31*, 825 (1966).
191. Wang, Y., Tai, H.-I.: *Hua Hsüeh Hsüeh Pao, 24*, 368 (1958); *C. A., 53*, 19 896 (1959).
192. Wang, Y., Tsai, H.-I.: *Hua Hsüeh Hsüeh Pao, 25*, 50 (1959); *C. A., 54*, 6561 (1960).
193. Weidmann, H., Tartler, D., Stöckl, P., Hönig, H.: *Monatsh. Chemie, 103*, 883 (1972).
194. Weidmann, H., Zimmerman, H. K., Jr.: *Chem. Ber., 92*, 1523 (1959).
195. Weidmann, H., Zimmerman, H. K., Jr.: *Chem. Ber., 92*, 2828 (1959).
196. Weidmann, H., Zimmerman, H. K., Jr.: *Ann., 633*, 198 (1960).
197. Weidmann, H., Zimmerman, H. K., Jr., Monk, J. R.: *Ann., 628*, 255 (1959).
198. Weissmann, B.: *J. Org. Chem., 31*, 2505 (1966).
199. Weissmann, B., Frederici, D.: *Biochim. Biophys. Acta, 117*, 498 (1966).
200. Werries, E., Buddecke, E.: *Z. Physiol. Chem., 351*, 1089 (1970).
201. Westphal, O., Schmidt, H.: *Ann., 575*, 84 (1952).
202. White, T.: *J. Chem. Soc., 1940*, 428.
203. Wolfrom, M. L., Bhat, H. B.: *Chem. Commun., 1966*, 146.
204. Wolfrom, M. L., Bhat, H. B.: *J. Org. Chem., 32*, 1821 (1967).
205. Wolfrom, M. L., Bhat, H. B.: *J. Org. Chem., 32*, 2757 (1967).
206. Wolfrom, M. L., Conigliaro, P. J.: *Carbohydr. Res., 11*, 63 (1969).
207. Wolfrom, M. L., Conigliaro, P. G., Soltes, E. J.: *J. Org. Chem., 32*, 653 (1967).
208. Wolfrom, M. L., Cramp, W. A., Horton, D.: *J. Org. Chem., 28*, 3231 (1963).
209. Wolfrom, M. L., Garg, H. G., Horton, D.: *Chem. and Ind. ,1964*, 930.
210. Wolfrom, M. L., Garg, H. G., Horton, D.: *J. Org. Chem., 30*, 1556 (1965).
211. Wolfrom, M. L., Olin, S. M., Polglase, W. J.: *J. Am. Chem. Soc., 72*, 1724 (1950).
212. Wolfrom, M. L., Shen Han, T. M.: *J. Org. Chem., 26*, 2145 (1961).
213. Wolfrom, M. L., Winkley, M. W.: *J. Org. Chem., 31*, 3711 (1966).
214. Wolfrom, M. L., Winkley, M. W.: *Chem. Commun., 1966*, 533.
215. Wolfrom, M. L., Winkley, M. W.: *J. Org. Chem., 32*, 1823 (1967).
216. Wolfrom, M. L., Winkley, M. W., Inouye, S.: *Carbohydr. Res., 10*, 97 (1969).
217. Wolfrom, M. L., Wurmb, R.: *J. Org. Chem., 30*, 3068 (1965).
218. Yamamoto, K., Matsushima, Y.: *Bull. Chem. Soc. Japan, 40*, 194 (1967).
219. Yoshimura, J., Ando, H., Takahashi, Y., Ono, H., Sato, T.: *Nippon Kagaku Zasshi, 85*, 142 (1964); *C. A., 62*, 623 (1965).
220. Zehavi, U., Jeanloz, R. W.: *Carbohydr. Res., 6*, 129 (1968).
221. Zehavi, U., Pollock, J. J., Teichberg, V. I., Sharon, N.: *Nature, 219*, 1152 (1968).
222. Zehavi, U., Pollock, J. J., Teichberg, V. I., Sharon, N.: *Israel J. Chem., 6*, 119p (1968).
223. Zervas, L., Konstas, S.: *Chem. Ber., 93*, 435 (1960).
224. Zilliken, F., Rose, C. S., Braun, G. A., György, P.: *Arch. Biochem. Biophys., 54*, 392 (1955).
225. Zurabyan, S. E., Antonenko, T. S., Khorlin, A. Ya.: *Izv. Akad. Nauk SSSR, Ser. Khim., 1969*, 2043.
226. Zurabyan, S. E., Antonenko, T. S., Khorlin, A. Ya.: *Carbohydr. Res., 15*, 21 (1970).
227. Zurabyan, S. E., Pimenova, V. V., Shashkova, E. A., Khorlin, A. Ya.: *Khim. Prirod. Soedinen., 1971*, 689.
228. Zurabyan, S. E., Volosyuk, T. P., Khorlin, A. Ya.: *Izv. Akad. Nauk SSSR, Ser. Khim., 1968*, 1812.
229. Zurabyan, S. E., Volosyuk, T. P., Khorlin, A. Ya.: *Carbohydr. Res., 9*, 215 (1969).

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