Syntheses of the Benzo[c]Phenanthridine Alkaloids

by
I. NINOMIYA
and
T. NAITO

The Chemistry of the Vancomycin Group of Antibiotics

by
F. SZTARICSKAI
and
R. BOGNÁR

AKADÉMIAI KIADÓ, BUDAPEST

RECENT DEVELOPMENTS
IN THE CHEMISTRY
OF NATURAL
CARBON COMPOUNDS

Vol. 10

Edited by

R. Bognár and *Cs. Szántay*

Two interesting groups of biologically active natural products are discussed in the present volume.

I. Ninomiya and *T. Naito* give a survey of recent results in the research of benzo[c]phenanthridine alkaloids. Synthetic methods, grouped in a well arranged system, constitute the main part, followed by descriptions of the syntheses of prominent representatives of the group, such as nitidine, a compound with antitumour activity.

Authenticity of the report on vancomycin antibiotics by *F. Sztaricskai* and *R. Bognár* is guaranteed by the authors' own achievements in the pertaining research. The isolation, activity, details of structural studies and syntheses of these glycopeptide antibiotics are surveyed and their uses (medical, agricultural or diagnostic) mentioned.

The volume is of primary interest to researchers, organic and pharmaceutical chemists, biochemists and pharmacologists.

AKADÉMIAI KIADÓ
BUDAPEST

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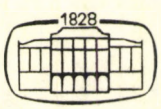
VOLUME X



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THE CHEMISTRY OF THE VANCOMYCIN GROUP OF ANTIBIOTICS

by

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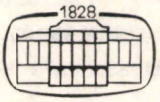

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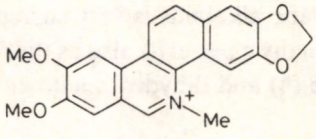
SYNTHESES OF THE BENZO[c]PHENANTHRIDINE ALKALOIDS

1. INTRODUCTION

Benzo[c]phenanthridine alkaloids, which are represented [1] by two types, nitidine **(1)** and chelidonine **(2)**, occupy an increasingly important position in alkaloid chemistry, though they consist of a relatively small number of alkaloids, found only in a relatively narrow range of plants, mainly in Fumariaceae, Papaveraceae, Rutaceae, and seldom in Rhamnaceae.



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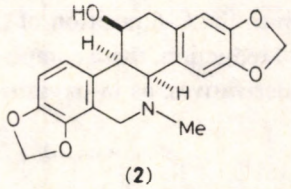


(2)

Recently, by the finding of marked potencies [2] of some of these alkaloids against leukemia, their chemistry and syntheses have become one of the most challenging problems, which stimulated synthetic chemists to try to conquer the field of this group; the result is a major progress achieved during the last five years.

The purpose of the present review is to summarize the chemistry and syntheses of benzo[c]phenanthridine alkaloids for the convenience of application in future development.

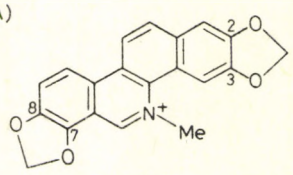
1.1. CLASSIFICATION OF BENZO[c]PHENANTHRIDINE ALKALOIDS

Apart from biogenetic considerations, the about thirty species of benzo[c]phenanthridine alkaloids* so far isolated [1a, b] can be divided into two main groups; the fully aromatic alkaloids (A) and the B/C-hexahydrobenzo[c]phenanthridine

* Nomenclature and numbering of the benzo[c]phenanthridines seem to have been in a rather confusing situation. We adopt here the IUPAC rules [3] as shown in the formula *(a)*, and the

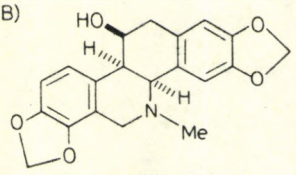
alkaloids (B), as exemplified by sanguinarine **(3)** or nitidine **(1)** (A) and chelidonine **(2)** or corynoline **(4)** (B), of which the former group (A) has the major role with respect to their number occurring in nature and also as regards their pharmacological activity.

(A)



(3)

(B)



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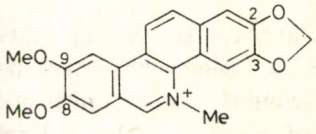

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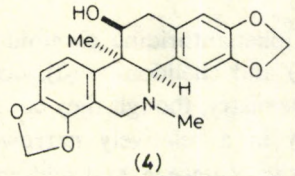
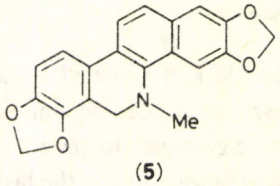
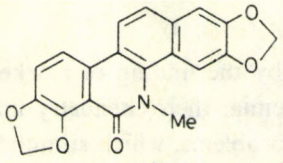


(4)

Further, since the C-6 position of the aromatic alkaloids is very susceptible to oxidation and reduction, these compounds in many cases exist also as the 6-oxo or 5,6-dihydro derivatives, as in oxysanguinarine **(6)** and dihydrosanguinarine **(5)**.



(5)



(6)

These alkaloids, which may contain tetra-, penta- and hexasubstituted components, can also be classified according to the pattern of substitutions into two basic groups, i.e. the 2,3,7,8-tetrasubstituted alkaloids as sanguinarine **(3)**, chelidonine **(2)**, and corynoline **(4)**, and the 2,3,8,9-tetrasubstituted ones as nitidine

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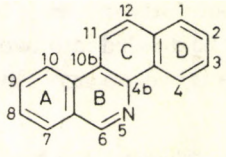
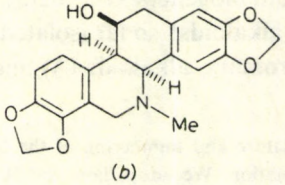
stereochemistry of (+)-chelidonine as depicted in the structure *(b)*.



(a)



(b)

(**1**), along with a small number of 2,3,7,8,10-pentasubstituted alkaloids in which an additional methoxyl group is found either in ring A or ring C.

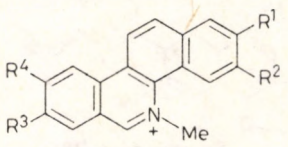
Interestingly enough from the chemotaxonomical point of view, it appears [1a, b, c] that rutaceous plants produce only alkaloids having the 2,3,8,9-tetrasubstituted structure, while papaveraceous plants contain alkaloids with the 2,3,8,9-tetrasubstitution pattern as the major components, in addition to a small amount of the 2,3,7,8-tetrasubstituted compounds.

With these characteristics of their structural features in mind, we have classified the benzo[c]phenanthridine alkaloids as shown below (Schemes 1, 2).

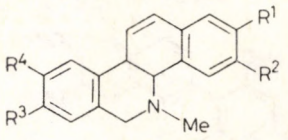
1.1.1. FULLY AROMATIC AND RELATED ALKALOIDS

(a) Tetrasubstituted Alkaloids

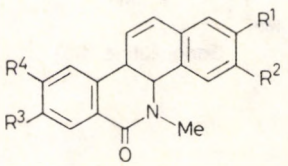
2,3,8,9-Substituted



Avicine (**7**) $R^1+R^2=OCH_2O$; $R^3+R^4=OCH_2O$
Nitidine (**1**) $R^1+R^2=OCH_2O$; $R^3=R^4=OMe$
Fagaronine (**15**) $R^1=OH$; $R^2=R^3=R^4=OMe$

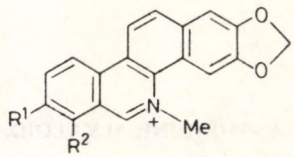


Dihydroavicine (**8**) $R^1+R^2=OCH_2O$; $R^3+R^4=OCH_2O$
Dihydronitidine (**13**) $R^1+R^2=OCH_2O$; $R^3=R^4=OMe$

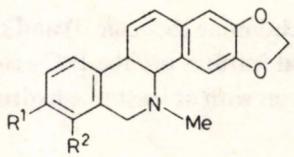


Oxyavicine (**9**) $R^1+R^2=OCH_2O$; $R^3+R^4=OCH_2O$
Oxynitidine (**14**) $R^1+R^2=OCH_2O$; $R^3=R^4=OMe$

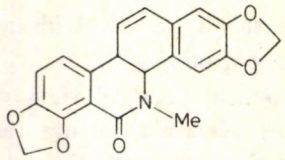
2,3,7,8-Substituted



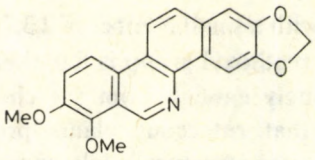
Sanguinarine (**3**) $R^1+R^2=OCH_2O$
Chelerythrine (**10**) $R^1=R^2=OMe$



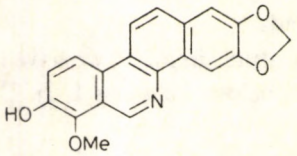
Dihydrosanguinarine (**5**) $R^1+R^2=OCH_2O$
Dihydrochelerythrine (**12**) $R^1=R^2=OMe$



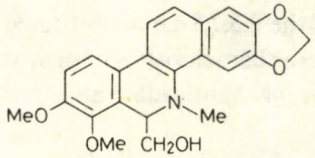
Oxysanguinarine (**6**)



N-Norchelerythrine (**11**)



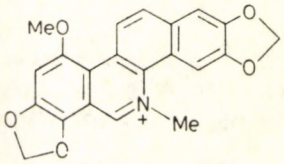
Decarine (**16**)



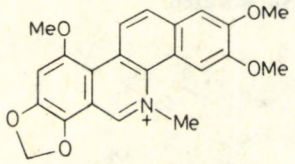
Bocconoline (**17**)

Scheme 1

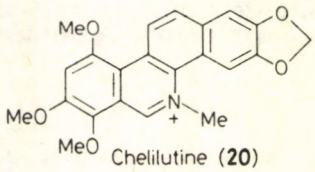
(b) Penta- or More Substituted Alkaloids



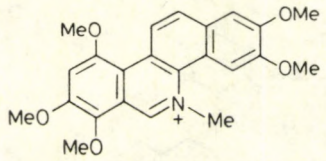
Chelirubine (**18**)



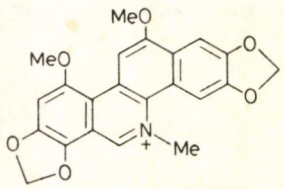
Sanguirubine (**19**)



Chelilutine (**20**)



Sanguilutine (**69**)



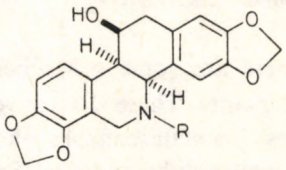
Macarpine (**21**)

Scheme 2

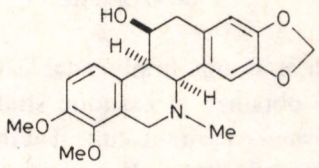
1.1.2. B/C-HEXAHYDROBENZO[c]PHENANTHRIDINE ALKALOIDS

Only two groups of these alkaloids, the chelidonine (Scheme 3) and corynoline groups (Scheme 4), are known. Their structural features are the B/C-*cis*-fused and B/C-hexahydrobenzo[c]phenanthridine skeleton with at least one hydroxyl group in the 11-position.

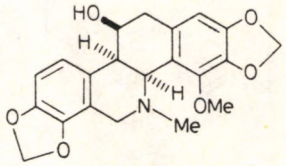
(a) Chelidonine group



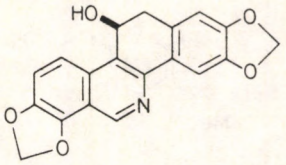
R=Me: Chelidonine (**2**)
R=H : Norchelidonine (**22**)



Homochelidonine (**23**)



Methoxychelidonine (**24**)

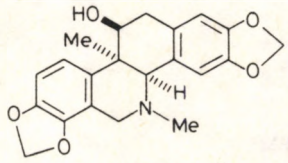


Luguine (**25**)*

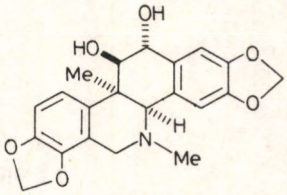
Scheme 3

(b) Corynoline group

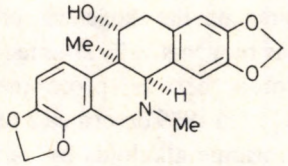
In addition to the above structural features of chelidonines, the alkaloids of this group have an additional methyl group at the ring junction (10b-position).



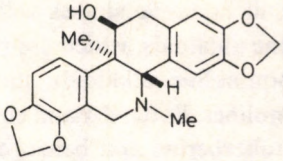
Corynoline (**4**)



12-Hydroxycorynoline (**26**)



11-Epicorynoline (**27**)



Isocorynoline (**28**)
(14-Epicorynoline)

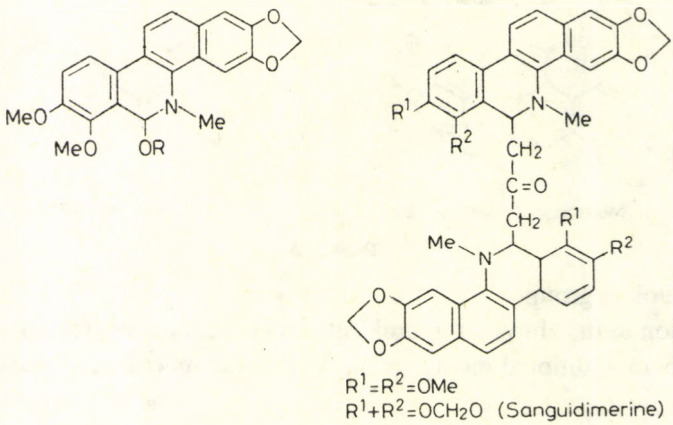
Scheme 4

The only exception in this group is isocorynoline **(28)**, which has a B/C-*trans*-fused ring system.

* Luguine **(25)** [4] is the newest addition to the benzo[c]phenanthridine alkaloids and is structurally situated between the fully aromatic and hexahydro types.

1.1.3. DERIVED ANALOGUES OF BENZO[c]PHENANTHRIDINE ALKALOIDS

Although it is not firmly established, there are some other benzo[c]phenanthridines obtained in isolation studies of plants. These derivatives, probable artefacts, always have a substituent at the 6-position of the benzo[c]phenanthridine skeleton, which is the position amenable to nucleophilic addition (Scheme 5).



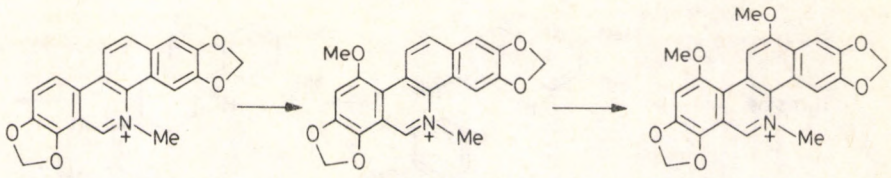
Scheme 5

1.2. BIOSYNTHESIS OF BENZO[c]PHENANTHRIDINE ALKALOIDS

Although still extensive studies will be required on the biogenesis of benzo[c]-phenanthridine alkaloids, which are regarded as biogenetically situated very close to other isoquinoline alkaloids such as protoberberines, protopines, and 1-benzylisoquinolines, Battersby and co-workers [5] in 1979 described a relationship between protoberberine and benzo[c]phenanthridine alkaloids by using isotope experiments as in the following biosynthetic pathway (Scheme 6).

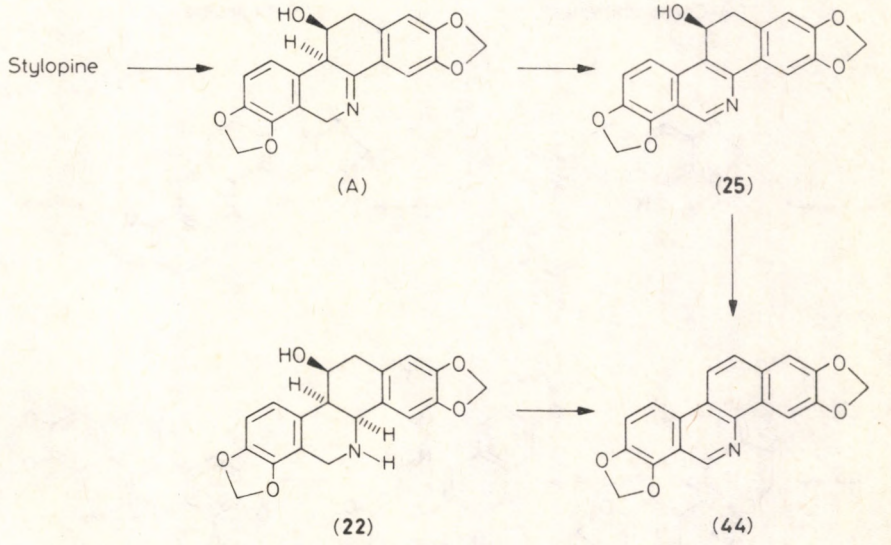
Nonaka and Nishioka [6] in 1975 also proposed a biogenetic relationship among alkaloids isolated from *Corydalis* plants. They also regarded the protoberberine alkaloids, such as coptisine and corysamine as the most plausible precursors in the biosynthesis of the corynoline group (Scheme 7).

Recently, Takao *et al.* [7] proposed a biogenetic conversion of the tetrasubstituted alkaloids into the corresponding penta- and hexasubstituted compounds by assuming introduction of oxygen functions into the aromatic ring at a later stage of the biosynthesis as shown below.



Sanguinarine (**3**) Chelirubine (**18**) Macarpine (**21**)

On the other hand, Castedo and co-workers [4] in 1978 isolated a new type of alkaloid, luguine **(25)**, and suggested that (+)-luguine might represent an important intermediate to explain the biosynthetic origin of the fully aromatic alkaloids such as norsanguinarine **(44)**, which would be formed as a result of either transformation of (−)-norchelidonine **(22)** or from a common intermediate (A) formed from stylopine.

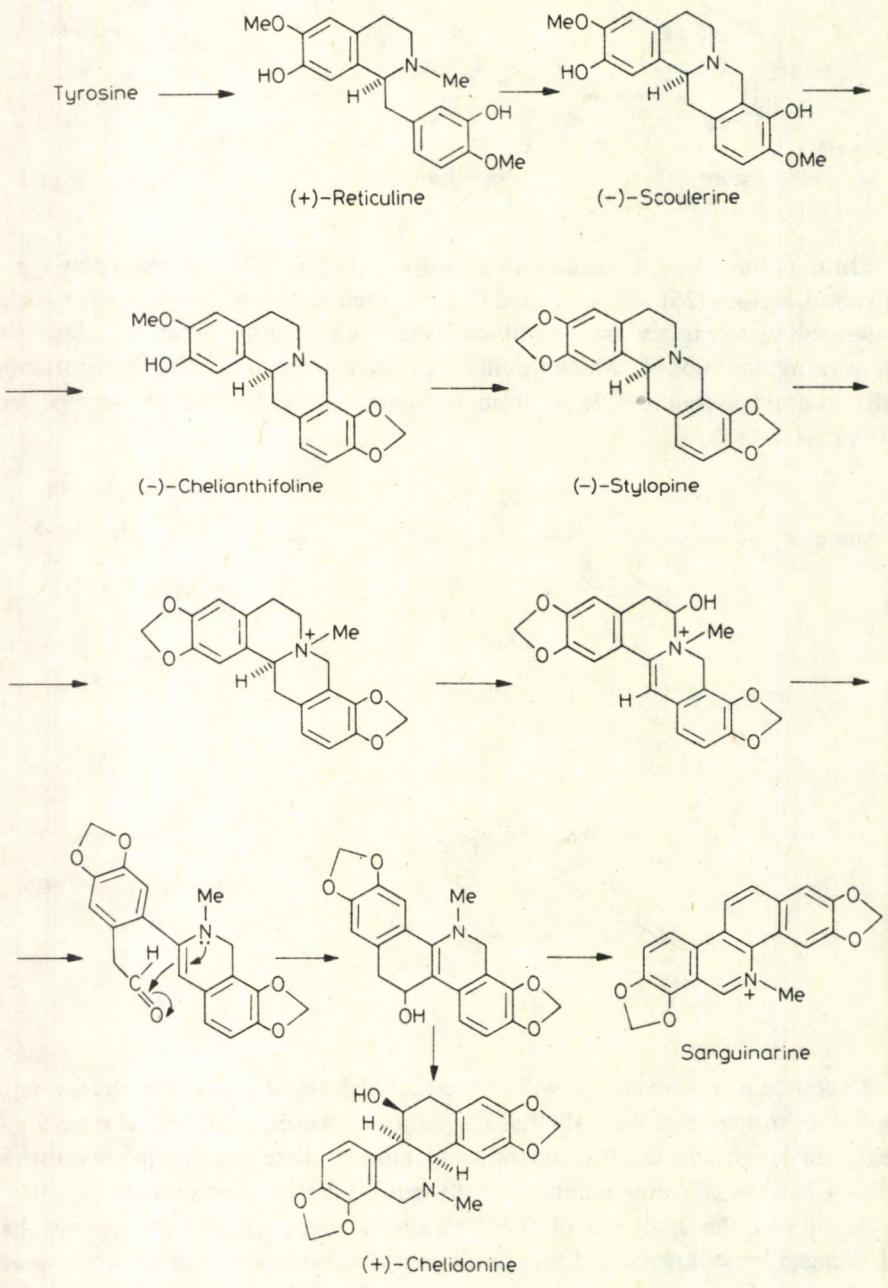


(A) (**25**)

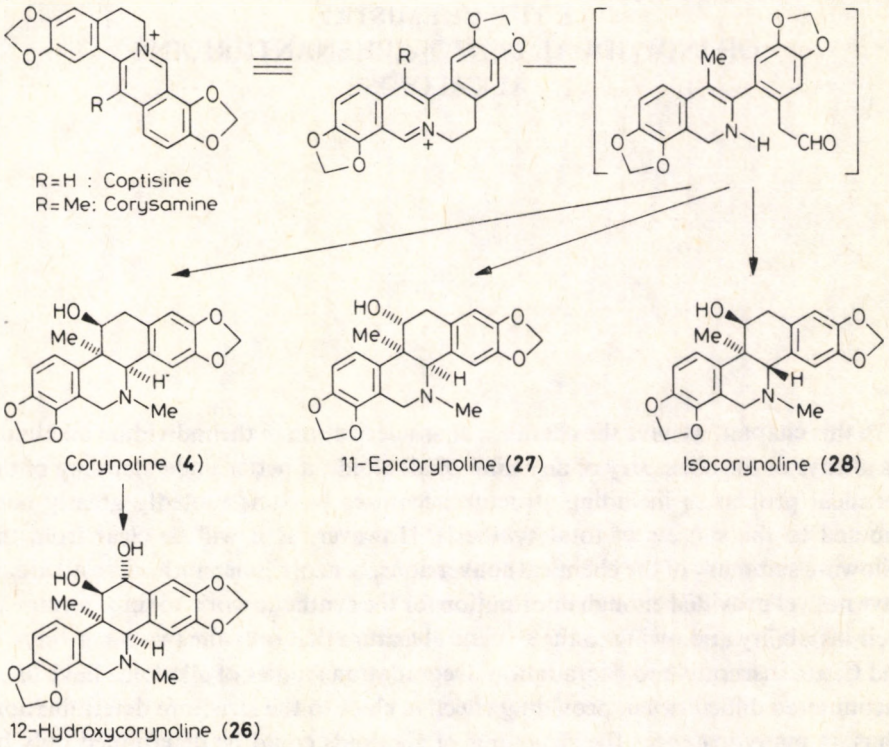
(**22**) (**44**)

Of course, extensive studies will be needed for the establishment of the formation and transformation of these alkaloids in plants. However, the above schemes may at least shed light on a gross relationship among these benzo[c]phenanthridine alkaloids, thus affording stimulus to efficient synthetic investigations.

Aiming at the synthesis of these alkaloids, many groups of chemists have challenged these targets, and as a result considerable knowledge has accumulated on the synthetic routes to benzo[c]phenanthridines, and this has also been successfully applied to the total synthesis of almost each alkaloid of this group.


Tyrosine
(+)-Reticuline
(-)-Scoulerine
(-)-Chelianthifoline
(-)-Stylopine
Sanguinarine
(+)-Chelidonine

Scheme 6



Scheme 7

In the following chapters, we intend to summarize synthetic works on benzo[c]phenanthridines, irrespective of the natural or artificial origin of the compounds, thus providing a good look at the patterns of synthetic approaches and their applications in the total syntheses of these alkaloids.

2. THE CHEMISTRY OF INDIVIDUAL BENZO[c]PHENANTHRIDINE ALKALOIDS

In this chapter, we give the chemical characterization of the individual alkaloids. As always in the chemistry of any kind of alkaloids, a better understanding of the chemical properties including structural features has undoubtedly greatly contributed to the success of total synthesis. However, as it will be clear from the following summary of the chemical conversions, benzo[c]phenanthridine alkaloids have not yet provided enough information for the synthetic work, mainly because of their instability and owing to the structural feature that only the two inner rings, B and C, are susceptible to degradation. Degradation studies of alkaloids have often encountered difficulties in providing effective clues to the structure determination. Thus, in many instances the structures of alkaloids could be determined only by total synthesis, particularly in the cases of the fully aromatic alkaloids, such as macarpine **(21)**, chelirubine **(18)**, and others, especially with respect to the positions and arrangement of substituents.

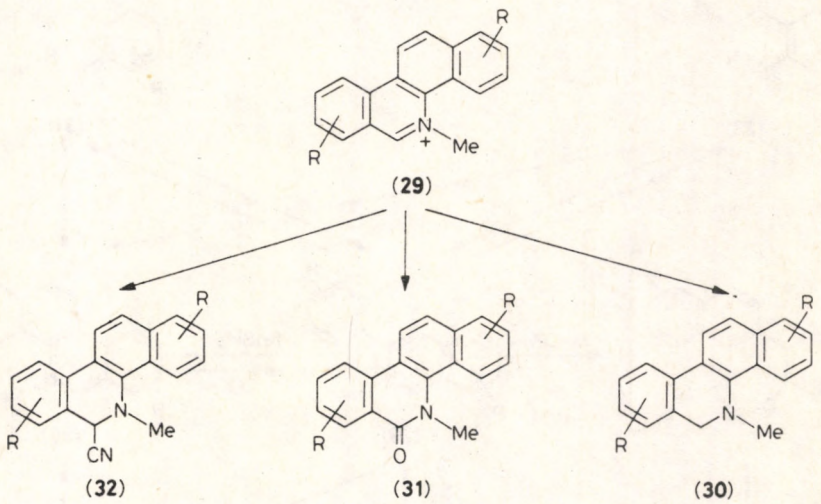
2.1. THE CHEMISTRY OF THE FULLY AROMATIC ALKALOIDS

This group of alkaloids is found in plants mostly in the form of the quaternary salts. Hence chemists have met with difficulties in isolating these aromatic alkaloids as they exist in nature. Therefore, conversions of the quaternary salts **(29)** into the isolable forms such as the corresponding 5,6-dihydrobenzo[c]phenanthridines **(30)** by reduction with sodium borohydride, the 6-oxo-derivatives **(31)** by oxidation with ferric cyanide, or the pseudocyanides **(32)** by reaction with potassium cyanide have been successfully applied and developed (Scheme 8).

Recently, introduction of a new chromatographic separation technique, the droplet countercurrent chromatography (DCCC) [8], has made a great contribution to the direct isolation and separation of the quaternary salts.

These quaternary salts are isolated as their chlorides, iodides, perchlorates or sulfates depending on their suitability for subsequent treatment.

The chemistry of the alkaloids possessing fully aromatic structure can be summarized as shown in Scheme 9, where the quaternary salt is placed in the centre, thus stressing interconversions to and from this compound in the course of the synthetic work.



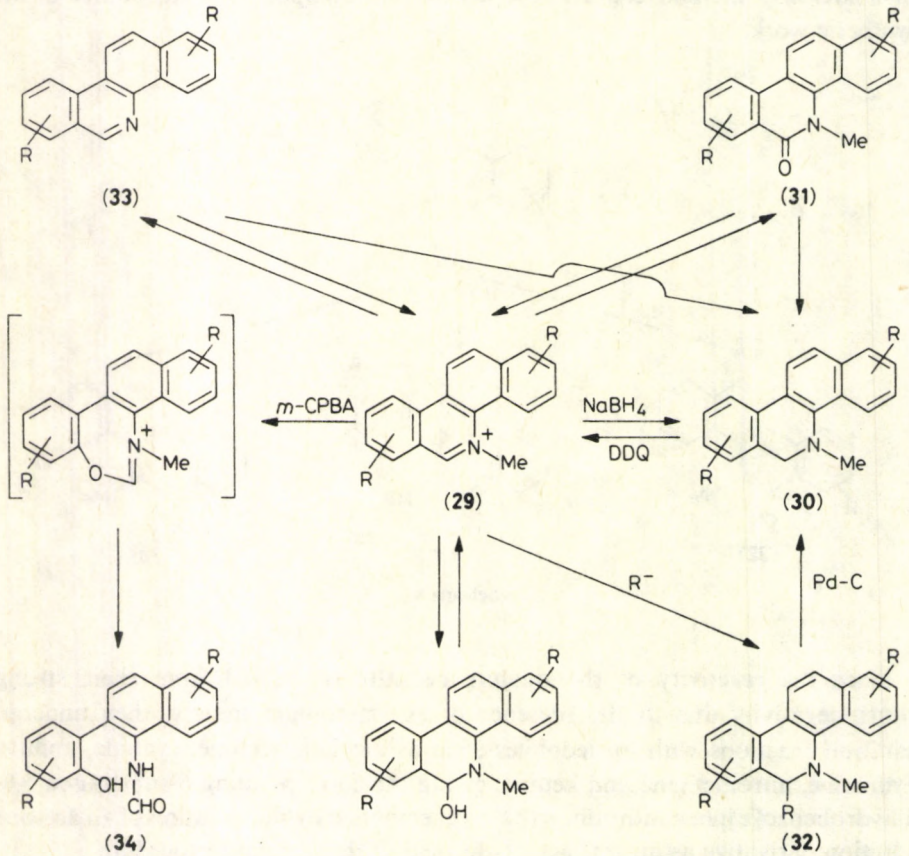
Scheme 8

Since the reactivity of the quaternary salts is derived from their strong electronegativity due to the presence of an immonium moiety, they undergo addition reactions with nucleophiles such as alcohol, acetone, cyanide, aniline, hydrazine, nitromethane and ketone, giving the corresponding 6-substituted 5,6-dihydrobenzo[c]phenanthridines **(32)** as exemplified by the isolation of an acetone addition derivative as an artefact [9] formed in the isolation procedure.

The quaternary salts **(29)** are also susceptible to oxidation and reduction to afford the corresponding 6-oxo- **(31)** and 5,6-dihydro **(30)** derivatives. On the way back to **29**, reduction [10] of the 6-oxo derivatives **(31)** with Vitride (Sodium *bis*(2-methoxyethoxy)aluminium hydride) readily afforded the 5,6-dihydro derivatives **(30)**, which then underwent dehydrogenation with DDQ or mercuric diacetate to restore the quaternary salts **(29)**, thus establishing a standard synthetic route for the final steps in the total synthesis of these alkaloids.

The quaternary salts **(29)** could undergo dealkylation under thermolytic conditions to afford the N-nor derivatives **(33)** which, in turn, could be converted into the quaternary salts **(29)** under conventional conditions using alkyl halide or

dialkyl sulfate in a protic solvent; however, yields are poor. Recently, Ishii *et al.* [11] have extensively investigated this process and established an apparently general procedure for this conversion; the N-nor derivatives **(33)** are first converted into the N-alkyl-5,6-dihydro derivatives **(30)** by the treatment with formic acid in the



Scheme 9

presence of sodium borohydride; dehydrogenation with DDQ converted then **30** into the quaternary salts **(29)**. Ishii [12] also described the procedure of choice for the conversion of the N-nor derivatives **(33)** into the tertiary amine **(30)** by the treatment with sodium borohydride and dimethyl sulfate in HMPA.

The immonium bond in the quaternary salts **(29)** is susceptible to addition reactions with nucleophiles as mentioned above, thus affording 6-substituted-5,6-dihydro derivatives **(32)**, of which some 6-alkyl- and 6-alkoxy derivatives are shown to have considerable antitumour activity.

When peracid was used as a nucleophile [13], the quaternary salts **(29)** underwent a Baeyer–Villiger-type oxidation (cleavage of a C=N$^+$ bond followed by insertion of an oxygen function into the 6a-position) to afford the 2-aryl-1-naphthylamines **(34)**, which were recently isolated from plants (e.g. arnottianamide and iso-arnottianamide) and synthesized [14] from the benzo[c]phenanthridine alkaloid nitidine **(1)**.

2.2. THE CHEMISTRY OF THE B/C-HEXAHYDROBENZO[c]PHENANTHRIDINE ALKALOIDS

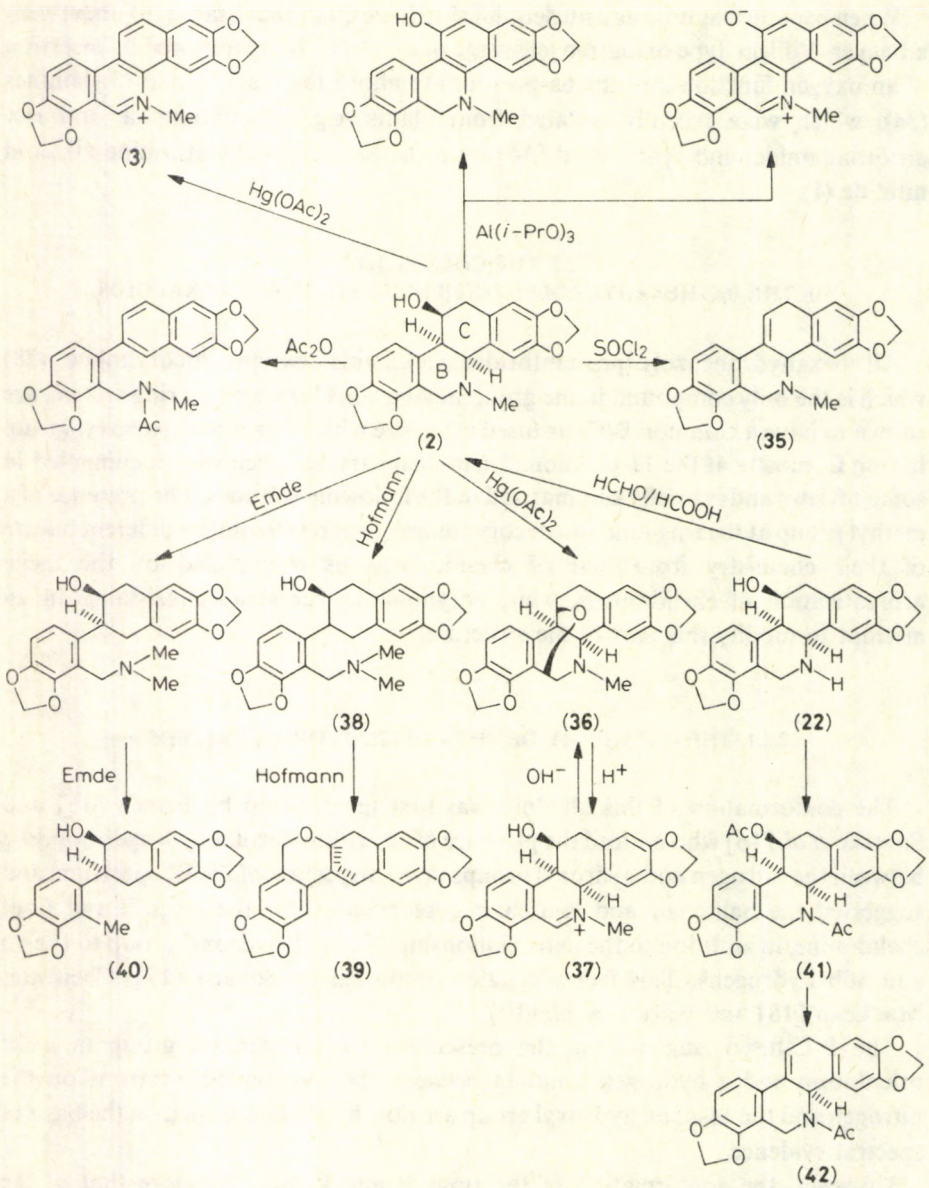
All hexahydrobenzo[c]phenanthridine alkaloids, except isocorynoline **(28)** which is the only compound in the group having a B/C-*trans*-fused ring system, are shown to have a common B/C-*cis*-fused structure with at least one hydroxyl group in ring C, mostly at the 11-position. Their chemistry has been well documented in some reviews and is briefly summarized in the following schemes. The presence of a methyl group at the ring junction in corynolines gives rise to quite a different nature of their chemistry from that of chelidonines, as exemplified by the facile aromatization of chelidonines, while corynolines offer serious resistance in an attempt to modify this part of the structure.

2.2.1. THE CHEMISTRY OF THE CHELIDONINE ALKALOIDS

The conformation of this alkaloid was first investigated by Bersch [15] and Snatzke *et al.* [16] who noticed the presence of an intramolecular hydrogen bonding between the nitrogen and hydroxyl groups upon inspection of the IR spectrum, and suggested the half-chair and half-chair conformation for the rings B and C of chelidonine, in addition to the *anti*-relationship of the 11-hydroxyl group to the 4b and 10b hydrogens. This fact was later supported by Seoane [17], Chen and MacLean [18] and Naruto *et al.* [19].

The B/C-fused ring system, the presence of a 11-hydroxyl group in *axial* orientation and a hydrogen bonding between the lone-paired electrons on the nitrogen and the 11-*axial* hydroxyl group are now firmly established on the basis of spectral evidence.

However, the conformation of the rings B and C, and therefore that of the chelidonine molecule had been disputed by many researchers and finally Cushman and Choong [20] suggested a B/C half-chair-envelope conformation from the inspection of the NMR spectrum at 360 MHz. It was also shown [17] that acetylation of the 11-hydroxyl group caused a change in the conformation of the molecule, thus making the problem even more difficult.


(3)
Hg(OAc)2
Al(i-PrO)3
Ac2O
SOCl2
(2)
(35)
Emde
Hofmann
Hg(OAc)2
HCHO/HCOOH
(38)
(36)
(22)
Emde
Hofmann
OH−
H+
(40)
(39)
(37)
(41)
(42)

Scheme 10

The absolute configuration of (+)-chelidonine was established [21] by examination of its crystalline *p*-bromobenzoate.

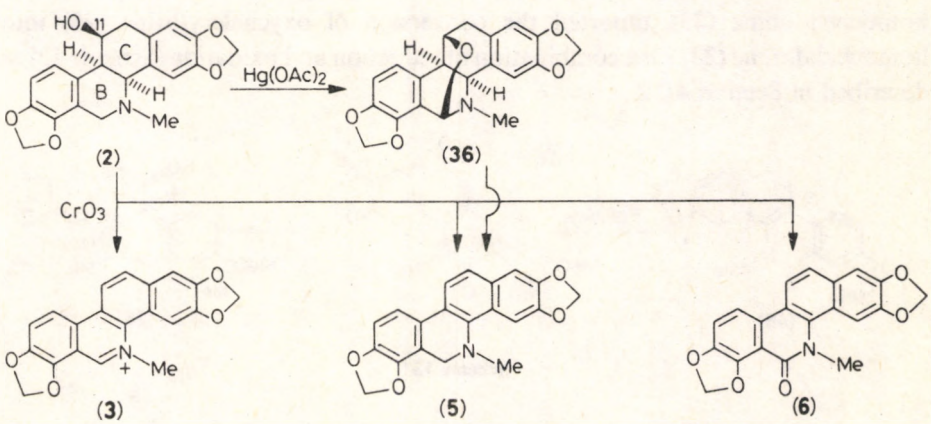
The 11-hydroxyl group of chelidonine **(2)** (Scheme 10) was readily eliminated [16] by treatment with thionyl chloride to give anhydrochelidonine **(35)**, which, however, has not been reconverted to chelidonine **(2)**. Oppolzer and co-workers [22, 23] reported the conversion of the N-acetyl- and N-ethoxycarbonyl derivatives into chelidonine **(2)** on their way to the total synthesis of chelidonine.

Chelidonine **(2)** was susceptible to oxidation [15] with mercuric diacetate to yield sanguinarine **(3)**. However, under mild conditions, using the same reagent or potassium permanganate in acetone solution, chelidonine **(2)** was converted into dehydrochelidonine **(36)** which has an intramolecular ether bond between the 11- and 6-positions and was shown to undergo cleavage of the ether linkage under acidic condition to give the 11-hydroxy derivative **(37)**, which was recyclized to the ether **(36)** by alkali.

The cleavage of ring B has been the most studied reaction in the investigation of the structure of chelidonine. The quaternary methyl iodide underwent ring opening under the conditions of Hofmann degradation to afford the corresponding methine derivative **(38)**, which was further degraded to give the des-N-derivative **(39)**. When chelidonine **(2)** was treated twice under the Emde degradation conditions, de-N-chelidonine **(40)** was obtained as a result of loss of nitrogen.

Norchelidonine **(22)** underwent methylation to give chelidonine **(2)**, and was acetylated to give the N,O-diacetate **(41)**, which was converted into the unsaturated N-acetate **(42)**.

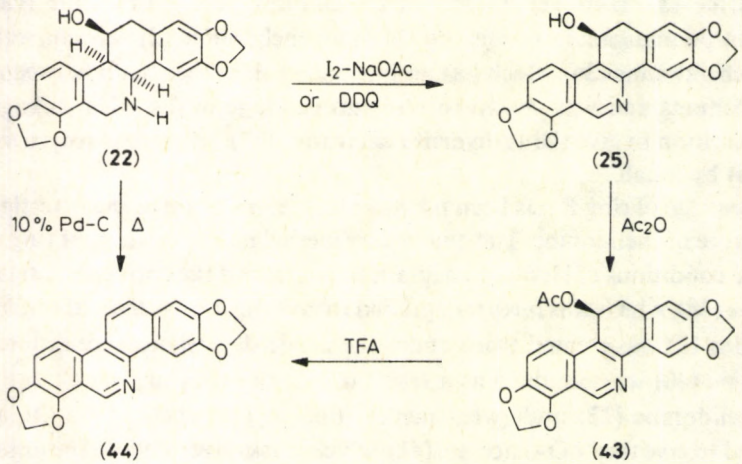
Interconversions of the chelidonine group of alkaloids are summarized in Scheme 11.



Scheme 11

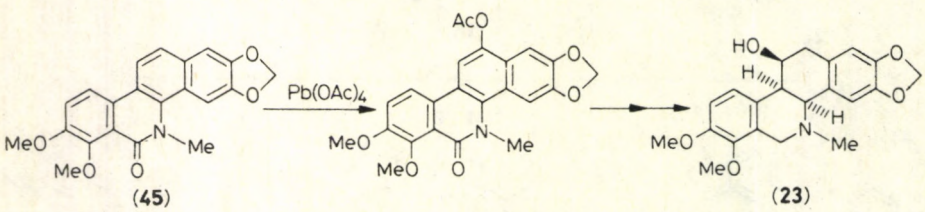
The instability of chelidonine **(2)** is mainly due to the presence of a labile 11-hydroxyl group in *axial* orientation, which is readily eliminated to cause aromatization, thus hampering studies on the conversion into other alkaloids. The only conversion reported [15] was oxidation to sanguinarine **(3)**, dihydrosanguinarine **(5)** and oxysanguinarine **(6)**.

Recently, Castedo *et al.* [4] isolated a new alkaloid, luguine **(25)**, and reported the one-step conversion of norchelidonine **(22)** into this alkaloid **(25)** and norsanguinarine **(44)**, which was also obtained from **(25)** *via* the acetate **(43)** (Scheme 12).



Scheme 12

On the other hand, Ninomiya and co-workers [24], in their total synthesis of homochelidonine **(23)**, reported the conversion of oxychelerythrine **(45)** into homochelidonine **(23)** *via* a combination of reduction and oxidation (Scheme 13) as described in Section 4.2.2.



Scheme 13

2.2.2. THE CHEMISTRY OF THE CORYNOLINE ALKALOIDS

The configuration of the B/C-*cis*-fused ring system and the 11-*axial* hydroxyl group of corynoline **(4)** was determined [25] in similar manner as in the case of chelidonine **(2)**, using various pieces of spectral evidence. The conformation of crystalline (±)-corynoline *p*-bromobenzoate was established [26] by X-ray analysis.

The absolute configuration of the (+)-alkaloid was suggested [27] from the inspection of the CD spectra of corynoline and corynolone **(52)** by applying the helicity and octant rules.

Corynoline **(4)** has much greater stability and inactivity than chelidonine **(2)** due to the presence of the 10b-methyl group at the ring junction, though thermolysis above 300°C causes dehydration and demethylation to yield norsanguinarine **(44)** [25] (Scheme 14).

The 11-*axial* hydroxyl group in corynoline **(4)** resists oxidation to some extent, yet it can be oxidized under the conditions of the potassium *tert*-butoxide–benzophenone method to obtain corynolone **(52)** [25]. The behaviour toward other oxidizing agents roughly corresponds to that of chelidonine **(2)**. Oxidation with mercuric diacetate yielded corynoloxine **(47)** as a result of oxidation at the 6-position followed by cyclization with the 11-hydroxyl group, while oxidation [25] with potassium permanganate afforded 6-oxocorynoline **(48)**.

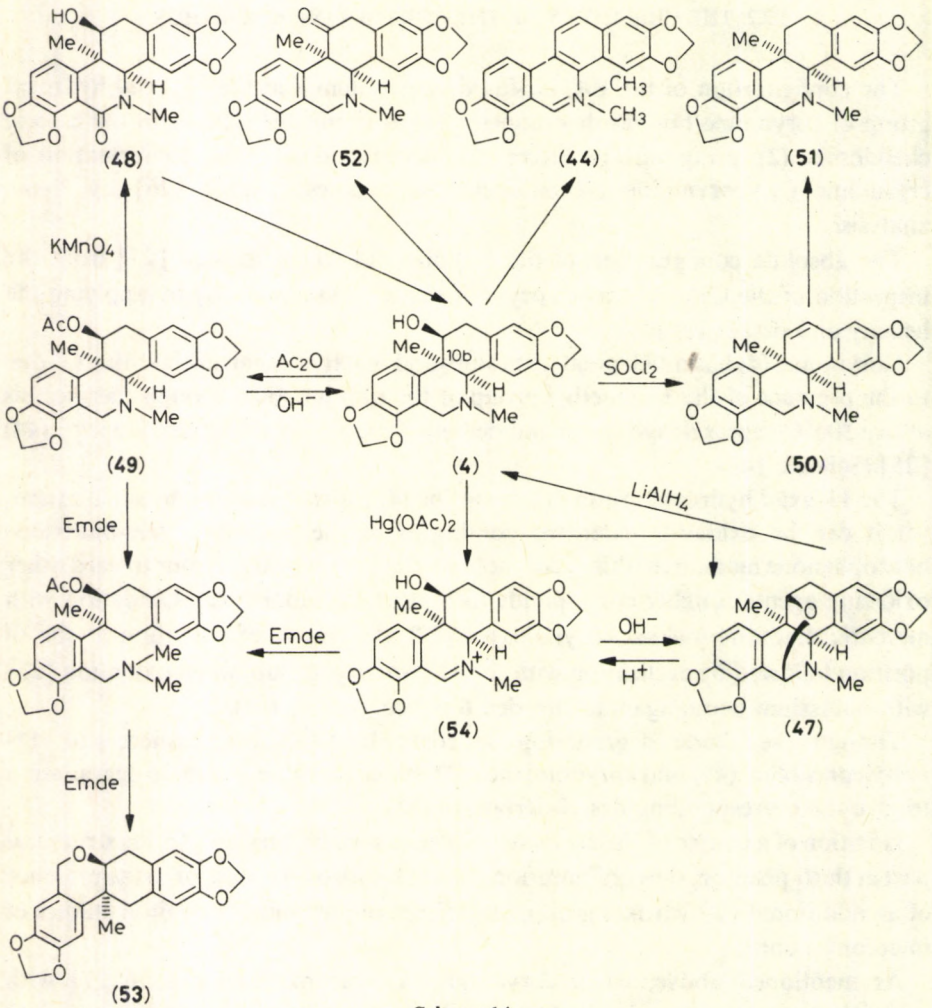
Though the Emde degradation of corynoline **(4)** was unsuccessful, 11-acetylcorynoline **(49)** and corynoloxine **(47)** underwent double Emde degradation to give the corresponding des-N-derivative **(53)**.

Isolation of a couple of closely related alkaloids which vary only in the structural part at the 6-position, the configuration of the 11-hydroxyl group, or in the presence of an additional 12-hydroxyl group, has offered opportunity for fruitful studies of interconversions.

As mentioned above, when corynoline **(4)** was oxidized [25, 26, 27] with mercuric diacetate, corynoloxine **(47)** was obtained, while oxidation with potassium permanganate in acetone solution afforded 6-oxocorynoline **(48)**. On the other hand, lithium aluminium hydride reduction of these derivatives (**48** and **54**) gave the alkaloid **(4)**, thus completing the chain of interconversions.

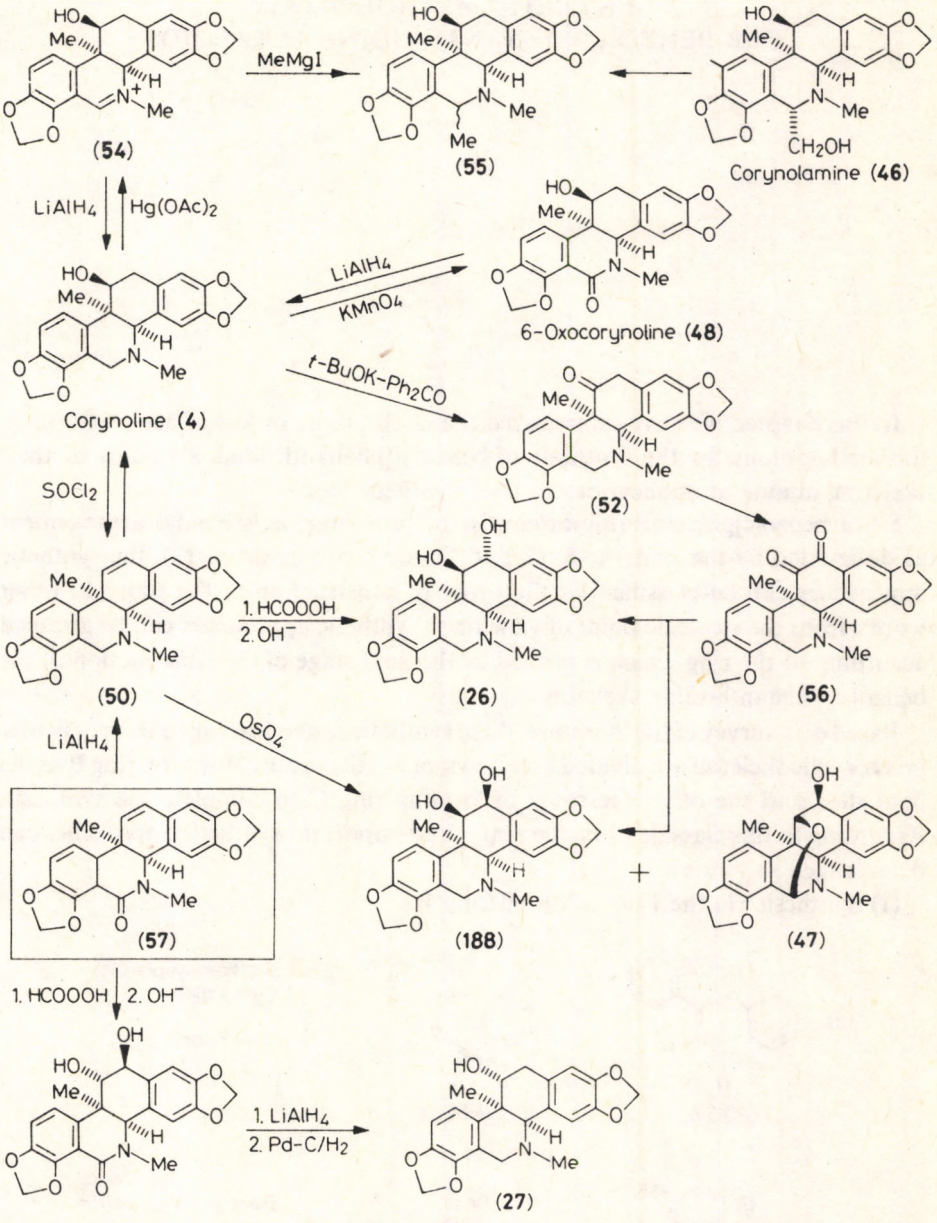
The 6-substituted corynolamine **(46)** [28] was converted into the corresponding 6-methyl derivative **(55)** (Scheme 15), which was also obtained from corynoline **(4)**.

Nonaka and Nishioka [6] described the conversion of corynoline **(4)** into 12-hydroxycorynoline **(26)** *via* two routes; the one by dehydration of **4** with thionyl chloride followed by hydroxylation of the 11,12-double bond, and the other, *via* the route involving the steps of oxidation to the 11,12-dioxo derivative **56** followed by reduction.



Scheme 14

By utilizing the above conversions, Ninomiya *et al.* [29] succeeded in achieving total syntheses of the corynoline group of alkaloids; first deoxy-6-oxocorynoline **(57)** was made *via* enamide photocyclization, and this was followed by stereoselective introduction of hydroxyl groups into the 11- and 12-positions, thus completing the first total synthesis of 11-epicorynoline **(27)** [29b, c], corynoline **(4)** [29a, b] and 12-hydroxycorynoline **(26)** [29a, b] (*cf.* Section 4.2.1.).


(54)
MeMgI
(55)
Corynolamine (46)
LiAlH4
Hg(OAc)2
LiAlH4
KMnO4
6-Oxocorynoline (48)
Corynoline (4)
t-BuOK-Ph2CO
(52)
SOCl2
1. HCOOH
2. OH⁻
(50)
(26)
(56)
LiAlH4
OsO4
(57)
(188)
(47)
1. HCOOH
2. OH⁻
1. LiAlH4
2. Pd-C/H2
(27)

Scheme 15

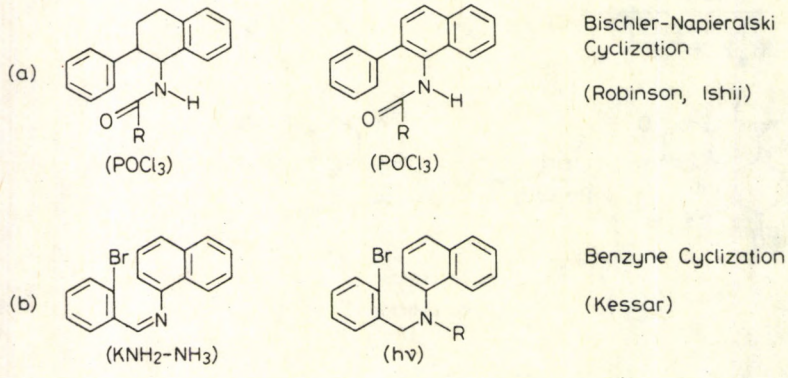
3. SYNTHETIC METHODOLOGY FOR BENZO[c]PHENANTHRIDINE ALKALOIDS

In this chapter, we have summarized the works, more or less of basic nature, on the methodology for the synthesis of benzo[c]phenanthridine alkaloids or their skeleton, aiming at applications in total synthetic studies.

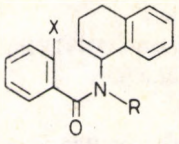
Since benzo[c]phenanthridines consist of four rings in steroidal arrangement as designated by the rings A, B, C, and D (see footnote on p. 12), the synthetic approaches can be classified by the order of construction of the rings. In other words, from the strategic point of view these synthetic approaches can be grouped according to the ring which is formed as the final stage of the construction of the benzo[c]phenanthridine skeleton.

Based on a survey of the literature, these synthetic routes leading to this particular heterocyclic skeleton are divided as follows; one is the route of forming ring B as the final step, and the other the route of forming ring C to complete the synthesis. According to this classification, the major and important synthetic approaches can be arranged as follows.

(1) Synthesis *via* the Formation of Ring B

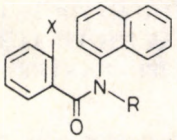


(c)



X=H, OMe, Br

(hν)

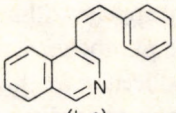


(hν)

Enamide Photocyclization
(Ninomiya, Kessar)

(2) Syntheses *via* the Formation of Ring C

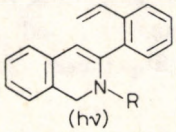
(a)



(hν)

Stilbene-type Cyclization
(Onda, Sainsbury)

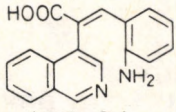
(b)



(hν)

Anhydroprotopine Cyclization
(Onda)

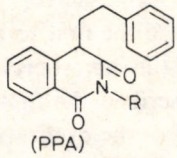
(c)



(HNO_2-Cu)

Pschorr Cyclization
(Abramovitch)

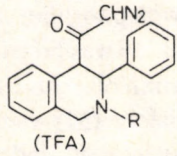
(d)



(PPA)

Isocarbostyril Cyclization
(Robinson)

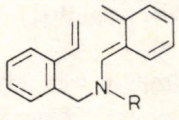
(e)



(TFA)

Dieckmann-Thorpe Cyclization
(Shamma, Cushman)

(3) Other Approach



Cycloaddition
(Oppolzer)

3.1. SYNTHETIC METHODS INVOLVING THE FORMATION OF RING B AS THE FINAL STEP IN THE CONSTRUCTION OF THE BENZO[c]PHENANTHRIDINE SKELETON

In the construction of this particular ring system, the routes involving the formation of the heterocyclic ring B as the final step have been considered the most orthodox approach leading to benzo[c]phenanthridines. Therefore, it is no wonder that the well-known Bischler–Napieralski cyclization of isoquinoline synthesis, which had been thoroughly studied and established as one of the most useful synthetic tools in heterocyclic chemistry, caught the attention of chemists from the beginning of researches in this field and it may appear still attractive.

On the other hand, as knowledge on organic photochemistry has accumulated and gained popularity, it has also been introduced into this particular field of heterocyclic chemistry, helping to develop a new methodology for the construction of the benzo[c]phenanthridine skeleton, as in the cases of benzyne cyclization and enamide photocyclization, which are now being regarded as the most widely applicable synthetic tools in this field, permitting the accomplishment of total syntheses of a number of alkaloids.

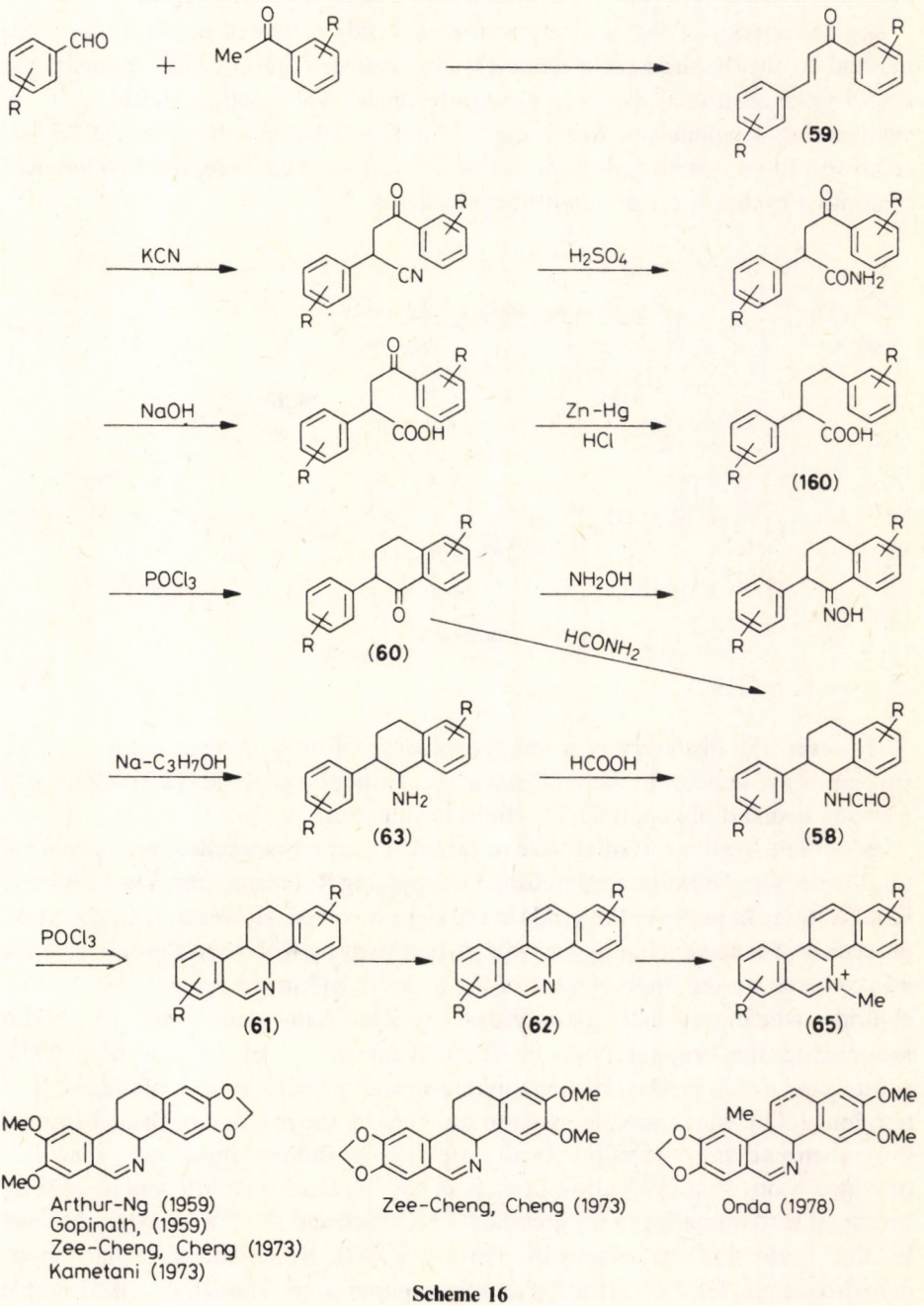
3.1.1. THE APPLICATION OF THE BISCHLER–NAPIERALSKI CYCLIZATION

Robinson *et al.* [30] dominated synthetic works on benzo[c]phenanthridines at the dawn of research in this field, and they were the first to apply the Bischler–Napieralski cyclization for the construction of this heterocyclic ring system.

In 1937, Robinson and co-workers [30] described the first application of the Bischler–Napieralski reaction, which consisted of the cyclization of N-(2-phenyl-1,2,3,4-tetrahydro-1-naphthyl)-formamide **(58)** with phosphoryl chloride in toluene under reflux (Scheme 16). The aliphatic formamide **58** was prepared by the sequence of reactions shown in the scheme. The condensation of a substituted benzaldehyde with substituted acetophenone afforded the chalcone **(59)** which was treated with potassium cyanide to give the keto-nitrile. Hydrolysis under acidic and basic conditions yielded the keto-acid, which was subjected to Clemmensen reduction to obtain the 2,4-diphenylbutyric acid. Friedel–Crafts-type cyclization gave the 2-aryl-1-tetralone **(60)**, which was converted into the aliphatic formamide **(58)** either by the Leuckart reaction or through the oxime and the naphthylamine **(63)**, which was formylated to give **58**.

The product of the Bischler–Napieralski cyclization was 4b,10b,11,12-tetrahydrobenzo[c]phenanthridine **(61)**. Therefore, successive dehydrogenation to remove

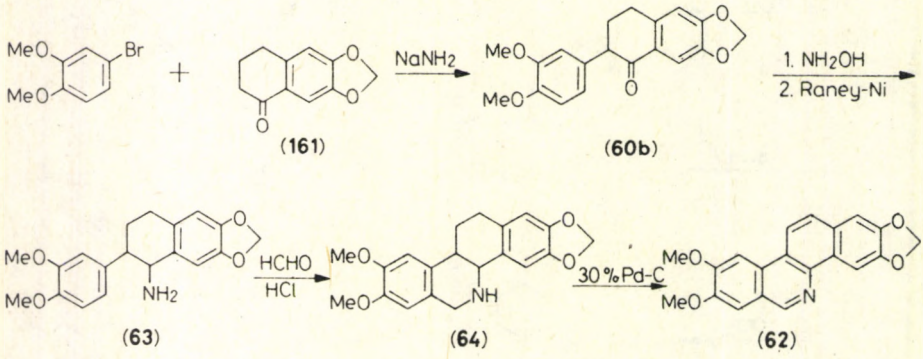
Robinson's Synthesis *via* the Bischler–Napieralski Reaction



Scheme 16

four hydrogens leading to the aromatic compounds **(62)** was required. By using this cyclization, the total syntheses of nitidine-type alkaloids were accomplished.

Regioselectivity of the cyclization provided only a limited application of this method to the synthesis of nitidine **(1)** and avicine-type alkaloids, namely, the 2,3,8,9-tetrasubstituted benzo[c]phenanthridines. The method failed [30] in synthesizing sanguinarine and chelerythrine-type alkaloids, namely, the 2,3,7,8-tetrasubstituted benzo[c]phenanthridine derivatives. Therefore, the development of another cyclization reaction attracted interest.



Scheme 17

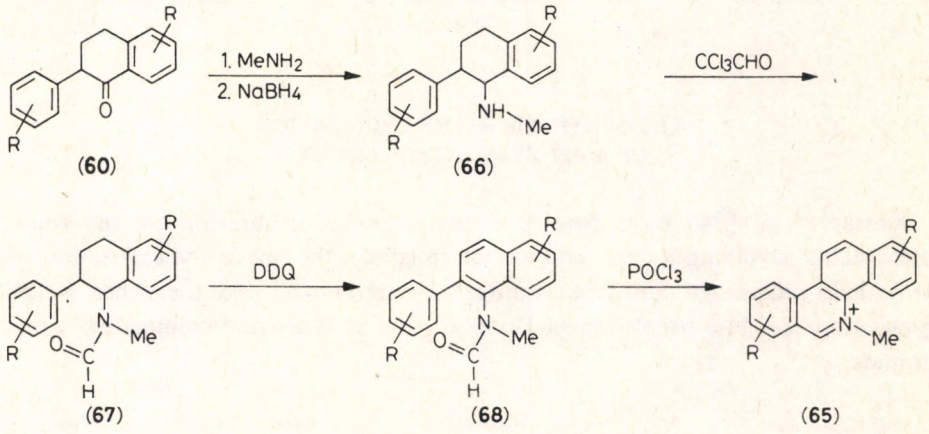
However, the discovery of a marked potency of nitidine against tumour and leukemia gave impetus to the synthesis of this particular alkaloid **(1)**, in response to a strong demand of supply for its clinical evaluation.

As a result, synthetic studies were restarted in the early seventies and a couple of total syntheses of nitidine accomplished by applying Robinson's method with some modifications to improve the yields in the steps involved. However, the cyclization procedure remained actually the same as that used by Robinson's group. Interest was centered on the improvement of the overall yield in the synthesis of nitidine chloride, which was first accomplished by Zee-Cheng and Cheng [31], who rationalized the original synthetic steps. Kametani *et al.* [32], also in 1973, contributed to this method by developing a new short-cut synthesis of the 2-aryl-1-tetralone **(60b)**, which was prepared in one step by the reaction of the substituted bromobenzene and 1-tetralone in the presence of sodium amide, and they also provided a one-step cyclization process of the tetrahydronaphthylamine **(63)** by heating it with formaline in the presence of HCl (Scheme 17). The product obtained by this route was the tetrahydro derivative **(64)**, which therefore had to be dehydrogenated for aromatization and then quaternized. Though it looked simple

and conventional, these two processes are actually tedious and often give only poor yields, particularly in the chemistry of benzo[c]phenanthridines.

Though there were a number of attempts to improve the overall yield of the total synthesis of the alkaloid, these efforts resulted only in some improvements of some of the steps.

Then in 1977, Ishii and co-workers [14a] made a major contribution to this synthesis by effecting the Bischler–Napieralski cyclization of the N-methyl-N-naphthylformamide **(68)** as shown below (Scheme 18).

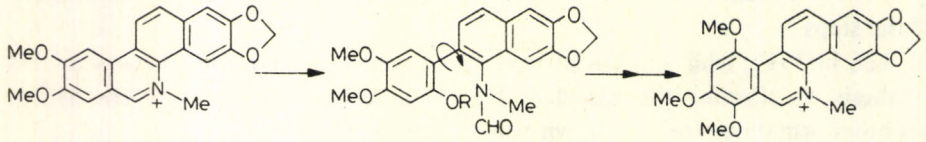


Scheme 18

They prepared the 2-aryl-1-tetralone **(60)** according to Robinson's procedure [30] and then introduced their major modifications. Treatment of the 1-tetralone **(60)** with methylamine in the presence of titanium tetrachloride followed by sodium borohydride reduction afforded the 2-aryl-1-methylaminotetralin **(66)**, which was then converted into the N-methylformamide **(67)** by treatment with chloral. Dehydrogenation with DDQ afforded the N-(2-aryl-1-naphthyl)--N-methylformamide **(68)**. The Bischler–Napieralski cyclization [14] of this aromatic N-methylformamide **(68)** afforded directly the quaternary salt **(65)**. By applying this modification, Ishii *et al.* accomplished the total syntheses of the following alkaloids in high yields of cyclization (in parentheses); chelirubine **(18)** (30.3%) [14a], chelilutine **(20)** (55.9%) [14a], sanguirubine **(19)** (42.4%) [33], and sanguilutine **(69)** (60.8%) [33].

Three main advantages can be mentioned in Ishii's modification [14a, 33]; (1) it is suitable for the large scale preparation of alkaloids; (2) N-methylation is effected at an early stage of the synthesis, and (3) there is a wide applicability to a variety of benzo[c]phenanthridines with different substitution patterns in rings A and D.

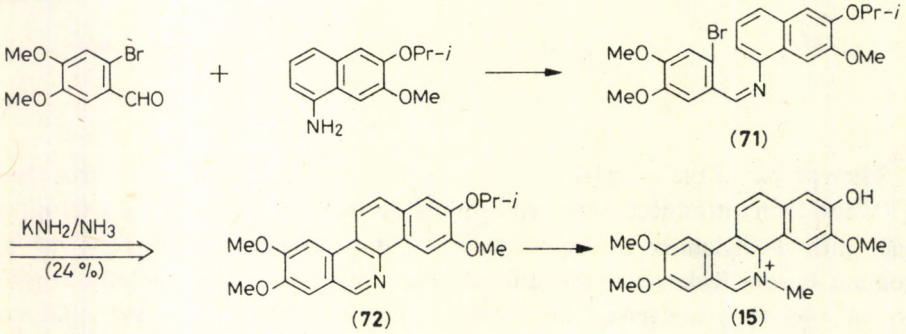
Their most beautiful application of the method can be seen [14a] in the conversion of nitidine **(1)** to chelilutine **(20)** *via* the newly isolated open-ring alkaloid, isoarnottianamide **(70)**, in only three steps.



Nitidine (**1**) Isoarnottianamide (**70**) Chelilutine (**20**)

3.1.2. SYNTHESIS *via* THE CYCLIZATION OF A BENZYNE INTERMEDIATE

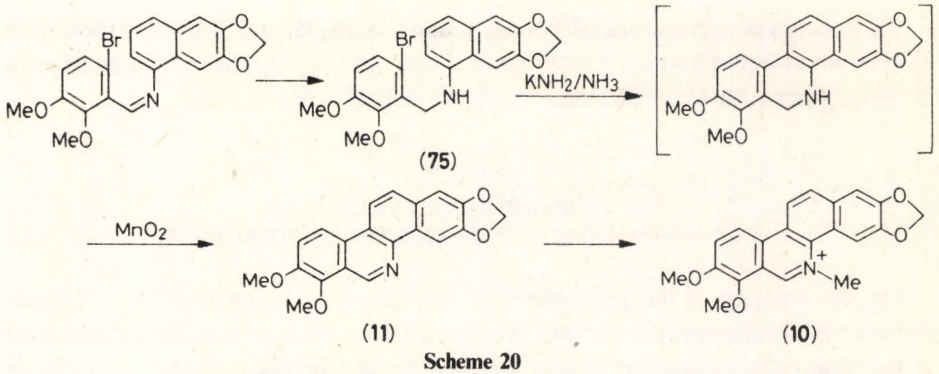
Kessar *et al.* [34] have developed two types of cyclization by the route presumably involving a benzyne-type intermediate; the one is the cyclization of *ortho*-halo-substituted anil-type conjugated compounds, and the other is the cyclization of N-*o*-bromobenzyl-1-naphthylamine-type non-conjugated compounds.



Scheme 19

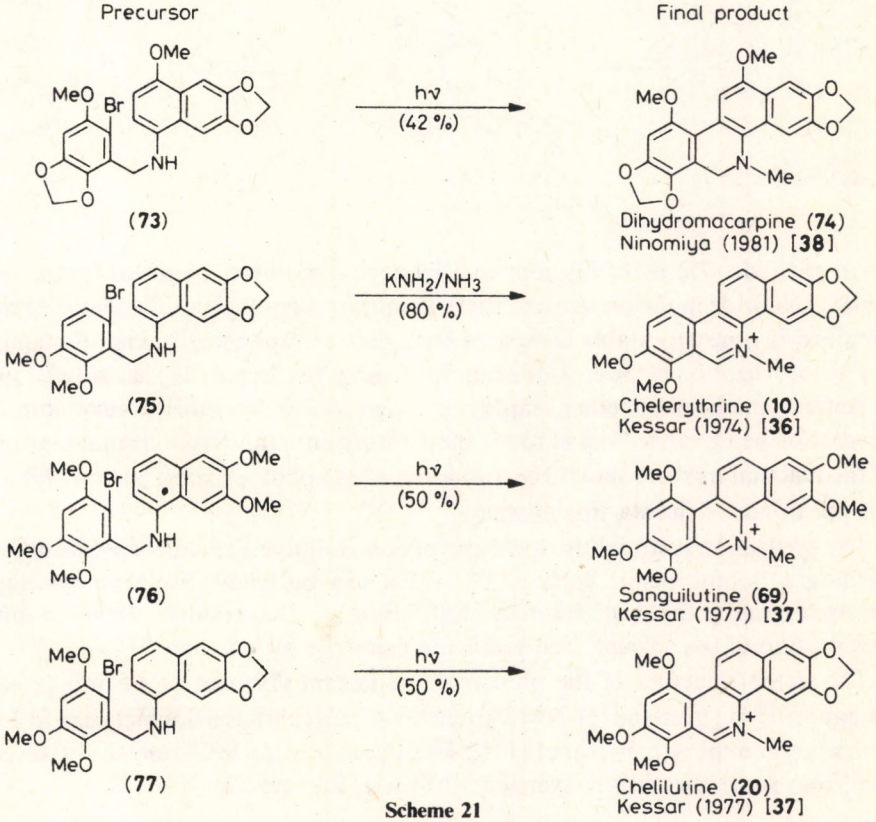
Treatment of the *o*-haloanil-type compounds, which are readily prepared from *o*-halobenzaldehyde and 1-naphthylamine, with potassium amide in liquid ammonia directly leads to the formation of the benzo[c]phenanthridine skeleton. This stilbene-type cyclization of anils was first carried out in concentrated sulfuric acid under irradiation, giving only poor yields of the product with cumbersome work-up. Kessar and co-workers then introduced the procedure using potassium amide in liquid ammonia, thus making this cyclization to attain a great practical value.

In 1974 Gillespie *et al.* [35a, b] successfully applied the method in the total synthesis of a potent antitumour alkaloid, fagaronine **(15)** (Scheme 19).



Scheme 20

Later Kessar *et al.* [36] found that sometimes the overall yields of the above *ortho*-haloanil cyclizations could be improved when the corresponding Schiff base was reduced into **75** prior to cyclization; thereby a new, widely applicable cyclization of *o*-bromobenzyl-1-naphthylamines was developed (Scheme 20).

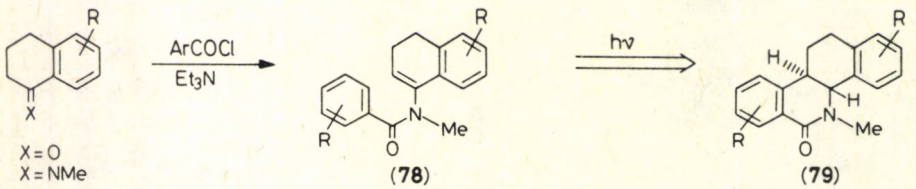


Scheme 21

These benzyne cyclizations of the compounds **73**, **75**, **76**, and **77** by treatment with potassium in liquid ammonia resulted in successful total syntheses of a number of benzo[c]phenanthridine alkaloids as shown below (Scheme 21).

3.1.3. ENAMIDE PHOTOCYCLIZATION FOR THE SYNTHESIS OF BENZO[c]PHENANTHRIDINES

On the analogy of the photochemical conversion of hexatrienes to cyclohexadienes, which is basically a reaction occurring from a photochemically excited state of the conjugated 6π electron system, Ninomiya *et al.* [39] discovered in 1969 a facile and general photocyclization of α,β-unsaturated acylenamines (enamides) and extended the reaction [40] to obtain a general method for the synthesis of various nitrogen-containing heterocyclic compounds, mainly alkaloids, including phenanthridines, benzo[a]-, [c]- or [i]-phenanthridines, protoberberines, yohimbines and azayohimbines.

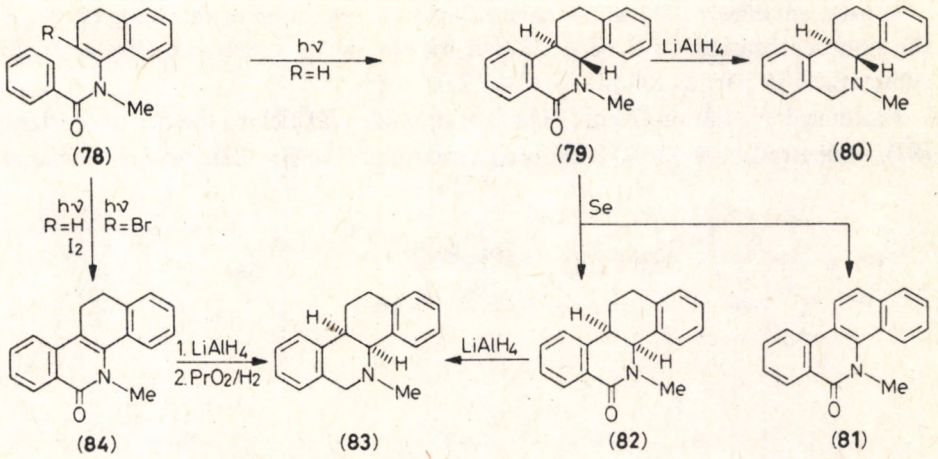


An enamide **(78)** is readily prepared by acylation of the 1-tetraloneimine with benzoyl chloride in the presence of triethylamine in a good yield. The enamide thus obtained is generally stable compared with the corresponding imine or enamine.

Photocyclization of the enamide can also readily be effected [41] in a solvent such as benzene, methanol, or ether, employing a high- or low pressure mercury lamp, the irradiation being carried out at room temperature until thin-layer chromatography of the reaction mixture shows the formation of the photocyclized lactam **(79)** and disappearance of the starting enamide.

The cyclization is straightforward and of nonoxidative and stereospecific nature, yielding a homogeneous lactam **(79)** which can be readily isolated by simple chromatography or just by recrystallization of the residue obtained after evaporation of the solvent. The yields are generally higher than 50%.

The stereo structure of the photocyclized lactam **(79)** can be unambiguously determined [41] from the ^{1}H-NMR spectrum which exhibits a doublet peak at δ 4.7 with a large coupling constant of 11–12 Hz, thus indicating B/C-*trans* structure, and also from some chemical conversions shown in Scheme 22.

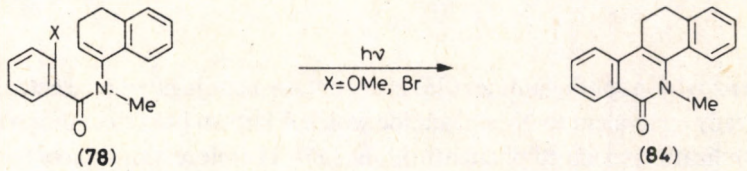


Scheme 22

Lithium aluminium hydride reduction of the lactam **(79)** gave the corresponding *trans*-amine **(80)**. When the lactam **(79)** was heated with selenium at an elevated temperature in order to prove the skeleton of the photocyclized product **(79)** as being benzo[c]phenanthridine by converting it into the known aromatic lactam **(81)**, the major product was the *cis*-lactam **(82)**, isomeric with the photoproduct **(79)**, along with a small amount of the expected lactam **(81)**. Reduction of the *cis*-lactam **(82)** with lithium aluminium hydride afforded the *cis*-amine **(83)**, which was also obtained from the oxidative photocyclization of the enamide and successive reductions. Comparison of the NMR spectra of these pairs of isomers **(79** and **82)** and **(80** and **83)** proved their stereochemistry.

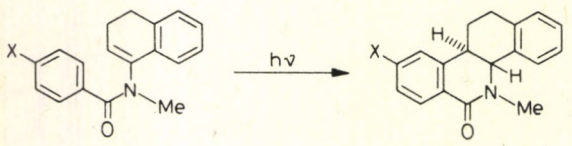
Regioselectivity [43] of the enamide photocyclization can be controlled by the use of an *ortho*-substituent which plays the part of a leaving group upon cyclization.

A methoxy- or bromo-substituted enamide **(78)** can undergo oxidative photocyclization [41] in the presence of iodine to afford the corresponding dehydrolactam **(84)** homogeneously and in a good yield; the product is particularly useful for further conversions into aromatic benzo[c]phenanthridines, the basic structures of many aromatic alkaloids [10, 29b, 29d, 41, 42]. As a result of elimination of the *ortho*-substituent, the products obtained in this case are always the dehydrolactams **(84)**.

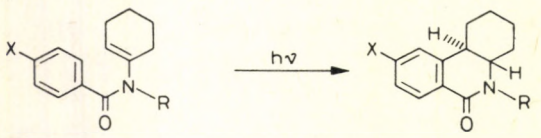


Substituent effects [43] which have either an accelerating or retarding effect on the photocyclization have been studied by Ninomiya and co-workers and are summarized in part as follows (Scheme 23).

Prolonged irradiation (70 h) [43] of the enamide **(78)** yielded the B/C-*cis*-lactam **(82)**, while irradiation for 40 h afforded a mixture of the *cis*- **(82)** and *trans*-lactams



X	Yield (%)
H	51-55
OMe	74
CN	20
COOMe	20

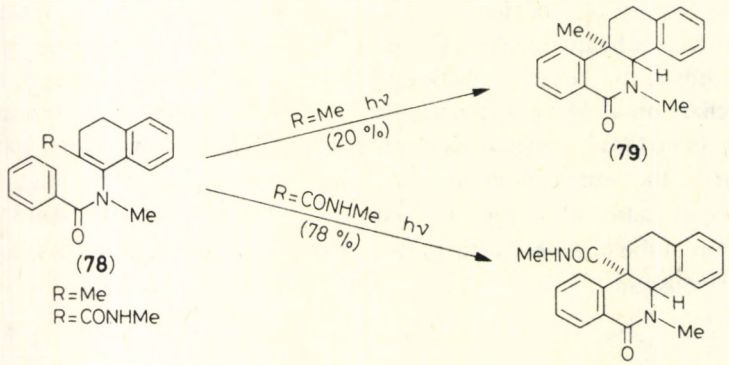


X	Yield (%)
H	55
OMe	29
COOMe	10

Scheme 23

(79) (7 and 1%). This result clearly suggested that *trans*-benzo[c]phenanthridones **(79)** were susceptible to photo-induced isomerization to afford the stable *cis*-isomers **(82)**.

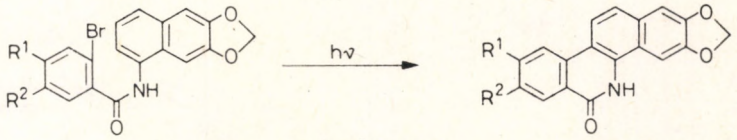
The presence of a substituent on the enamide double bond lowered the yield of cyclization presumably due to steric hindrance, but an electron-withdrawing group such as ester and amide groups raised the yield considerably [29b, d].



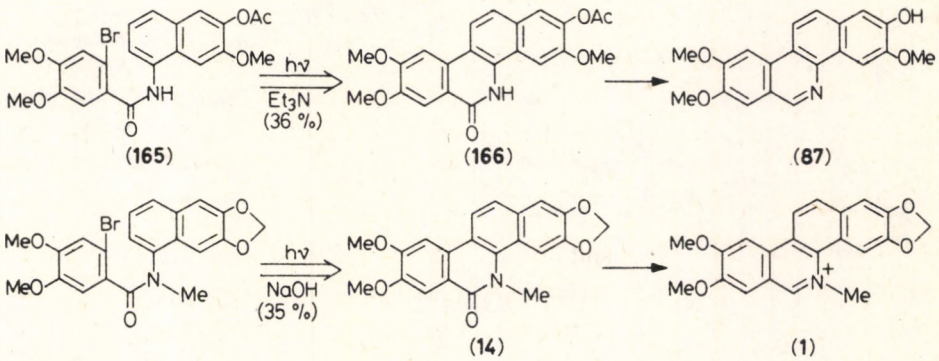
N-Benzoyl-1-naphthylamines **(85)** also contain a conjugated 6π electron system, structurally equivalent to N-benzanilide which is known [44] to undergo oxidative photocyclization to afford phenanthridone **(86)**. Therefore, this type of benzamides

can be regarded as a kind of enamides having a potentiality to undergo photocyclization.

Kessar and co-workers [45] described the first photocyclization of this type of compounds **(85)** which contained an *o*-bromo substituent as the leaving group.



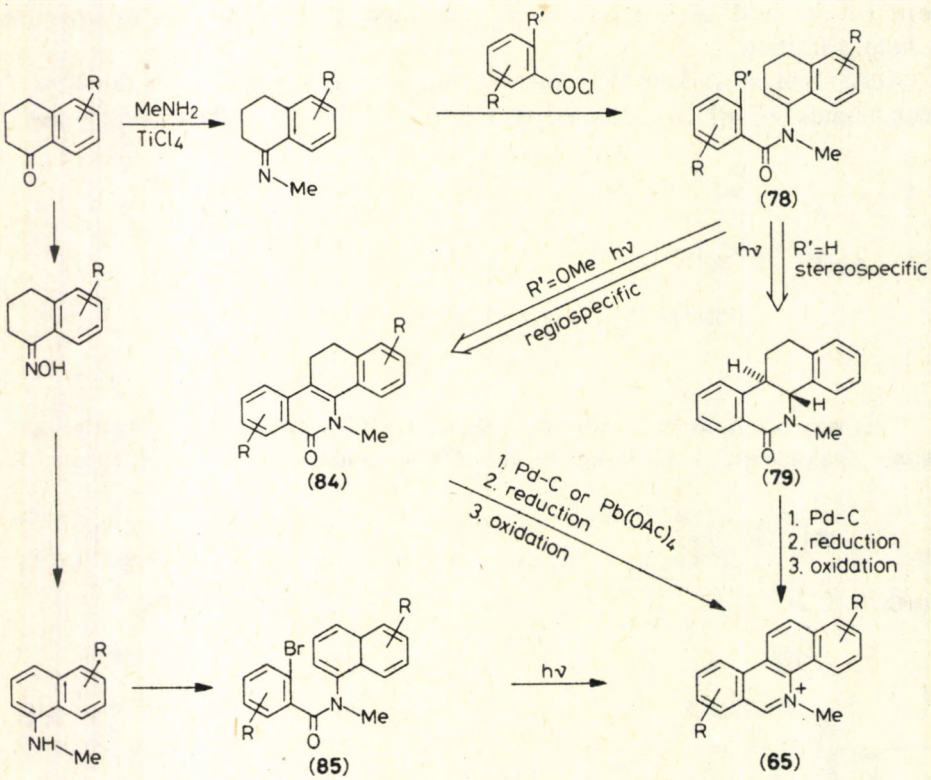
This way of photocyclization has been successfully applied to the synthesis of some alkaloids such as N-norfagaronine **(87)** [46] and nitidine **(1)** [47] (Scheme 24).



Scheme 24

It seems to be essential that hydrogen bromide formed as the cyclization is proceeding should be trapped by adding a base, such as triethylamine, to the reaction mixture.

Enamide photocyclization can be regarded as one of the most useful methods for the synthesis of benzo[c]phenanthridines. On the basis of the accumulated evidence on this cyclization, one can summarize the strategy for the construction of the target molecule as shown in Scheme 25.

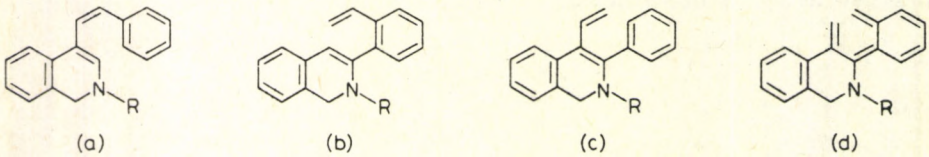


Scheme 25

3.2. SYNTHESIS *via* THE FORMATION OF RING C AS THE FINAL STEP

3.2.1. PHOTOCYCLIZATION OF STILBENE TYPE TRIENES

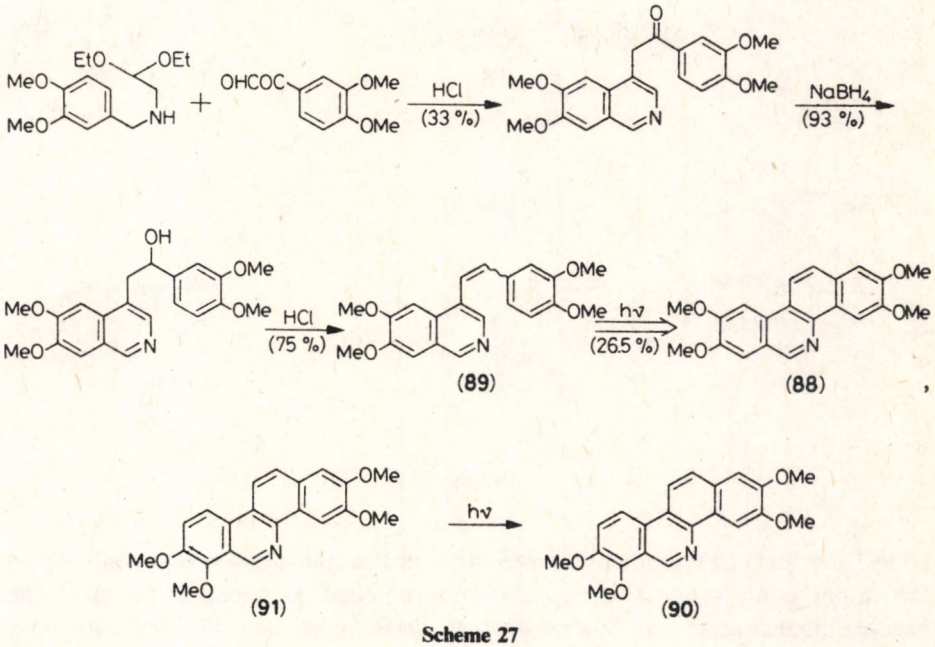
On the analogy of the well-studied hexatriene-cyclohexadiene photocyclization, several triene cyclizations (a, b, c, and d) seem promising to attain the synthesis of benzo[c]phenanthridines (Scheme 26).



Scheme 26

Routes (c) and (d) have not been studied, apparently owing to the inaccessibility of the starting triene; the investigation of routes (a) and (b) encountered some difficulties when applied to the total synthesis of the alkaloids.

Among these approaches to benzo[c]phenanthridines by routes involving triene intermediates, it is natural that the stilbene-type cyclization was the first to have been investigated.



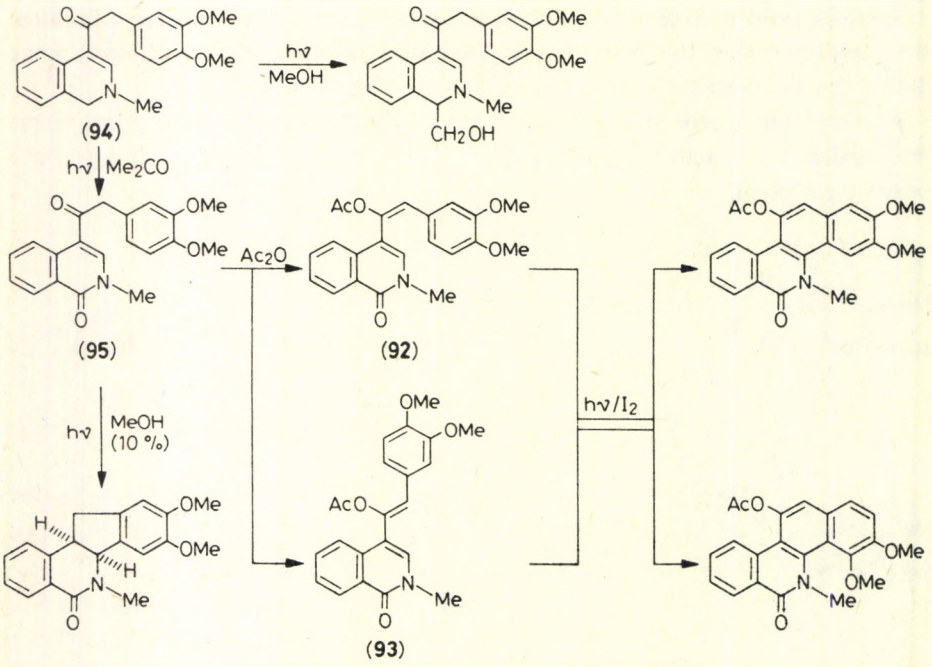
Scheme 27

In 1967 Dyke and Sainsbury [48] described the first instance of the construction of this particular ring system **(88)** by route (a). However, owing to the insufficient reactivity of the 3-position of the isoquinoline moiety **(89)**, they only obtained a poor yield of the cyclic product (Scheme 27).

This cyclization was applied [48] to the synthesis of the compound of the 2,3,7,8-tetrasubstituted-type **(90)** *via* the stilbene **(91)**, but yields seem to be very poor.

In 1977 Onda *et al.* [49] reported the photocyclization of the enol acetate-type trienes **(92** and **93)** (Scheme 28), but further applications to the synthesis of multi-substituted benzo[c]phenanthridines have not been described.

The enolizable ketones **94** and **95** were prepared and irradiated [49]; however, cyclization to benzo[c]phenanthridines could not be achieved in this way. The ketone **(95)** was then converted into a mixture of the corresponding enol acetates **92**

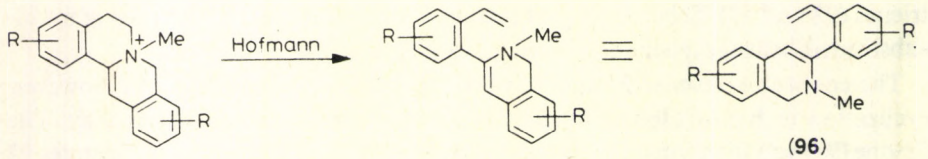


Scheme 28

and **93**, in which the *E*-isomer **(92)** preferable for the cyclization was present only as the minor component. Photocyclization proceeded as expected to yield the benzo[c]phenanthridines, but no further development of this approach was reported.

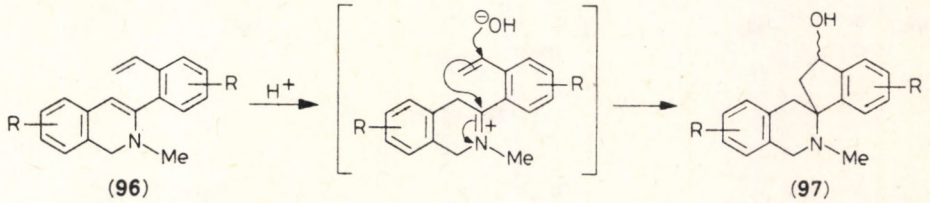
3.2.2. PHOTOCYCLIZATION OF TRIENES FORMED IN THE HOFMANN DEGRADATION OF PROTOBERBERINE ALKALOIDS

Photocyclization of this type of trienes **(96)** has an advantage in the preparation of the starting compounds, as they are generally obtainable by the one-step Hofmann degradation of the methoiodides of naturally abundant protoberberine alkaloids.



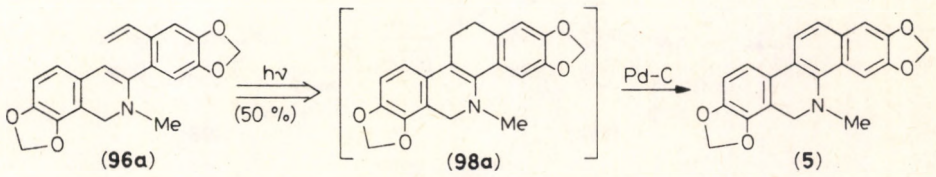
Because of the favourable structural features of these trienes, many researchers have attempted their application to benzo[c]phenanthridine synthesis. As early as 1916, Perkin [50] published a number of papers on the chemistry of anhydrocryptopines (as he called compound **96**) but failed in constructing the target skeleton.

Dyke and Brown [51] and Onda *et al.* [52] also studied the cyclization of anhydrocryptopine **(96)** by acid treatment, but they only obtained products with cyclopentane ring **(97)**.



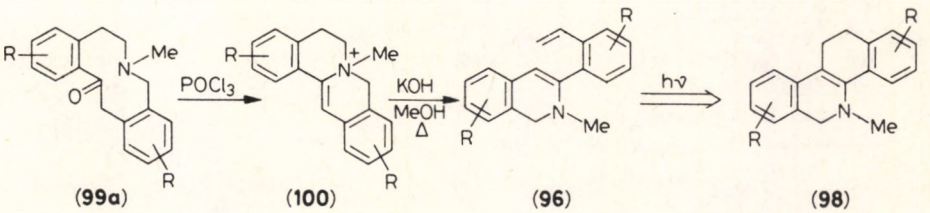
(96) (97)

The first cyclization of this type of triene to benzo[c]phenanthridines was reported in 1969 by Onda and co-workers [53] who irradiated the triene **(96a)** derived from coptisine and obtained the benzo[c]phenanthridine **(98a)** which, without isolation, was dehydrogenated with palladium-on-charcoal to yield dihydrosanguinarine **(5)** as the final product.



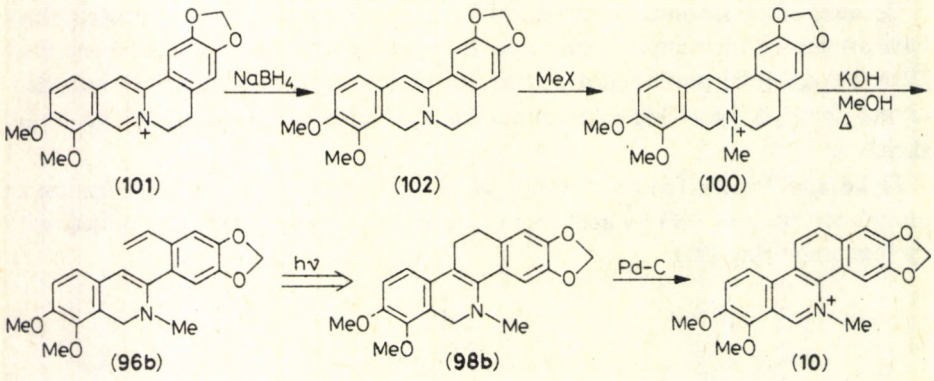
(96a) (98a) (5)

Since the above success Onda *et al.* [54] have described the cyclization of several trienes of this type, and established the method as a useful synthetic route.

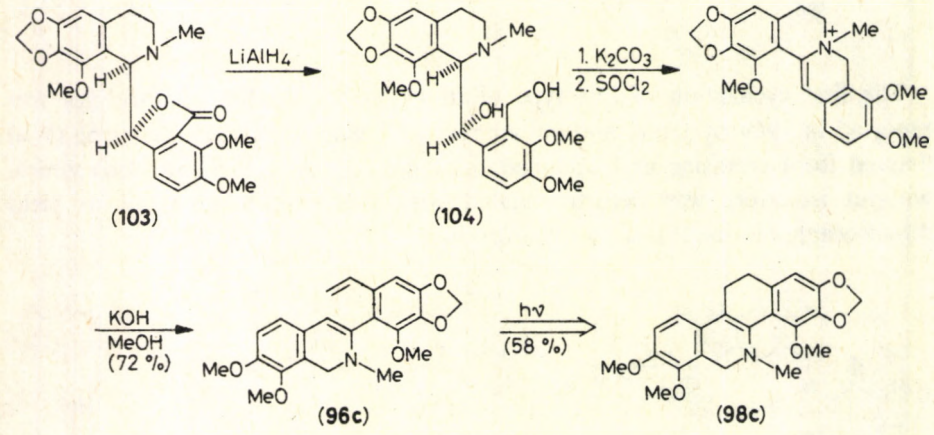


(99a) (100) (96) (98)

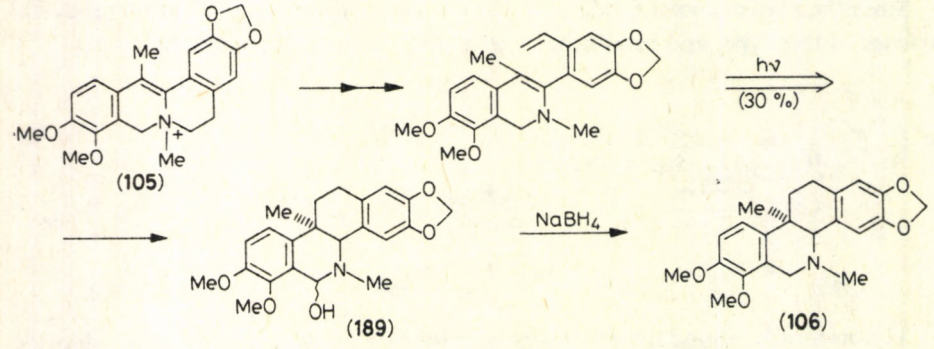
The naturally abundant protopine alkaloid **(99a)** gave isoprotopine chloride **(100)** on treatment with phosphorus oxychloride, and the product was then



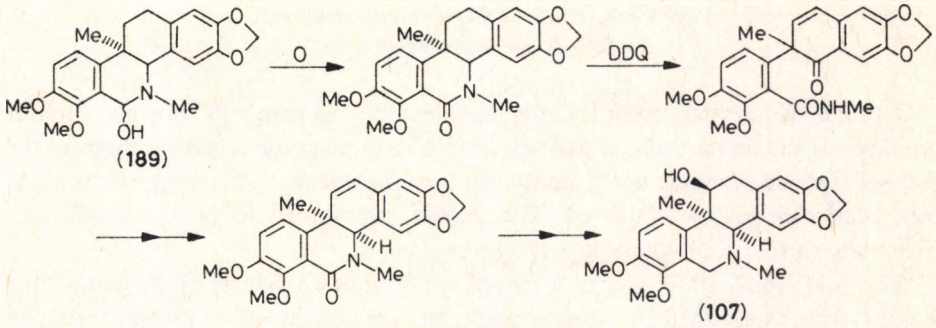
Scheme 29



Scheme 30



Scheme 31



Scheme 32

converted into anhydroprotopine **(96)** under the conditions of Hofmann degradation.

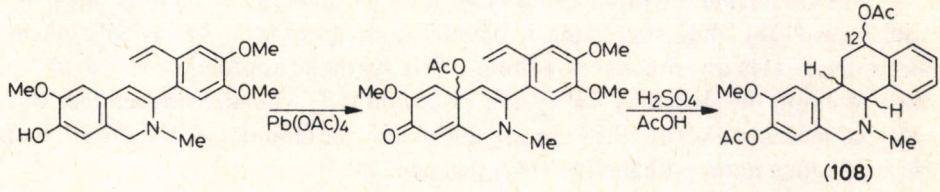
In their further work, Onda *et al.* [54] utilized the most abundant alkaloid, berberine chloride **(101)**, which was first reduced with sodium borohydride to obtain dihydroberberine **(102)**; this gave the corresponding methosulfate **(100)** upon treatment with dimethyl sulfate. Hofmann degradation of the quaternary salt **(100)** afforded the triene **(96b)** (anhydromethylberberine), which underwent smooth photocyclization to yield the benzo[c]phenanthridine **(98b)** and then natural chelerythrine **(10)** (Scheme 29).

A further extension of this triene photocyclization [55] was the conversion of (−)-α-narcotine **(103)** into benzo[c]phenanthridine **(98c)** *via* the route involving narcotinediol **(104)** (Scheme 30).

Also by utilizing the 13-methylberberine derivative **(105)**, Onda *et al.* [56] developed the route of synthesizing the 10b-methylbenzo[c]phenanthridine **(106)**, which is the basic structure of the corynoline group of alkaloids (Scheme 31).

Finally, Onda *et al.* [57] succeeded in converting the naturally abundant protoberberine alkaloid **(105)** *via* **189** into the corynoline analogue **(107)**, which differs from corynoline **(4)** only by the presence of 7,8-dimethoxy substituents instead of the 7,8-methylenedioxy group (Scheme 32).

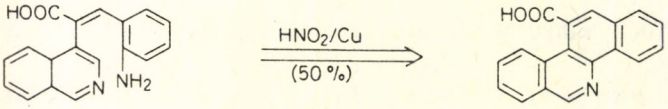
Recently, Kametani and co-workers [58] reported a facile introduction of an oxygen function into the 12-position of the benzo[c]phenanthridine skeleton **(108)** using special triene cyclization.



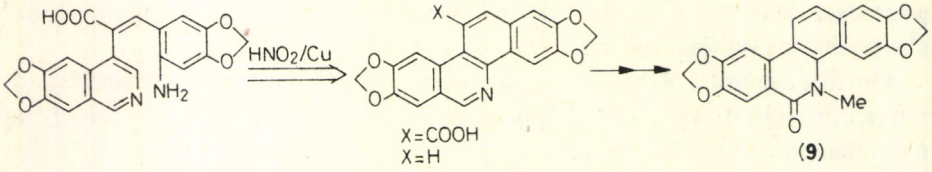
3.2.3. PSCHORR CYCLIZATION LEADING TO BENZO[c]PHENANTHRIDINES

Though the intermolecular Pschorr reaction [59] has been well known as a useful method for the preparation of various diaryls, its application to the synthesis of the benzo[c]phenanthridine skeleton, that is, intramolecular Pschorr cyclization, has not been thoroughly evaluated. The reason is assumed to be the insufficient reactivity of the isoquinoline ring.

The first application of the Pschorr cyclization to benzo[c]phenanthridine synthesis was described by Abramovitch and Tertzakian [60], who reported the synthesis of this ring system, though only in a poor overall yield.



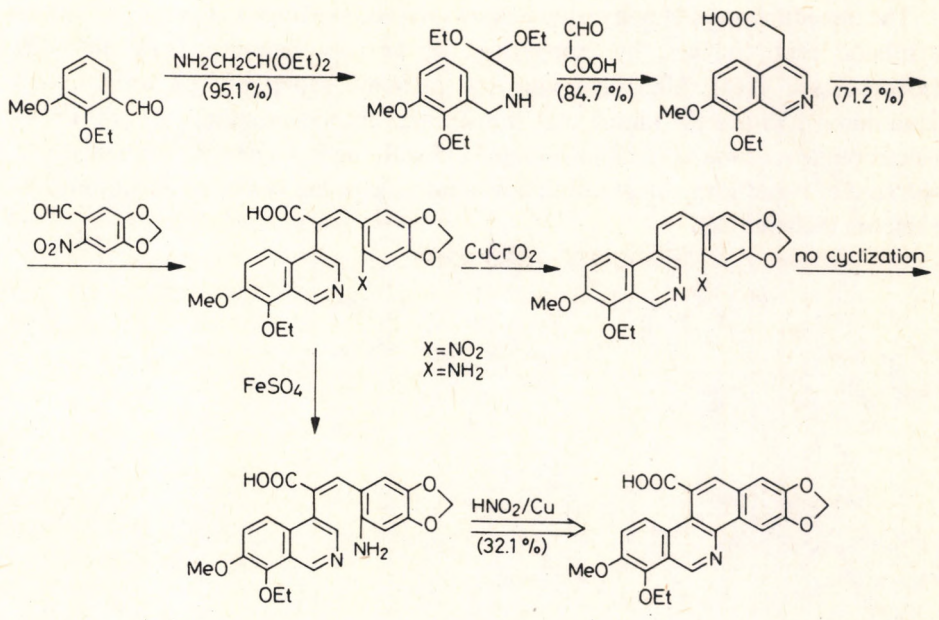
Dyke and co-workers [61] applied Abramovitch's route and synthesized oxyavicine **(9)**, in only 5% overall yield, from piperonal.



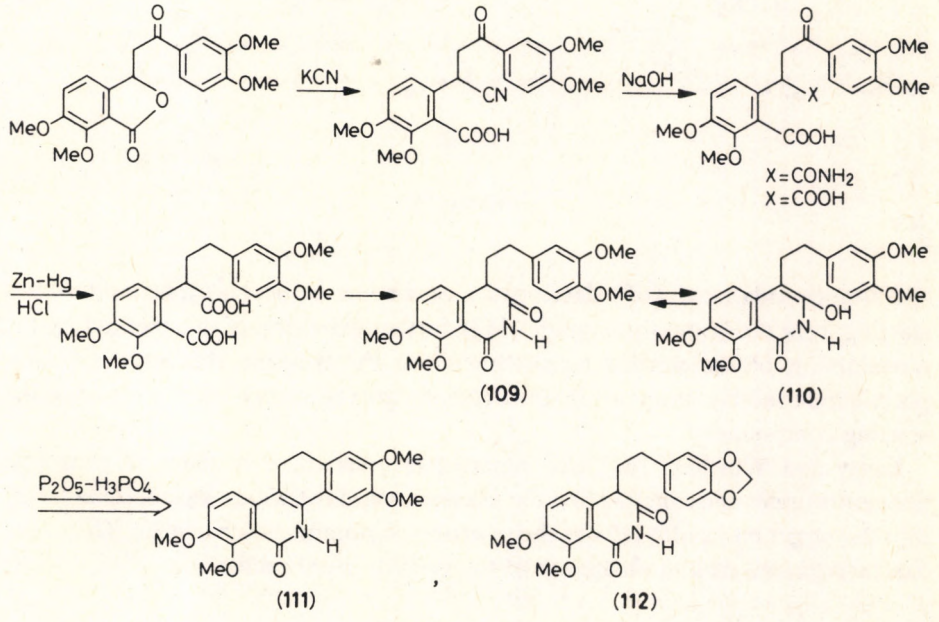
Ishii *et al.* [62] carefully investigated the reaction on the synthesis of ethyl isodecarine, and established the optimal conditions of all steps to the benzo[c]phenanthridine, showing the requirement of a carboxyl group in the Pschorr cyclization (Scheme 33).

3.2.4. SYNTHESIS OF BENZO[c]PHENANTHRIDINES *via* ISOCARBOSTYRILS

4-(2-Phenethyl)homophthalimides **(109)** exist as tautomeric mixtures with the enol form **(110)**, thus suggesting a possibility of cyclization to benzo[c]phenanthridines **(111)** under acidic conditions. This synthetic approach was one of the early attempts developed by Bailey and Robinson [63] who also noticed that this cyclization was of a very limited use, as indicated by the failure [64] of cyclization of the analogous homophthalimide **(112)** (Scheme 34).

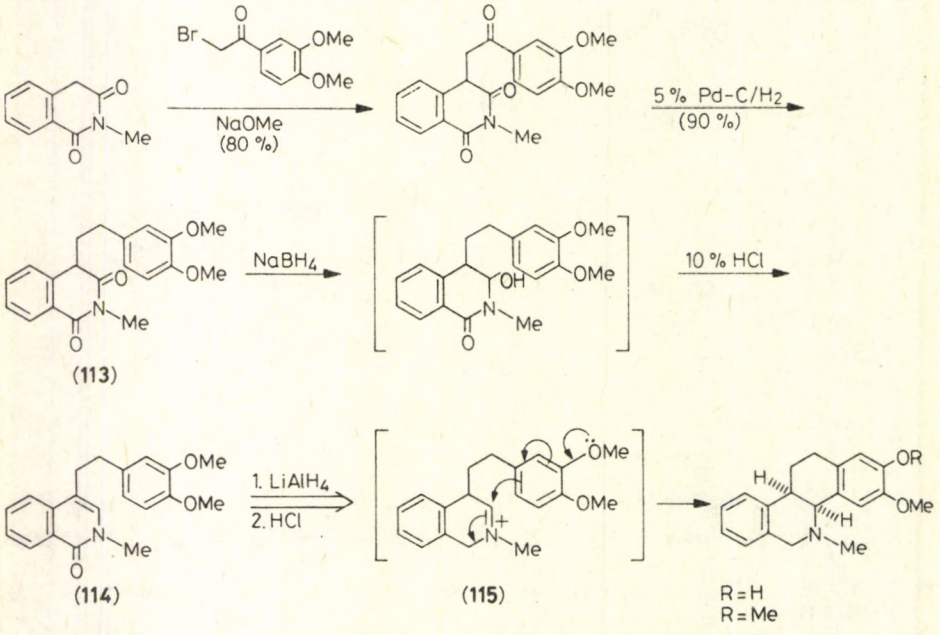


Scheme 33



Scheme 34

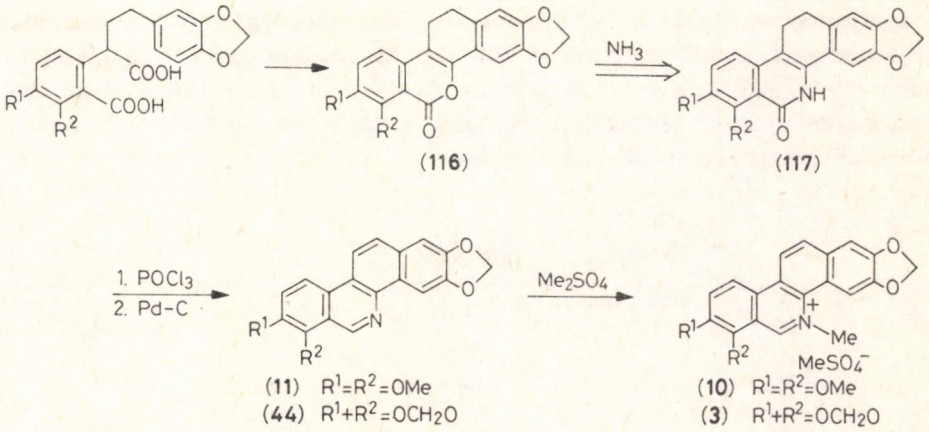
The introduction [65] of hydride reagents offered a change to develop the above synthetic route further, by converting the homophthalimide **(113)** into the isocarbostyril **(114)**. After removing the carbonyl group of **114** with lithium aluminium hydride the amine was transformed into immonium salt **(115)**. The electrophilic reaction of the 3-position of **115** with the benzene ring, particularly in the case of *p*-methoxy substitution, did occur to give the benzo[c]phenanthridine skeleton (Scheme 35).



Scheme 35

Although Iida *et al.* [65] developed a new route to benzo[c]phenanthridines starting from the homophthalimide **(113)**, further extension of this method by the introduction of the desired substituents into the benzene ring of the homophthalimide moiety seems to involve considerable difficulty when preparing the starting compounds.

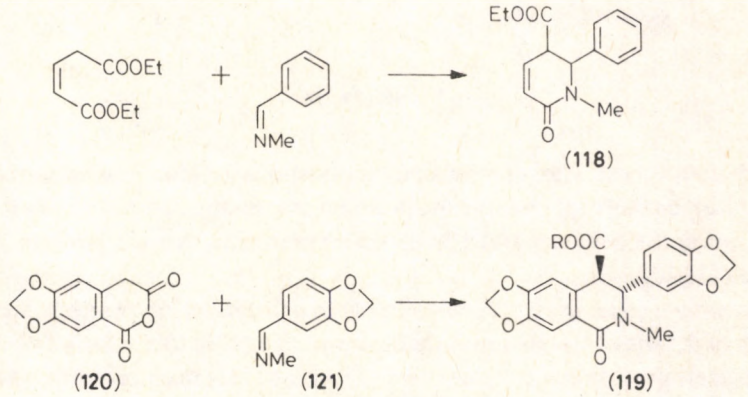
Bailey and Worthing [64] also reported an alternative synthesis of benzo[c]-phenanthridines constructing first the isocoumarin **(116)**, which was then converted into the target molecule **(117)** by the insertion of ammonia (Scheme 36). This route can be regarded as a modification of the isocarbostyril cyclization.



Scheme 36

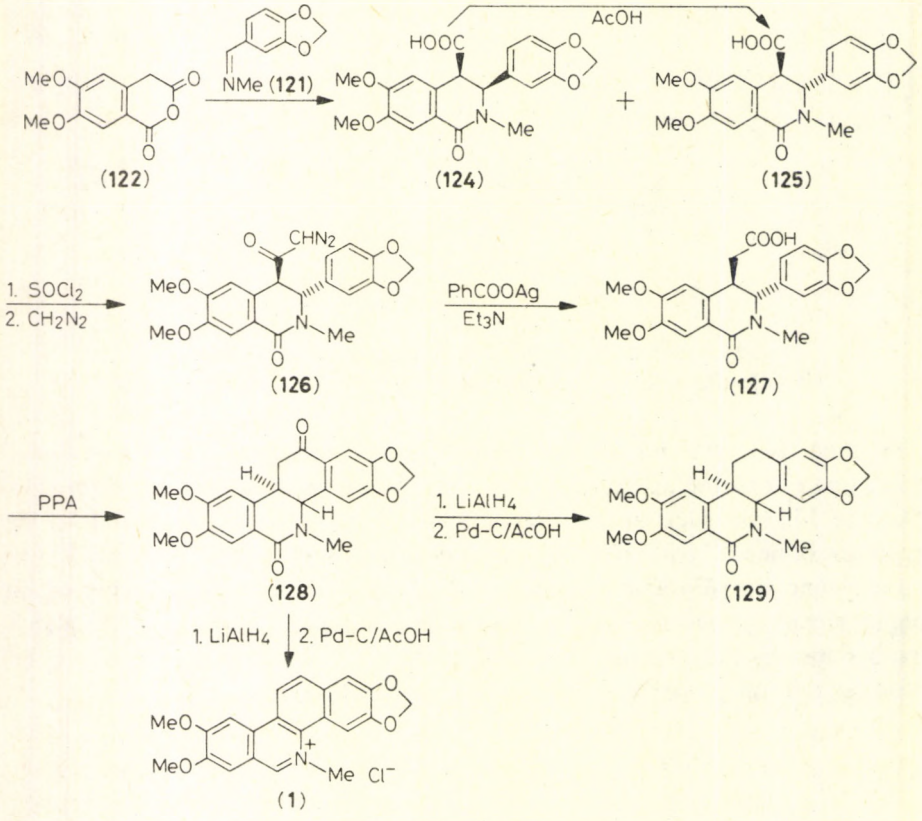
3.2.5. SYNTHESIS OF BENZO[C]PHENANTHRIDINES *via* THE CONDENSATION OF HOMOPHTHALATE WITH A SCHIFF BASE

In 1965, Shamma and co-workers [66] reported the condensation of diethyl glutaconate with the appropriate imine for the preparation of the lactam **(118)** (Scheme 37), and suggested the potentiality of using this condensation for the synthesis of heterocyclic compounds; this was elegantly accomplished in 1977 by both Haimova's [67] and Cushman's [68] groups. Haimova *et al.* reported the highly stereoselective synthesis of 3,4-dihydro-1(2*H*)-isoquinolones **(119)** and 8-oxoberbines by the condensation of homophthalic anhydride **(120)** with a Schiff base such as **121** prepared from piperonal and methylamine.



Scheme 37

Independently, Cushman and co-workers [68] thoroughly investigated the stereochemistry and the reaction conditions of the condensation of homophthalic anhydride with imines and established that it is a very useful method for the synthesis of isoquinoline alkaloids; they achieved the stereoselective total synthesis of the 13-methylberbine alkaloid, cavidine.

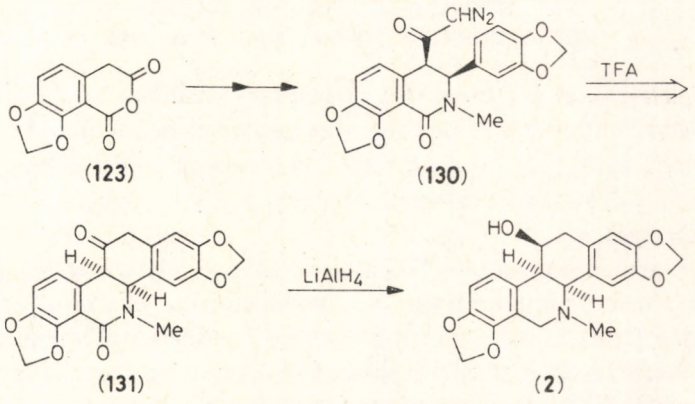


Scheme 38

Based upon these studies, the first applications of this condensation to the synthesis of benzo[c]phenanthridines appeared in the works of Shamma and Tomlinson and Cushman and Cheng on oxysanguinarine **(3)** [69] and nitidine chloride **(1)** [70].

The synthetic route used by Cushman's group [70] is as follows (Scheme 38). The addition of 4,5-dimethoxyhomophthalic anhydride **(122)** to a solution of the Schiff base, 3,4-methylenedioxybenzylidenemethylamine **(121)** in chloroform resulted in a rapid exothermic condensation which afforded a diastereomeric mixture of the *cis*-

and *trans*-substituted isoquinolones (**124** and **125**); these were separable by recrystallization but, more conveniently, were isomerized to the thermodynamically more stable *trans* isomer (**125**) by heating in refluxing acetic acid for 16 h. In this way the desired *trans*-isoquinolone (**125**) was obtained in 92% yield. Treatment of the acid chloride with diazomethane gave the crystalline diazoketone (**126**), which readily underwent Wolff rearrangement on the addition of a silver benzoate in triethylamine to give the corresponding homologous acid (**127**) upon hydrolysis.



(123) (130) (131) (2)

Scheme 39

Intramolecular Friedel–Crafts cyclization with polyphosphoric acid gave the B/C-*trans*-12-oxo-benzo[c]phenanthridone (**128**). Further modification of the structure by conventional reactions resulted in the total synthesis of nitidine chloride (**1**), a supply of which was in strong demand for clinical evaluation.

Shamma and Tomlinson [69] described a similar synthesis of oxysanguinarine (**6**), in which they improved the preparation of the homophthalic acid in an eight-step sequence of reactions and employed its diester for the condensation with the Schiff base **121**; this required refluxing in methanolic sodium methoxide solution for a week to obtain the *trans*-lactam (**125**) in 61% yield.

The main feature of this synthetic approach is its high stereoselectivity giving only the *trans*-isoquinolone (**125**), which can be readily converted into the B/C-*trans*-benzo[c]phenanthridine (**129**). By making a good use of this feature, Cushman and co-workers [2b, 71] succeeded in a smart total synthesis of (±)-chelidonine (**2**), by developing a way to cyclize the intermediary diazoketone (**130**) directly to the 11-oxo-benzo[c]phenanthridone (**131**) by treatment with trifluoroacetic acid (Scheme 39).

3.3. ANOTHER APPROACH FOR THE SYNTHESIS OF BENZO[c]PHENANTHRIDINES

There is one other approach for the synthesis of the benzo[c]phenanthridine skeleton which must be mentioned, the synthesis involving the reaction of benzocyclobutenes, developed by Oppolzer and co-workers.

3.3.1. INTRAMOLECULAR CYCLOADDITION OF *o*-QUINODIMETHANES TO ACETYLENES

The application of the chemistry of benzocyclobutenes to the synthesis of alkaloids has recently made remarkable progress, which had actually been initiated by Oppolzer's work [22, 23] on the total synthesis of (±)-chelidonine **(2)**. The synthetic utility of *o*-quinodimethanes for the synthesis of polycyclic ring systems is very considerable.

Benzocyclobutenes are known [72] to undergo thermolytic ring opening to form the assumed reactive intermediates, *o*-quinodimethanes, as a result of thermally induced conrotatory ring opening. It is assumed that these *o*-quinodimethanes are present in the thermodynamically more stable *E*-form rather than in the unstable *Z*-form owing to the influence of the substituent on the cyclobutene ring, thus showing a high potentiality of undergoing a Diels–Alder-type cycloaddition, as the diene component, to either an olefinic or acetylenic unsaturated bond.

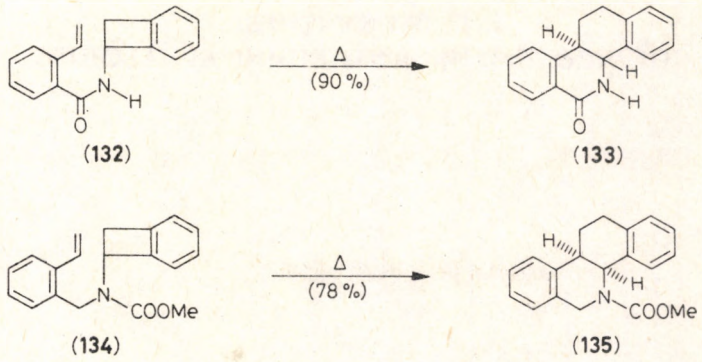
The application [72] of this thermolysis of benzocyclobutenes have caught the attention of organic chemists, thus leading to a new synthetic method which consists of an intramolecular and intermolecular cycloaddition to various unsaturated bonds.

Upon investigating this possibility, Oppolzer and Keller [22] discovered a new intramolecular cycloaddition of *o*-quinodimethanes to the unsaturated bonds in the side chain and applied this reaction to the total synthesis of the structurally most complicated alkaloid (±)-chelidonine **(2)**.

For the synthesis of this B/C-hexahydrobenzo[c]phenanthridine alkaloid, a simple stereoselective approach to the chelidonine framework was first investigated [22], by using the amide **(132)**. Surprisingly, simple heating of the amide **132** in a boiling bromobenzene solution for 16 h yielded the *trans*-fused benzo[c]phenanthridine **(133)** in 90% yield. Thermolysis of the more flexible urethane **134** afforded the desired *cis*-fused **135** in 78% yield (Scheme 40).

The above mentioned method (starting from the two building blocks **136** and **137** was exploited for the synthesis of chelidonine-type alkaloids (Scheme 41).

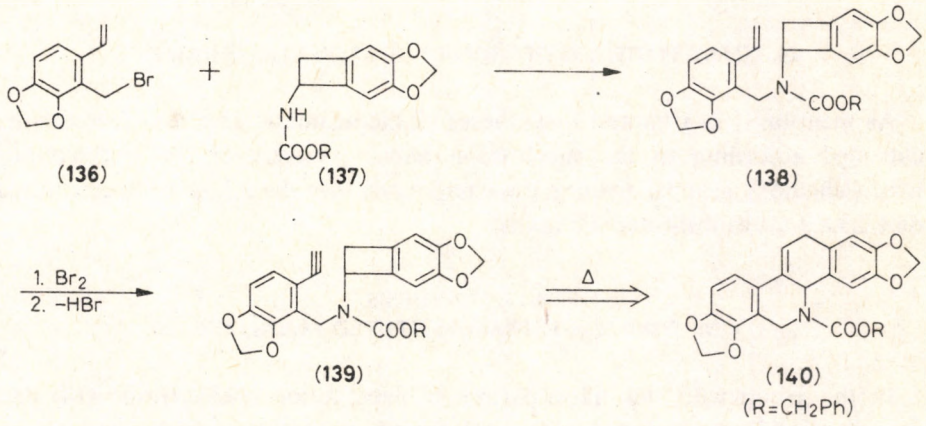
The condensation of the two components was accomplished by the conversion of **137** to its sodium salt, followed by addition of the bromide of **136** in the presence of



(132) (133) (134) (135)

Scheme 40

sodium iodide and subsequent stirring at 25 °C for 16 h to obtain the styrene **(138)**. In order to make room for the introduction of the 11-hydroxyl group and the B/C-*cis*-fused ring system, the styrene was converted into the acetylene derivative **(139)**



(136) (137) (138) (139) (140)
(R=CH_2Ph)

Scheme 41

by bromination and dehydrobromination. The intermolecular cycloaddition of the acetylene **(139)** was carried out by heating the compound in *o*-xylene at 120 °C for 1 h, which gave the crystalline tetrahydrobenzo[c]phenanthridine **(140)** in 73% yield. Usual modification of the structure completed the first total synthesis of chelidonine **(2)** [22, 23].

4. TOTAL SYNTHESIS OF BENZO[c]PHENANTHRIDINE ALKALOIDS

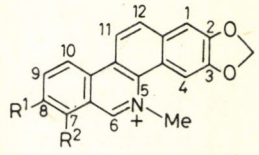
In this chapter total synthetic works on the benzo[c]phenanthridine alkaloids are summarized and some comments are given on their characteristics. Useful information on the isolation and structure determination of alkaloids is conveniently summarized in Kametani's book [73a, b].

4.1. TOTAL SYNTHESIS OF THE FULLY AROMATIC ALKALOIDS

As mentioned in Chapter 3, syntheses of the aromatic alkaloids can also be classified according to the substitution pattern, mostly of the methoxyl or methylenedioxy groups, dividing the compounds into the 2,3,7,8-tetrasubstituted and 2,3,8,9-tetrasubstituted alkaloids.

4.1.1. SYNTHESIS OF THE 2,3,7,8-TETRASUBSTITUTED ALKALOIDS

In the group with this basic pattern of substitution chelerythrine **(10)** and sanguinarine **(3)** are included, along with their 6-oxo and 5,6-dihydro derivatives.

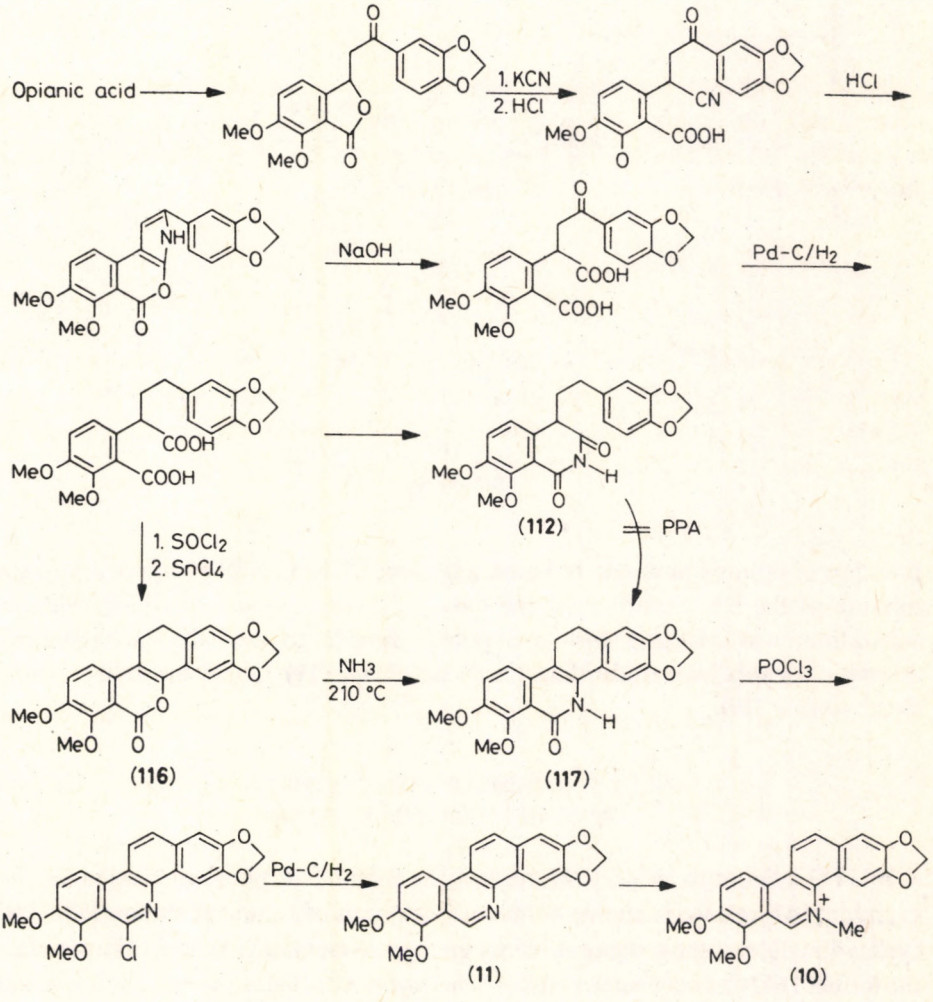


$R^1 = R^2 = OMe$: Chelerythrine (**10**)
$R^1 + R^2 = OCH_2O$: Sanguinarine (**3**)

These two alkaloids are the most common and best studied [1] compounds in the group. Therefore, many research teams have synthesized them *via* various routes. Since their structures differ only in the 7,8-substituents, common synthetic approaches were made.

4.1.1.1. SYNTHESIS OF CHELERYTHRINE *via* CYCLIZATION OF A HOMOPHTHALIC ANHYDRIDE

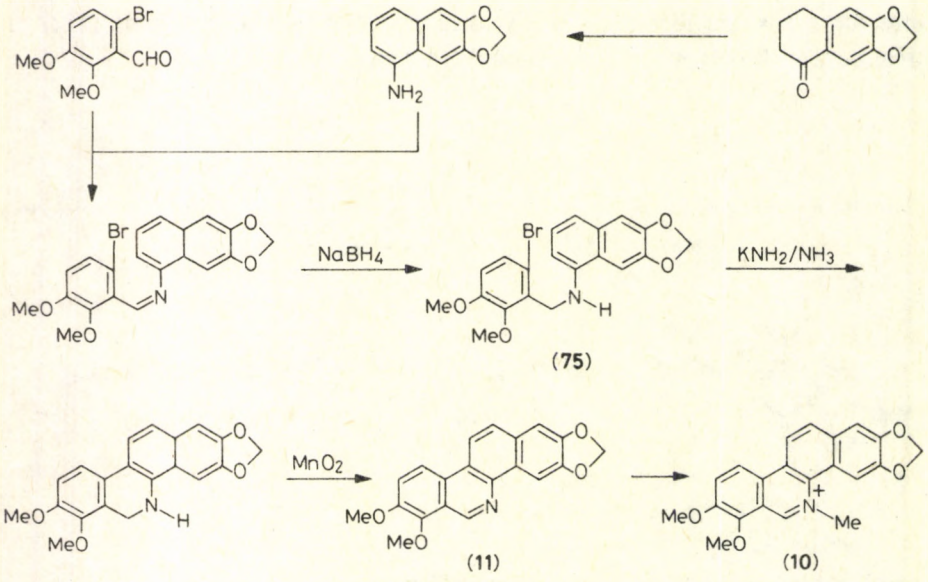
In 1956 Bailey and Worthing [64] reported the first total synthesis of chelerythrine **(10)** by a route involving the cyclization of the homophthalic anhydride prepared from opianic acid, to given the isocoumarin **(116)**, which was then converted into the benzo[c]phenanthridone **(117)** by the insertion of ammonia into the 5-position (Scheme 42). Cyclization of the homophthalimide **(112)** on treatment with polyphosphoric acid failed to occur.



Scheme 42

4.1.1.2. SYNTHESIS OF CHELERYTHRINE *via* BENZYNE CYCLIZATION

In 1974 Kessar *et al.* [36] reported a convenient synthesis of chelerythrine starting from 6,7-methylenedioxy-1-naphthylamine and 2-bromo-5,6-dimethoxybenzaldehyde, *via* the cyclization of the *o*-bromobenzylnaphthylamine **(75)** in the

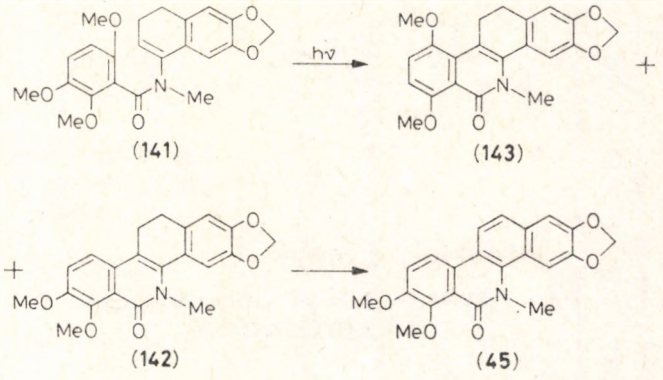


Scheme 43

presence of potassium amide in liquid ammonia (Scheme 43). The product was a mixture of the 5,6-dihydro- and the fully aromatic compounds which, without separation, was oxidized with manganese dioxide to give the homogeneous aromatic benzo[c]phenanthridine, norchelerythrine **(11)**. Quaternization afforded chelerythrine **(10)**.

4.1.1.3. SYNTHESIS OF CHELERYTHRINE *via* ENAMIDE PHOTOCYCLIZATION

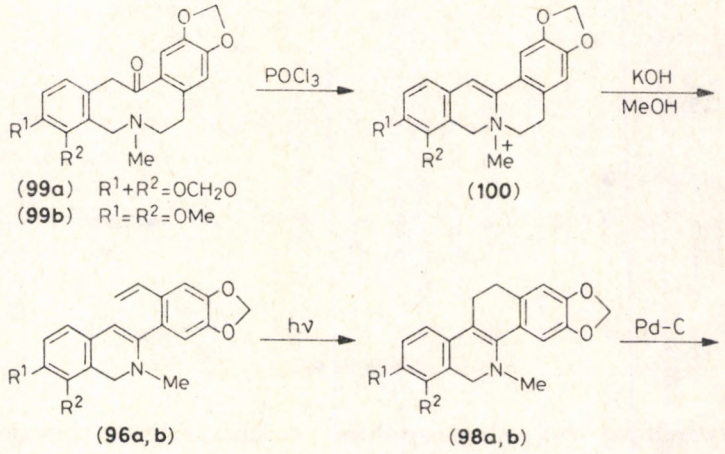
In 1977 Ninomiya *et al.* [24] applied the method of photocyclization to the enamide **(141)** in a work aiming at the total synthesis of homochelidonine **(23)**. The cyclization did not show regioselectivity giving two lactams **(142** and **143)**, of which the former **(142)** was converted into oxychelerythrine **(45)** for the elucidation of the structure (Scheme 44).



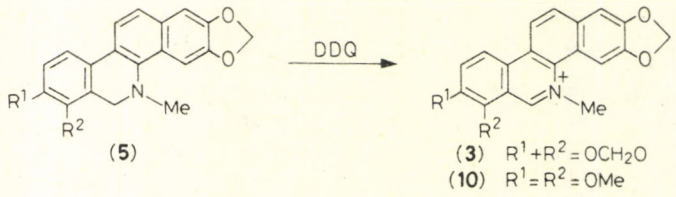
Scheme 44

4.1.1.4. CONVERSION OF PROTOPINES INTO BENZO[c]PHENANTHRIDINE ALKALOIDS

Onda and co-workers [53] (1969) successfully converted protopine **(99a)** and allocryptopine **(99b)** into the corresponding benzo[c]phenanthridine alkaloids, sanguinarine **(3)** and chelerythrine **(10)** [54], respectively, *via* the Hofmann degradation of the quaternary salts **(100)** to the trienes **(96)**, followed by photocyclization and dehydrogenation (Scheme 45).

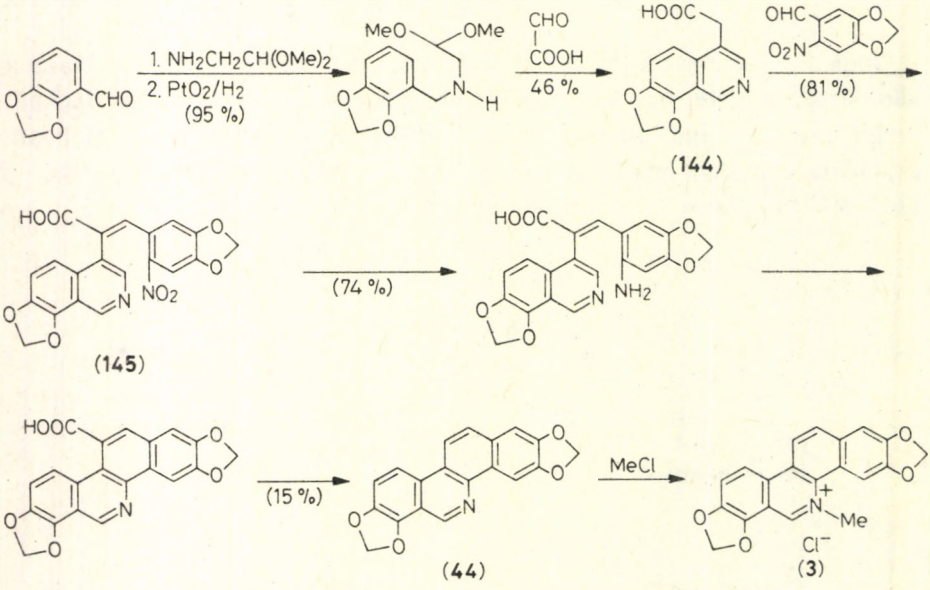


Scheme 45



4.1.1.5. SYNTHESIS OF SANGUINARINE *via* PSCHORR CYCLIZATION

Although there was a possibility of applying the synthetic route adopted for the synthesis of chelerythrine **(10)** to the synthesis of sanguinarine **(3)**, the first total synthesis was reported in 1970 by Sainsbury *et al.* [74], who applied the Pschorr cyclization to achieve this synthesis (Scheme 46).



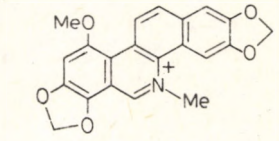
Scheme 46

They developed convenient syntheses of the starting compounds, the isoquinoline-4-acetic acid **(144)** and 4,5-methylenedioxy-2-nitrobenzaldehyde, which were combined to give the nitrostilbene **(145)**. Reduction, diazotization and the Pschorr reaction followed by decarboxylation afforded the benzo[c]phenanthridine, norsanguinarine **(44)**.

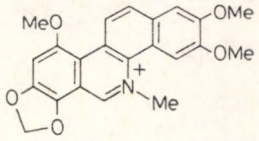
4.1.2. SYNTHESIS OF THE 2,3,7,8,x-PENTASUBSTITUTED ALKALOIDS

Alkaloids having one or two more substituents in addition to the 2,3,7,8-tetrasubstitution can be synthesized by the extension of the methods described in the previous section for the synthesis of sanguinarine **(3)** or chelerythrine **(10)**.

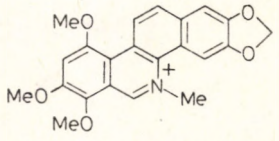
The target alkaloids in this category are the following (Scheme 47).



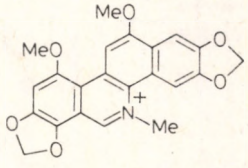
Chelirubine (**18**)



Sanguirubine (**19**)



Chelilutine (**20**)



Macarpine (**21**)

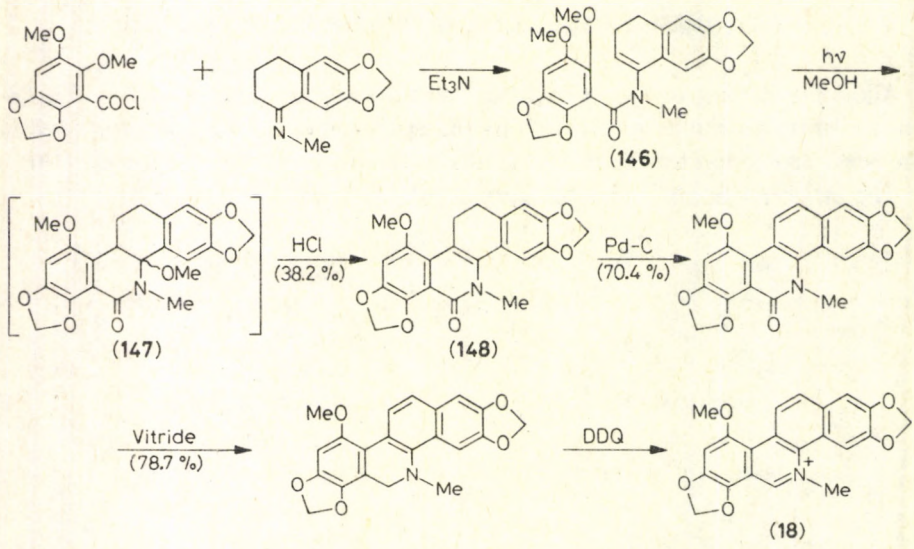
Scheme 47

All these alkaloids have been synthesized very recently; earlier synthetic work had been hampered by ambiguities concerning the structures of these compounds, particularly with respect to the positions of the substituents in the rings, which were finally established by direct total syntheses.

The first breakthrough in the problems of the structures came from a proposal of the structure of chelirubine **(18)** by Ishii and co-workers [10] who upon thorough inspection of various spectral data revised the previously proposed structures, and proved the validity of the new structure by the first total synthesis, applying the enamide photocyclization [10].

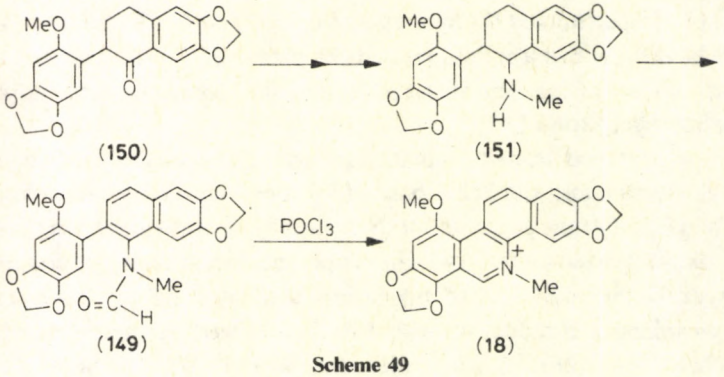
The synthesis started from two building blocks, 2,3-dimethoxy-5,6-methylenedioxybenzoyl chloride and the Schiff base of 6,7-methylenedioxy-1-tetralone, which were combined to give the enamide **146** (Scheme 48). This enamide was irradiated to afford the photocyclized lactam **147** which upon acid treatment was converted into the didehydrolactam **148**. Dehydrogenation with 30% palladium-on-carbon in refluxing *p*-cymene and reduction with Vitride followed by treatment with DDQ afforded chelirubine **(18)**, identical with the natural alkaloid, thus proving the structure proposed by Ishii's group.

An alternative total synthesis of chelirubine **(18)** was reported in a more practical manner by Ishii *et al.* [14a] who applied the modified Bischler–Napieralski cyclization to the aromatic formamide **(149)** as shown in Scheme 49.



Scheme 48

The starting 2-aryl-1-tetralone **(150)** was prepared according to Robinson's process and converted into the aliphatic formamide **(151)** by treatment with methylamine in the presence of titanium tetrachloride, reduction with sodium borohydride and formylated with chloral. Dehydrogenation with DDQ afforded the aromatic formamide **(149)**. The refluxing of this formamide with phosphoryl chloride in acetonitrile solution afforded the cyclized quaternary chloride in one

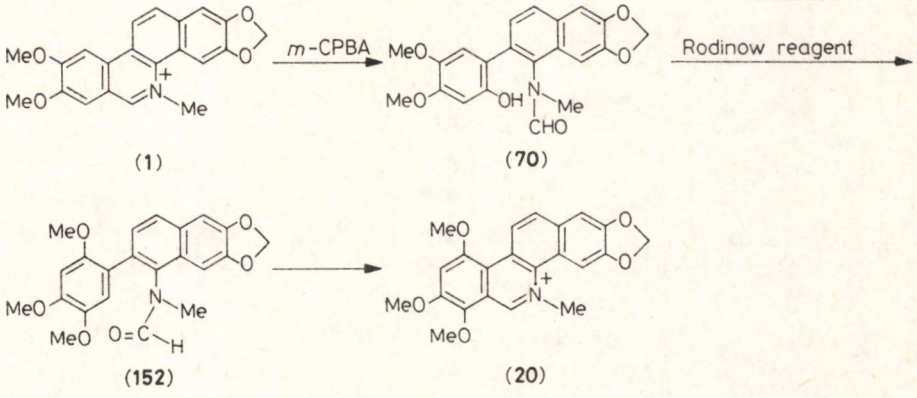


Scheme 49

step, which was identical with natural chelirubine **(18)**. This modified Bischler–Napieralski reaction of the aromatic formamides offers a convenient way for the large scale preparation of various alkaloids for biological evaluation.

4.1.2.1. SYNTHESIS OF THE PENTASUBSTITUTED ALKALOIDS, CHELILUTINE (20), SANGUIRUBINE (19) AND SANGUILUTINE (69)

In 1977 Ishii *et al.* [14a] isolated the new alkaloids arnottianamide and isoarnottianamide **(70)**, which are the ring-opened derivatives of benzo[c]phenanthridine alkaloids, and synthesized them by conversions from chelerythrine **(10)** and nitidine **(1)**. Through the intermediate isoarnottianamide **(7)** they succeeded in converting nitidine **(1)** into chelilutine **(20)** as shown in Scheme 50.



(1) (70) (152) (20)

Scheme 50

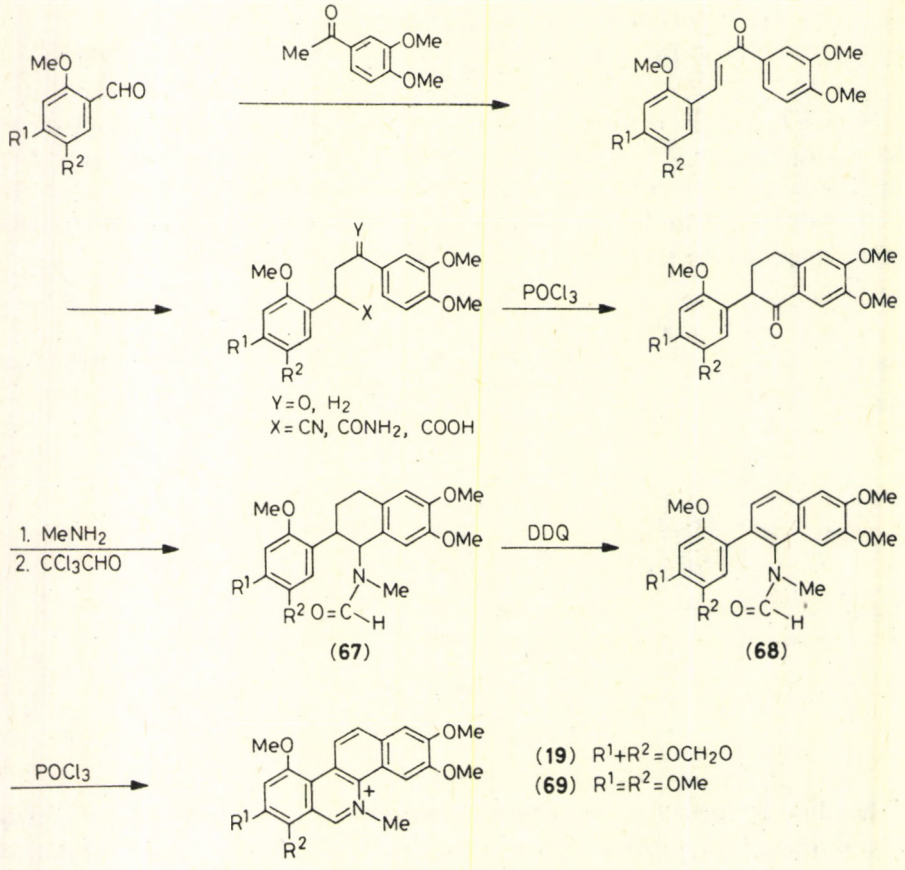
Nitidine **(1)** was oxidized with *m*-chloroperbenzoic acid in hexamethylphosphoric triamide (HMPA) to afford isoarnottianamide **(70)**, which was methylated to give the starting N-methylformamide **(152)**. The refluxing of this aromatic formamide with phosphoryl chloride in acetonitrile for 1 h afforded the cyclized quaternary chloride, which was identical with chelilutine; the yield was 55.9%.

Ishii *et al.* [14a] also succeeded in the Bischler–Napieralski cyclization of the formamide derivative **(153)** to give norchelirubine **(154)** (Scheme 51).

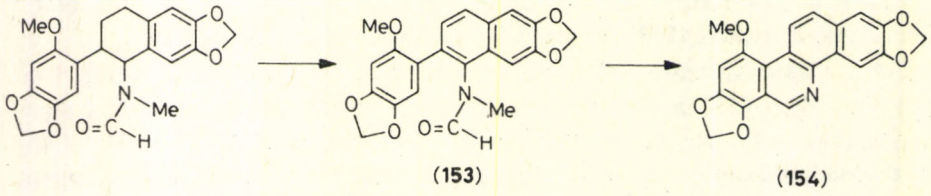
Further, as an extension of the modified Bischler–Napieralski cyclization, Ishii and co-workers [33] in 1978 synthesized two pentasubstituted alkaloids, sanguirubine **(19)** and sanguilutine **(69)**, by the route involving the synthesis of the corresponding 2-aryl-1-tetralones according to Robinson's process, followed by the conversion of these products into aromatic N-methylformamides **(68)** and subsequent cyclization (Scheme 52).

These total syntheses also confirmed the correctness of the proposed structures of these alkaloids.

The total syntheses of the two alkaloids, chelilutine **(20)** and sanguilutine **(69)**, were also achieved by Kessar *et al.* [37] in 1977; they applied the benzyne photo-

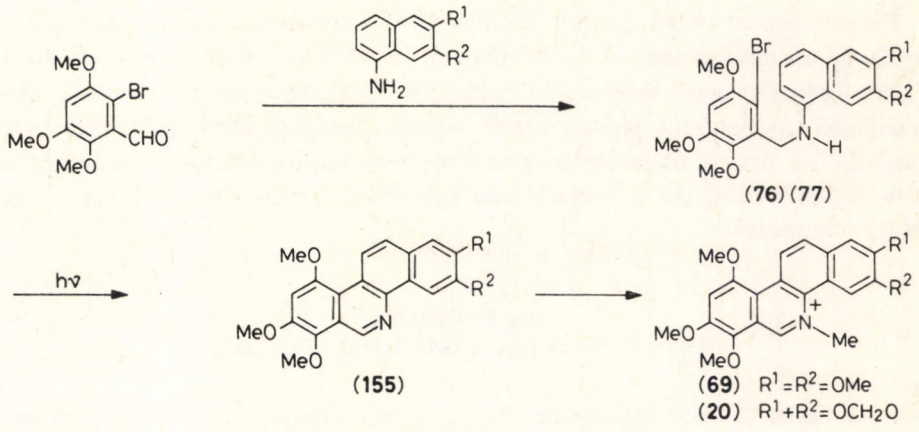


Scheme 51



Scheme 52

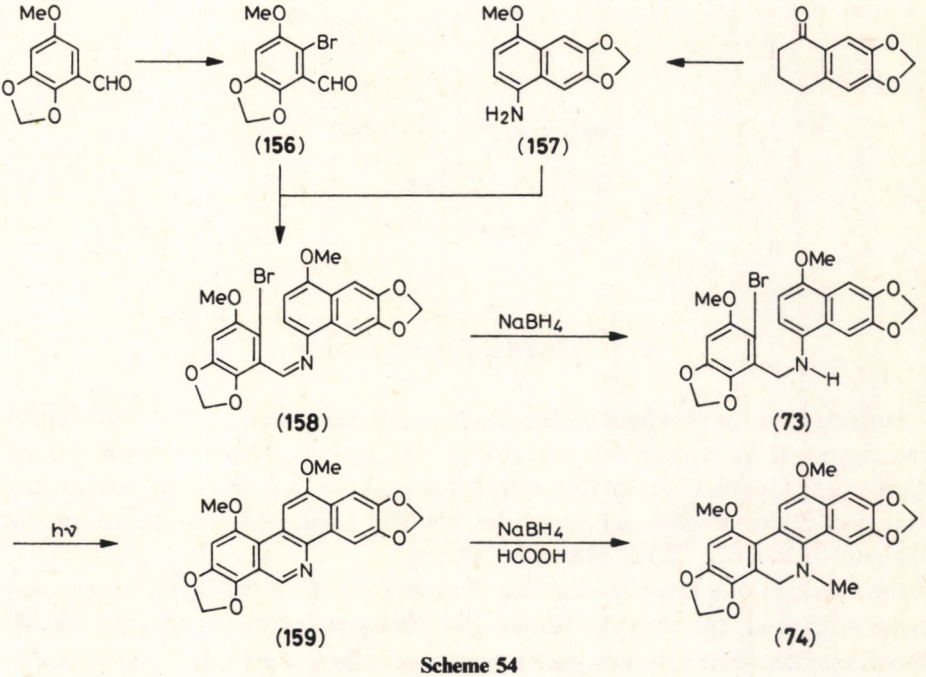
cyclization to the appropriately substituted N-(*o*-bromobenzyl)naphthylamines **(76, 77)** to obtain the corresponding N-norbenzo[c]phenanthridines **(155),** which were converted into the alkaloids, **20** and **69**, respectively (Scheme 53).



Scheme 53

4.1.3. SYNTHESIS OF THE HEXASUBSTITUTED ALKALOID MACARPINE

Ambiguity on the structure of macarpine **(21)** was finally clarified in 1981 by the total synthesis of this alkaloid, applying the benzyne photocyclization [38] (Scheme 54).

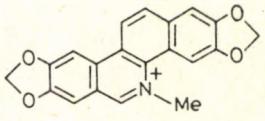


Scheme 54

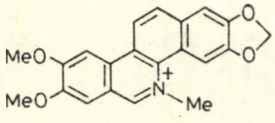
Kessar's process was applied to combine the 2-bromobenzaldehyde **(156)** and 4-methoxy-6,7-methylenedioxy-1-naphthylamine **(157)** to obtain the Schiff base **(158)**. Reduction gave the N-(2-bromobenzyl)-1-naphthylamine **(73)**, which was irradiated to afford the photocyclized benzo[c]phenanthridine **(159)**. Reductive methylation yielded dihydromacarpine **(74)** which was identical with an authentic sample derived from the natural alkaloid, thus ending the dispute over the structure of this compound.

4.1.4. SYNTHESIS OF THE 2,3,8,9-TETRASUBSTITUTED ALKALOIDS

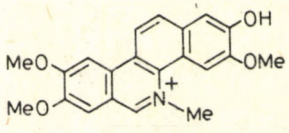
As nitidine **(1)** and fagaronine **(15)** have been known to have very potent antileukemic activity, benzo[c]phenanthridine alkaloids with this substitution pattern have been considered very important, becoming targets of synthetic efforts. Avicine **(7)** also belongs to this group.



Avicine (**7**)



Nitidine (**1**)



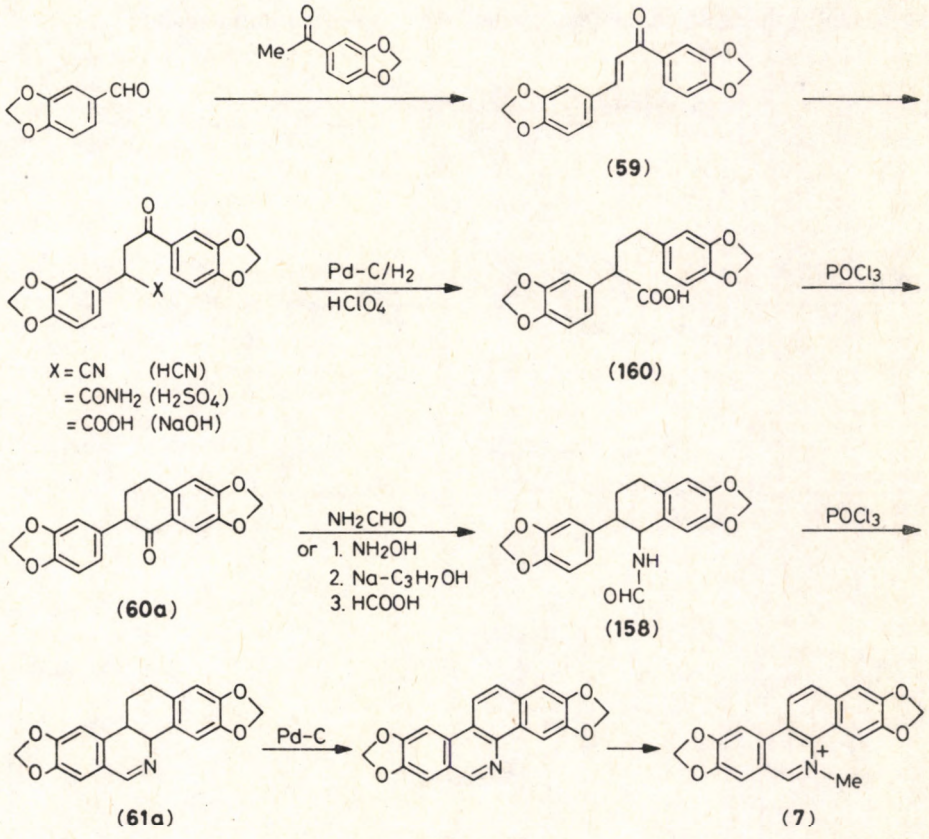
Fagaronine (**15**)

4.1.4.1. SYNTHESIS OF AVICINE (7)

Isolated from the root bark of *Zanthoxylum avicennae*, the structure of avicine **(7)** was proposed by Arthur and co-workers [75] and confirmed by its first total synthesis by Gopinath *et al.* [76] in 1961. The synthesis was accomplished starting from the chalcone **(59)** and using the Bischler–Napieralski cyclization of the aliphatic formamide **(58)** as shown in Scheme 55.

Introduction of a cyano group into the β-position followed by hydrolysis first under acidic and then basic conditions, and subsequent reduction afforded the 2,4-diarylbutyric acid **(160)**, which was cyclized to give the key intermediate, the 2-aryl-

1-tetralone **(60a)**. Introduction of a formamido group was effected by the Leuckart reaction to give the aliphatic formamide **(158)**. Bischler–Napieralski cyclization with phosphoryl chloride furnished the 4b,10b,11,12-tetrahydrobenzo[c]-



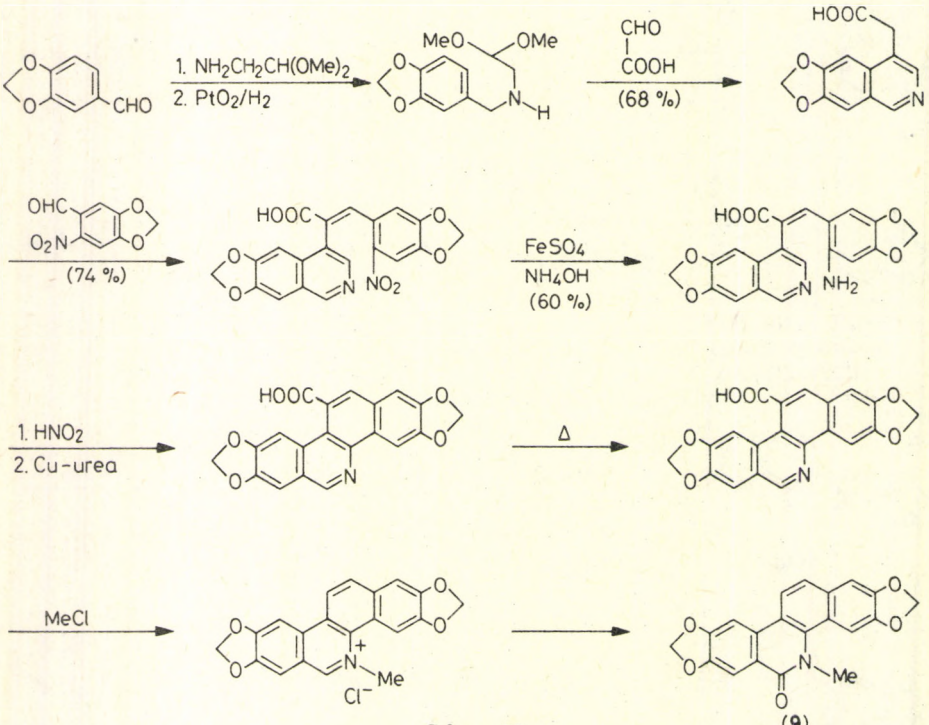
Scheme 55

phenanthridine **(61a)** which was dehydrogenated and quaternized to obtain the alkaloid avicine **(7)**. This synthesis of avicine is a typical example of application of the classical Robinson route, which has been investigated and applied to many other alkaloids by many groups.

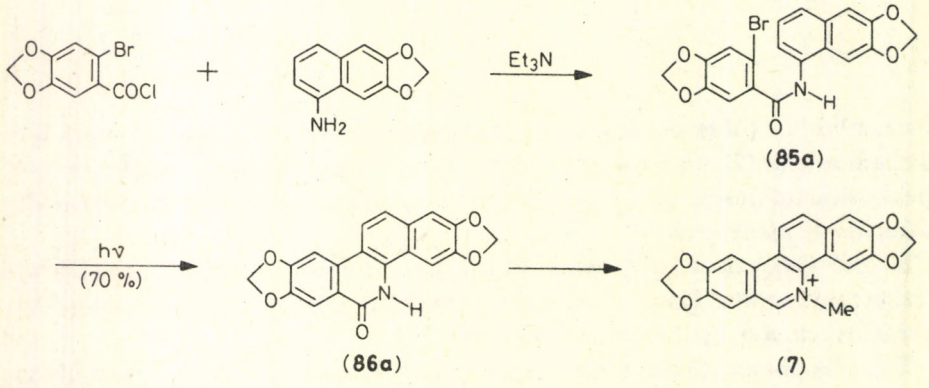
In 1968 Dyke *et al.* [61] reported the synthesis of oxyavicine **(9)** by applying Pschorr cyclization. This synthesis was virtually an application of the method of Abramovitch and Tertzakian [60] (Scheme 56).

Enamide photocyclization has played an important part in the synthesis of the alkaloid.

Kessar and co-workers [45] started their synthesis of avicine from 2-bromo-3,4-methylenedioxybenzoyl chloride and 6,7-methylenedioxynaphthylamine, which were combined to give the *o*-bromobenzoylnaphthylamine **(85a)** (Scheme 57). Photocyclization of this enamide **(85a)** proceeded smoothly to give a good yield of the benzo[c]phenanthridone **(86a)**, which was converted into avicine **(7)**.

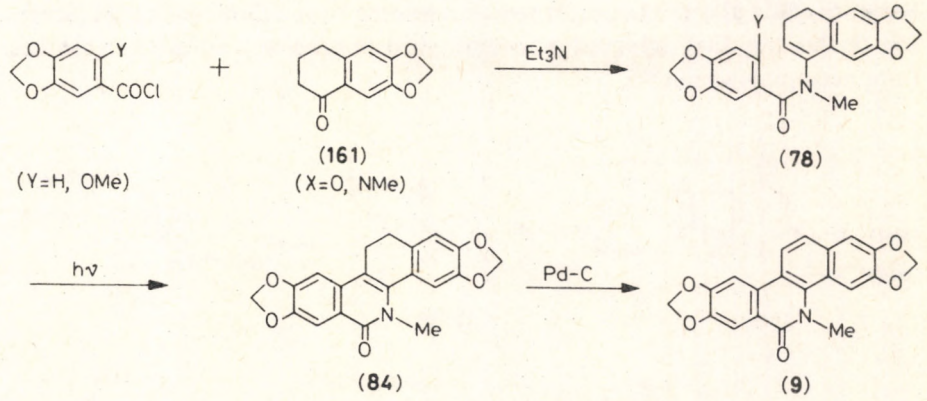


Scheme 56



Scheme 57

Ninomiya *et al.* [42] also carried out the photocyclization of enamides, such as N-(3,4-dihydronaphthyl)-N-methylbenzamides **(78)** and obtained the corresponding 11,12-dihydrobenzo[c]phenanthridone **(84)**, which was dehydrogenated to afford oxyavicine **(9)** (Scheme 58).



Scheme 58

The synthesis of avicine **(7)**, which is an analogue of the potent antileukemic alkaloid nitidine **(1)**, has not attracted much interest because it has no biological activity.

4.1.4.2. SYNTHESIS OF NITIDINE (1)

Nitidine **(1)** has been the most investigated alkaloid, the purpose being the large scale preparation of the compound.

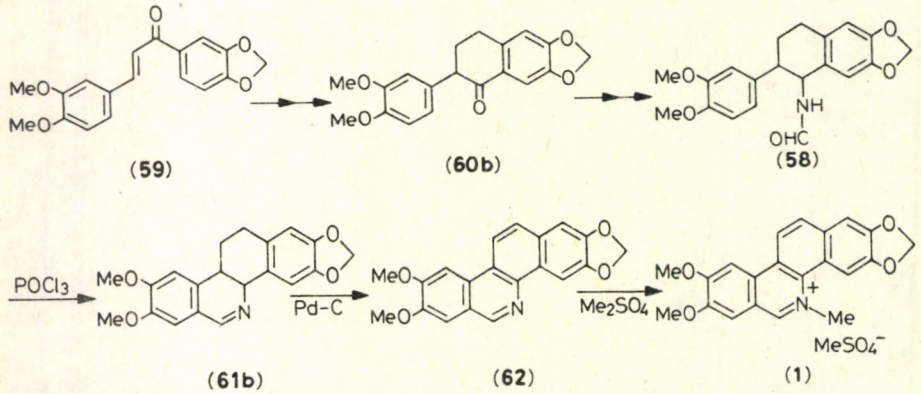
Isolated first from *Xanthoxylum nitidum*, Arthur and Ng [77], in 1959, proposed the structure. The alkaloid occurs in nature as the quaternary salt, and also as oxynitidine **(14)** and dihydronitidine **(13)**.

The first synthesis was accomplished by two groups, Arthur and Ng [77], and Gopinath *et al.* [78] in 1959. They followed the synthetic route designed by Bailey and Robinson [63], and also used by Bailey and Worthing [64] for the synthesis of chelerythrine **(10)**. This is the classical synthesis *via* the chalcone **(59)**, 2-aryl-1-tetralone **(60b)**, the aliphatic formamide **(58)**, the cyclized product 4b,10b,11,12-tetrahydrobenzo[c]phenanthridine **(61b)**, nornitidine **(62)**, and nitidine methosulfate **(1)** (Scheme 59).

More than ten years later, triggered by the finding of a marked potency against leukemia, studies toward the total synthesis of nitidine became a challenging task.

Accordingly, Zee-Cheng and Cheng [31], Kametani [32], and many other researchers described their achievements on the synthesis of nitidine.

A practical preparation of nitidine chloride was first reported by Zee-Cheng and Cheng [31] in 1973, who virtually followed the route of Arthur and Ng but improved the steps which had given only poor yields, such as the formation of the tetralone **(60b)** and the Leuckart reaction leading to the formamide **(58)** (Scheme 59). In this way they achieved an overall yield of nitidine methosulfate synthesis from acetopiperone in 9%.

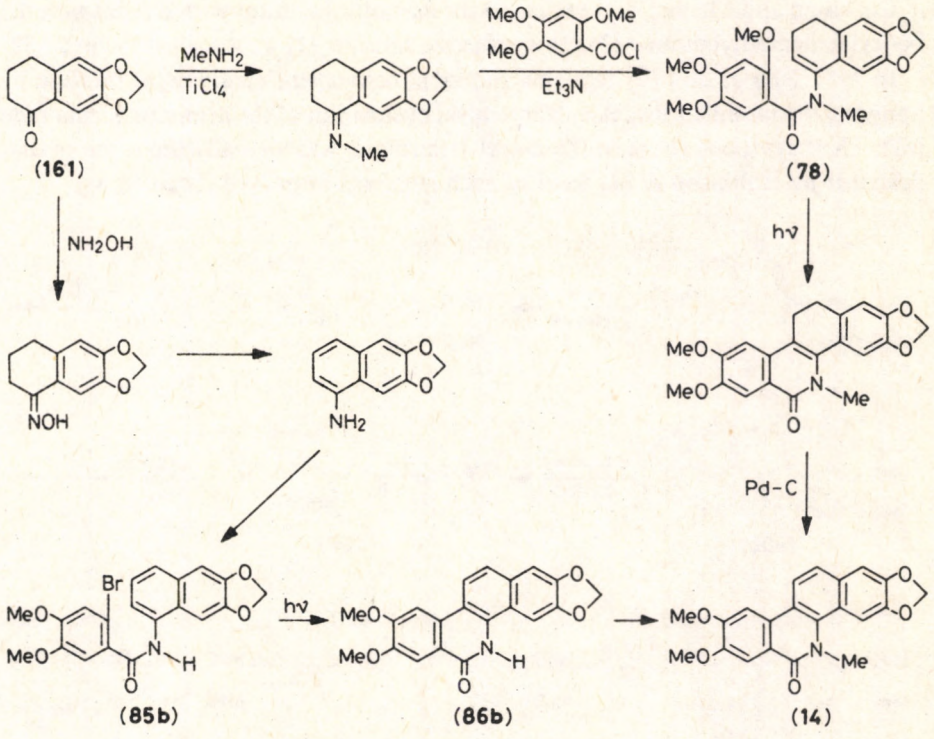


Scheme 59

In 1973 Kametani *et al.* [32] also described an alternative synthesis of nitidine. They reported the preparation of the 2-aryl-1-tetralone **(60b)** by the direct condensation of the appropriately substituted 1-tetralone **(161)** with the bromobenzene derivative in the presence of sodium amide (Scheme 17) and prepared the hexahydrobenzo[c]phenanthridine **(64)** by the Mannich reaction of the tetrahydronaphthylamine **(63)** with formaldehyde and hydrochloric acid; however, the yields were not high.

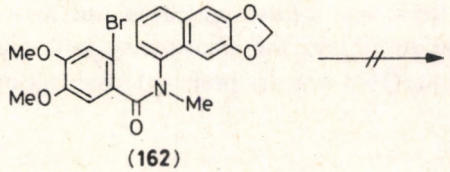
Both Kessar's [45] and Ninomiya's [42] groups applied enamide photocyclization to achieve the synthesis of nitidine, though they used different types of enamides **(78** and **85)**, as summarized in Scheme 60.

6,7-Methylenedioxy-1-tetralone **(161)** was the common starting compound in both syntheses and, in order to attain regioselective cyclization, an additional *o*-substituent, a bromo or methoxyl group, was introduced in the enamides **(78** or **85b)**. With the assistance of such a leaving group, the photocyclization of the enamides occurred only at the point of attachment of these *o*-substituents; the products were then dehydrogenated to regain the aromaticity of ring C.



Scheme 60

In 1977 Begley and Grimshaw [47] reported an attempt at the modified synthesis of nitidine by shortening the steps in Kessar's synthetic route using the N-methylbenzamide **(162)**; however, they failed in completing the synthesis, since this N-methylbenzamide **(162)** gave no benzo[c]phenanthridone on electrochemical treatment.

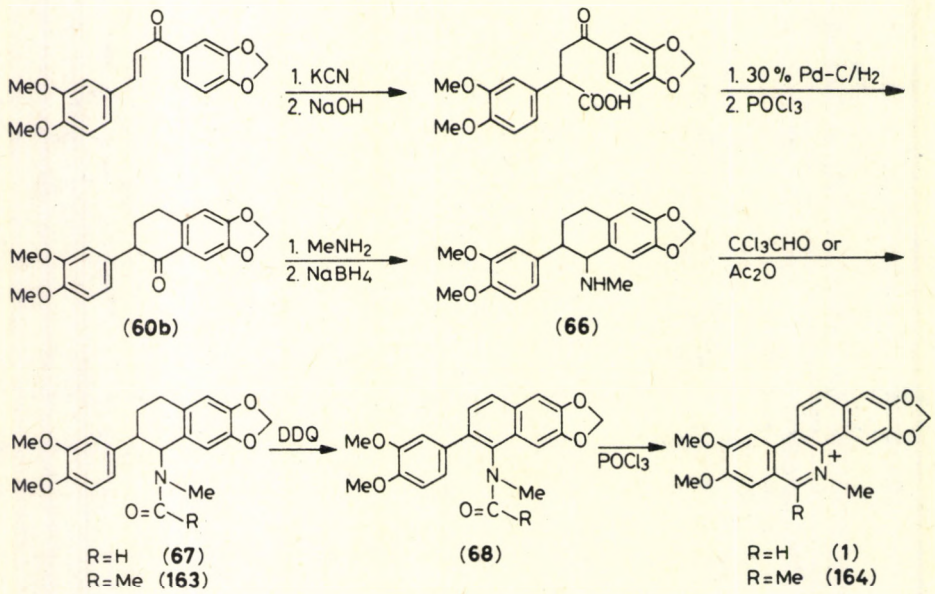


(162)

A strong demand for the compound for clinical trials intensified research on a more convenient syntheses of nitidine. As a result, two syntheses, which can be regarded as the most preferable ones at the moment, have been reported by Cushman and Cheng [70] and Ishii *et al.* [79].

Cushman and Cheng [70] condensed the homophthalic anhydride **(122)** with the benzylidenemethylamine **(121)** to synthesize nitidine **(1)** as shown in Scheme 38.

In 1981 Ishii *et al.* [79] reported the most convenient synthesis of nitidine by applying the modified Bischler–Napieralski cyclization of the aromatic formamide **(67)**. Their synthetic route to the 2-aryl-1-tetralone was virtually the same as that designed by Robinson *et al.*, yield of each step was improved (Scheme 61).



Scheme 61

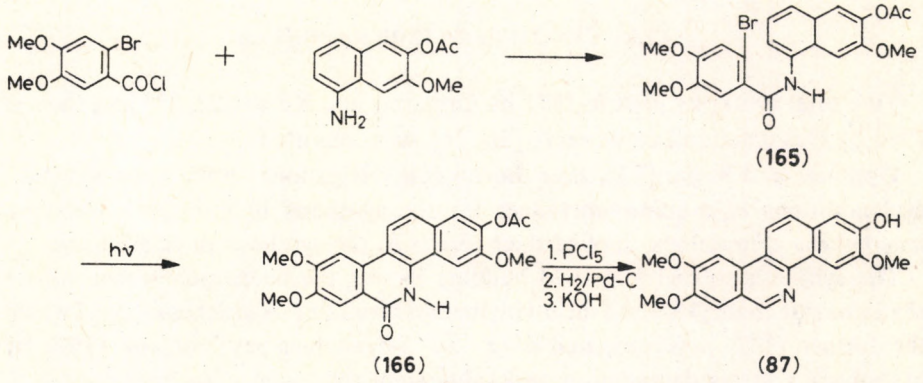
Treatment of the 2-aryl-1-tetralone **(60)** with methylamine, followed by reduction with sodium borohydride, yielded the *trans*-2-aryl-1,2,3,4-tetrahydronaphthylmethylamine **(66)**, which was formylated with chloral or acetylated with acetic anhydride to give the aliphatic N-methylformamide **(67** or **163)**. Dehydrogenation with DDQ afforded the aromatic formamide **(68)**, and this was cyclized by means of phosphoryl chloride to obtain the quaternary salt, nitidine chloride **(1)**. 6-Methylnitidine chloride **(164)** was also prepared from **163** for biological evaluation.

4.1.4.3. SYNTHESIS OF FAGARONINE (15)

Fagaronine **(15)** [80] is an alkaloid having a potent antileukemic activity, similarly to nitidine. However, the presence of a hydroxyl group at the 2-position has made the synthetic approach very difficult.

Fagaronine **(15)** was isolated in 1972 by Messmer and co-workers [80] from *Fagara zanthoxyloids*. Though a structure was proposed, there remained some doubts concerning its validity.

In 1974 Gillespie *et al.* [35] applied the Kessar cyclization of anils to the anil **71** prepared from two starting blocks, 6-isopropoxy-7-methoxynaphthylamine and 2-bromo-4,5-dimethoxybenzaldehyde, which were combined to give the required



Scheme 62

Schiff base **(71)** (Scheme 19). Treatment of this product with potassium amide in liquid ammonia effected cyclization to the aromatic benzo[c]phenanthridine **(72)**. Quaternization *via* the methosulfate and deisopropylation completed the total synthesis of fagaronine **(15)**. In this synthesis the isopropyl group played the part of a protective for the hydroxyl; it was then readily removable after quaternization on the nitrogen atom.

In 1975 Ninomiya and co-workers [46] applied enamide photocyclization in their attempt at synthesizing fagaronine. The enamide **(165)** was prepared from 6-acetoxy-7-methoxynaphthylamine and 2-bromo-4,5-dimethoxybenzoyl chloride and irradiated according to Kessar's procedure to give the lactam **(166)**, which was aromatized and hydrolyzed to obtain norfagaronine **(87)** (Scheme 62).

4.2. TOTAL SYNTHESIS OF THE B/C-HEXAHYDROBENZO[c]PHENANTHRIDINE ALKALOIDS

As mentioned previously, hexahydrobenzo[c]phenanthridine alkaloids consist of two groups, the corynoline and chelidonine alkaloids. They differ only in the presence or absence of a 10b-methyl group; otherwise they have the common structural feature of the B/C-*cis*-4b,5,6,10b,11,12-hexahydrobenzo[c]phenanthridine skeleton, with at least one hydroxyl group at the 11-position.

4.2.1. TOTAL SYNTHESIS OF THE CHELIDONINE ALKALOIDS

Chelidonine **(2)** and homochelidonine **(23)** are two representative alkaloids isolated from *Chelidonium majus* [1] and they have B/C-*cis*-4b,5,6,10b,11,12-hexahydrobenzo[c]phenanthridine skeleton with a hydroxyl group at the 11β-position.

4.2.1.1. SYNTHESIS OF CHELIDONINE (2)

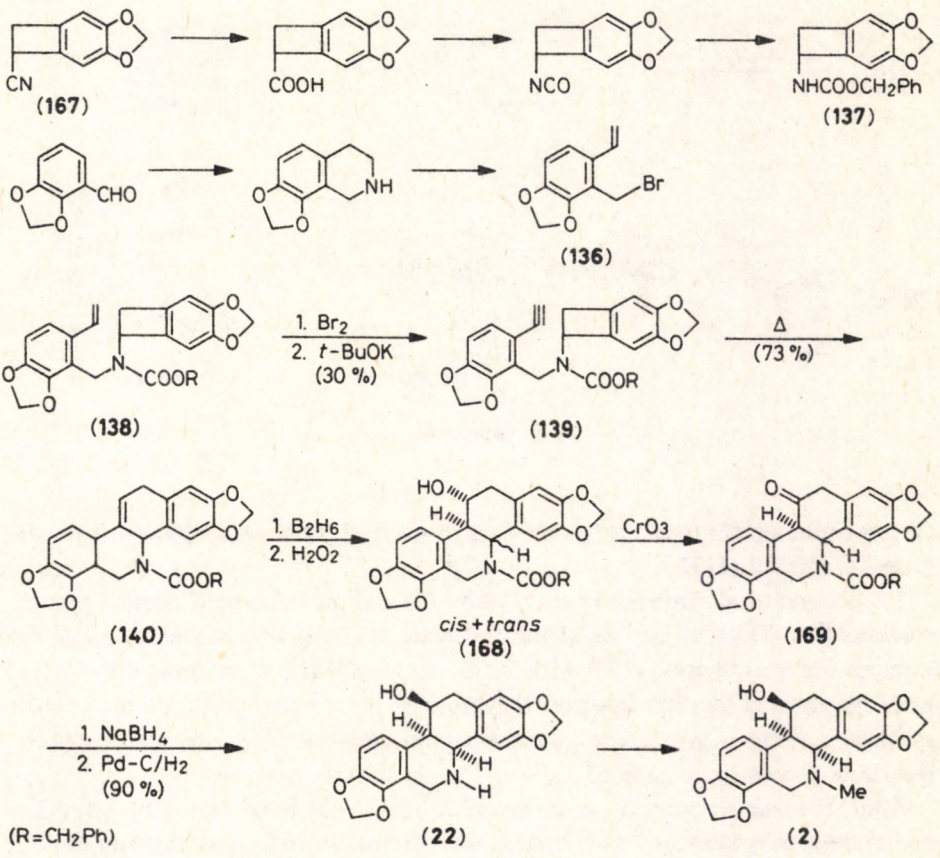
Two total syntheses, first in 1971 by Oppolzer and Keller [22, 23] and then in 1980 by Cushman and co-workers [2b, 71], were reported.

Oppolzer and Keller [22], after thorough investigations on the intramolecular cycloadditions of *o*-quinodimethanes for the synthesis of nitrogen containing heterocyclic compounds, applied their results to the synthesis of chelidonine.

The synthesis started from two building blocks, the benzocyclobutene with a urethane side chain **(137)** and the *o*-vinylbenzyl bromide **(136)** (Scheme 63), of which the former **(137)** was prepared from the 5-cyanobenzocyclobutene **(167)** by hydrolysis, Curtius degradation and subsequent treatment with benzyl alcohol, while the latter compound **(136)** was made from 2,3-methylenedioxybenzaldehyde through cyclization of the tetrahydroisoquinoline, followed by Hofmann and von Braun degradations.

The condensation of these two components (**136** and **137**) was achieved by converting **137** into the sodium salt by means of sodium hydride, and then by the addition of the bromide **(136)** and sodium iodide, to obtain the styrene **(138)** in 77% yield (Scheme 63). In order to leave a double bond after cyclization, bromination, followed by treatment with alkali was used to convert styrene **(138)** into the acetylenic compound **(139)**. Thermal cyclization was effected by heating the **139** at 120 °C in *o*-xylene for 1 h. A crystalline benzo[c]phenanthridine **(140)** was obtained in 73% yield. Introduction of a hydroxyl group into the 11β-position was accomplished by hydroboration of **140**, followed by oxidation with hydrogen peroxide, to obtain a mixture of the B/C-*cis*- and *trans*-alcohols **(168)** which, after separation, could be converted into the ketone **(169)** by Jones oxidation and then reduced with sodium borohydride in methanol–dioxane solution at 0 °C for 1 h, furthermore the N-benzyloxycarbonyl group was removed by catalytic hydrogenation. In this way N-norchelidonine **(22)** was obtained in 90% yield from **168**. Finally, N-methylation of **(22)** completed the first total synthesis of (±)-chelidonine **(2)**.

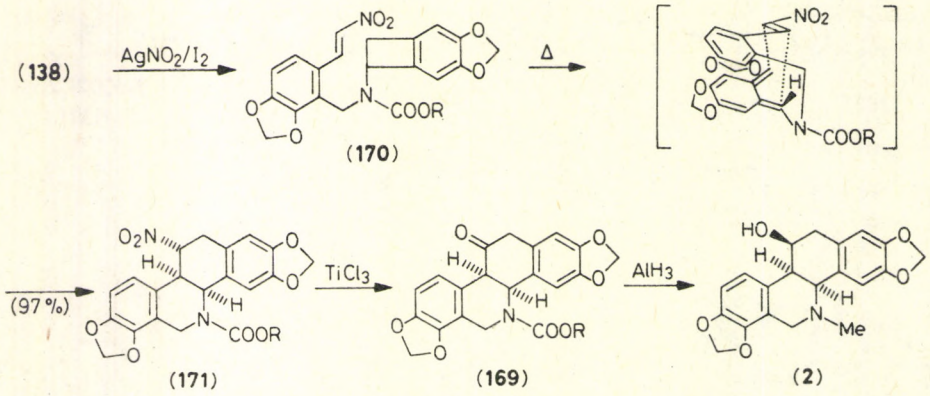
Although the above synthesis included the key step **(139)**→**(140)** as an efficient construction of the benzo[c]phenanthridine skeleton, the transformations **(138)** →**(139)** and **(168)**→**(169)** gave only poor yields, making this synthesis less attractive.



Scheme 63

Recently, Oppolzer [23] reported a modification of the above synthesis in converting **138** into **169** as shown in Scheme 64.

First, a nitro group was introduced into the styrene **(138)** by reaction with silver nitrite in the presence of iodine and potassium acetate, giving the nitrostyrene **(170)**, in order to control regioselectivity of the following cycloaddition. Heating of the nitrostyrene **(170)** in xylene at 120 °C for 2 h gave the *cis*-fused adduct **(171)** in 97% yield. This remarkable regio- and stereoselectivity of the cycloaddition is indicative of a transition state such as depicted in the scheme, showing that the nitro group is in the unusual *exo*-orientation, thus becoming a driving force for the stereoselective cycloaddition. Treatment of the nitro compound **171** with titanium chloride under mild conditions afforded the ketone **169**. The simultaneous reduction of the carbonyl and urethane groups of the ketone **169** with aluminium hydride yielded

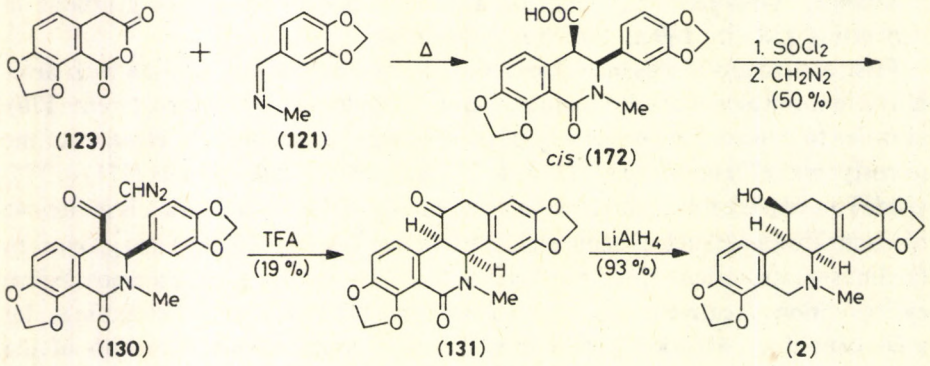


Scheme 64

(±)-chelidonine **(2)** in one step; an overall yield of 54% was achieved from the nitro compound **(171).**

The second total synthesis of chelidonine **(2)** was reported by Cushman and co-workers [2b, 71] who exploited the synthetic route of the condensation of the homophthalic anhydride **(123)** with the Schiff base **(121).** After completion of the total synthesis of nitidine chloride, they noticed the stereoselective nature of their synthesis and made good use of this in the total synthesis of (±)-chelidonine **(2)** by the route shown in Scheme 65.

After the developing of a convenient preparation of the 2-carboxy-3,4-methylenedioxyphenylacetic acid which was used as one of the starting compounds, Cushman and co-workers [2b, 71] started their short synthesis of the alkaloid. The condensation of two components (**123** and **121**) afforded the 3,4-*cis*-substituted



Scheme 65

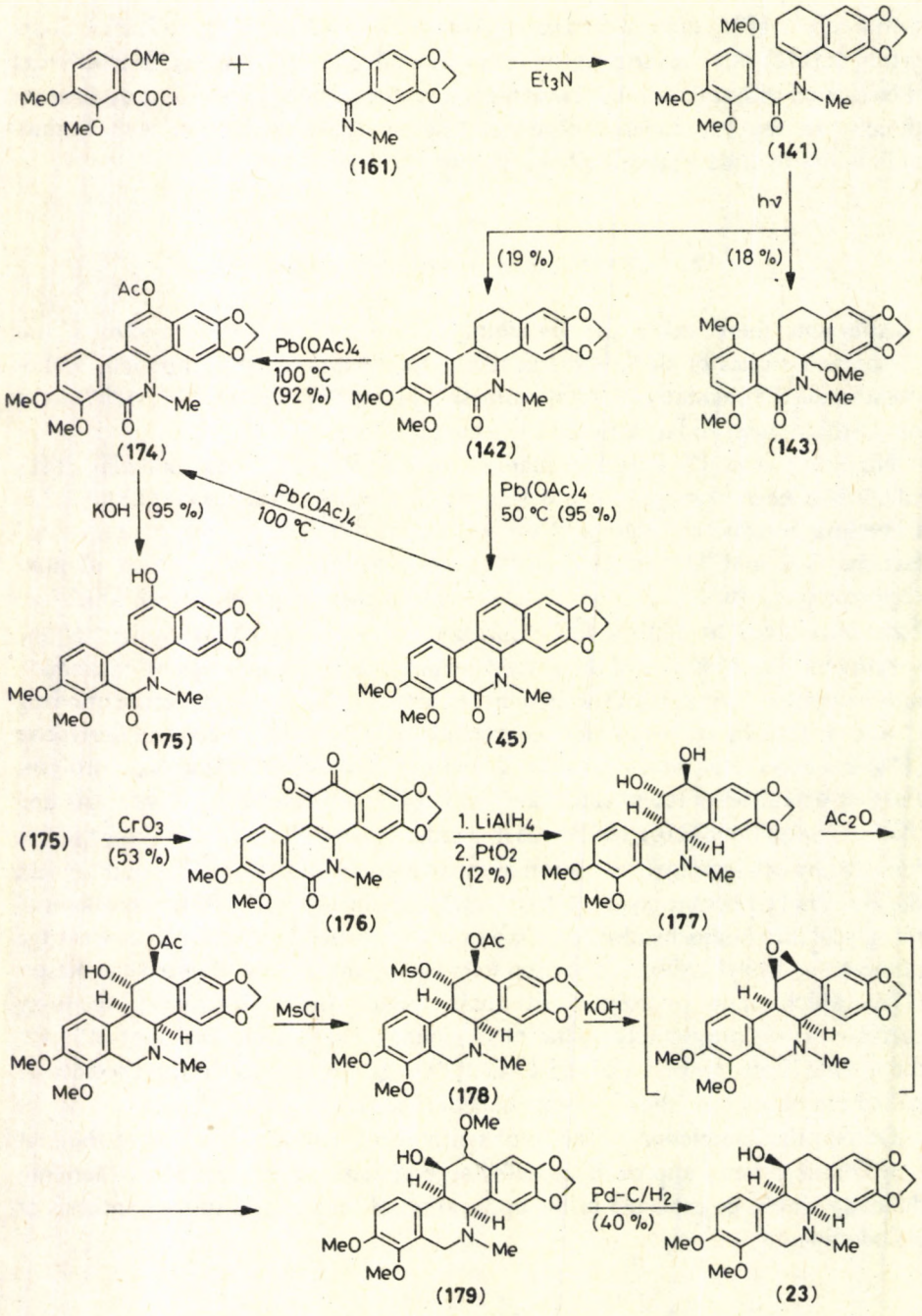
isoquinolone **(172)** upon refluxing in acetonitrile solution for 1 h. The carboxyl group of **(172)** was converted into the corresponding diazoketone **(130)** which was then treated with trifluoroacetic acid for a very short time to bring about cyclization directly to the 11-oxobenzo[c]phenanthridone **(131)**. Reduction with lithium aluminium hydride yielded (±)-chelidonine **(2)**.

4.2.1.2. SYNTHESIS OF HOMOCHELIDONINE (23)

Although there exists the possibility of extending both Oppolzer's and Cushman's routes of chelidonine synthesis to the analogous compound, homochelidonine **(23)**, actually the total synthesis by Ninomiya's group [24] (1977) is the only achievement so far reported (Scheme 66).

Ninomiya *et al.* [24] applied enamide photocyclization to the enamide **(141)**, which had been prepared from 6,7-methylenedioxy-1-tetralone **(161)** and 2,3,6-trimethoxybenzoyl chloride, and obtained a mixture of the two photocyclized lactams (**142** and **143**) in 19 and 18% yield, respectively, as a result of non-regioselectivity due to the presence of two *ortho*-methoxyl groups, one of which had been introduced to control the cyclization to the point of attachment of the substituent. The structure of the desired lactam **(142)** was confirmed by conversion into oxychelerythrine **(45)**. Oxidative introduction of the oxygen function into ring C was effected by means of lead tetraacetate to obtain the 12-acetoxy derivative **(174)** in a good yield, which gave the 12-hydroxy compound **(175)** upon hydrolysis. Further oxidation of the phenolic derivative **(175)** with chromic trioxide afforded the 11,12-*o*-quinone **(176)**, which was first reduced with lithium aluminium hydride followed by catalytic hydrogenation of the remaining 4b,10b-double bond to yield the B/C-*cis*-11,12-*trans*-glycol **(177)**, though only in 12% yield. The conversion of this glycol into homochelidonine **(23)** was accomplished by the solvolytic cleavage of the 12-hydroxyl group; acetylation followed by mesylation afforded the diester **(178)**, which upon hydrolysis with base yielded the 11-hydroxy-12-methoxy derivative **(179)**, presumably *via* the intermediary formation and cleavage of a 11,12-β-epoxide. Hydrogenolysis of the 12-methoxyl group of **(179)** in the presence of palladium on carbon afforded homochelidonine **(23)**.

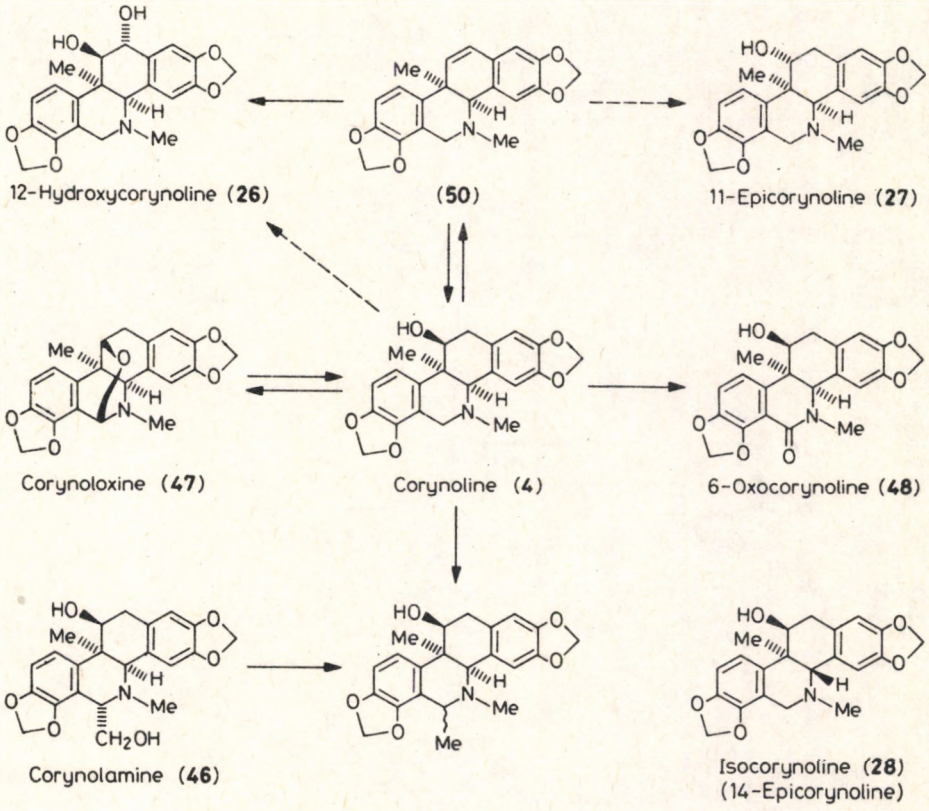
Despite the completion of the total synthesis of homochelidonine for the first time, this synthetic approach contains several steps to be improved, therefore difficulty would be expected in the application of the method to the synthesis of chelidonine.


Et3N
(161)
(141)
hν
(19 %)
(18 %)
Pb(OAc)4
100 °C
(92 %)
(174)
(142)
(143)
KOH
(95 %)
Pb(OAc)4
100 °C
Pb(OAc)4
50 °C (95 %)
(175)
(45)
CrO3
(53 %)
(176)
1. LiAlH4
2. PtO2
(12 %)
(177)
Ac2O
MsCl
(178)
KOH
(179)
Pd-C/H2
(40 %)
(23)

Scheme 66

4.2.2. TOTAL SYNTHESIS OF THE CORYNOLINE ALKALOIDS

Corynoline **(4)**, 12-hydroxycorynoline **(26)**, 11-epicorynoline **(27)**, corynoloxine **(47)**, corynolamine **(46)**, and isocorynoline **(28)** are the alkaloids isolated from the *Corydalis* plants, and they form a structurally related group. In the course of their structure determinations they were chemically interconverted as shown in Scheme 67 [6].



Scheme 67

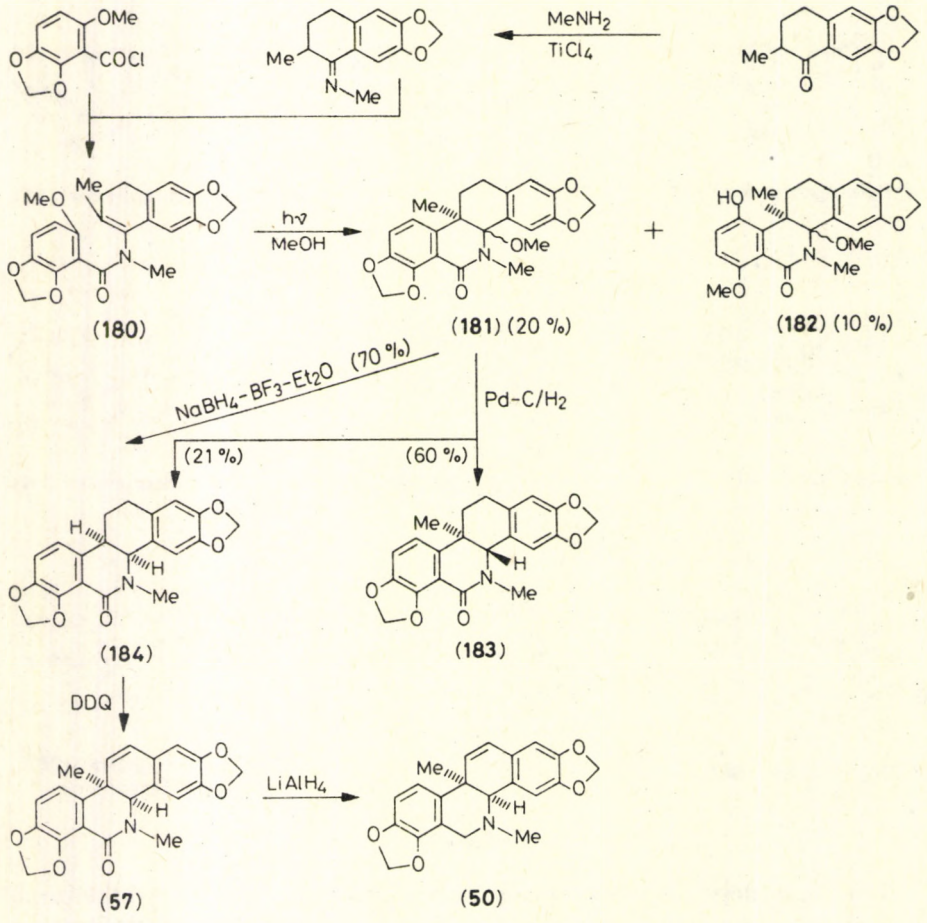
From the strategic point of view, one can readily draw up a plan to synthesize all these compounds, except isocorynoline, by first preparing the 11,12-dehydro derivative **(50)**, which would play the part of an important key compound for the different alkaloids (*cf.* Scheme 67).

The above proposition was materialized by Ninomiya and co-workers [29] who accomplished the total syntheses of the corynoline group of alkaloids (**4**, **26**, and **27**) by applying enamide photocyclization.

4.2.2.1. SYNTHESIS OF THE KEY INTERMEDIATE 11,12-DEHYDRO DERIVATIVES

The synthetic route to the key intermediates, the 11,12-dehydro derivatives (**50** and **57**) is as follows [29a, b] (Scheme 68).

The enamide (**180**) was readily prepared from two starting blocks, 2,3-methylenedioxy-6-methoxybenzoyl chloride and 6,7-methylenedioxy-2-methyl-1-



Scheme 68

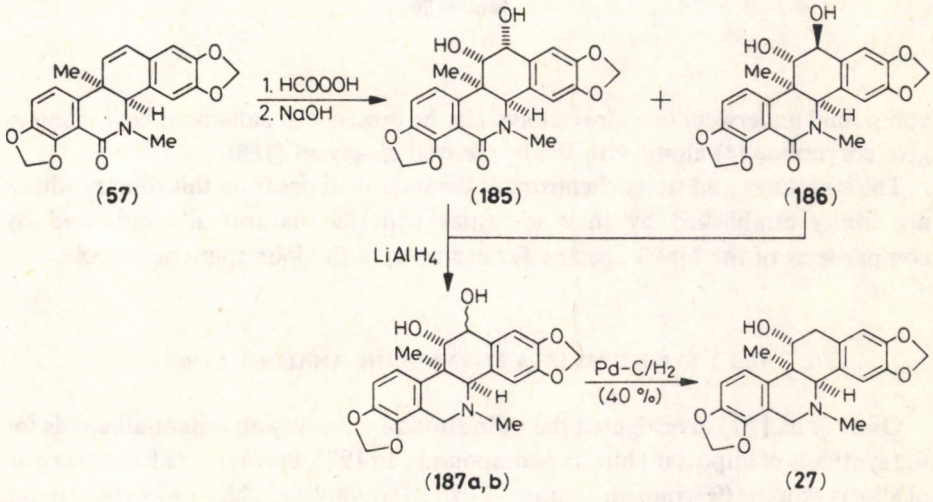
tetraloneimine. Irradiation of the enamide (**180**) in methanol solution afforded a mixture of two lactams (**181** and **182**) in 20 and 10% yield, respectively, as a result of little regioselectivity in the cyclization. The *o*-methoxyl group migrated in a 1,5-

manner to the 4b-position and was readily eliminated by hydrogenolysis in the presence of palladium-on-carbon. The product of hydrogenolysis was a mixture of the B/C-*trans*- and *cis*-lactams (**183** and **184**) in 60 and 21% yields, of which the former (**183**) can be used for the synthesis of isocorynoline (**28**).

The desired B/C-*cis*-lactam (**184**) was solely obtained by hydrogenolysis with sodium borohydride in the presence of boron trifluoride ethereate in 70% yield. Dehydrogenolysis with DDQ converted the lactam (**184**) into the 11,12-dehydrolactam (**57**), albeit in only 28% yield; this was further reduced with lithium aluminium hydride to the corresponding dehydroamine (**50**).

4.2.2.2. SYNTHESIS OF CORYNOLINE

Employing the above 11,12-dehydro derivatives (**50** and **57**), introduction of the oxygen functions was achieved as follows. First the 11,12-dehydrolactam (**57**) was converted to 11-epicorynoline (**27**) [29b, c] (Scheme 69).

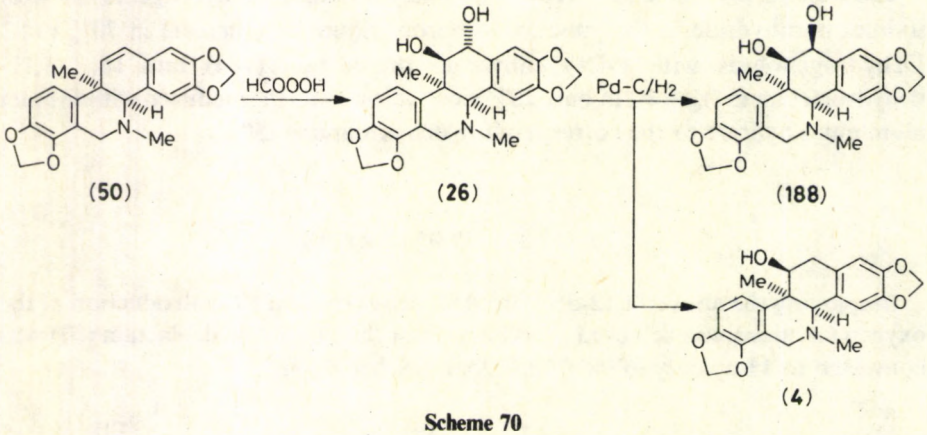


Scheme 69

Then glycol formation from the dehydrolactam (**57**) by oxidation with performic acid followed by hydrolysis afforded a mixture of the 11,12-*cis*- (**185**) and *trans*-(**186**) glycols, which were reduced with lithium aluminium hydride to give the corresponding dihydroxyamines; **187a** and **187b**, respectively. Elimination of the 12-hydroxyl group of (**187a**) and (**187b**) was accomplished by hydrogenolysis with palladium-on-carbon to afford 11-epicorynoline (**27**).

From the 11,12-dehydroamine **(50)** corynoline **(4)**, 12-hydroxycorynoline **(26)** [29a, b] were prepared as shown in Scheme 70.

The dehydroamine **(50)** was converted by treatment with performic acid into the 11,12-*trans*-glycol **(26)**, which was identical with 12-hydroxycorynoline; this



Scheme 70

compound underwent dehydrogenolysis in the presence of palladium-on-carbon to give corynoline **(4)** along with the isomerized *cis*-glycol **(188)**.

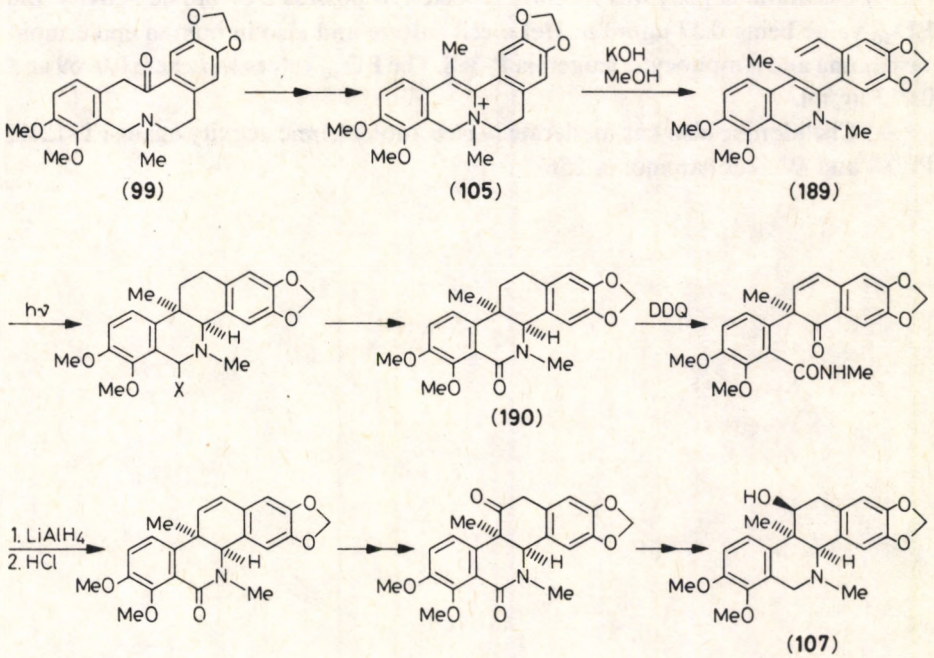
The structures and stereochemistry of the intermediates from this total synthesis are firmly established by their identities with the natural alkaloids and by comparisons of the NMR spectra, for example, of the four epimeric glycols.

4.2.2.3. SYNTHESIS OF A CORYNOLINE ANALOGUE (107)

Onda *et al.* [57] investigated the utilization of naturally abundant alkaloids for the synthesis of important but rare compounds. In 1975, they reported a conversion of allocryptopine **(99)** into an analogue **(107)** of corynoline, which differs only in the 7,8-substituents, that is in having 7,8-dimethoxy groups **(107)** instead of the 7,8-methylenedioxy group of natural corynoline **(4)**.

The synthetic strategy (Scheme 71) involved the conversion of allocryptopine **(99)** into the quaternary berbine **(105)**, which on Hofmann degradation afforded the triene **(189)**. Photocyclization of this triene as mentioned previously (Section 3.2.2.), afforded the benzo[c]phenanthridone **(190)**, which was further converted into the target analogue **(107)** by a route similar to that used by Ninomiya's group in the synthesis of corynoline.

Some of the fully aromatic benzo[c]phenanthridine alkaloids were found to be highly cytotoxic and displayed considerably strong antileukemic activities in both leukemia L-1210 and P-388 systems in mice, and also inhibited Lewis lung carcinoma.



Scheme 71

It is said that nitidine chloride is one of the most remarkable alkaloid of this group and it was regarded as the most hopeful compound for curing leukemia, it also proceeded to the stage of clinical evaluation. However, because of its intense toxicity it was removed from the market. Fagaronine seems to be still under investigation and now under clinical trial for evaluation as an antileukemic drug.

Studies have also been carried out to determine structure–activity relationships in the case of benzo[c]phenanthridine derivatives. Zee-Cheng and Cheng [82] suggested the importance of a triangular arrangement of the electron rich centres in the molecule to possess antileukemic activity.

Apart from the study of the antileukemic activity, only fragmentary researches have been reported. Therefore, there is much to be done in the future on the establishment of the general pharmacology of this group of compounds.

Sanguinarine, chelerythriene, and chelirubine have been reported to possess some nematocidal activity [52].

Very few reports are available on the activity of the chelidonine and corynoline alkaloids.

(−)-Chelidonine [2b] was recently reported to possess a cytotoxic activity, the ED_{50} value being 0.27 μg/ml in HeLa cell culture and also in human epidermoid carcinoma and lymphocytic leukemia P-388. The ED_{50} values were here 0.0069 and 0.052 μg/ml.

(+)-Chelidonine also has moderate *in vivo* antileukemic activity against L-1210, P-388 and Walker carcinoma 256.

5. PHARMACOLOGICAL ACTIVITY OF BENZO[c]PHENANTHRIDINE ALKALOIDS AND RELATED COMPOUNDS

From a comparison of the amount of work on these alkaloids, which is indicative of the demand for them, it is obvious that nitidine and fagaronine have been in the centre of interest. Some of the benzo[c]phenanthridine alkaloids have shown high potency against leukemia as listed in Table I.

Table I

	Active against	Inactive against
Fagaronine	PS(270), LE	BI, KB
O-Methylfagaronine	PS(260), LE	BI, KB
Nitidine chloride	PS(200), LD, KB	BI, LL (new)
Oxynitidine	KB	PS
Methoxydihydronitidine	PS(263), LE, KB	BI, LL (new)
Chelidimerine	KB	
Sanguidimerine	KB	LE, PS, WA
Camptothecin	LE, PS(250), WA, KB	
Ellipticine	WA, LE, BI, KB	LL
9-Methoxyellipticine	PS(200), LE, SA, LE, KB	WA

The above bioassays were conducted in the National Cancer Institute, Washington, and the tumour systems employed were as follows:

BI	B16 melanocarcinoma; mouse	ILS	=40%
CA	Adenocarcinoma 755; mouse	TWI	=58%
HE	HeLa human carcinoma; cell culture	ED_{50}	=1.0%

KB	Human epidermoid carcinoma of the nasopharynx; cell culture	ED_{50}	=1.0%
LE	Lymphoid leukemia L-1210; mouse	ILS	=25%
LL	Lewis lung carcinoma; mouse	TWI	=58%
		ILS	=40%
LL (new)	Lymphocytic leukemia −388; mouse	ILS	=25%
SA	Sarcoma 180; mouse	TWI	=58%
WA	Walker carcinoma 256; rat	TWI	=58%

TWI	tumour weight inhibition
ILS	increase in life span
ED_{50}	dose level in g/ml at which 50% inhibition of the growth of cells is obtained

6. FUTURE PERSPECTIVES

In the past five years major progress has been achieved in the development of the methodology of the syntheses of benzo[c]phenanthridine alkaloids and related compounds.

Of course, the motivation of these synthetic studies was not only the establishment of the structures and the chemistry of these compounds; it was also the chemist's response to the rising demands for the supply of pharmacologically active derivatives. However, it can be said that the time is ready for going more deeply into projects on the exploitation of drugs based on this group of compounds.

Also, as suggested by the natural occurrence of optically active alkaloids in this group, such as chelidonine and corynoline, there should be research coming up on asymmetric syntheses of these alkaloids and also studies on the optically active components.

Very recently, Naito and co-workers [83] reported an asymmetric synthesis of xylopinine, an alkaloid closely related to benzo[c]phenanthridines, by using enamide photocyclization in the presence of a chiral reagent. This work hopefully would trigger a possibility of a new asymmetric syntheses of benzo[c]phenanthridine alkaloids.

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THE CHEMISTRY OF THE VANCOMYCIN GROUP OF ANTIBIOTICS

1. INTRODUCTION

In recent scientific researches considerable interest has been devoted to the structural investigation of glycopeptides and glycoproteins of biological importance. Besides enzymes, immunological substances, cell-wall components of microorganisms and skeletal constituents of animal organisms, several antibiotics have also been involved in these studies.

Vancomycin-type antibiotics are also biologically active substances of glycopeptide character having medium molecular weight and complicated aglycone moieties. However, these compounds significantly differ from other glycopeptide antibiotics, such as the representatives of the bleomycin–phleomycin [1], tallisomycin [2] or the minosaminomycin [3] groups, with respect both to their structural properties and biological effect. While the antibiotics of the latter groups are built from linear peptide chains and possess — first of all — anticancer properties, each antibiotic of the vancomycin group has been shown to have cyclic heptapeptide structure and antibacterial activity.

The representatives of this group of antibiotics, in chronologic order of their discovery, are:

1956 Vancomycin (Vancocin, Vancocina)
1957 Actinoidin
1957 Ristocetin (Spontin)
1963 Ristomycin
1968 Avoparcin (Avotan)
1969 Antibiotic LL-AM 374
1978 Antibiotic complex A-35512

According to the name of vancomycin, discovered first, these antibiotics are cited in the literature as the vancomycin group of antibiotics [4, 5]. These compounds had been introduced into medical practice much earlier than their chemical

structure was elucidated in every detail. They possess *in vivo* activity particularly against Gram-positive microorganisms, and practically no strains of bacteria resistant to them have emerged during their application [6]. Therefore, against β-lactam resistant infections and for the treatment of patients sensitive to penicillins, the vancomycin-type antibiotics may be more advantageously used than semisynthetic penicillins and cephalosporins.

Studies on the vancomycin-type antibiotics are still in progress. Intensive work is being done to discover and isolate further representatives of this group. This research is stimulated by the growing interest in the practical applications of the members (with the exception of actinoidin*) of this family of antibiotics.

Vancomycin (termed Vancocin) and ristomycin are produced on industrial scale by the Eli Lilly Co. (USA) and the National Research Institute of Antibiotics (Moscow, USSR), respectively, for clinical purposes. Although the Abbott Laboratories have stopped the marketing of *Spontin* (ristocetin) as an antibacterial antibiotic, it is now used as a laboratory diagnostic agent in haematology under the trade name *Aggrecetin* for the diagnosis of von Willebrand's disease, a type of bleeding disorder of genetic origin.

Application of ristomycin for the same purpose is now being in progress in Hungary [7, 8]. As structure investigations, to be discussed in the following chapters, have proved the identity of ristocetin and ristomycin, the two antibiotics must be considered to have identical diagnostic value.

Apart from the importance of antibiotics in human therapy, their veterinary application and utilization as feed additives have also aroused growing interest [9, 10].

In 1978 the American Cyanamid Co. (USA) introduced avoparcin in England as feed additive under the trade name *Avotan.*

A period of more than twenty years, which passed between the isolation and complete structure elucidation of the vancomycin-type antibiotics, was necessary to elaborate satisfactory methods for the purification and selective chemical degradation of these complicated natural compounds. The results permitted then the application of the modern physicochemical techniques suitable for the identification of the composition of the unusual structural elements. Up to now ristocetin (ristomycin) is the only glycopeptide antibiotic with a molecular weight higher than 2000 (2063) dalton and with completely known structure. These studies have shown that — in addition to several natural amino acids (phenylalanine, aspartic acid) — the building blocks of the aglycone moieties in these antibiotics are hitherto unknown amino acids of phenolic character, such as actinoidinic acid, ristomycinic acid and vancomycinic acid, arranged in a tri- or tetracyclic

* In the Soviet Union actinoidin was used for the external treatment of purulent wounds.

heptapeptide structure. Some of these cyclic peptides also contain the relatively rare *cis*-amide bonds. The aglycones are glycosylated by simple or complex carbohydrates in one, two, three or four positions. Amongst the carbohydrate constituents of the vancomycin-type antibiotics several common neutral sugars (D-glucose, D-mannose, D-arabinose, L-rhamnose), as well as unusual new amino sugars have been found. Acosamine, actinosamine, ristosamine and vancosamine became known as natural compounds for the first time as a result of just these structural studies. All of the latter sugars are 3-amino-2,3,6-trideoxyhexopyranoses, and later some of their derivatives have also been found as constituents of other antibiotics [10a, 10b, 11, 12,] (megalomycin, kidamycin, hedamycin, sporaviridin). None of the oligosaccharides ristobiose, ristotriose and ristriose — isolated in the degradation studies — had been known preceding these investigations. Later the structures of the carbohydrate constituents of the vancomycin group of antibiotics were also proved by syntheses and these studies greatly promoted the development of new semisynthetic anthracycline glycoside-type antibiotic analogues [13].

Information obtained about the structure of the vancomycin-type antibiotics permitted the explanation of their biogenesis and structure–activity relationship. The biological activity of vancomycin is due to a specific complex formation between the antibiotic and the cell-wall mucopeptide having N-acyl-D-Ala-D-Ala moiety at the C-terminus, and a similar mechanism has been established for the action of ristocetin A (ristomycin A) [28, 56].

During the past years several research groups have been studying the constitution of the vancomycin group of antibiotics in the Soviet Union, in the USA, in Great Britain and in Hungary. The present review aims at summarizing the recent results of this international co-operation and scientific competition, in the expectation of promoting the work of those performing scientific activities in related fields of structural research.

2. GENERAL CHARACTERIZATION OF THE VANCOMYCIN GROUP OF ANTIBIOTICS

The members of the vancomycin group of antibiotics are stable, glycopeptide-type compounds of relatively high molecular weight.

These antibiotics are produced by actinomycetes belonging to the family of *Streptomyces* and *Actinomyces;* during the fermentation the biologically active components are biosynthesized. The antibiotics form derivatives with picric acid, Reinecke salt or salicylaldehyde. However, due to their relatively high molecular weight, they cannot be readily obtained in crystalline form. Therefore they are usually purified by repeated ion-exchange chromatography and isolated from the concentrated eluate by freeze-drying. Paper- and thin layer chromatography are the most suitable methods for the identification of these antibiotics [14, 15].

The molecular weights marked with asterisk in Table I were determined by californium plasma desorption mass spectrometry [21, 28]. The molecular weight of vancomycin was calculated on the basis of the molecular formula obtained by structural elucidation. The other molecular weights are approximate values, based on elemental nitrogen analysis and reducing carbohydrate content [24, 33, 34], or estimated from the combining weights, determined by ion-exchange or the thermistor–osmometric method [31] and potentiometric titration [32].

The pure antibiotics are strongly laevorotatory compounds. Their UV spectra, recorded in acidic or neutral medium, have an absorption maximum at 280 nm which is shifted to 292–300 nm under alkaline conditions. The IR spectra of the antibiotics are fairly complex, therefore only the most characteristic data are discussed. The bands of weak or medium intensity at 820 and 840 cm^{-1} can be unequivocally assigned to the C—H out of plane bending vibration of the trisubstituted aromatic ring, and the band of medium intensity at 980 cm^{-1} is characteristic of sugars. The wide and intense bands at 1060 and 1120 cm^{-1} (and also the complex band between 2400–3700 cm^{-1}) indicate the polyhydroxyl content of the components. The latter intensive band may be assigned to the stretching vibration [ν(≡C—OH)] of several phenolic hydroxyl groups. The

Table I

Physical data of the members of vancomycin group of antibiotics

Antibiotic	Producing strain	Molecular weight	$[\alpha]_D^{20}$	UV-spectrum λ_{max} (nm)		References
				in acid	in base	
Vancomycin	*Streptomyces orientalis* n. sp.	1420		278	300	4, 5, 16–19, 37
Actinoidin A	*Streptomyces actinoides*	2050 (2150)	−62° (0.34; H_2O)	280	300	4, 5, 22, 23, 33
Actinoidin B		2010	−93° (0.30; H_2O)	280	300	
Avoparcin α (antibiotic L-AV 290)	*Streptomyces candidus (NRRL 3218)*	1907*		280	300	20, 21, 57*
Avoparcin β		1942*	−86° (0.92; H_2O)			
Ristocetin A		2063*	(−120°)–(−130°)	280	300	4, 5, 24–28*, 56
Ristocetin B	*Nocardia lurida*		(−144°)–(−149°)	280	300	
Ristomycin A	*Proactinomyces fructiferi var. ristomycini*	2063*	−131° (0.5; H_2O)	280	300	4, 5, 28–32, 34
Ristomycin B		(1900–2000)	−145°	280	300	
Antibiotic complex A-35512	*Streptomyces candidus (NRRL 8156)*					
A-35512 A		2106	−100° (1.0; H_2O)	282	292	54, 55b
A-35512 B		2143 1954*	−123° (1.0; H_2O)	282	292	54, 55, 55a, 55b
A-35512 C		1862	−161° (1.05; H_2O)	282	292	54, 55b
A-35512 E		2018	−108.3° (1.0; H_2O)	286 (310)	270 (300.354)	54, 55b
A-35512 H		1660	−123.5° (1.0; H_2O)	282	292	54, 55b

* Determined by californium plasma desorption mass spectrometric method.

spectrum of each antibiotic contains a band at 1220 cm^{-1} known to be characteristic of the ν(C—O) vibration of amino acids. The $\nu(COO^-)_s$ signal at 1400 cm^{-1}, as well as the ν(C=O) of the carboxyl group (appearing as a well-defined shoulder at ca. 1690 cm^{-1}) can be found only in the spectra of vancomycin and the variants of actinoidin, which possess terminal carboxyl group. The bands between 1610–1620 cm^{-1} can be attributed to the symmetrical N—H in-plane bending vibration [$\beta_s(NH_2)$] of the free primary amino group. The weak band at 1730 cm^{-1} and the weak shoulder occasionally appearing at 1740–1745 cm^{-1} indicate the presence of the ester group ($COOCH_3$) in ristocetin and ristomycin. No similar band can be found in the spectra of vancomycin and actinoidin. The very wide intense band above 2000 cm^{-1} including several shoulders (with a main maximum at 3300 cm^{-1}), is characteristic of the representatives of the vancomycin group of antibiotics, and can be attributed to the presence of phenolic and polyhydroxyl structural elements and amino acid moieties.

Mild acid hydrolysis of the antibiotics gave simple sugars (D-glucose, D-mannose, D-arabinose, L-rhamnose and L-fucose) and biologically active aglycones (see Table II). It is to be noted that while neutral sugars are frequently occurring structural elements of natural compounds, they are relatively rare as constituents of antibiotics, either in simple or complex form. The new amino sugar acosamine (3-amino-2,3,6-trideoxy-L-*arabino*-hexopyranose), actinosamine (4-O-methyl--acosamine), ristosamine (3-amino-2,3,6-trideoxy-L-*ribo*-hexopyranose) and vancosamine (3-amino-3-C-methyl-2,3,6-trideoxy-L-*lyxo*-hexopyranose) were isolated for the first time from these antibiotics [35, 44, 47]. Most recent studies have shown that antibiotic A-35512 B contains 3-amino-3-C-methyl-2,3,6-trideoxy-L-*xylo*-hexopyranose [55a, 58, 61]. All the above trideoxy-aminohexoses belong to the L-series of sugars, and only vancosamine and its C-3 epimer isolated from antibiotic A-35512 B are branched-chain amino sugars. These results are also remarkable from the point of view of the important recent research work on deoxy amino sugars, which has opened up new prospects in the investigation of other biologically active compounds containing carbohydrates (immunopolysaccharides, amino- and anthracycline glycoside antibiotics). For example the synthesis and clinical application of the deoxy analogues of aminoglycoside antibiotics led to significant new results, both theoretical and practical, in the treatment of diseases caused by resistant bacteria [59, 60].

The aglycones, obtained on removal of the carbohydrates, retained — in part — the biological activity of the parent antibiotics, indicating that the structural units, responsible for the biological effect, are included primarily in the aglycones. The microbiological investigation of the acid hydrolysate of ristocetin A and B led to particularly surprising results. Both in animal experiments and *in vitro* studies using the serial dilution method Philip *et al.* [25] found the hydrolysates of ristocetin A

Table II

Structural units of the members of vancomycin-type antibiotics

Antibiotic	Carbohydrates		Amino acids	References
	Neutral	Deoxy-amino		
Vancomycin	D-glucose	L-vancosamine	L-aspartic acid N-methyl-D-leucine Actinoidinic acid Vancomycinic acid	4, 5 17, 24, 35 37–39
Avoparcin α	D-glucose D-mannose L-rhamnose	L-ristosamine	D-α-amino-4-hydroxyphenylacetic acid Actinoidinic acid Monodechlorovancomycinic acid	40, 21
Avoparcin β	D-glucose D-mannose L-rhamnose	L-ristosamine	D-α-amino-4-hydroxyphenylacetic acid D-α-amino-3-chloro-4-hydroxyphenylacetic acid Actinoidinic acid Monodechlorovancomycinic acid	40, 21
Actinoidin A	D-glucose D-mannose	L-acosamine L-actinosamine	D-phenylalanine D-α-amino-4-hydroxyphenylacetic acid Actinoidinic acid Monodechlorovancomycinic acid	4, 5, 22 33, 41–45
Actinoidin B	D-glucose D-mannose	L-acosamine L-actinosamine	D-phenylalanine D-α-amino-3-chloro-4-hydroxyphenylacetic acid Actinoidinic acid Monodechlorovancomycinic acid	4, 5, 22, 33, 41–45
Ristocetin A (Ristomycin A)	D-glucose D-mannose D-arabinose L-rhamnose	L-ristosamine	Actinoidinic acid Ristomycinic acid Didechlorovancomycinic acid	4, 5, 26, 27 46–53
Ristocetin B (Ristomycin B)	D-glucose D-mannose L-rhamnose	L-ristosamine	Actinoidinic acid Ristomycinic acid Didechlorovancomycinic acid	4, 5, 26, 27 46–53
A-35512 B	D-glucose D-mannose L-rhamnose L-fucose	3-amino-2,3,6-trideoxy-3-C-methyl-L-*xylo*-hexopyranose (C-3 epimer of L-vancosamine)	Glycine and four unknown amino acids; one of the latter is an analogue of ristomycinic acid: 6,3′-diglycyl-3-chloro-5,6′-dihydroxydiphenyl ether	54, 55, 55a, 61

and ristocetin B five and four times, respectively, as active as the parent antibiotics. This observation, however, has not been satisfactorily explained even in the knowledge of the structure of the antibiotics, though Kuwahara and Chambers [62] were able to confirm these results.

The aglycone of each representative of these antibiotics has heptapeptide-type structure. Besides the usual amino acids and their derivatives the peptide chain is built from hitherto unknown aromatic amino acids of phenolic character (Table II). Of the known amino acids, L-aspartic acid and N-methyl-D-leucin are present in vancomycin, and L-phenylalanine has been isolated from the actinoidin variants. On the other hand, D-α-amino-4-hydroxyphenylacetic acid — the amino acid of actinoidin A and avoparcin α —, and also, D-α-amino-3-chloro-4-hydroxyphenylacetic acid, present in actinoidin B and avoparcin β, were isolated for the first time from these antibiotics [40, 42].

As shown in Table II actinoidinic acid [45] is a common building unit occurring in each vancomycin-type antibiotic. Similarly, either vancomycinic acid [39] or its dechloro analogues [21, 27, 43, 50] are essential elements of the structure in each representative, whereas ristomycinic acid [48, 49] was isolated exclusively from the variants of ristomycin [48–51] and ristocetin [26–28]. The new aromatic diaminodicarboxylic acids, named after actinoidin and ristomycin, are built from C-phenylglycine derivatives attached to each other with diphenyl [21, 27, 38, 50, 51] or diphenyl ether linkages [27, 48–51].

In the molecule of vancomycinic acid having triamino-tricarboxylic acid structure, the two phenylserine units are symmetrically linked to the central *p*-hydroxyphenylglycine moiety through diphenyl ether bonds [21, 27, 39, 43, 50]. An additional characteristic of these antibiotics — with the exception of ristocetin (ristomycin) — is that the aromatic ring of the C-phenylglycins and vancomycinic acid contains chlorine substituents.

Recently, a chlorinated amino acid, similar to ristomycinic acid, has been found in the molecule of antibiotic A-35512 B [54, 55, 55a, 58].

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3. VANCOMYCIN

3.1. ISOLATION

Vancomycin was first isolated by McCormick and co-workers [1] from a submerged culture of *Streptomyces orientalis* n. sp. and characterized as an amorphous product. Later antibiotic K-288, isolated from a culture of *Streptomyces haranomachiensis* by Matsumoto [2], was shown to be identical with vancomycin. After pre-treatment of the ferment liquor, the antibiotic was adsorbed on a Permutit DR (OH^-) or Amberlite IRC-50 ion-exchange resin column, then eluted with 30% aqueous acetone or ethanol containing 1% of acetic acid, and precipitated in the form of the required salt with a mineral or organic acid. Vancomycin can also be precipitated with Orange II, Methyl Orange, or with *p*-(*p'*-hydroxyphenyl)azobenzenesulfonic acid; however, recovery of the antibiotic from these complexes is fairly tedious [1].

The crystallization of vancomycin was reported by Higgins and co-workers [3]. Vancomycin sulfate was crystallized from aqueous methanol containing 10% urea, whereas crystallization of the vancomycin free base was achieved from aqueous solution containing 25% dimethyl sulfoxide or acetamide. These authors found the slightly cross-linked Dowex-50 (H^+ or Na^+) resin preferable for the purification of the antibiotic.

Later, vancomycin preparations, previously believed to be homogeneous, were separated into three components (CM-1, CM-2 and CM-3 vancomycin) on CM-Sephadex C-50 column by Best *et al.* [4]. The biological activities of these variants differed significantly: CM-3 was fourteen times as active as CM-1 and twice as active as CM-2. By an improved purification process, commercial vancomycin was separated into four components by Nieto and Perkins [5]. However, the investigations of the latter two research groups were centred primarily on the mechanism of action of the antibiotic and not on its purification or structure elucidation.

3.2. BIOLOGICAL ACTIVITY, APPLICATION

Vancomycin is a narrow spectrum bactericidal antibiotic introduced in medical practice in the years 1956–58 [6]. However, after the discovery and general use of semisynthetic penicillins and cephalosporins, its therapeutic importance has decreased to find application only for the treatment of infections when β-lactam antibiotics are ineffective. Owing to the emergence of significant resistance and the sensitivity of patients to penicillins, it is well possible that therapy with vancomycin — in combination with various aminoglycoside antibiotics — will come into prominence again in the future [7].

Vancomycin is particularly active against Gram-positive *Staphylococcus* and *Streptococcus* strains and some spirochetes [1], and also effective against *Neisseria*-type Gram-negative cocci. The value of its effectivity is almost equal to that of penicillin. At the same time, most of the Gram-negative bacteria, *Mycobacterium tuberculosis* and fungi are insensitive to vancomycin. Cross-resistance between vancomycin and other antibiotics has not been reported.

The most important clinical indications of vancomycin include the treatment of acute staphylococcal infections [9], cases of endocarditis caused by streptococci or other microorganisms, and also when the administration of penicillins or cephalosporins is contraindicated. In such cases vancomycin is given in combinations with aminoglycoside antibiotics (streptomycin, gentamycin).

The antibiotic activity of vancomycin is subject to slight variation between pH 6.5–8.0 [6, 8].

Vancomycin is poorly absorbed from the gastrointestinal tract. Since intramuscular injection causes moderate pain, in clinical practice the antibiotic is administered intravenously in cases of systemic infection. Vancomycin is relatively non-toxic; neurotoxicity is mentioned as its most important reported side effect.

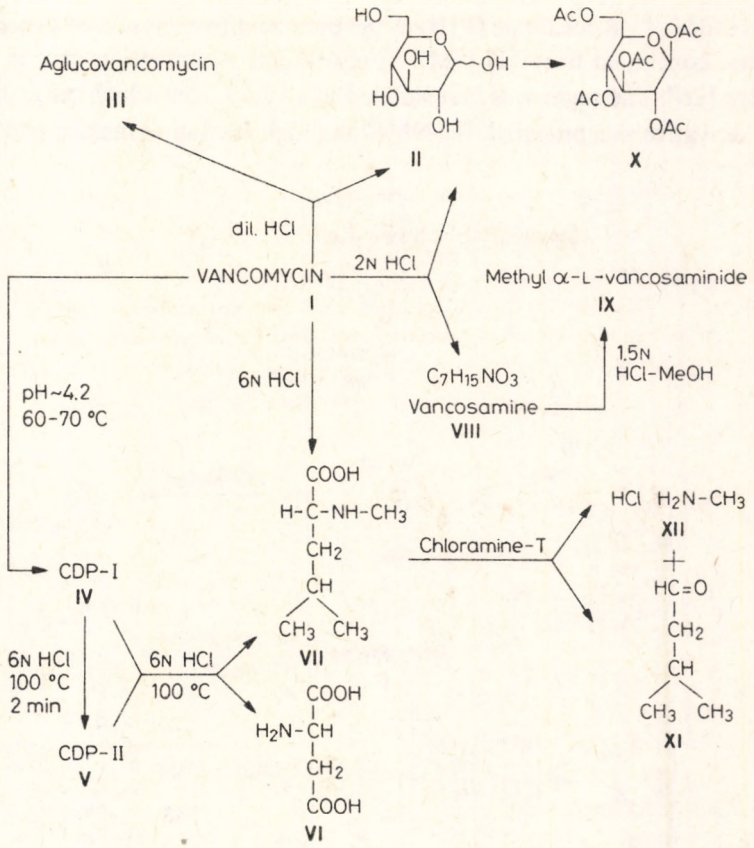
3.3. STRUCTURAL STUDIES

3.3.1. HYDROLYSIS

Chemical characterization of vancomycin has been given as early as in the first publications on its structural studies, however, an incorrect molecular weight (about 3000) was assumed [1, 3]. At that time the antibiotic was probably inhomogeneous, since — contrary to later reports — alanine was detected as one of the hydrolysis products [10]. Later Soviet authors [4a] reported a molecular weight of about 1600, estimated on the basis of potentiometric titration, and this value was corrected to 1700–1800 by Nieto and Perkins [5]. Mild acid hydrolysis of vancomycin (**I**; Scheme 1) resulted in D-glucose (**II**) and the aglycone of vancomycin (**III**) which retained 75% of the original antibiotic activity.

Compound **II** was identified in the form of penta-O-acetyl-β-D-glucose (**X**) by Higgins *et al.* [3].

Stability and hydrolysis studies on vancomycin led to the isolation of two crystalline degradation products (**IV–V**) [11], and these were of considerable help in the further structural studies. On short treatment with 0.6 N hydrochloric acid compound **IV** smoothly transformed into **V**, which — similarly to the antibiotic itself — split into smaller structural units under more drastic acidic conditions. Of the ninhydrine-positive components L-aspartic acid (**VI**) and N-methyl-D-leucine (**VII**) were first isolated. As **VI** is a frequently occurring natural compound, it was readily identified. The reaction of **VII** with chloramine T yielded isovaleraldehyde (**XI**) and methylamine (**XII**). The D-configuration of **VII** was proved on the basis of its negative specific optical rotation. In the beginning of the 1970's the structural investigation of vancomycin excited the interest also of research groups in Cambridge and Brighton. Weringa *et al.* [26] isolated D-glucose and a new



Scheme 1

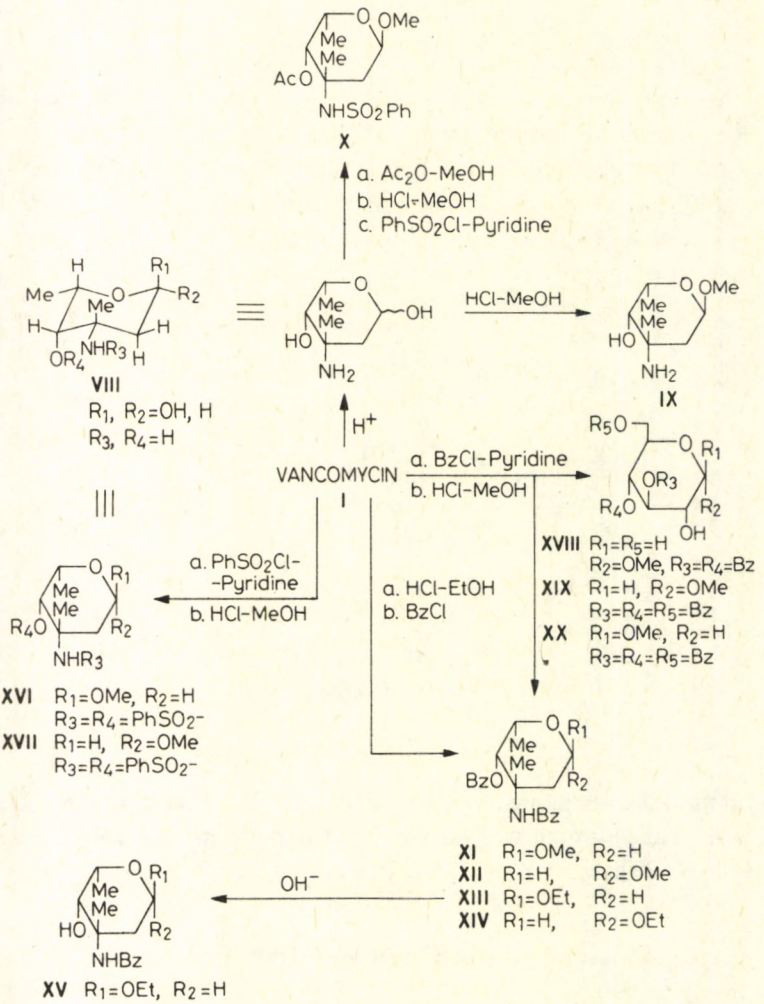
deoxyamino hexose, named vancosamine (**VIII**) from the acid hydrolysate of the antibiotic. Vancosamine was also isolated and studied by Johnson and his co-workers [14, 20].

3.3.2. CARBOHYDRATES

As it has been mentioned, the presence of D-glucose in the molecule of vancomycin was demonstrated by researchers at Eli Lilly Co. during their early studies [3] immediately following the discovery of the antibiotic.

The isolation and identification of the structure of the novel branched-chain trideoxy amino sugar, vancosamine, resulted from the ingenious work of English chemists.

The presence of vancosamine (**VIII**) in the mild acid hydrolysate of vancomycin (**I**) was first concluded from ^{1}H-NMR spectroscopic examination carried out in Cambridge [26]. The sugar was first isolated as a syrup from which the crystalline triacetyl derivative was prepared. ^{1}H-NMR and high resolution mass spectroscopic



Scheme 2

investigation then proved that the chemical structure of vancosamine — having the molecular formula $C_7H_{15}NO_3$ — was 3-amino-3-C-methyl-2,3,6-trideoxy-L-*lyxo*-hexopyranose. It was also unequivocally established that 3-methyl-4-oxohexane-

carboxylic acid* was not a building unit of the antibiotic, but a secondary product formed from vancosamine. However, these authors did not report if vancosamine belonged to the D- or L-sugar series.

In parallel studies Smith *et al.* [14] isolated a crude mixture of unknown amino compounds from the 2 N hydrochloric acid hydrolysate of vancomycin (**I**) by ion-exchange chromatography on Amberlite IR-120 (H^+) resin. Treatment of this mixture with methanolic hydrochloric acid, followed by thin layer chromatography led to the isolation of methylvancosaminide (**IX**).

The negative absorption at about 600 nm in the CD spectrum of **IX** clearly demonstrated a negative chirality between the *cis*-oriented hydroxyl and amino groups, and hence the L-configuration of the amino sugar [15]. The *cis* relation of the above groups in **VIII** was also proved by the N→O acyl migration reaction, first observed by Fodor and Ötvös [16]. Thus, acetylation of **VIII** with acetic anhydride in methanol, followed by methanolysis and subsequent treatment with benzenesulfonyl chloride in pyridine gave methyl N-benzenesulfonyl-O-acetyl-α L-vancosaminide (**X**). This result could be explained by the migration of the N-acetyl group, formed during acetylation, to the adjacent *cis*-oriented hydroxyl group under the conditions of the methanolysis step, allowing the liberated amino group to react with benzenesulfonyl chloride in the next step. Benzoylation of vancomycin (**I**) followed by methanolysis resulted in methyl N,O-dibenzoyl-α- and β-L-vancosaminide (**XI** and **XII**), and the ^{1}H-NMR spectrum of **XI** showed the *cis-diaxial* orientation of H-5 and the C-3 methyl group. Acid hydrolysis of vancomycin, followed by evaporation of the crude sugar fraction with added ethanol and benzoylation of the product yielded a mixture of the corresponding α- and β-ethyl glycosides (**XIII** and **XIV**). Partial alkaline hydrolysis of **XIII** led to the N-benzoyl analogue **XV**. Similarly crystalline methyl N,O-dibenzenesulfonyl-α- and β-vancosaminide were prepared by acylation of **I** with benzenesulfonyl chloride and subsequent methanolysis.

The optical rotation of each N,O-diacyl-α-glycoside was more negative than that of the corresponding β-isomer. This observation, and also the application of Hudson's isorotation rules confirmed the assignment of the L-configuration to vancosamine.

The mass spectra of all vancosamine derivatives had prominent peaks assignable to the ions involving the C-1, C-2 and C-3 atoms of the original compound. The interpretation of the ^{1}H-NMR spectra of the α- and β-N,O-diacyl glycosides was not difficult using the known literature data of methyl N,O-diacetyl-α-L-daunosaminide [17] and 1,4-di-O-acetyl-β-L-arcanose [18].

* According to Marshall [11] this compound was first incorrectly named vancomycinic acid.

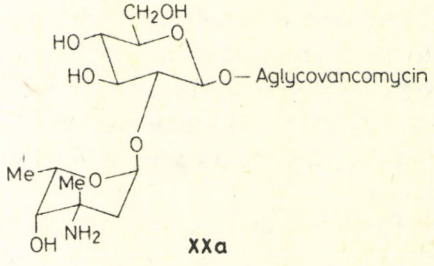
The above results and ^{13}C-NMR studies with compounds **VIII** and **XVI** [19] confirmed the 3-amino-3-C-methyl-2,3,6-trideoxy-L-*lyxo*-hexopyranose structure and $^1C_4$ conformation of vancosamine.

Besides compounds **XI** and **XII** Johnson *et al.* [20] isolated methyl 3,4-di-O-benzoyl-α-D-glucoside (**XVIII**) and also the α- and β-anomers of methyl 3,4,6-tri-O-benzoyl-D-glucoside (**XIX** and **XX**) from vancomycin (**I**). The formation of these derivatives (**XVIII–XX**) suggests that the vancosamine moiety is linked to the aglycone probably through the C-2 hydroxyl group of D-glucose. However, the assumption of these authors according to which another labile group may be attached to the C-6 hydroxyl group of D-glucose, proved to be incorrect.

Later Roberts *et al.* [25] confirmed the presence of the 2-O-L-vancosaminyl-D-glucosyl moiety in the molecule of vancomycin by two different methods.

Vancomycin was first permethylated using Hakomori's method and then subjected to methanolysis. The resulting partially methylated products were then deuteromethylated; the mass spectral investigations demonstrated the formation of 2-O-$^2H_3$-permethyl-D-glucose.

The other method involved the reduction of the partially methylated glucose with sodium borohydride and subsequent acetylation to give 1,2,5-tri-O-acetyl-3,4,6-tri-O-methyl-D-glucitol, which was identified by its mass spectrum. These experiments unequivocally prove that vancosamine is linked to the C-2 position of D-glucose in vancomycin, since the molecule does not contain any other reducing sugar.

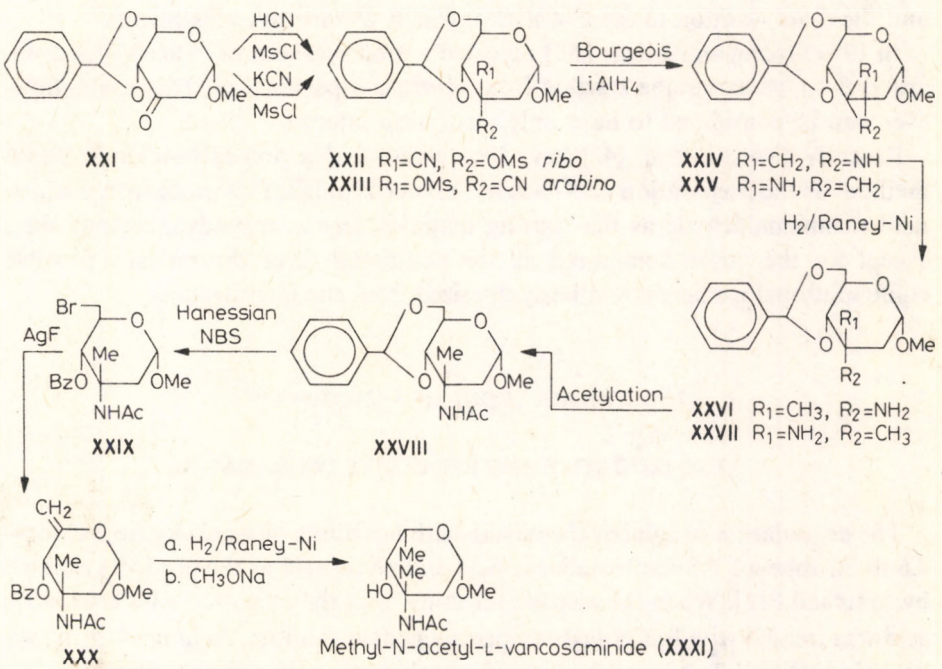


XXa

These studies, however, gave no information about the configuration of the glycosidic linkages between D-glucose and the aglycone, and between the two monosaccharides. This problem was finally solved by Williams and Kalman [35]. ^{1}H-NMR investigations at 270 MHz established that the above disaccharide was present in **XXa** in the form of a 2-O-α-L-vancosaminyl-β-D-glucosyl unit.

3.3.2.1. DEFINITIVE SYNTHESIS OF VANCOSAMINE

Besides the generation of the C-2 and C-6 deoxy functions, one of the key steps in the synthesis of vancosamine is the introduction of the *axial* methyl and *equatorial* amino group to the C-3 position. Although the preparation of hexoses having such C-3 branch is, in principle, possible by the Baer–Fischer dialdehyde–nitromethane cyclization method, the separation and identification of the resulting theoretically expected eight stereoisomers would present great difficulty [21]. A recent method, elaborated by Bourgeois [22] and involving the generation and reduction of spiro–aziridine derivatives, has significantly improved the synthesis of branched-chain amino sugars of the above type. This procedure was applied by Lukács *et al.* [23] for the first synthesis of vancosamine derivatives (Scheme 3).



Scheme 3

Addition of hydrogen cyanide to the starting methyl 4,6-O-benzylidene-2-deoxy-α-D-*erythro*-hexopyranoside-3-ulose (**XXI**) under kinetic control, followed by mesylation afforded **XXII** with *ribo* configuration. Treatment of this latter compound with lithium aluminium hydride resulted in the spiro–aziridine derivative **XXIV** the catalytic reduction of which, performed under pressure, gave

the amine **XXVI**. The steric positions of the substituents at the quaternary centre of **XXVI** were determined by comparative ^{13}C-NMR examination of the model compounds **XXV** and **XXVII** and also **XXIII** — obtained by addition of potassium cyanide to **XXI**. Acetylation of **XXVI** gave **XXVIII** which was treated with N-bromosuccinimide according to the Hanessian procedure to obtain methyl 3-acetamido-4-O-benzoyl-6-bromo-2,3,6-trideoxy-3-C-methyl-α-D-*ribo*-hexopyranoside (**XXIX**).

The desired L-*lyxo* analogue, methyl N-acetyl-β-L-vancosaminide (**XXXI**), was obtained by inversion of the configuration at C-5 involving dehydrobromination of **XXIX** with silver fluoride [24] followed by catalytic hydrogenation of the resulting exo-methylene derivative (**XXX**) in the presence of Raney nickel and subsequent O-debenzoylation. The ^{13}C-NMR spectroscopic investigation of **XXXI** unambiguously demonstrated the *equatorial* orientation of the anomeric methoxyl group and the *axial* position of the C-4-hydroxyl and C-3-methyl substituents.

In 1979 Dyong and Friege [36] reported a total synthesis of N-acetyl-1,4-di-O-acetyl-β-D,L-vancosamine starting from *cis-trans*-3-penten-2-ol. This route, however, can be considered to have only theoretical interest.

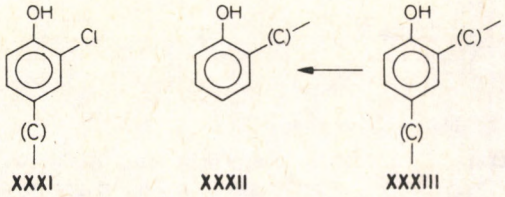
Recently Fronza *et al.* [42] have also elaborated a non-carbohydrate based method for the preparation of N-benzoyl-L-vancosamine. This procedure, using α-methylcinnamaldehyde as the starting material, seems very advantageous since except for the L-*ribo* compound all the additional three theoretically possible configurational isomers could be synthesized from the intermediates.

3.3.3. THE AGLYCONE OF VANCOMYCIN

3.3.3.1. OXIDATIVE AND REDUCTIVE DEGRADATION

The degradation of aglucovancomycin with hot dilute nitric acid gave 2-chloro-4,6-dinitrophenol, 3,5-dinitrosalicylic acid and picric acid as the products isolated by Marshall [11]. When the mixture resulting from the oxidation with cold nitric acid was treated with hot potassium permanganate solution, 3-chloro-4-hydroxybenzoic acid and 3-chloro-4-hydroxy-5-nitrobenzoic acid were obtained.

The oxidation of vancomycin with potassium permanganate followed by methylation with diazomethane yielded dimethyl 4-hydroxyisophthalate [13]. These results led to the assumption that 3-chloro-4-hydroxybenzoic acid and 3,5-dinitrosalicylic acid originated from the structural units **XXXI** and **XXXII**, respectively, of the aglycone moiety.



XXXI XXXII XXXIII

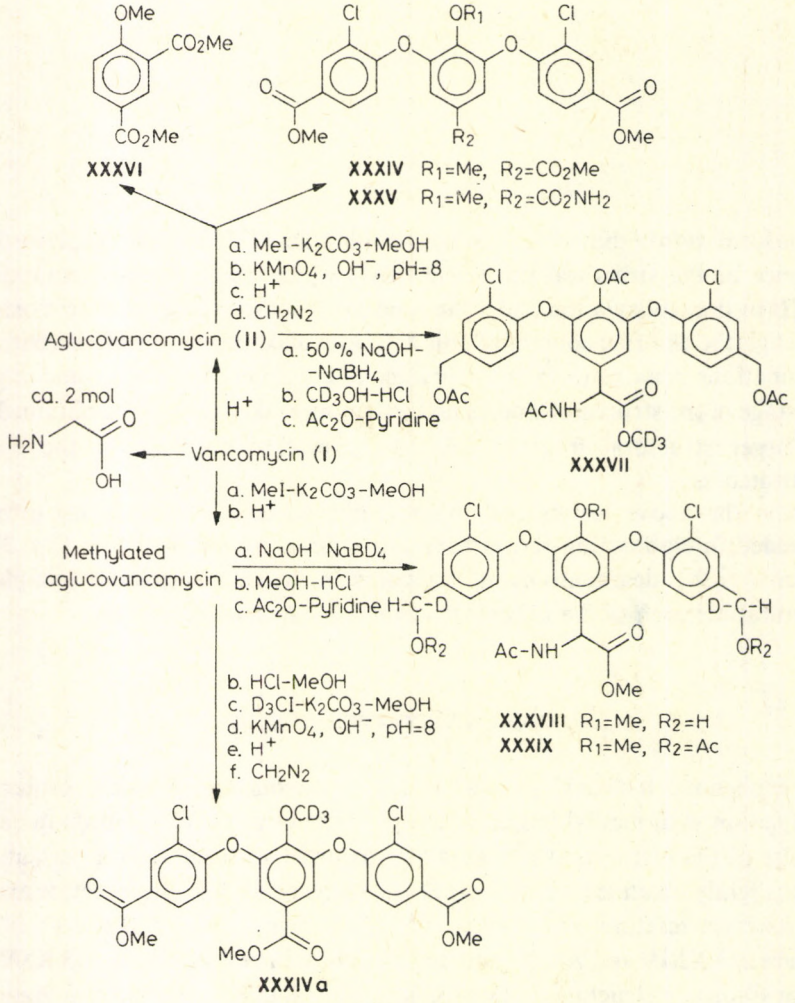
The formation of dimethyl 4-hydroxyisophthalate (**XXXVI**) was explained by the presence of the structural portion **XXXIII** which could easily decompose into **XXXII** by decarboxylation under the conditions of the drastic nitric acid oxidation. Although the chlorine content of unit **XXXI** justified the results of microanalytical examinations demonstrating the presence of covalent chlorine in vancomycin, at that stage of the structural studies no further information could be obtained about the larger structural fragment of the antibiotic incorporating the chlorine-substituted unit.

Using the above results and other published data, the Williams group has succeeded in elaborating satisfactory procedures for the oxidative [25, 27] and reductive [28] degradations of vancomycin which remarkably simplify the structural research of the aglycones of the vancomycin antibiotics.

3.3.3.2. VANCOMYCINIC ACID

The phenolic hydroxyl groups of aglucovancomycin (**II**) were protected by methylation with methyl iodide in the presence of potassium carbonate, and the mixture of the methylated products was oxidized with potassium permanganate under slightly alkaline conditions (pH ~ 8) (Scheme 4). The ethyl acetate extract of this reaction mixture was further methylated with diazomethane to obtain a mixture of **XXXIV** and **XXXV** and dimethyl 4-methoxyisophthalate (**XXXVI**). The composition and structure of the products were established by microanalytical and spectroscopic investigations. This reaction sequence proved to be useful also for the determination of the exact place of attachment of the sugars to the aglycone moiety [25, 27]. In this case the antibiotic was first methylated, the sugars were liberated by methanolysis and the partially methylated aglycone was deuteromethylated. The product was oxidized with potassium permanganate followed by methylation with diazomethane.

This reaction sequence yielded the deuteromethylated **XXXIVa** having the same structure as **XXXIV**. Since the deuteromethyl group had substituted the hydroxyl group carrying the disaccharide moiety of the parent antibiotic, it was unambiguously established that the 2-O-α-vancosaminyl-β-D-glucosyl unit was attached to the central pyrogallol ring of **XXXIV** in the molecule of vancomycin.



Scheme 4

The earlier studies of Johnson *et al.* [10] revealed that the alkaline hydrolysis of vancomycin or its crystalline degradation products afforded about two molecular equivalents of glycine, although this amino acid could not be detected upon acid-catalyzed hydrolysis of the antibiotic.

To avoid the undesired oxidation of the phenolic hydroxyl groups, the alkaline hydrolysis of vancomycin was performed under reductive conditions by Smith *et al.* [28]. Such reactions have been widely used in the structural investigation of glycopeptides and proteins containing carbohydrate–serine or –threonine linkages [29].

Accordingly aglucovancomycin (**II**) was hydrolyzed in concentrated sodium hydroxide solution in the presence of sodium borohydride. Chromatography of the crude product gave 3-chloro-4-hydroxybenzyl alcohol and 3-chloro-4-hydroxybenzaldehyde, although no aldehyde proton could be detected in the ^{1}H-NMR spectra of **II** and its derivatives.

When the above alkaline hydrolysis was carried out in the presence of sodium borodeuteride, the corresponding deuterated benzyl alcohol was detected, whereas the isolated benzaldehyde did not contain deuterium. These experiments provide strong evidence that glycine and 3-chloro-4-hydroxybenzaldehyde are liberated simultaneously and the benzyl alcohol must be produced by the reduction of the aldehyde.

The above reaction, performed in the presence of sodium borohydride, gave a more polar product also, which was esterified with deuteromethanol and then acetylated to give **XXXVII**, in which two molecules of 3-chloro-4-hydroxybenzyl alcohol were linked to the central ring *via* ether bonds; therefore, the appearance of this alcohol in the reaction mixture was not surprising.

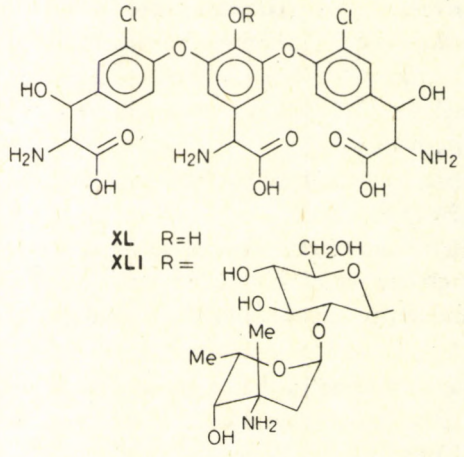
The following reactions were accomplished to provide evidence for the presence of the phenylglycine unit.

In parallel experiments, the methylated aglucovancomycin was subjected to alkaline hydrolysis in the presence of a large excess of sodium borohydride and a large excess of sodium borodeuteride in order to avoid the aldehyde production and prevent the oxidation of the sensitive pyrogallol ring. The crude product was then esterified with methanol–hydrochloric acid, and compound **XXXVIII** was isolated as the major product by chromatography. Acetylation of the latter gave **XXXIX** indicating the presence of three acetylable groups. As expected, two deuterium atoms were found to be incorporated into **XXXIX** on reduction with sodium borodeuteride.

The formation of compounds **XXXVII–XXXIX** served as indirect evidence for the presence of the novel aromatic triamino–tricarboxylic acid (**XL**, named vancomycinic acid*) in the molecule of vancomycin. The isolation of **XL** remained unsuccessful, since the two β-phenylserine moieties, linked to the central phenylglycine unit, underwent retro–aldol cleavage under alkaline conditions, resulting in two molecular equivalents of glycine and the aldehyde. The aldehyde group of the latter benzaldehyde derivative was then reduced into the corresponding benzyl alcohol on the action of the large excess of the reducing agent.

According to the results described above, vancomycinic acid (**XL**) is present in vancomycin in the glycosylated form **XLI**, as shown in Scheme 5.

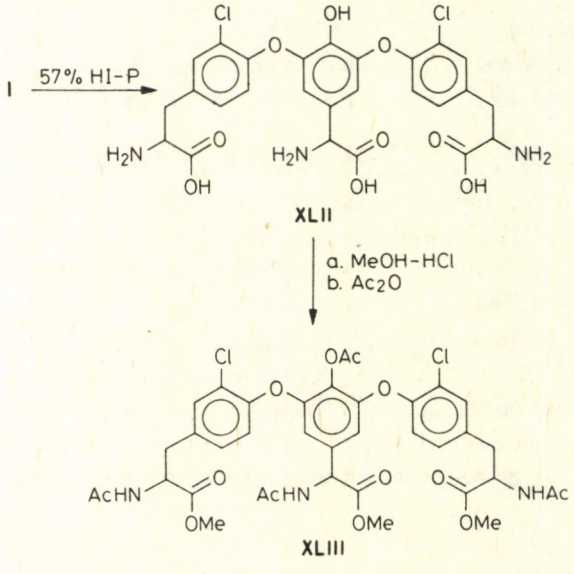
* The name vancomycinic acid was recently suggested for **XL** by Harris *et al.* [30].



Scheme 5

Recently Trifonova *et al.* [31] have found direct evidence for the presence of the extremely acid- and alkali-sensitive vancomycinic acid **(XL)** through the isolation of dideoxyvancomycinic acid **(XLII**, Scheme 6).

Vancomycin was treated with hydrogen iodide and red phosphorus to afford bis-*m,m'*-O-(*m*-cholorophenylalanyl)-*p*-hydroxyphenylglycine **(XLII)** *via* the elimina-

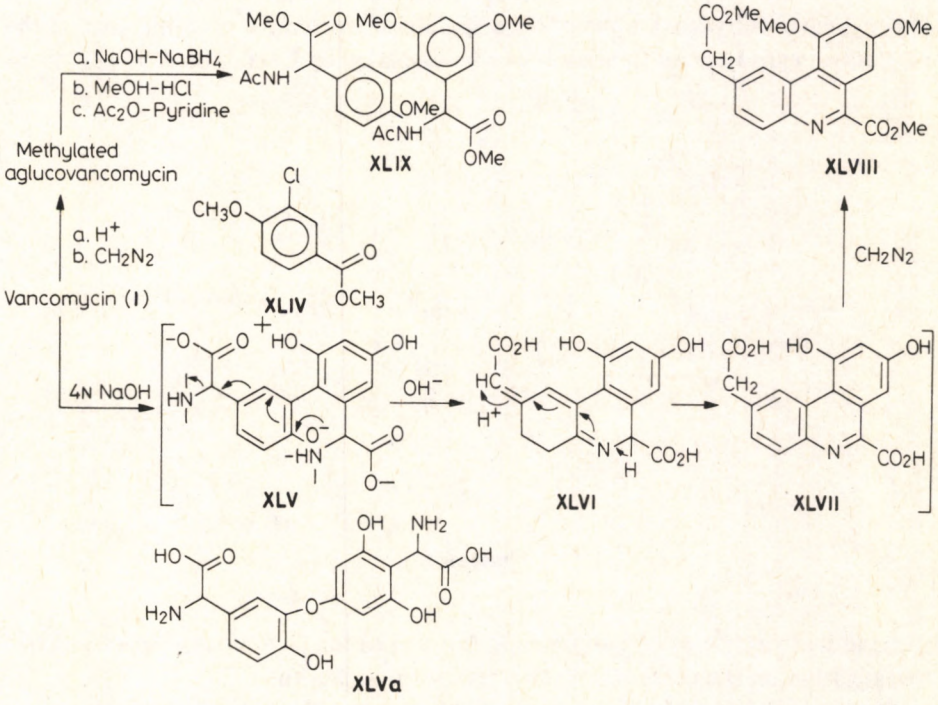


Scheme 6

tion of the β-hydroxyl groups of the serine unit. The IR and mass spectral analyses of the tetraacetyl-trimethyl ester **XLIII** — obtained from **XLII** — unequivocally proved the proposed structure.

3.3.3.3. ACTINOIDINIC ACID

The ^{1}H-NMR spectrum of methylated aglucovancomycin, obtained by treatment with diazomethane, showed the presence of five methyl groups. One of these was assigned to the phenolic hydroxyl group liberated after the removal of the disaccharide moiety, and the second belonged to the methoxycarbonyl group. The remaining three phenolic hydroxyl groups must be included in a novel unknown aromatic structural unit **XLV** (Scheme 7).

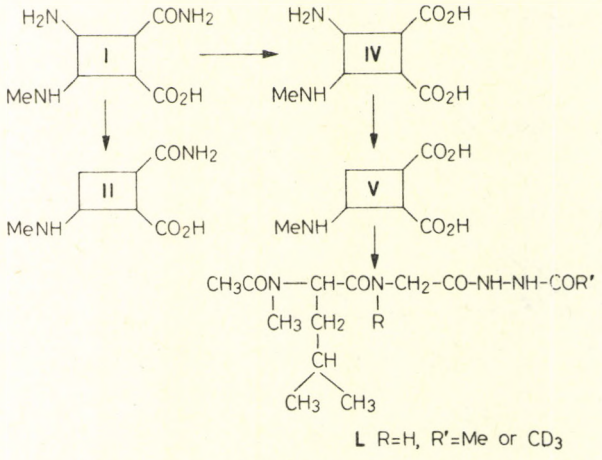


Scheme 7

Methylation of the product, obtained by hydrolysis of vancomycin **(I)** with 4 N sodium hydroxide, with diazomethane afforded methyl 3-chloro-4-methoxybenzoate **(XLIV)** and a novel unknown product with composition $C_{20}H_{19}NO_6$. The formation of **XLIV** was in good accordance with the structure of vancomycinic

acid (**XL**) suggested on the basis of oxidative and reductive degradations. The ^{1}H-NMR spectrum of the other product indicated the presence of 19 protons, a 1,2,3,5-tetrasubstituted- and a 1,2,4-trisubstituted aromatic ring, and it was concluded that dimethyl 4-methoxyisophthalate (**XXXVI**) — obtained from the antibiotic (**I**) by oxidation — originated from the degradation of this latter trisubstituted ring. Further spectroscopic studies have demonstrated that the structure of the unknown product corresponds to the phenanthridine derivative **XLVII**, containing the two hydroxyl groups of the tetrasubstituted ring in *meta* position. However, fragment **XLVII** is not present in the molecule of vancomycin but it is formed by cyclization during the alkaline hydrolysis *via* the sequence **XLV→XLVI→XLVII.**

Later this assumption was proved by the reductive alkaline hydrolysis of the methylated aglucovancomycin, which resulted in **XLIX**, the di-N-acetyldimethyl ester of the new amino acid **XLV**, named actinoidinic acid. Actinoidinic acid (2,3′-diglycyl-4,6,6′-trihydroxydiphenyl) is a structural element of each member of the vancomycin group of antibiotics. It was first isolated by Lomakina *et al.* [32] from



Scheme 8

the acid hydrolysate of actinoidin A, B, but due to the lack of mass spectrometric investigation its structure was incorrectly given as **XLVa**.

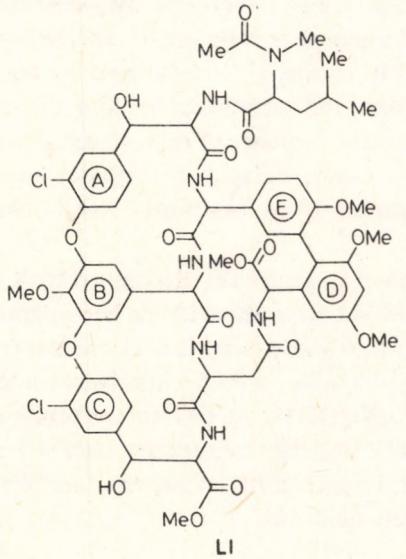
The investigations discussed above led to the identification of all the structural units present in the skeleton of vancomycin, and also the positions of the alcoholic and phenolic hydroxyl groups attached to this skeleton were established. Moreover, these studies also demonstrated that vancomycin (**I**) contained one of each primary amino, secondary amino, carboxyl and carboxamido group (Scheme 8). Aglucovancomycin (**II**) prepared by removal of the carbohydrate functions, contained

no primary amino group, indicating the presence of this group in the vancosamine unit.

At the same time, the secondary amino group of the antibiotic could be detected in **II** as well as in its crystalline degradation products **IV** and **V**, and was therefore assigned to the N-methyl-D-leucine unit. Direct evidence to support this assumption was obtained by the mass spectral analysis of the N-acetylhydrazide of N-acetyl-N-methylleucylglycine, isolated from the mild hydrazinolysis of **V** [33]. This result also demonstrated that the carboxyl group of N-methyl-D-leucine is involved in a peptide bond with the α-amino group of one of the β-hydroxytyrosine units of vancomycinic acid, from which the glycine part of dipeptide **L** is formed by secondary cleavage, as discussed above.

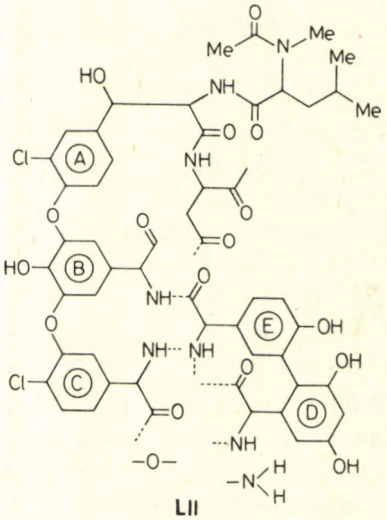
Nieto and Perkins [5] observed that the electrometric titration of the free carboxyl group of vancomycin is accompanied by an increase in the UV extinction coefficient at 294 nm. Since this 294 nm band is appropriate to the biphenyl ring system, it has been assumed that this carboxyl group must be near the actinoidinic acid moiety in the molecule of the antibiotic.

It was shown that while vancomycin contained only a single carboxyl group, two carboxyl functions were detected in its degradation products **IV** and **V**. Earlier Johnson [10] supposed that the transformation **I**→**IV** was accompanied by the loss of one molecular equivalent of ammonia, as a result of the transformation of asparagine — present in vancomycin — into aspartic acid. At the same time the Williams group [27] first concluded that each functional group of L-aspartic acid in **V** was involved in amide linkages. The structure of the hexa-O-methyl-N-acetyl derivative of the latter was given as **LI** on the basis of ^{13}C- and ^{1}H-NMR



LI

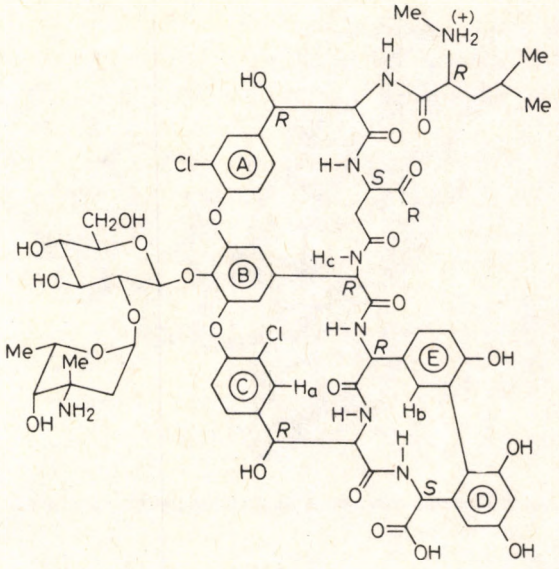
spectroscopic examinations and several pieces of indirect chemical evidence. These results were later corrected on the basis of ^{1}H-NMR spectral data obtained at 270 MHz, and the positions of the L-aspartic acid and actinoidinic acid units were exchanged as shown in structure **LII** [34, 35]. However, the exact structure of the peptide bonds of the bis-glycyl residue of actinoidinic acid still remained unknown at this stage of the structural research.



LII

The presence of the secondary hydroxyl groups of the two β-hydroxytyrosine units in vancomycinic acid was established by spectroscopic data and also by dinitrobenzoylation. The complete structure of vancomycin was finally determined by Sheldrick *et al.* [37] by means of X-ray analysis of the crystalline degradation product **IV** formed from the antibiotic by loss of ammonia. These studies unequivocally confirmed the presence of each structural unit of the antibiotic (D-glucose, L-vancosamine, L-aspartic acid, N-methyl-D-leucine, vancomycinic acid, actinoidinic acid) deduced from chemical, NMR- and mass spectrometric investigations.

However, comparison of structure **IV**, determined by X-ray measurements, with that of **XLI** clearly showed that in the tricyclic heptapeptide unit of the latter the sequence of actinoidinic acid and L-aspartic acid was incorrectly given. Namely, the structure **XLI**, supposed earlier, would entirely exclude the possibility of the experimentally established $CONH_2 \rightarrow COOH$ transformation during the hydrolysis of vancomycin. Consequently, the L-asparagine unit of **I** is replaced by L-aspartic acid in structure **IV**. In respect of the other structural features the two supposed structures are completely identical.



I Vancomycin R=NH_2
IV Crystalline degradation product R=OH

The free carboxyl group of vancomycin belongs to the tetrasubstituted phenylglycine unit of actinoidinic acid. It is an interesting feature that the structure also contains a relatively rare *cis* amide bond.

The three-dimensional actual structure of the antibiotic (Fig. 1) is extremely compact, carrying a cleft on the side of the molecule bearing the chlorine atoms and also a less well-defined indention on the other side [37].

The 33 water molecules are linked in the extended crystal structure by a complex system of hydrogen bonds, but there is no space for any water molecules inside the tricyclic ring system. There are only five hydrogen bonds in **IV** which do not involve water. Thus, there are hydrogen bonds between the carboxyl group of L-aspartic acid and the hydroxyl group of the trisubstituted ring of actinoidinic acid; the NH_3^+ group of N-methyl-D-leucine and the carboxylate of actinoidinic acid; between the C-4-hydroxyl group of D-glucose and the NH of the glycine part of vancomycinic acid, and also between the C-4-hydroxyl group of vancosamine and the carboxylate and NH of L-aspartic acid. The remaining hydrogen bonds are between the molecule **IV** and water molecules, and between the water molecules themselves, constituting a complicated three-dimensional system.

In 1981 English chemists [47] have somewhat modified this three-dimensional structure determined on the basis of the results of X-ray measurements. The ^{1}H-

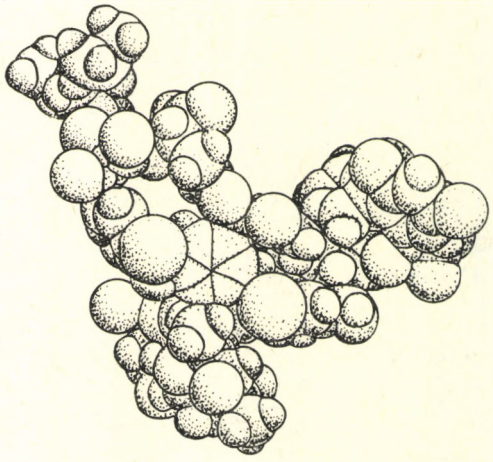


Fig. 1. Three-dimensional structure of antibiotic vancomycin

NMR (^{1}H n.O.e. difference spectroscopy) studies, carried out with the antibiotic itself, suggested an orientation of ring A as shown in formula **I**. It was concluded that during the transformation **I**→**IV** ring A undergoes a rotation of ca. 180° and this assumption was justified by the presence (in a ratio of 2 : 1, based on the NMR data) and isolation of two crystalline isomers of **IV**.

Comparative studies on these two isomers have led to the conclusion that the earlier X-ray measurements had been performed with the isomer of **IV** having different stereochemistry (rings A and C in the same rotational position) from that of vancomycin.

Accordingly, the correct structure of vancomycin can be rather given by formula **I**. It is quite natural and understandable that the above results prompted and helped, both theoretically and practically the structure elucidation of the other members of the vancomycin group of antibiotics. It is unlikely that the structure of such a complex molecule like vancomycin **(I)** could have been entirely determined without X-ray analysis.

3.4. MODE OF ACTION

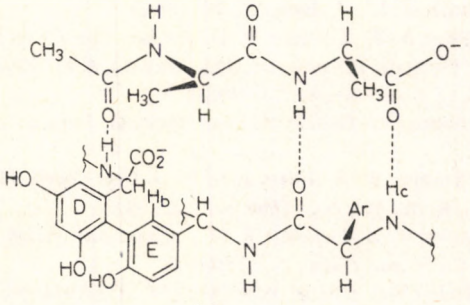
Reynolds [38] and Jordan [39] were the first to find independently that vancomycin significantly inhibited the biosynthesis of cell-wall peptidoglycan, but the mechanism of action was not exactly known. Later Hancock and Fitz-James [40] showed that although this inhibitory effect was very similar to that of several

other antibiotics, which completely differ from vancomycin (bacitracin, D-cycloserine, penicillin, ristocetin), the site of action might be different.

Of the members of the vancomycin antibiotics only the partial structure of ristomycin was known in the beginning of the 1970's as a result of the structural research work of Soviet and Hungarian chemists [41]. After various other hypotheses, Nieto and Perkins [5] supposed that the inhibitory effect of vancomycin is due to a binding between the antibiotic and the N-acyl-D-Ala-D-Ala unit of the cell-wall peptidoglycan. They suggested that — similarly to ristomycin A — the phenolic hydroxyl groups, also present in the aglycone of vancomycin, were involved in the complex formation with the peptide through hydrogen bonds.

It was presumed that — owing to the reduced conformational flexibility of vancomycin — the greatest part of the peptide was present in the cleft of the antibiotic molecule in a way corresponding to a "key–lock" relation, similar to that known from enzymology [43].

On the basis of model studies with synthetic peptides, Nieto and Perkins [44] have established the following conditions of the formation of stable vancomycin–peptide complexes: (a) three amide linkages and (b) free terminal carboxyl group are required; (c) the carboxyl terminal and subterminal residues must be either glycine or of the D-configuration; (d) the size of the chain in these residues has a great influence on the affinity for vancomycin and a methyl group is the optimum in each case; (e) the nature of the side chain in the third and fourth amino acid units has less pronounced influence on the complex formation, but an L-configuration is somewhat better than a D-configuration on the third position. According to ^{1}H-NMR spectroscopic investigations, Brown *et al.* [45, 46] demonstrated that the C-terminal alanine methyl group of the N-Ac-D-Ala-D-Ala peptide was strongly shielded in the presence of vancomycin. Williams and Kalman [35] observed that binding of N-Ac-D-Ala-D-Ala to vancomycin **(I)** resulted in significant downfield shifts of the H_b and H_c protons and a marked upfield shift of H_a. This observation is explained by that these protons are close to the peptide unit in the antibiotic–peptide complex and the proton of NH_c is bonded to one of the carbonyl groups of the peptide.



LIII

On the basis of this evidence, and studies with space-filling models, it has been concluded that vancomycin is associated with the backbone of N-Ac-D-Ala-D-Ala as shown by structure **LIII**. In **LIII** Ar represents the central ring B of vancomycinic acid carrying the disaccharide moiety, which faces the C-terminal methyl group of N-Ac-D-Ala-D-Ala residue. The methyl group of the second D-alanine unit, facing ring D of actinoidinic acid (bearing the two phenolic hydroxyl groups), is not quite centrally located, therefore it is not similarly shielded during the complex formation.

Although this model is consistent with the structure determined by X-ray analysis and with the spectroscopic results, it cannot be considered the only possible one. For the determination of the exact mode of binding of the antibiotic–peptide complex further experimental data are necessary.

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4. ACTINOIDIN

4.1. ISOLATION

Actinoidin, produced by *Proactinomyces actinoides*, was first isolated in 1957 by Sorin *et al.* [1]. Later Lomakina, *et al.* [2] separated the antibiotic into several biologically active components and decomposition products, by means of electrophoresis and chromatography. The antibiotic was adsorbed from the mycelium-free ferment broth of pH 2.5 on Sulfocationite-type ion-exchange resin (SZDV-3*) and then eluted with 0.25 N ammonium hydroxide in 25% aqueous acetone. The eluate was adjusted to pH 3 with ion-exchange resin in the hydrogen form, and the hydrochloride of actinoidin was precipitated from the concentrated solution by the addition of acetone. The crude antibiotic is generally purified by chromatography using the gradient elution technique with 0.2 M ammonium acetate as the eluent. In the pH interval 8.5–9.5 three biologically active variants (A, B and C) were isolated in 60–70, 20–30 and 3–5% yields, respectively. Occasionally these components could be crystallized from a 5 : 1 water–*n*-propanol mixture [3].

Yurina and Lomakina reported [4] that actinoidin A and B transformed into biologically less active degradation products of lower molecular weight under mild acidic conditions.

4.2. BIOLOGICAL ACTIVITY

Soviet authors reported [1] that actinoidin inhibited the growth of the most common Gram-positive microorganismus, and it was most active against staphylococci and pneumococci. According to antibacterial tests, the antibiotic is effective against microorganisms which have developed resistance to penicillin, streptomycin, albomycin, chloramphenicol and chlorotetracycline. These studies suggest that the mode of action of actinoidin is different from that of the above antibiotics, and according to biological results obtained sor far, microorganisms hardly develop resistance to actinoidin [5]. Earlier, actinoidin was used in the Soviet Union for the external treatment of purulent wounds.

Since orally administered actinoidin is poorly adsorbed from the gastro-intestinal tract, and subcutaneous or intramuscular injections caused inflammation and other undesired effects, the antibiotic could not find use in medical practice.

The biological effects of the actinoidin A and B variants were found identical on *Bac. mycoides*, whereas variant C was only half as active as the two other components [3].

It was shown first by Hungarian researchers in Debrecen (Boda *et al.* [10]) that actinoidin A and B, similarly to vancomycin, inhibited platelet agglutination induced by ristocetin or ristomycin. At the same time, it did not inhibit aggregation induced by other compounds (thrombin, adrenalin, ADP or collagen),

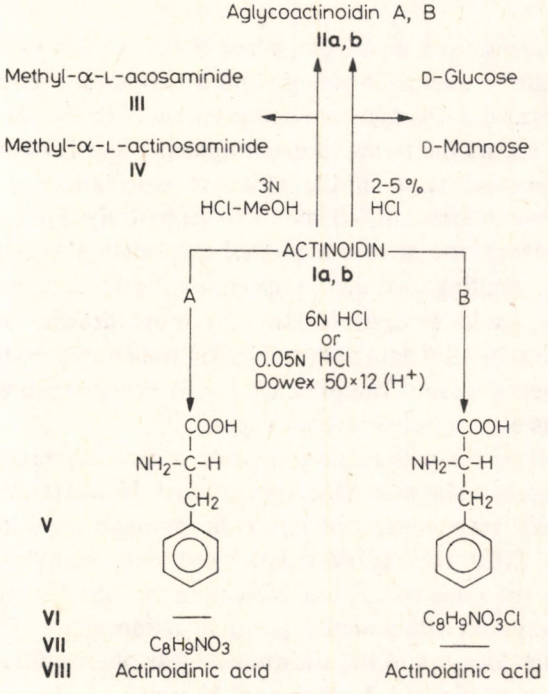
* Made in USSR and corresponding to Dowex 50.

and did not considerably affect platelet agglutination induced by Bovine Factor VIII, either. Of the members of the vancomycin group of antibiotics vancomycin was the most, and actinoidin the least effective in causing plasma protein precipitation. However, in the light of the results obtained so far, no correlation seems to exist between the ability of these antibiotics precipitate plasma proteins and their inductory or inhibitory effects on platelet agglutination.

4.3. STRUCTURAL STUDIES

4.3.1. HYDROLYSIS

The mild acid hydrolysate of actinoidin A and B **(Ia, b)** gives positive Fehling reaction, and contrary to the cases of vancomycin and avoparcin, the hydrolysates have been shown by Sztaricskai *et al.* [6] to contain mannose besides glucose. The antibiotic variants contain these neutral sugars in 1 : 1 ratio. The aglycones **(IIa, b)** retained the antibiotic activity of the parent compounds in a significantly smaller degree than the aglycone of vancomycin; an activity of about 10% was detected with both variants. The methyl glycosides of two novel trideoxy-amino-hexoses, methyl α-L-acosaminide **(II)** and methyl α-L-actinosaminide **(IV)** were also isolated from the methanolysate of actinoidin by Lomakina *et al.* [7] (Scheme 9). Several other



Scheme 9

ninhydrin-positive components were also detected in the course of acid hydrolysis experiments [8]. In parallel studies, the antibiotic was hydrolyzed with 0.05 N hydrochloric acid in the presence of Dowex 50×12 (H^+) ion-exchange resin. The amino acids adsorbed on the resin were eluted with dilute ammonia solution and some of them could be isolated as crystalline products by repeated ion-exchange chromatography on Dowex 50×4 (H^+) resin [3].

On the basis of its paper chromatographic behaviour, specific optical rotation and elemental analysis, amino acid **V** was identified as L-phenylalanine [8, 32]. The structural composition of amino acids **VI** and **VII** was found $C_8H_8NO_3Cl$ and $C_8H_9NO_3$, respectively, and it was also shown that **VI** was not present in variant A, whereas variant B did not contain amino acid **VII**. At the same time **V** and actinoidinic acid **(VIII)** are present in the molecules of both variants. The name actinoidinic acid indicates that this compound has been identified for the first time as a structural unit of the antibiotic actinoidin.

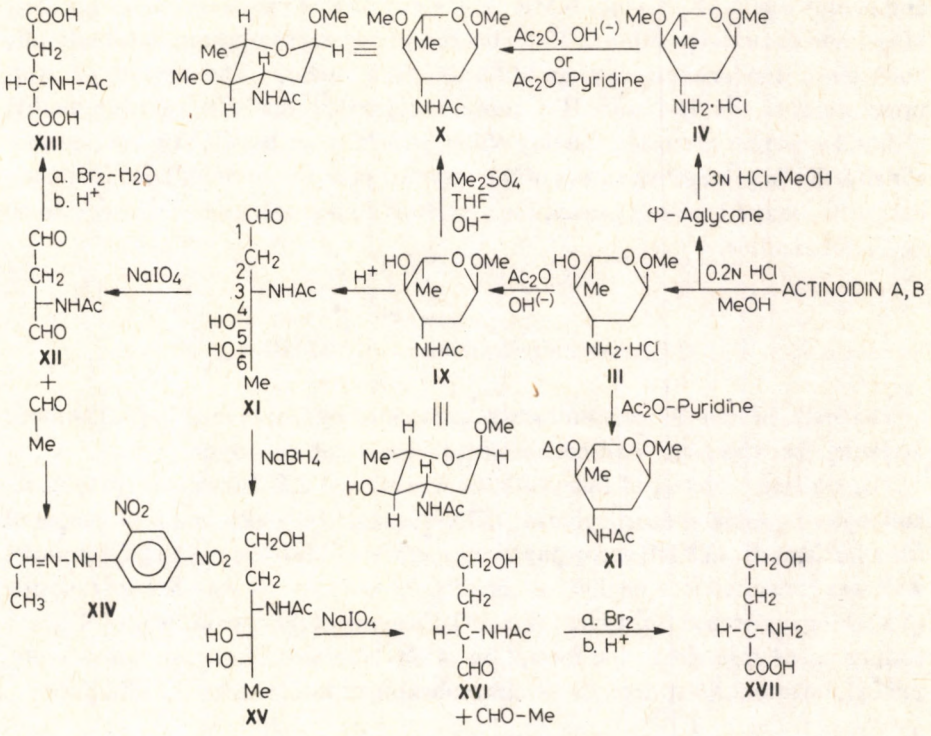
Yurina *et al.* [9] reported that actinoidin A and B contained one molecular equivalent of each of the above amino acids.

4.3.2. L-ACOSAMINE AND L-ACTINOSAMINE

As it has been mentioned, actinoidin A and B were shown by Soviet authors in 1973 [7] to contain — besides D-glucose and D-mannose — two novel trideoxy-amino hexoses, named acosamine and actinosamine. For the early designation of these two amino sugars the terms "aminofragment-I and II" were also used.

The most important steps in the structure elucidation of acosamine and actinosamine are summarized in Scheme 10. In the hydrolysis of actinoidin A, B **(Ia, b)** with 0.2 N hydrochloric acid in methanol only methyl acosaminide **(III)** was liberated, and the resulting so-called ψ-aglycone still contained actinosamine **(IV)**. This latter sugar could be split off only by more drastic methanolysis, and comparison of the analytical data obtained for **III** and **IV** showed that **IV** contained one additional methyl group. The presence of one primary amino group and one C—CH_3 unit was also detected in both sugars.

Treatment of **III** and **IV** with acetic anhydride under alkaline conditions resulted in methyl N-acetylacosaminide **(IX)** and methyl N-acetylactinosaminide **(X)**. Acetylation of **III** with acetic anhydride in pyridine gave methyl N,O-diacetylacosaminide **(XI)**, whereas **IV** transformed into methyl N-acetylactinosaminide **(X)** under the same conditions, indicating that the free hydroxyl group of acosamine is substituted with a methyl group in actinosamine. The most valuable information for the location of the substituents was obtained from the oxidation experiments with periodic acid. Treatment of N-acetylacosamine **(XI)** — obtained



Scheme 10

from **IX** by acid hydrolysis — with sodium periodate gave acetaldehyde and compound **XII**. Further oxidation of **XII** with aqueous bromine, followed by hydrolysis, resulted in N-acetylaspartic acid **(XIII)**. Acetaldehyde was identified in form of its 2,4-dinitrophenylhydrazone **(XIV)**. Accordingly, compound **XIII** contains the C-1–C-4 carbon chain of N-acetylacosamine **(XI)**, and acetaldehyde is formed from the C-5 and C-6 atoms of **XI**, indicating the location of the acetamido group at C-3, and the presence of the two hydroxyl groups at C-4 and C-5.

Periodate oxidation of N-acetylacosaminitol **(XV)**, obtained from **XI** by reduction with sodium borohydride, gave **XVI** and acetaldehyde. Further oxidation of **XVI** with aqueous bromine and subsequent hydrolysis resulted in homoserine **(XVII)**. According to these experiments acosamine was shown to have a 3-amino-2,3,6-trideoxyaldohexose structure. Since methylation of methyl N-acetylacosaminide **(IX)** with dimethyl sulfate led to the formation of methyl N-acetylactinosaminide **(X)**, actinosamine was identified as 4-O-methylacosamine.

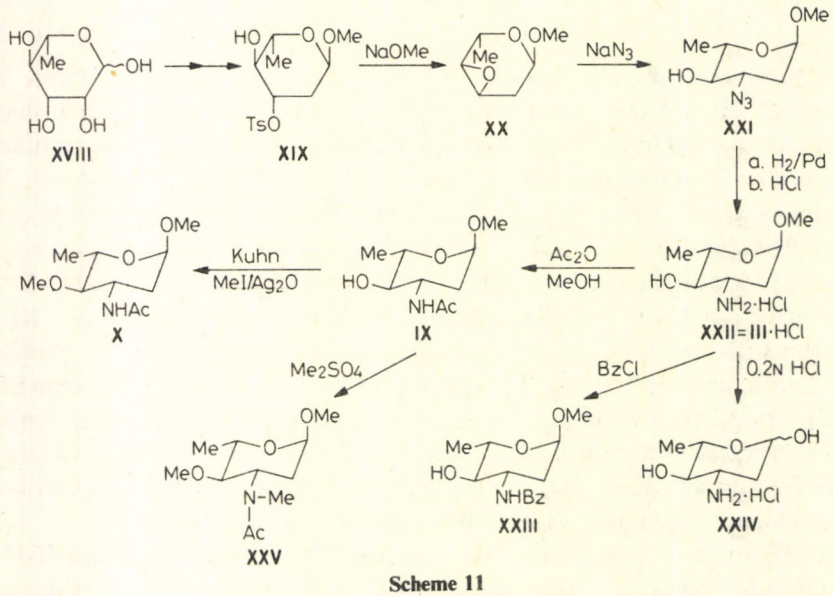
The ^{1}H-NMR spectra of **IX** and **X** were found to be exceptionally similar, the only difference being the appearance of two methoxyl signals in the spectrum of **X**,

and of only one in **IX**. Further NMR studies established the *equatorial* orientation of H-1 and the *axial* position of H-3, H-4 and H-5 in acosamine and actinosamine, and hence the *arabino* configuration of both sugars. The *equatorial–axial* orientation of the H-1 and H-5 protons suggested that the isolated methyl glycosides were α-glycosides having either α-D-($^4C_1$)- or α-L-($^1C_4$) conformation. Finally, the large negative values of the specific optical rotation obtained for each derivative unambiguously proved the L-*arabino* configuration of both acosamine and actinosamine.

4.3.2.1. SYNTHESES OF L-ACOSAMINE AND L-ACTINOSAMINE

The first synthesis of L-acosamine was elaborated by Gupta in 1974 [12] without knowing that this sugar had been isolated from a natural compound.

The starting material of this synthesis was methyl 2,6-dideoxy-3-O-*p*-toluenesulfonyl-α-L-*arabino*-hexopyranoside **(XIX)** (Scheme 11) which had been prepared from L-rhamnose **(XVIII)** according to the method of Marsh *et al.* [13]. Compound **XIX** was converted into methyl 3,4-anhydro-2,6-dideoxy-α-L-*ribo*-hexopyranoside **(XX)** using the procedure of Jarý *et al.* [14]. Cleavage of the epoxide ring of **XX** with sodium azide resulted in the formation of an "abnormal" *diequatorial* product, methyl 3-azido-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside **(XXI)** in 89% yield.



Scheme 11

Contrary to these results of Gupta [12], we have found [12a] that the cleavage of the epoxide ring of **XX** with sodium azide resulted in 50% of the L-*arabino* compound **(XXI)** and ca. 10% of the isomeric L-*xylo* azide derivative.

Opening of the epoxide ring of hexopyranosides, having half-chair conformation, with nitrogen nucleophiles leads to the formation of deoxy amino hexose derivatives [15], and of the products of half-chair conformation the major component is the one which contains the new functional groups in *trans-diaxial* orientation [16]. Owing to their conformational flexibility [15, 12], the monocyclic 2,3- or 3,4-anhydrohexopyranosides can readily transform into the alternative conformer, and thus the ratio of the *diaxial* and *diequatorial* ring-opened products is more dependent on the properties of the nucleophile and on the reaction conditions than that of the products prepared from the conformationally more rigid bicyclic sugar epoxides [18].

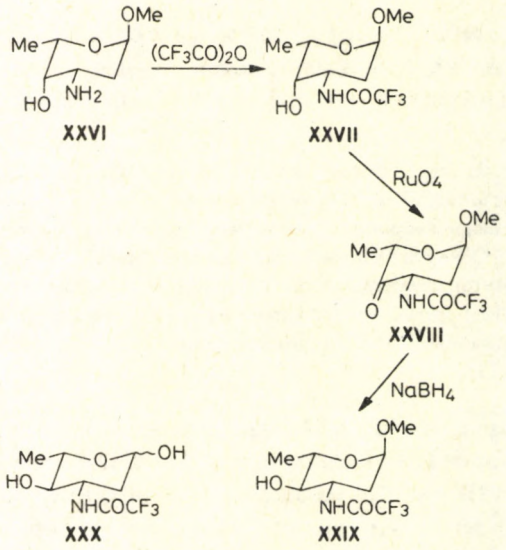
Catalytic hydrogenation of **XXI** yielded methyl 3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside **(III)**, isolated in the form of its hydrochloride salt **(XXII)**. Characterization of **III** was also accomplished by preparing the N-acetyl **(IX)** and N-benzoyl **(XXIII)** derivatives on treatment with acetic anhydride and benzoyl chloride, respectively, in methanol solution.

Methyl α-L-acosaminide **(III)** was also synthesized by Lee *et al.* [19] in an essentially similar way. Compound **III** was then acetylated with acetic anhydride in pyridine to obtain the diacetate **XI**, the physical data of which were in good accordance with those of the sample isolated from the antibiotic [7]. Mild acid hydrolysis of **III**, followed by freeze-drying gave the hydrochloride of free acosamine **(XXIV)**. The authors observed that methylation of **IX** with dimethyl sulfate did not give the expected **X**, but the dimethylated derivative **XXV** or, presumably, its dimeric analogue. Therefore, **X** was prepared by methylation of methyl N-acetyl-α-L-acosaminide **(IX)** according to Kuhn's procedure. Although the melting point and value of specific optical rotation of the product was somewhat higher than those reported for **X**, the IR and ^{1}H-NMR spectral data were practically identical [19]. The above differences in the physical data might have arisen from the different anomeric contents of the synthetic and natural samples.

The Arcamone group [20] transformed methyl α-L-daunosaminide **(XXVI)** into methyl N-trifluoroacetyl-α-L-acosaminide **(XXIX)** by oxidation of the N-trifluoroacetyl derivative **XXVII** with ruthenium tetroxide and subsequent reduction of the resulting uloside **XXVIII** with sodium borohydride (Scheme 12).

Mild acid hydrolysis of **XXIX** gave 2,3,6-trideoxy-3-trifluoroacetamido-L-*arabino*-hexopyranose **(XXX)** which was then used for the synthesis of 4′-epidaunorubicin and 4′-epiadriamycin.

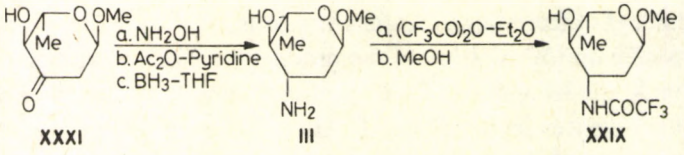
Another synthesis of L-acosamine from 3,4-di-O-acetyl-L-rhamnal using C-3 epimeric azide intermediates has been published by Heyns *et al.* [21]. This method will be discussed in connection with the synthesis of L-ristosamine.



Scheme 12

Perhaps the most simple route so far reported to L-acosamine derivatives has been recently elaborated by Pelyvás and Whistler [11] (Scheme 13).

The acetylated oxime* prepared from methyl 2,6-dideoxy-α-L-*erythro*--hexopyranoside-3-ulose **(XXXI)** was stereospecifically reduced with the borane–tetrahydrofuran complex to obtain, after trifluoroacetylation, methyl N-



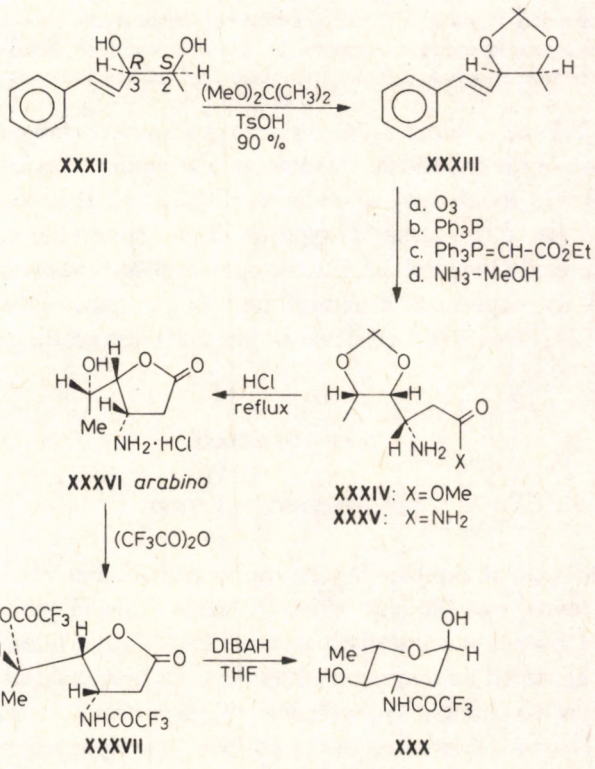
Scheme 13

trifluoroacetyl-α-L-acosaminide **(XXIX)**. Only traces of the corresponding L-*ribo* epimer (L-ristosamine) could be detected in the reaction mixture. Compound **XXIX** was obtained in 67% overall yield, based on the uloside **XXXI**.

In 1980 Fronza *et al.* [22] reported an ingenious chiral synthesis of N-trifluoroacetyl-L-acosamine **XXX** from non-carbohydrate precursors. The starting compound of this method (Scheme 14) was cinnamaldehyde, readily obtainable

* Details of the preparation of this compound is given in Chapter 6, pp. 166–167.

from D-glucose with baker's yeast. Reaction of cinnamaldehyde with acetaldehyde gave the key intermediate diol **XXXII**, in which the absolute configurations of the C-2 and C-3 carbon atoms (2*S*, 3*R*) were identical with that of the C-5 and C-4 carbons of L-acosamine. Treatment of **XXXII** with 2,2-dimethoxypropane in the presence of



XXXII **XXXIII**

XXXVI *arabino*

XXXIV: X=OMe
XXXV: X=NH2

XXXVII **XXX**

Scheme 14

p-toluenesulfonic acid afforded the isopropylidene derivative **XXXIII**. This latter was converted into a mixture of **XXXIV** and **XXXV** by sequential ozonolysis, reduction with triphenylphosphine, treatment with ethoxycarbonylmethylenetriphenylphosphorane and ammonia in methanol. Acid hydrolysis of this mixture resulted in the lactone hydrochloride **XXXVI**. Finally, reduction of the N,O-ditrifluoroacetyl derivative of **XXXVI** with diisobutylaluminium hydride (DIBAH) in tetrahydrofuran led to N-trifluoroacetyl-L-acosamine (**XXX**).

Another chiral synthesis of 3-acetamido-2,3,6-trideoxy-L-*arabino*-hexose (N-acetyl-L-acosamine) starting from sorbic acid was reported by Dyong and Bendlin [23].

Grete *et al.* [24] used cyclopentadiene as starting material for still another chiral synthesis of L-acosamine.

L-acosamine has been used for the preparation of 4'-epiadriamycin, a novel semisynthetic anticancer antibiotic of therapeutical importance. Since the L-rhamnose-based methods for the production of this amino sugar are expensive for industrial purposes, the latter chiral syntheses, based on noncarbohydrate precursors, have become important from both the theoretical and practical points of view.

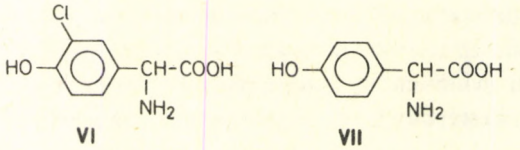
Canadian [25] and American [26] research groups have elaborated methods for the preparation of the enantiostereoisomer of acosamine. This latter amino sugar (D-acosamine) was isolated by Harada *et al.* [23a] in 1979 as a carbohydrate component of the oligosaccharide-type antibiotic sporaviridin. Angolosamine (3-dimethylamino-3-C-methyl-2,3,6-trideoxy-D-*arabino*-hexopyranose) has been shown to be an important structural unit of the macrolide-type antibiotic, angolamycin [27]. This branched-chain amino sugar was synthesized by Bear *et al.* [28] in 1977.

4.3.3. AGLYCONE

4.3.3.1. PHENYLGLYCINES

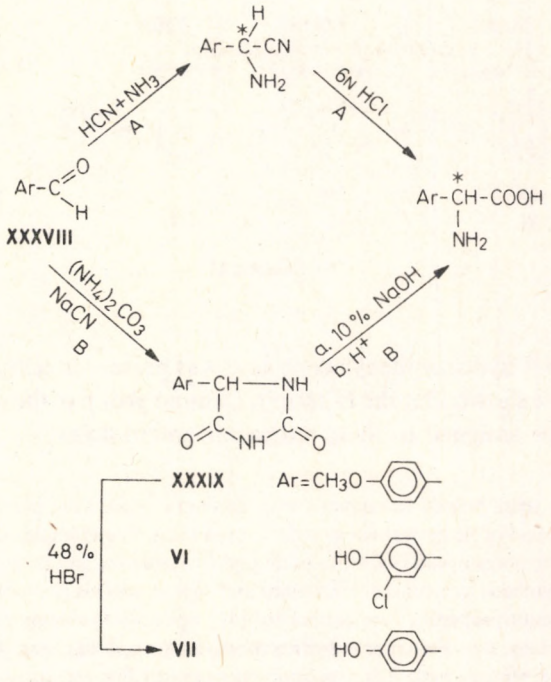
The only difference found between the amino acids **VI** and **VII**, isolated from the actinoidin variants, was the lack of one chlorine atom in the molecule of **VII**. Compound **VI** gave characteristically positive Pauly- and Millon tests; the yellow colour which appeared on treatment with ninhydrin first changed into brown and then violet. Like α-aminophenylacetic acid, **VI** gave blue colour reaction with 4-nitrobenzoyl chloride. According to the positive Van Slyke reaction and also the formation of 2,4-dinitrophenyl derivative, one primary amino group was concluded to be present in the molecule.

The ultraviolet spectrum of **VI** had an absorption maximum at 280 nm, and the analysis of the infrared spectra suggested the presence of a 1,2,4-trisubstituted aromatic ring. On the basis of these results the structure of **VI** and **VII** was supposed to be α-amino-3-chloro-4-hydroxyphenylacetic acid and α-amino-4-hydroxyphenylacetic acid, respectively [8].



VI **VII**

The definitive synthesis of these amino acids was first accomplished by the Bognár group [29] by two different routes. Using the Strecker synthesis (Scheme 15, method A) D,L-α-amino-4-methoxyphenylacetic acid **(XXXIX)** was prepared from *p*-methoxybenzaldehyde **(XXXVIII)** and then demethylated with 48% hydrogen bromide to obtain **VII**. This method, however, could not be successfully used when

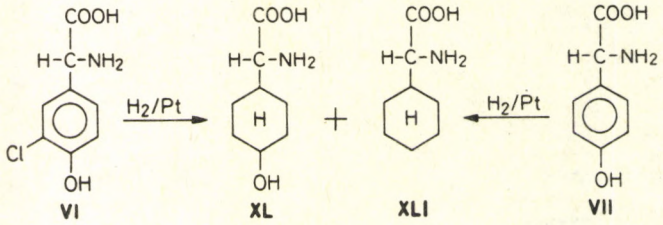


Scheme 15

starting directly from *p*-hydroxybenzaldehyde; therefore amino acids of such type were prepared by the "hydantoin-method" (method B). In this way both amino acids **(VI** and **VII)** have been synthesized.

UV and IR spectroscopic analyses and paper chromatographic examination established the identity of D,L-α-amino-3-chloro-4-hydroxyphenylacetic acid with **VI** isolated from actinoidin B, and also D,L-α-amino-4-hydroxyphenylacetic acid was identified with amino acid **VII** obtained from actinoidin A. The above preparative method was later successfully applied by Asai *et al.* [30] for the synthesis of **VII** and α-amino-3,5-dichloro-4-hydroxyphenylacetic acid, isolated from the antibiotic enduracidin. Ten years later compound **VI** was also identified by American chemists [31] as a structural unit of avoparcin *β* (see Chapter 5).

The configurations of **VI** and **VII** were established by Lomakina *et al.* [32] by determinations of the specific optical rotations; of *p*-hydroxy-D-cyclohexylglycine **(XL)** and D-cyclohexylglycine **(XLI)**, obtained from **VI** and **VII**, respectively, by catalytic hydrogenation over Adams catalyst. These data unambiguously showed that actinoidin A contained D-α-amino-4-hydroxyphenylacetic acid, whereas D-α-



Scheme 16

amino-3-chloro-4-hydroxyphenylacetic acid was present in actincoidin B. Further studies [38] have shown that the N-terminal amino group of the actinoidin A and B aglycones can be assigned to these particular amino acids.

During the past years several compounds with antibiotic effect have been shown to contain α-aminophenylacetic acid or its derivatives as structural element. For example, etamycin (viridogrisein) [44] and ostreogricin (mikamycin) [45, 46] contain L-phenylsarcosine and L-α-aminophenylacetic acid, respectively. Semisynthetic penicillines (ampicillin) and cephalosporins (cephalexin, cephaloglycin) of outstanding therapeutic properties were synthesized [47] using D-(—)-α-aminophenylacetic acid as the side chain. Additionally, D-(—)-α-amino-4-hydroxyphenylacetic acid has been recently applied for the preparation of novel semisynthetic β-lactam antibiotics (amoxicillin, cefadroxil, cefatrizin) [33, 48].

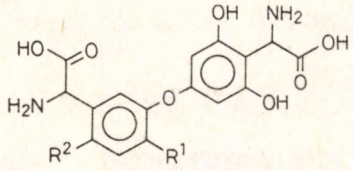
4.3.3.2. ACTINOIDINIC ACID (AMINO ACID B*)

The presence of the new aromatic diamino-dicarboxylic acid, actinoidinic acid in actinoidin- and ristomycin variants was first reported by Lomakina *et al.* [9, 34]. However, for the lack of mass spectrometric examination, the Soviet authors [35] proposed an incorrect structure **(VIIIa)** for actinoidinic acid (Scheme 17).

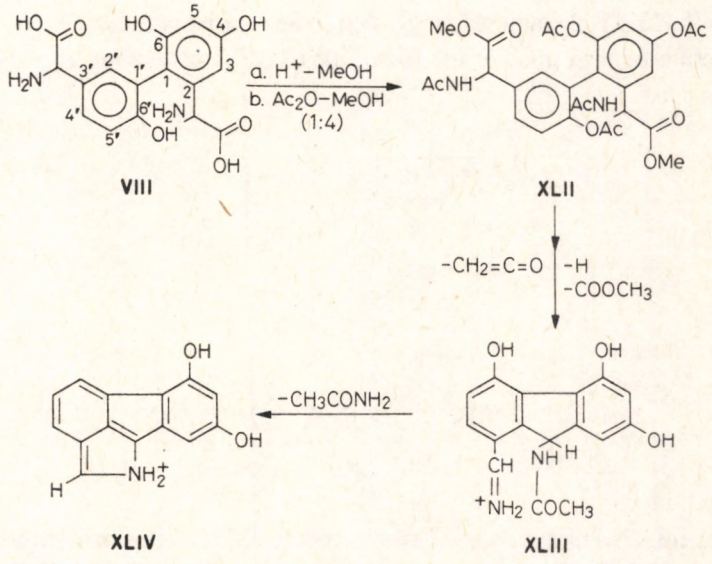
Later this compound was isolated also from vancomycin by English chemists and on the basis of detailed studies its structure was corrected to **VIII** (see Chapter 3, pp. 115–116). This revised structure has been supported by chemists at the Lomonosov University (Moscow) [36] by mass spectral investigations.

* In early communications appeared in Russian the name "amino acid B" was used for this compound.

The most suitable derivative of actinoidinic acid for the mass spectral studies was the dimethyl ester of N,O-peracetylactinoidinic acid (**XLII**). The spectrum showed a low-intensity peak of the molecular ion at *m/e* 568 (12%), corresponding to the molecular formula $C_{28}H_{30}N_2O_{12}$ (Scheme 17). In the first stage of fragmentation an



VIIIa R^1=OH; R^2=H
or R^1=H; R^2=OH



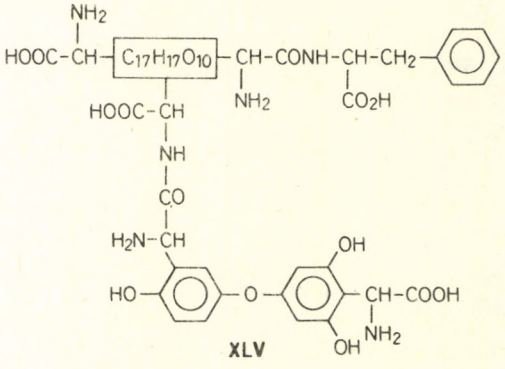
Scheme 17

ion $(M\text{-}COOCH_3—3CH_2CO)^+$ was detected, which then decomposed by the loss of fragments with mass numbers 60 and 59. The formation of these fragments can be explained by the elimination of one proton and one $COOCH_3$ group, leading to the formation of the tricyclic compound **XLIII**, which can finally transform into the tetracyclic ion **XLIV** by the loss of a CH_3CONH_2 unit. The formation of these latter two fragments is possible only if the glycine residue of the tetrasubstituted ring of actinoidinic acid is linked in *ortho* position with respect to the place of attachment of the two aromatic rings.

The determination of the exact structure of actinoidinic acid was essential, because this amino acid was known as a common building unit of each of the vancomycin-type antibiotics. Further evidence for the structure of this compound was obtained from chemical degradation studies carried out in other laboratories. These results and also the partial definitive synthesis of actinoidinic acid are discussed in Chapter 6 (pp. 178–179), dealing with the structural investigation of ristocetin and ristomycin.

4.3.3.3. MONODECHLOROVANCOMYCINIC ACID

The presence of an unknown building unit with triamino-tricarboxylic acid composition (Y*) in aglycoactinoidins was first suggested by Berdnikova *et al.* [37, 38] in 1976. This Y element was suggested to be located between the phenylalanine and actinoidinic acid units of the tripeptide **(XLV)** constituting about 80% of the aglycone.

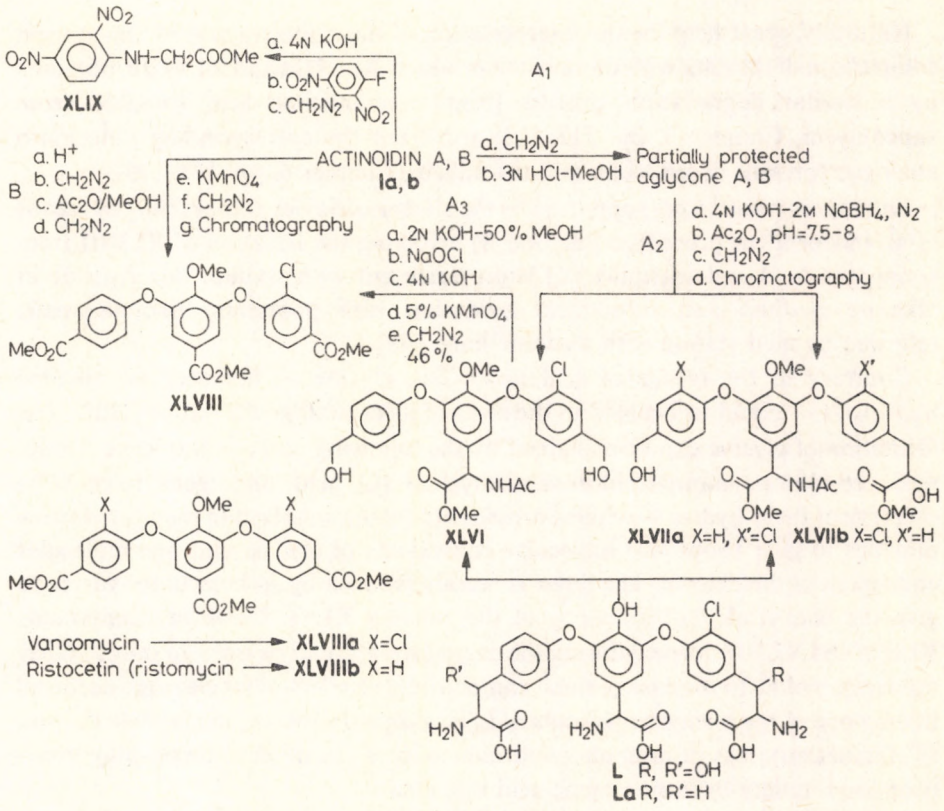


XLV

The constitution of this compound was concluded from the formation of Y-Phe in the course of Edman degradation and the structure of the tripeptide **XLV**. However, the framed structural unit of **XLV** was incorrectly given the chlorine atom being entirely omitted. Moreover, the structure involving diphenyl ether bond was also incorrectly written for actinoidinic acid.

The correct structure of monodechlorovancomycinic acid, present in both actinoidin variants, was finally determined by Sztaricskai *et al.* [39]. Accordingly, the composition of **L** is 3-(2-chloro-4-seryl-phenoxy)-5-(4-seryl-phenoxy)-*p*-hydroxyphenylglycine.

* Designation used earlier by Soviet authors [37, 38].



Scheme 18

The phenolic hydroxyl groups of the antibiotic **(Ia, b)** were first blocked by methylation with diazomethane and the carbohydrate moieties were removed by methanolysis (Scheme 18, method A_1). The partially protected aglycone was then hydrolyzed by treatment with 4 N potassium hydroxide in the presence of two molar equivalents of sodium borohydride under nitrogen atmosphere. The liberated amino acid derivatives were N-acetylated under slightly basic conditions, methylated again with diazomethane and finally chromatographed on silica gel. In this way, besides the O-methyl-N-acetyl esters of the formerly obtained amino acids **(V–VIII)**, the successful isolation of compounds **XLVI** and **XLVIIa, b**, was achieved. The presence of chlorine was confirmed by elemental analysis and by the detection of the $^{35}Cl:^{37}Cl$ isotope peaks in the mass spectrum. Degradation of **XLVI** with sodium hypochlorite, followed by alkaline hydrolysis, oxidation with potassium permanganate and methylation with diazomethane (method A_3, reaction steps *a–e*) gave **XLVIII** in 46% yield. The structure of this compound was unambiguously established by ^{1}H-NMR- and mass spectrometric investigation.

Naturally, great help for the interpretation of these spectra was obtained from comparison of the data with those of the products **XLVIIIa** and **XLVIIIb**, obtained by a similar degradation process from vancomycinic acid (isolated from vancomycin, Chapter 3, pp. 111–112) and from the corresponding didechloro analogue (present in ristocetin and ristomycin, Chapter 6, pp. 174–179).

Later these results were supported by the studies of Soviet authors [40] by means of an independent procedure (method B) involving the isolation of **XLVIII** from actinoidin A, B and tripeptide **XLV** on treatment with sodium borohydride in alkaline medium and subsequent oxidation with potassium permanganate, followed by methylation with diazomethane [49].

Contrary to the results of acid hydrolysis, glycine — liberated on alkaline hydrolysis — could be isolated in the form of its crystalline derivative **XLIX**. The formation of glycine can be explained by the following reaction sequence. Under basic conditions monodechlorovancomycinic (**L**) acid undergoes retro–aldol cleavage at the β-hydroxy-α-aminopropionate groups involved in the phenylserine moieties to give about two molecular equivalents of glycine and one molecular equivalent of dialdehyde. The latter is readily reduced by sodium borohydride to give the benzyl alcohol functions of the isolated **XLVI**. However, compounds **XLIIIa** and **XLIIIb**, formed during the degradation, could be isolated only in fairly moderate yields. In the case of these compounds the retro–aldol cleavage occurred in only one of the phenylserine units of **L**, accompanied by sequential dehydration (β-elimination), loss of ammonia and reduction of the other α-amino-β-hydroxypropionyl moiety to form a lactic acid side chain.

Additional proof for the structure of **L** [39] has been recently given by Berdnikova [49] by means of a study on the dideoxy derivative **La** obtained by the reductive acid hydrolysis (HI/P) of the partially protected aglycone. This method is essentially the same as one of the procedures applied successfully for the structure elucidation of vancomycin and ristomycin A (*cf.* Chapter 3, p. 114 and Chapter 6, pp. 173–174).

It is to be noted that independently of these results, American researchers have also detected monodechlorovancomycinic acid from the α- and β-variants of avoparcin (see Chapter 5, pp. 144–146).

As a result of the above studies, the structure of each building element of actinoidin has been unequivocally elucidated.

4.3.4. THE CARBOHYDRATE–AGLYCONE LINKAGES

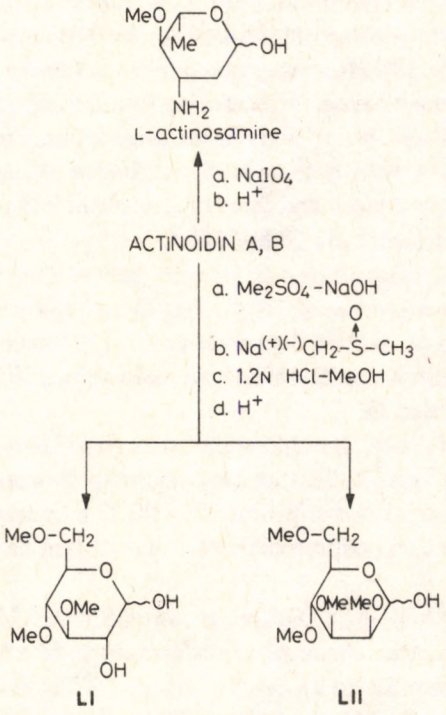
The sequence of attachment of the sugar units and the linkage of the carbohydrate moiety to the aglycone were investigated by Soviet and Hungarian researchers [41] by means of hydrolysis studies and also by methylation followed by hydrolysis. On

effecting the hydrolysis and methanolysis of the antibiotic with acids of various concentration, it was found that the monosaccharides were liberated in the following order:

L-acosamine > D-glucose > D-mannose > L-actinosamine,

indicating the acosamine linkage to be the most sensitive, and the bonding of actinosamine the least sensitive to acid hydrolysis.

With the exception of the latter trideoxy-amino-sugar, which contains no free hydroxyl groups, all the other monosaccharides could be oxidized by periodate. The methanolysis of permethyl actinoidin, prepared by methylation using Haworth's



Scheme 19

and Hakomori's methods, followed by hydrolysis, gave rise to 3,4,6-tri-O-methyl-D-glucose (**LI**) and 2,3,4,6-tetra-O-methyl-D-mannose (**LII**) [41] (Scheme 19). Since acosamine was liberated first, followed by D-glucose and D-mannose, it was concluded that D-mannose was separately attached to the aglycone, whereas the two other sugars were present in the form of a 2-O-(L-acosaminyl)-D-glucosyl

disaccharide moiety linked also to the aglycone. Later, partial hydrolysis studies with the products prepared by methylation of the actinoidin variants with diazomethane have shown that D-mannose is linked to one of the phenolic hydroxyl groups of the actinoidinic acid unit, and the disaccharide (named acobiose) is attached to the single hydroxyl group of monodechlorovancomycinic acid [42].

These findings, in accordance with the number of the free hydroxyl groups of the aglycones of both actinoidin variants, permit the conclusion that the L-actinosamine unit can be attached only to one of the alcoholic hydroxyl groups of the aglycones. This assumption is also supported by the experimental fact that this latter glycosidic bond is the most stable under the conditions of both acid hydrolysis and methanolysis [42].

The structural unit of actinoidin, built from acobiose and monodechlorovancomycinic acid, suggests some similarities to the structure of vancomycin (see Chapter 3, p. 108 and 119). However, vancomycin is known to be a compound of the monoglycoside type whereas — according to the results so far obtained — the actinoidin variants A and B are triglycoside antibiotics. Potentiometric titration experiments [43] have shown that both actinoidin variants contain one free carboxyl-, three primary amino- and three or four phenolic hydroxyl groups and the estimated molecular weights are 2000–2100.

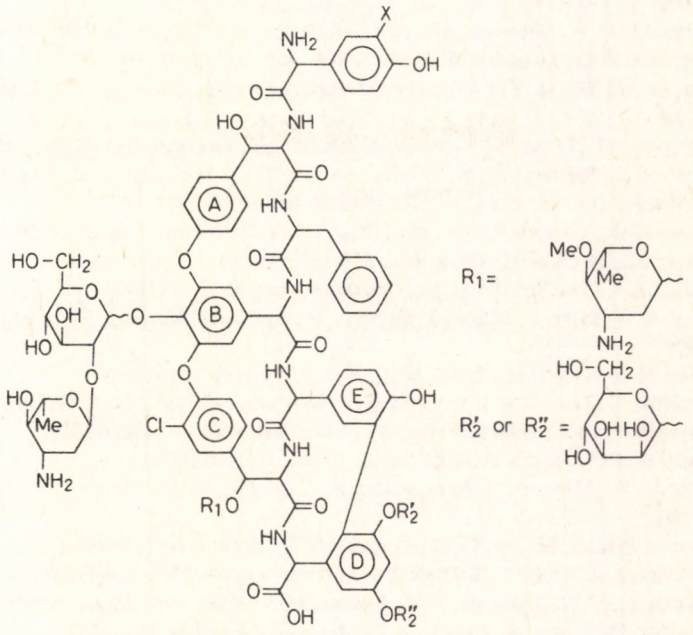
On the basis of the demonstrated similarities between the structural elements of actinoidin and the other members of the vancomycin-type antibiotics (vancomycin, and avoparcins α and β), in 1979 Sztaricskai *et al.* [39] have proposed hypothetic structures for actinoidin A and B which have been shown to be in good agreement with the formulae **Ia** and **Ib**.

According to this proposal, the chlorine atom of ring C may also be situated at the appropriate position of ring A. Further on, it could not be exactly determined which of the two phenylserine units is substituted by the L-actinosamine moiety at the β-hydroxyl group. The exact position of the D-mannose unit has not been determined either.

Based on the ^{1}H-NMR spectroscopic examination of **XLVIII**, **La** and also the product obtained by the chemical transformation of the L-phenylserine-free dipeptide prepared from **XLV** (Y), Soviet authors [50] concluded in 1982 that the chlorine atom of monodechlorovancomycinic acid was attached undoubtedly to ring C in **Ia** and **Ib**. It was also assumed that L-actinosamine was glycosidically attached to the hydroxyl group of the serine unit near to the C-terminus.

These results are in good agreement with the proposals given for the similar structural units of avoparcin (*cf.* Chapter 5, p. 149). However, the place of attachment of D-mannose to the aglycone has not been determined [42].

Naturally, these studies have not been finished as yet, since the exact configuration of the glycosidic linkages and some details of the stereochemistry of the heptapeptide unit require further chemical and instrumental studies.



Ia Actinoidin A, X=H
Ib Actinoidin B, X=Cl

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5. AVOPARCIN

5.1. ISOLATION

Antibiotic LL-AV 290, another member of the vancomycin group, was isolated by Kunstmann *et al.* [1] in 1968 from the culture of *Streptomyces candidus* (NRRL 3218) using absorption and ion-exchange chromatographic techniques. Later the antibiotic complex was named avoparcin [3] and shown to consist mainly of a 1 : 3 mixture of avoparcins α and β.

The presence of two other minor components has also been detected which differ from the major compounds in the absence of one of the trideoxy-amino-hexose units [5].

5.2. BIOLOGICAL ACTIVITY AND APPLICATION

Preliminary microbiological studies have shown that avoparcin is active against Gram-positive bacteria but inactive against Gram-negative organisms.

Avoparcins α and β significantly lose antibacterial activity when their aqueous solutions in the pH range 5–8 are heated at higher temperatures for a longer period of time; a mixture of epi-avoparcins α and β (70%) is formed [9].

Epi-avoparcin β is 10–100-fold less active against Gram-positive microorganisms than the intact antibiotic. Under similar conditions all the other minor avoparcin components also gave the corresponding epi-derivatives.

Avoparcin has been compared with vancomycin and methicillin for *in vitro* and *in vivo* activity [2]. These studies showed that against group A streptococci avoparcin was more active than vancomycin, but less active than methicillin. Against group D streptococci avoparcin and vancomycin were equally effective whereas methicillin was inactive. At the same time, against staphylococci avoparcin was found to be less active than vancomycin or methicillin. No difference was found in the antibiotic susceptibility of the pathogenic strains isolated from clinical sources in 1957 and those isolated in 1967. With respect to their antibacterial activities avoparcin and vancomycin are about equivalent preparations. However, it is a great advantage of the application of avoparcin, as compared to that of vancomycin or ristocetin (ristomycin), that avoparcin causes less local damage upon intramuscular administration. Practically it is not absorbed on oral administration; probably, this latter property played a part in introducing avoparcin to be used as a feed additive. The antibiotic has been marketed in Western Europa for this purpose by American Cyanamid Co. under the trade name *Avotan* [4, 5].

5.3. STRUCTURAL STUDIES

Prior to the isolation of avoparcin both the methodical and instrumental prerequisites of the structural studies of vancomycin-type antibiotics had been secured, and several structural fragments of these antibiotics (actinoidinic acid, 4-hydroxyphenyl-glycine, 3-chloro-4-hydroxyphenylglycine, L-ristosamine and neutral carbohydrates) had been identified as building units of other members of this group. Therefore it was a relatively short period of time required for the establishment of the structure of avoparcin.

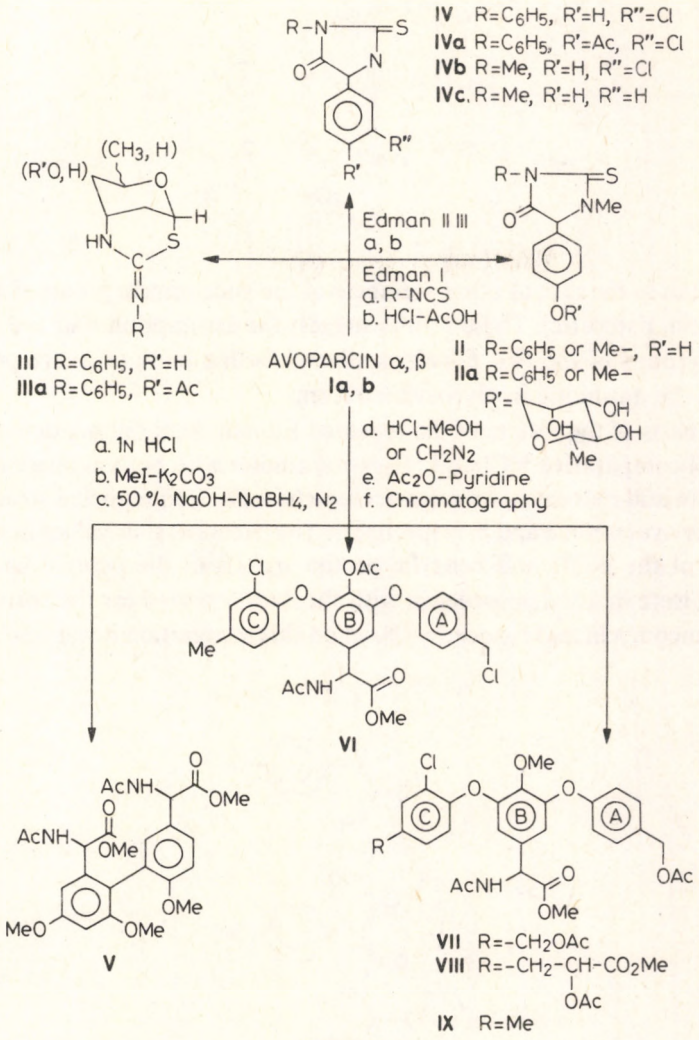
5.3.1. AGLYCONE

The molecular weights of the avoparcin antibiotics were determined by means of the McFarlane californium plasma desorption mass spectrometric method [6], and avoparcin α and β were characterized by the formulae and molecular weights $C_{89}H_{101}O_{36}N_9Cl$ (1907) and $C_{89}H_{100}O_{36}N_9Cl_{12}$ (1942), respectively.

In 1974 Hlavka *et al.* [3] found that the Edman degradation of avoparcin (**Ia, b**, Scheme 20) yielded *p*-hydroxyphenylsarcosine as the phenylthiohydantoin derivative **II** and a 3-amino-2,3,6-trideoxyhexose as the phenylisothiocyanate derivative **III**. In stage *b* of the Edman degradation, involving cyclization in acidic medium, the formation of the acyl derivative **IIIa** was also observed. Thin layer chromatographic examination of the reaction mixture led to the isolation of the rhamnoside derivative **IIa**. In fact, the formation of **IIa**, as an unexpected product, proceeded in stage *a* of the first Edman-cycle. The second and third Edman-cycles afforded the thiohydantoin derivative of 3-chloro-4-hydroxyphenylglycine (**IV–IVb**) in a well-known sequence of reactions (see Chapter 4, pp. 132–134). Besides **IV** the formation of the acetyl derivative **IVa** was also observed.

When the Edman degradation sequence was repeated using methyl isothiocyanate [4, 5] it was found that avoparcin β contained 3-chloro-4-hydroxyphenylglycine, whereas in the molecule of the minor antibiotic variant α it was replaced by 4-hydroxyphenylglycine. The ratio of these products (4 : 1) was in good accordance with the determined 3 : 1 ratio of avoparcin β and α in the antibiotic complex.

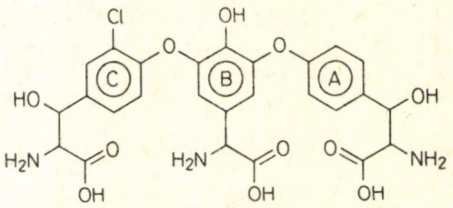
Based on the work of the researchers at Cambridge, the Ellestad group [4, 5] applied reductive alkaline hydrolysis for the elucidation of the structures of the aglycones of avoparcin α and β, (*cf.* Chapter 3, pp. 112–113). Following suitable derivatization and intensive chromatographic efforts compounds **V–IX** could be isolated. Compound **V** formed from the actinoidinic acid unit of avoparcin in the same fashion as observed in the degradation of the antibiotics vancomycin, ristocetin (ristomycin) and actinoidin. Product **VI** could arise by successive retro–aldol cleavage of monodechlorovancomycinic acid (**X**), present in the molecule of



Scheme 20

the antibiotic, reduction of the intermediary aldehyde and introduction of chlorine during treatment with hydrochloric acid. Such degradation products have been isolated only in the structural investigation of avoparcin α and β. Compound **VIII** was shown to form from one of the phenylserine units of **X** by β-elimination followed by deamination and reduction.

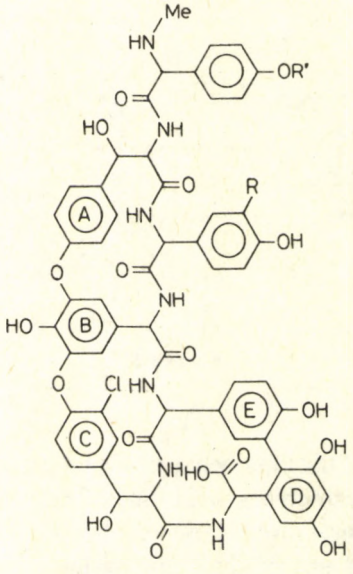
However, the amount of glycine, obtained from avoparcin and its crystalline degradation product on retro–aldol cleavage, was shown to be much less (0.55 and



X

0.5 mol) than in the case of other members of the vancomycin group of antibiotics (vancomycin, ristocetin). These results suggest the assumption that the secondary hydroxyl groups of the two β-hydroxyphenylalanine units of **X** are presumably present in the antibiotic in glycosylated form.

On the basis of the results of the repeated Edman degradation, detailed above, and also of comparative ^{13}C- and ^{1}H-NMR studies with both avoparcin variants, vancomycin and ristocetin, American researchers [4, 5, 9] reported structures **XIa** and **XIb** for avoparcin α and β, respectively. The chemical shift values and coupling constants of the α-CH and benzylic proton signals of the peptide backbone of avoparcin were in good accordance with the data reported for the corresponding units of vancomycin and ristocetin. Based on this recognition it was concluded [9]



XIa R=R'=H
XIb R=Cl, R'=H
XIIa R=H, R'=α-Rhap.
XIIb R=Cl, R'=α-Rhap.

that the stereochemistry of these centres of avoparcin — except those of the N-terminus — is the same as that of the two antibiotics mentioned above. Epimerization experiments, carried out with deuterium oxide, have shown that the formation of the diastereoisomeric mixture from the antibiotic is due to epimerization, indeed, at the α-methine of the N-terminal phenylsarcosine subunit. Additionally, in the knowledge of the composition of **IIa**, the rhamnoside of aglycoavoparcin α and β were characterized by structures **XIIa** and **XIIb** [4] in which the anomeric configuration of the sugar is α.

5.3.2. THE CARBOHYDRATE–AGLYCONE LINKAGES

Degradation studies on avoparcin demonstrated the presence of one molecule each of glucose, mannose and rhamnose as the neutral carbohydrates of the antibiotic. As it has been mentioned, the place of attachment of rhamnose could be determined by structural studies of the aglycone of the antibiotic. The new, unknown amino sugar, previously obtained in the form of derivatives **III** and **IIIa**, was shown later to be identical with L-ristosamine, isolated for the first time by Bognár *et al.* [7] from ristomycin (*cf.* Chapter 6, p. 161).

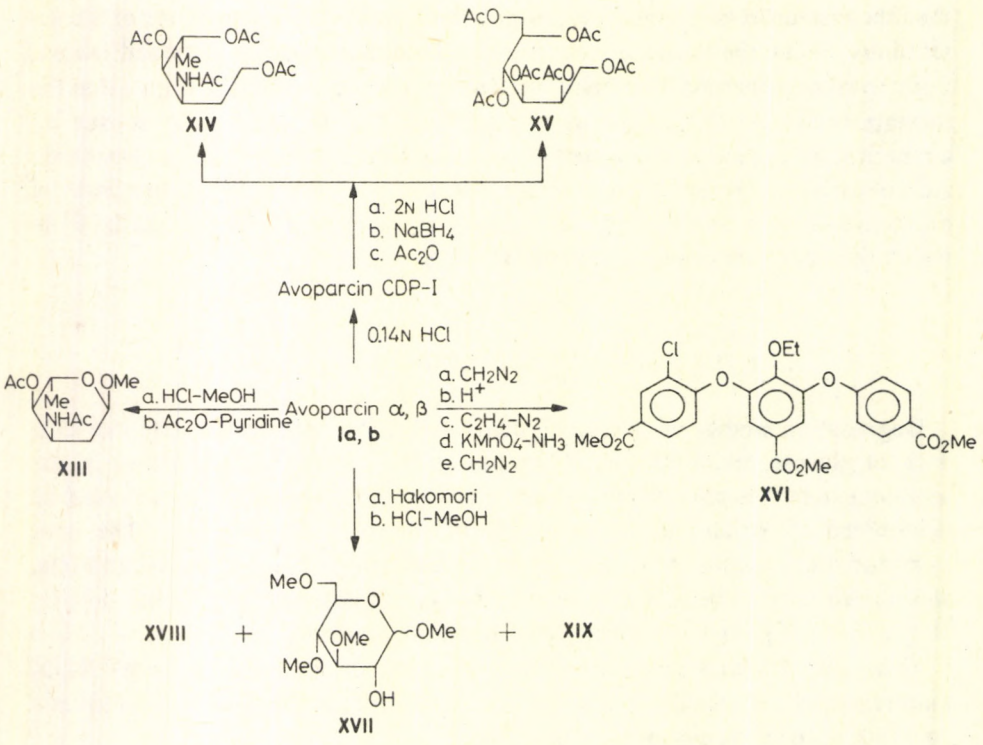
This novel trideoxy-aminosugar was identified in the form of methyl N,O-diacetyl-α-L-ristosaminide (**XIII**) and it was also established that the antibiotic contained two molecules of L-ristosamine.

Recently American chemists have reported [8] the formation of an undesired trimeric product from three molecules of ristosamine and one molecule of ammonia under certain conditions of the isolation process.

Similarly to vancomycin, mild acid hydrolysis of avoparcin yielded a crystalline degradation product designated as CDP-I, and this compound retained about 60% of the antibiotic activity of the parent variants. Studies on the carbohydrate content of CDP-I revealed that only one molecule of L-ristosamine and mannose were present, and these sugars were identified as ristosaminitol peracetate (**XIV**) and mannitol peracetate (**XV**).

Comparative ^{13}C-NMR studies [5] on avoparcin and CDP-I support the assumption that the β-hydroxyl groups of the phenylserine units of the aglycone are glycosylated by ristosamine and mannose. Recent studies [9] have shown that the configuration of both glycosidic linkages is α. Further studies aimed at determining the place of attachment of the other ristosamine and the glucose molecules, and also, the location of the unsubstituted hydroxyl groups.

Reductive alkaline degradation experiments with the permethylated and perethylated antibiotic complex have established that the phenolic hydroxyl groups of the actinoidinic acid unit, and those of the 3-chloro-4-hydroxy- and 4-

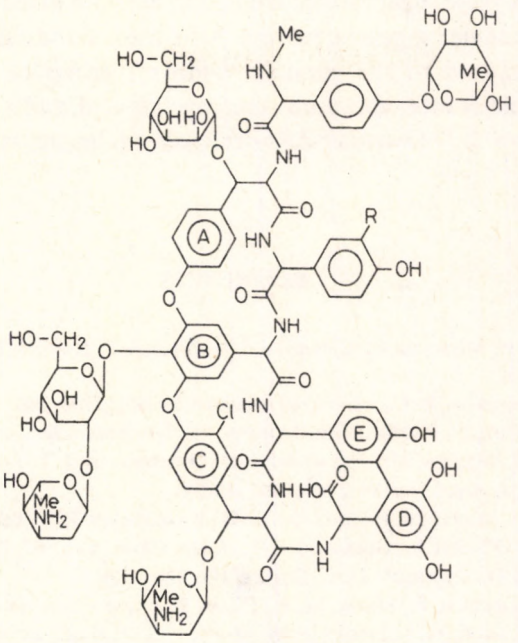


Scheme 21

hydroxyphenylglycine moieties are unsubstituted in avoparcin α and β. At the same time, when avoparcin was treated with excess diazomethane, then hydrolyzed to the aglycone, the latter subjected to treatment with diazoethane and the product oxidized with permanganate and treated again with diazomethane, compound **XVI** was isolated. This result clearly proves that the phenolic hydroxyl group of the central (B) ring of monodechlorovancomycinic acid must be in glycosylated form in the intact antibiotic.

Hakomori permethylation of avoparcin, followed by methanolysis resulted in methyl 3,4,6-tri-O-methyl-D-glucoside (**XVII**), methyl 2,3,4,6-tetra-O-methyl-D-mannoside (**XVIII**) and methyl 2,3,4-tri-O-methyl-L-rhamnoside (**XIX**); no methylated ristosamine was detected. These results indicate that D-glucose is the only nonterminal neutral sugar in the antibiotic, and it is glycosylated by the other L-ristosamine unit at the C-2 hydroxyl group. Based on spectral evidence and biogenetic analogies with vancomycin, American researchers [5, 9] suppose that this 2-O-α-L-ristosaminyl-β-D-glucosyl disaccharide is linked to the phenolic

hydroxyl group of the monodechlorovancomycinic acid moiety in avoparcin α and β (**Ia, b**). Namely, with the exception of the β-attachement between D-glucose and ring B, each glycosidic bond has α-configuration in both variants.



Ia Avoparcin α R=H
Ib Avoparcin β R=Cl

At the same time, however, the positions of the D-mannose and L-ristosamine moieties, attached to the β-hydroxyl groups of the two phenylserine units may be interchanged. Naturally, there are additional structural and stereochemical problems to be solved or made precise in the future.

Structures **Ia** and **Ib** are very similar to those given for vancomycin and even more for the actinoidin variants A and B. In the aglycones of all the three antibiotics the amino acids are involved in a heptapeptide chain forming a tricyclic structure. Instead of the phenylalanine unit of actinoidins A and B, avoparcin α and β contain 4-hydroxy- and 3-chloro-4-hydroxyphenylglycine, respectively. In the molecules of the actinoidin variants these latter two amino acids correspond structurally to the N-terminal phenylsarcosine unit of avoparcin.

On the other hand, ristocetin (ristomycin) and the recently isolated A-35512 B antibiotic contain ristomycinic acid with diphenyl ether linkage and its chloro-substituted analogue, respectively. It seems very likely that these structural units are biosynthesized also from *p*-hydroxyphenylglycine and its derivatives.

Avoparcin α and β are the only tetraglycoside representatives of the vancomycin group of antibiotics. Actinoidin A and B and avoparcin α and β are the only members containing two molecules each of trideoxy-amino-hexose. Although the configurations of these sugars are different, they are built in the antibiotic molecules according to the same biogenetic pattern. Also, the heterodisaccharide unit of each antibiotic is attached to the phenolic hydroxyl group of the central ring of vancomycinic acid or monodechlorovancomycinic acid, and all these disaccharides have a common 2-O-(3-amino-2,3,6-trideoxy-α-L-hexopyranosyl)-β-D-glucosyl-type structure.

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6. RISTOCETIN–RISTOMYCIN

As early as the very first stage of the structural research on the American ristocetin and Soviet ristomycin, isolated with a difference of a few years, these antibiotics were suspected to be identical. This was suggested by their similar names and by the results of early comparative microbiological and chemical investigations [1]. On the basis of the experimental evidence accumulated during the years of structural studies, the possibility of any difference between these antibiotics could be precluded [2]. In this chapter ristocetin and ristomycin, therefore, will be discussed together, and the different names are used rather to indicate the origin of the antibiotic samples.

6.1. ISOLATION

Ristocetin was first isolated at the Abbott Laboratories (USA) by Grundy *et al.* [3] in 1957 from the fermentation broth of *Nocardia lurida*. Six years later this antibiotic was also isolated by Brazhnikova *et al.* [4] at the Research Institute of New Antibiotics (Moscow, USSR) from cultures of *Proactinomyces fructiferi var. ristomycini* and named ristomycin. Later both ristocetin [5] and ristomycin [6] were separated into two biologically active variants, designated as components A and B*.

The isolation technique of crystalline ristocetin was reported by Philip *et al.* [7]and the ion-exchange chromatographic purification of ristomycin was elaborated by Lavrova *et al.* [6]. The sulfate salts of ristocetin A and B were crystallized from aqueous ethanol, whereas crystalline ristomycin and ristomycin sulfate were obtained from water–isopropanol mixture (Fig. 2).

The separation of the ristomycin variants was effected by means of gel-filtration on Sephadex G-25 (Samsonov and Etingov [8]). The industrial production of the antibiotic was elaborated by Ziserman *et al.* [9]. Besides biological activity measurements, polarimetric [10] and UV-photometric [11] methods were also developed for the identification and determination of the active components in the fermentation broth and in the intermediates of the industrial production.

* Earlier Soviet authors [6, 8] used the designation "antibiotic III" and "antibiotic IV" for ristomycin A and ristomycin B, respectively.

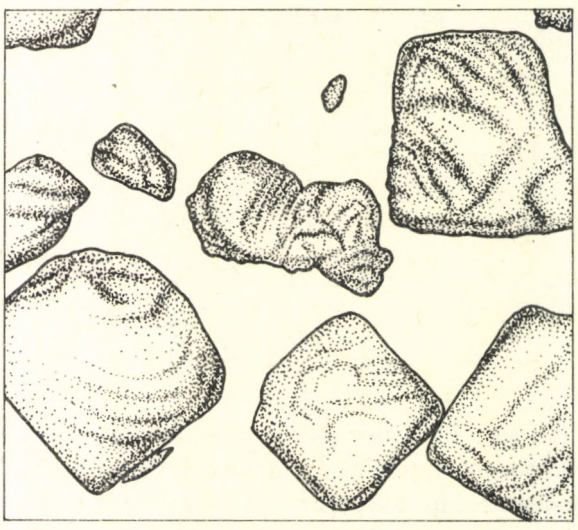


Fig. 2. Crystals of ristomycin A sulfate

6.2. BIOLOGICAL ACTIVITY AND APPLICATION

Ristocetin possesses both *in vitro* and *in vivo* specific antibiotic activity against Gram-positive microorganisms. Although early publications suggested that the antibiotic was also effective against *Mycobacterium tuberculosis*, this effect could not be utilized in therapy. With the exception of a few strains, ristocetin is inactive against Gram-negative bacteria, yeasts, fungi and protozoa [3]. In the end of the 1950's, this antibiotic was considered particularly important, since it was also active against *Staphylococcus aureus*, resistant to most antibiotics (erythromycin, penicillin, streptomycin, tetracyclines) commercially available at that time. Also, microorganisms slowly developed resistance to ristocetin [12]. Particularly quick recovery was observed on the administration of ristocetin in clinical cases of *Pneumococcus pneumoniae* infections [13].

Similarly to ristocetin, ristomycin was also found to be an effective antibiotic possessing practically the same sensitivity and spectrum of activity as ristocetin [14–16]. In animal experiments it was shown to have low toxicity, but caused infiltration on subcutaneous administration [17]. Therefore, in clinical practice it is used in the form of infusion [18] and has been beneficially applied for the treatment of septic endocarditis, purulent meningitis and, in surgery, for the after-treatment of postoperative infections [19].

Variants B of both antibiotics were found three or four times as active as variants A. However, owing to difficulties in the fermentation, only ristocetin A (ristomycin A) is industrially produced and marketed.

The microbiological investigation of the mild acid hydrolysate of ristocetin A and B led to a particularly interesting observation. Philip *et al.* [20] reported that the hydrolysates of ristocetin A and ristocetin B were five and four times, respectively, as active than the parent compounds in animal experiments using the serial dilution method. Recently this finding has been further confirmed by the studies of Kuwahara and Chambers [96]. Additionally, no development of full cross-resistance was observed.

Earlier ristocetin was marketed in Western countries by the Abbott Laboratories (USA) under the trade name "*Spontin*", but now it is no longer applied as an antibacterial agent. Ristomycin is still used for

medical purposes in the USSR under clinical control. Its most important side-effects are: intermittant fever, nausea, unification of the vein-wall, phlebitis and allergic reactions in patients with insufficient renal function.

In 1971 Howard and Firkin [21] observed the absence of ristocetin-induced platelet aggregation (a normal reaction of the antibiotic) in patients suffering from von Willebrand's disease. This disease is a kind of haemophilia of genetic origin and its detection is extremely important, particularly prior to surgical operations. The observation of this effect gave impetus to haematologic research also leading to the application of ristocetin as a laboratory diagnostic agent for characterizing von Willebrand's disease. For this purpose ristocetin has been put on the market by the firms Lundbeck (Denmark) and Bio-Data (USA), under the trade name "*Aggrecetin*". Ristomycin A has been introduced in Hungary for similar application by Boda *et al.* [21–24], demonstrating the identical diagnostic value of the antibiotic with that of ristocetin A.

Ristomycin A is being produced and marketed in Hungary as a laboratory diagnostic agent by the Reanal Fine Chemicals Co. (Budapest) under the trade name "*Aggristin*".

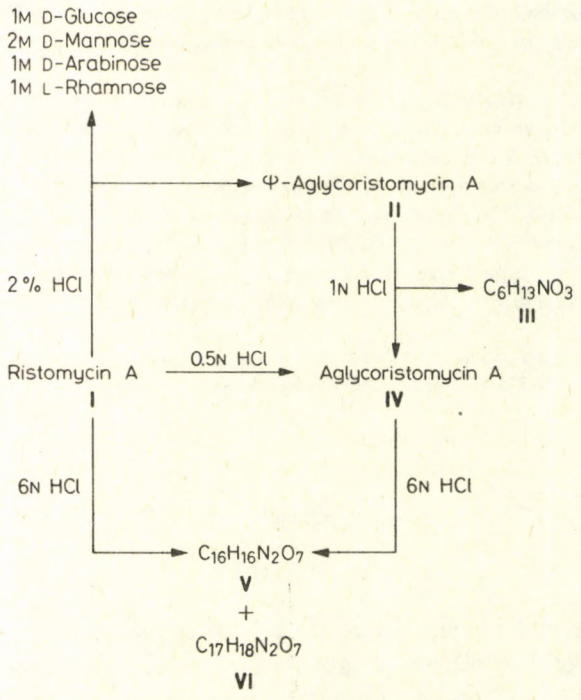
6.3. STRUCTURAL STUDIES

6.3.1. HYDROLYSIS

Although several attempts were made during the past two decades for the determination of the molecular weight of ristocetin [7, 10] and ristomycin [25, 26], the correct value and molecular formula have been given only recently. Californium plasma desorption mass spectral studies by McFarlane [27] showed the molecular weight to be 2063 daltons. If this high performance and rarely used method had not been applied, the exact molecular weight and composition of ristocetin A (ristomycin A) probably still would not be known with certainty.

The first hydrolysis studies on ristocetin (I) were performed by American chemists [7, 20]. Mild sulfuric acid hydrolysis of the antibiotic resulted in D-glucose, D-mannose, D-arabinose and L-rhamnose, their ratio being 2 : 4 : 2 : 2 in ristocetin A, and 2 : 2 : 1 : 2 in ristocetin B, according to quantitative paper chromatographic method [20]. Of the carbohydrates, liberated on hydrolysis, only arabinose was not decomposed by *Salmonella parathyphi*, indicating the D-configuration of this sugar. Contrary to these results, Soviet and Hungarian chemists [1, 28] found a molecular ratio of 1 : 2 : 1 : 1 for the above carbohydrate components of ristomycin A. Additionally, it was shown that D-arabinose was not present in ristomycin B, and also that only one molecule each of the other three neutral sugars was present in this variant. The results proved that ristocetin A and B contain the same neutral sugar components as involved in the molecules of ristomycin A and B [1].

Repeating these experiments with ristocetin A and B, the arabinose content of the antibiotic samples was found to be varying. The discrepancy, therefore, was due not only to the incorrect value of the molecular weight (about 4000) given by Philip *et al.*



Scheme 22

[20], but also to the inhomogeneity of the samples of ristocetin B being contaminated by variant A.

The Bognár group [29] observed the formation of two kinds of aglycones on acid hydrolysis of ristomycin (**I**), (Scheme 22) depending on the concentration of the acid and on the reaction conditions. Mild acid treatment led to the above mentioned neutral sugars and a so-called pseudo-aglycone* (**II**), the continued acid hydrolysis of which yielded one molecule of a new trideoxy-amino-hexose (**III**, L-ristosamine) and the aglycone **IV**. This aglycone could be obtained also directly from the antibiotic (**I**) under more vigorous acidic conditions. Compounds **II** and **IV**, however, were not homogeneous substances as shown by electrophoretic examination. Therefore their composition could be deduced only roughly by calculating the difference between the molecular weight of the intact antibiotic and those of the isolated homogeneous fragments. Later Williams *et al.* [85] isolated ψ-aglycoristocetin A by methanolysis (5% hydrochloric acid in methanol), the purity of which can be checked by HPLC examination. Most recently Nielson *et al.*

* A novel designation introduced by Williams *et al.* [85].

[97] have shown that compound **II** possesses both higher antibacterial activity and toxicity than the parent antibiotic.

More drastic acid hydrolysis of **I** and **IV** resulted in the formation of two aromatic diamino-dicarboxylic acids (**V** and **VI**).

Comparative structural studies on **V** proved the identity of this compound with actinoidinic acid, a component of other members (vancomycin, actinoidins, avoparcins) of the vancomycin group of antibiotics. Compound **VI**, isolated first from ristomycin, was named ristomycinic acid*. Early structural work, performed by Brazhnikova *et al.* [30] demonstrated that the amino acid composition of ristomycin A and B was identical, hence, the structural difference between the two variants arose from a difference in the carbohydrate moieties. The majority of the components of the antibiotic molecule could be explored by acid hydrolysis studies. However, the structure of the remaining building unit, monodechlorovancomycinic acid, could be identified only by reductive alkaline degradation experiments.

6.3.2. CARBOHYDRATES

The results detailed below will show that while the American and Soviet research groups concentrated their efforts primarily on the determination of the structure of the aglycone unit, the Hungarian researchers paid attention first of all to the structure elucidation of the carbohydrate moieties.

6.3.2.1. SEQUENTIAL ATTACHMENT OF NEUTRAL SUGARS

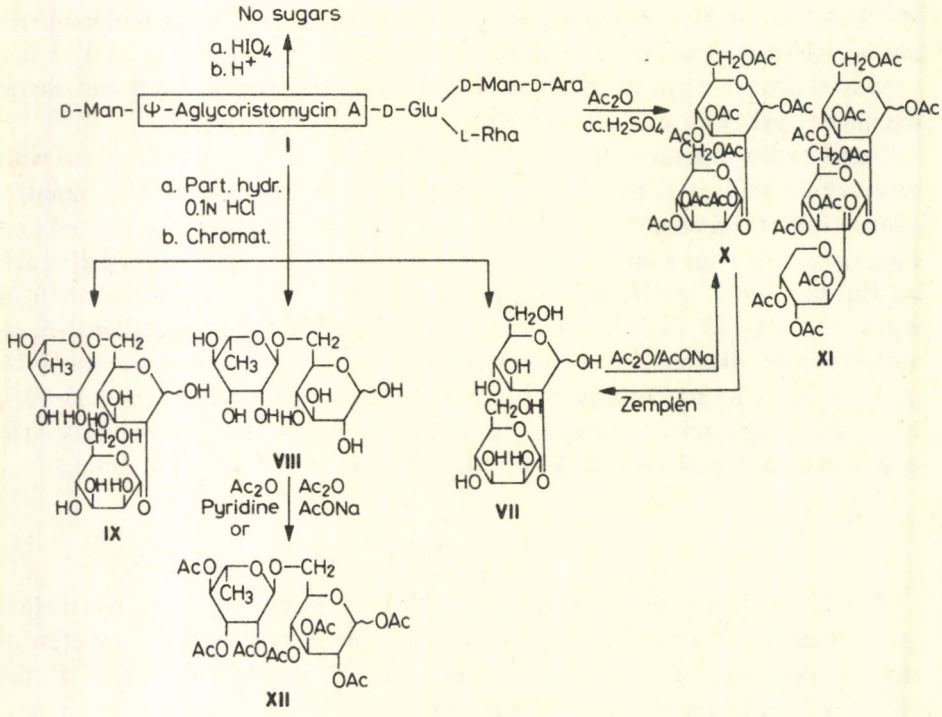
In 1968 Soviet and Hungarian chemists [25] isolated several partially hydrolyzed products by chromatography on Sephadex G-25 of the mild acid hydrolysates of ristomycin A and B.

The fragments obtained from ristomycin A were: D-Man-aglycone, D-Man-aglycone-D-Glu, aglycone-D-Glu-L-Rham, D-Glu-D-Man, D-Glu-L-Rham, L-Rham-D-Glu-D-Man and D-Glu-D-Man-D-Ara, whereas from the hydrolysate of variant B the aglycone moiety, aglycone-D-Man, D-Glu-aglycone-D-Man, aglycone-D-Glu-L-Rham and D-Glu-L-Rham were isolated.

Based on these results it has been concluded that the neutral sugars are attached to the aglycone at two different places in both variants. The structures of the fragments obtained by hydrolysis were established by Sztaricskai *et al.* [31].

Column chromatography on cellulose of the concentrated partial acid hydrolysate of **I** led to the isolation of two disaccharides (**VII** and **VIII**) and a trisaccharide

* Earlier ristomycinic acid was named "amino acid A" and actinoidinic acid was termed "amino acid B" and "amino acid C" by Soviet authors.

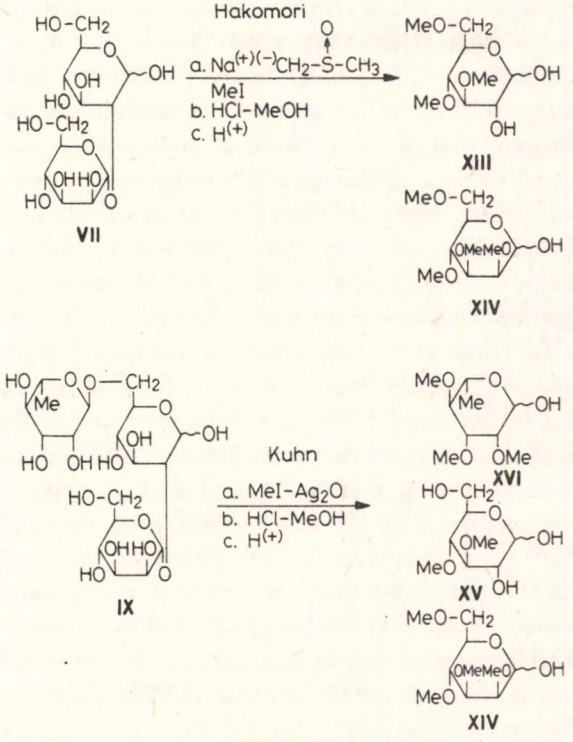


Scheme 23

(**IX**). Acetolysis of the antibiotic resulted in an octaacetyl disaccharide (**X**) and a decaacetyl trisaccharide (**XI**). The product obtained by Zemplén-deacetylation of **X** was found to be identical with **VII** — also isolated from the acid hydrolysate of the antibiotic — and this disaccharide was named *ristobiose*. Acetylation of **VIII** with acetic anhydride in pyridine, or in the presence of sodium acetate, gave **XII**, identified as hepta-O-acetyl-*rutinose* (6-O-α-L-rhamnopyranosyl-D-glucose). This observation and also those described by Gorin and Perlin [32] lead to the conclusion that L-rhamnose is linked by an α-glycosidic bond to the C-6 position of D-glucose in the heterotetrasaccharide side chain of ristomycin A.

In the acid hydrolysate of the product, isolated after the periodate oxidation of ristomycin A, the presence of monosaccharides could not be detected, indicating that the glucose moiety of pyranose structure — representing the branching unit of the heterotetrasaccharide side chain — also contained vicinal diol function oxidizable by periodate. Since L-rhamnose is linked to C-6 of D-glucose, the mannose unit must be attached either through the C-2 or the C-4 hydroxyl group of D-glucose. Enzymatic hydrolysis of **VII**, and Hakomori-methylation followed by

hydrolysis of the resulting octa-methyl derivative led to 3,4,6-tri-O-methyl-D-glucose (**XIII**) and 2,3,4,6-tetra-O-methyl-D-mannose (**XIV**), and hence to the assignation of the structure of *ristobiose* as 2-O-α-D-mannopyranosyl-D-glucose. The previously unknown trisaccharide **IX** was named *ristotriose*. In the hydrolysate of the methylated derivative of ristotriose, obtained by Kuhn's procedure [36], **XIV**,



Scheme 24

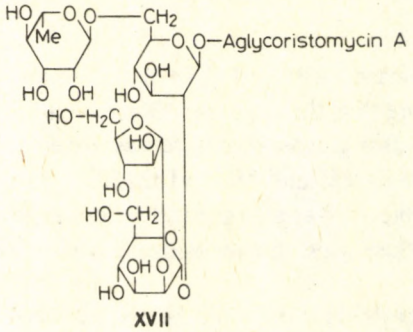
3,4-di-O-methyl-D-glucose (**XV**) and 2,3,4-tri-O-methyl-L-rhamnose (**XVI**) were detected demonstrating that the supposed O-α-L-rhamnopyranosyl-(1→6)-O-α-D-mannopyranosyl-(1→2)-D-glucose structure was correct. The 1→2 attachment of D-mannose to D-glucose in **VII** and **IX**, and therefore in the heterotetrasaccharide side-chain of the antibiotic, was also proved by the experimental fact that neither ristobiose nor ristotriose gave phenylosazone derivative with phenylhydrazine [31].

The other trisaccharide *ristriose* could be obtained only in traces by the partial acid hydrolysis of the antibiotic, since D-arabinose was split off during the isolation procedure. This fact is in good accordance with earlier observations [37, 38]

demonstrating the exceptional lability of β-arabinofuranosides under acidic conditions.

However, in the knowledge of the structures of **VII** and **IX** and also that of **XVI**, detected in the hydrolysate of permethylated ristomycin A, it was reasonable to conclude that the terminal unit of the heterotetrasaccharide side chain of the antibiotic was D-arabinose. If D-arabinose were attached to D-glucose, this latter could not undergo decomposition on periodate oxidation. On the basis of similar considerations a 1→3 type attachment of D-arabinose to D-mannose can also be precluded. The presence of 3,4,6-tri-O-methyl-D-mannose besides compounds **XIV–XVI** in the hydrolysate of the permethylated antibiotic suggested that D-arabinose, the terminal unit of the tetrasaccharide side-chain, was attached to D-mannose with a 1→2 linkage. At the same time it was shown that compound **XIV**, present in the hydrolysate, originated from the D-mannose unit attached separately to the aglycone, and it was not a product of accidental incomplete methylation. Four years later this result was supported also by Williams *et al.* [2] by means of mass spectroscopic investigation of the partial hydrolysis products of ristocetin A.

Comparative ^{13}C-NMR studies on model compounds by Neszmélyi *et al.* [34] have shown that the chemical structure of deca-O-acetylristriose (**XI**), obtained from ristomycin A by acetolysis, is O-(2,3,4-tri-O-acetyl-β-D-arabinopyranosyl)-(1→2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→2)-O-(1,3,4,6-tetra-O-acetyl-α-D-glucopyranose), having the D-arabinose unit in $^{1}C_{4}$ (D) conformation. On the basis of more recent comparative ^{13}C-NMR studies using the alkaline degradation product (S_4-Ph_3)* — containing the tetrasaccharide — and methyl α-D-arabinofuranoside and phenyl β-D-glucopyranoside as model compounds, Sztaricskai *et al.* [47] have concluded that the composition of *ristotetrose* — the branched heterotetrasaccharide side chain of the antibiotic — is O-α-D-Araf-(1→2)-O-α-D-Manp-(1→2)-O-[α-L-Rhap]-(1→6)-O-β-D-Glcp (**XVII**), which is attached to the aglycone *via* a β-glycosidic linkage:



XVII

* The structure investigation of S_4-Ph_3 (**CVI**) is detailed on pp. 186–187.

Based on the above results it may be suggested that the terminal α-D-Araf unit of **VII** is converted into a β-D-Arap moiety under the conditions of the acetolysis *via* a mechanism involving ring expansion and inversion of the anomeric centre, to afford deca-O-acetylristriose (**XI**).

This suggestion has been considerably supported by recent acetolysis experiments with ristocetin A [28], which resulted in the isolation of dodecaacetylristotetrose, containing the entire sugar side chain.

In the knowledge of the ratio of the neutral sugars and the mild acid hydrolysis products of ristomycin B [25], it can be proposed that the side chain of this latter variant of the same biological origin differs from **XVII** in ristomycin A only in the lack of the 2-O-α-D-arabinofuranosyl-α-D-mannopyranosyl unit [31]. Similar data have not been reported so far for ristocetin B.

6.3.2.2. SYNTHESIS OF RISTOBIOSE AND RISTOTRIOSE

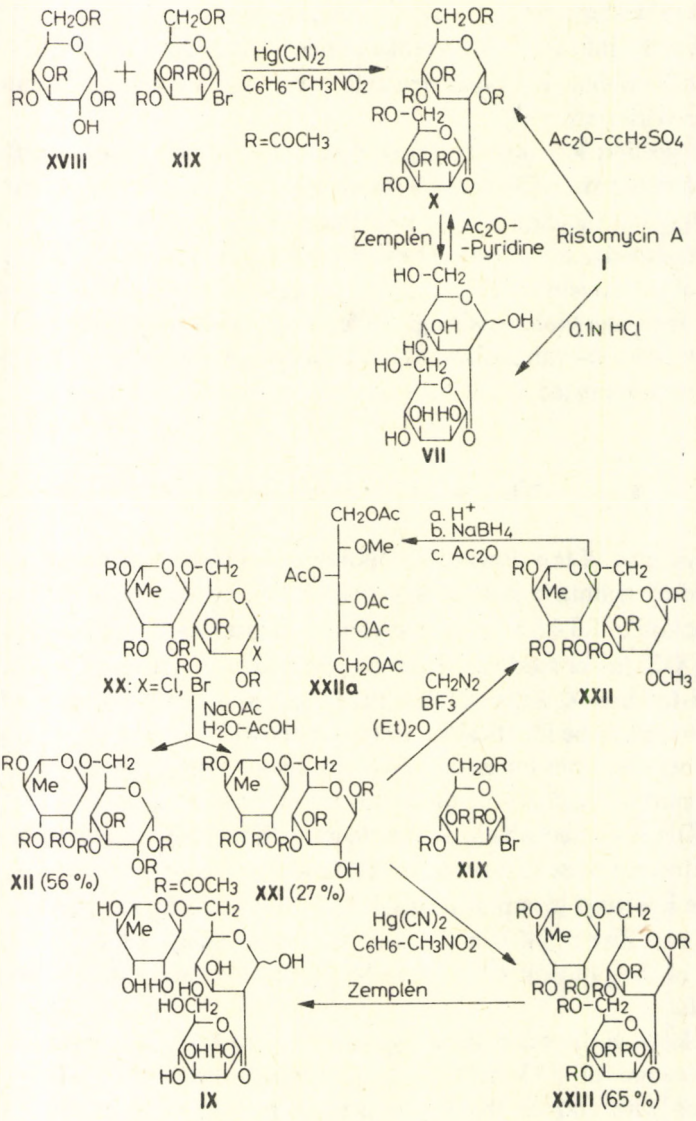
The structures of the previously unknown ristobiose and ristotriose were also established by definitive syntheses, accomplished by Sztaricskai *et al.* [33].

The reaction of 1,3,4,6-tetra-O-acetyl-α-D-glucose (**XVIII**) with α-acetobromo-D-mannose (**XIX**) under modified Helferich conditions [39] resulted in 74% of octa-O-acetyl-α-ristobiose (**X**, Scheme 24a). Product **X** and its O-deacetylated derivative **VII** were found to be identical in every respect with the corresponding authentic samples obtained from the antibiotic (**I**) by acetolysis and partial acid hydrolysis.

As the starting material of the synthesis of *ristotriose*, hexa-O-acetylrutinosyl halide (**XX**) was chosen which, on treatment with sodium acetate in aqueous acetic acid, gave hepta-O-acetyl-α-rutinose (**XII**) and also hexa-O-acetyl-β-rutinose (**XXI**) with a free hydroxyl group at position C-2. This method was previously used by Helferich and Zirner [40] in the case of octa-O-acetylcellobiose, however, the extension of the procedure for octa-O-acetyl-lactose and -maltose has remained unsuccessful.

The presence of the free hydroxyl group at C-2 in **XXI** was confirmed by the ^{1}H-NMR spectrum of 2-O-methyl-hexa-O-acetyl-β-rutinose (**XXII**), obtained on methylation with diazomethane–boron trifluoride etherate. Further evidence for the structure of **XXI** was obtained from the gas chromatographic identification of 2-O-methyl-1,3,4,5,6-penta-O-acetylsorbitol (**XXIIa**), prepared from **XXII** by successive acid hydrolysis, reduction with sodium borohydride and acetylation.

Mannosylation of **XXI** with **XIX** gave deca-O-acetyl-ristotriose (**XXIII**) in 65% yield. The physical data of this latter compound and also those of ristotriose (**IX**), prepared by O-deacetylation, were found to be identical with the data of the respective authentic samples isolated from the antibiotic.

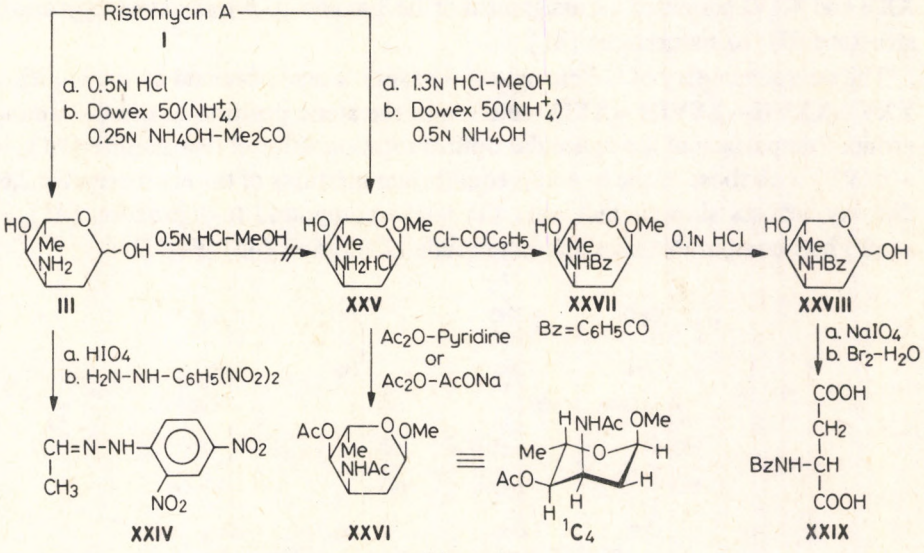


Scheme 24a

These results not only support the structure of ristobiose and ristotriose, but confirm, again that D-mannose is attached to the C-2 hydroxyl group of D-glucose *via* an α-glycosidic linkage in the tetrasaccharide side chain of the antibiotic. Both oligosaccharides have been first observed as natural compounds being the structural components of an antibiotic [33].

6.3.2.3. RISTOSAMINE

It has been mentioned that the continued mild acid hydrolysis of the ψ-aglycone (**II**), a fragment of the antibiotic containing none of the neutral sugars, results in an unknown trideoxy amino sugar. One molecule of this sugar occurs in both antibiotics, and because of the presence of the amino group it was first named aminofragment and later *ristosamine* (**III**). The isolation and structure investigation of **III** were first published by Bognár *et al.* [41–43] (Scheme 25).



Scheme 25

Ristosamine gives positive Fehling, Tollens, Keller–Kiliani and iodoform reactions, as well as the xanthydrol colour test. Oxidation of this new amino sugar with periodic acid leads to the consumption of about two moles of the reagent. The formation of formaldehyde could not be detected, however, acetaldehyde was isolated by distillation from the reaction mixture and identified as the 2,4-dinitrophenylhydrazone (**XXIV**). This observation, and also the positive iodoform test indicate that a methyl group is present at C-5 of the ristosamine molecule. Although direct conversion of **III** to methyl ristosaminide (**XXV**) could not be achieved with methanol–hydrochloric acid, methanolysis of the antibiotic directly produced the required methyl glycoside. The consumption of about 0.7 mol of periodate by methyl ristosaminide indicated a pyranoside ring with either C-2 or C-4 "deoxy function". The positive Keller–Kiliani and xanthydrol tests of ristosamine

(III) suggested that this compound was a 2-deoxy sugar derivative. Acetylation of XXV with acetic anhydride gave crystalline methyl N,O-diacetylristosaminide (XXVI) and benzoylation with benzoyl chloride afforded methyl N-benzoylristosaminide (XXVII). Mild acid hydrolysis of XXVII resulted in crystalline N-benzoylristosamine (XXVIII). Treatment of this latter with sodium periodate, followed by further oxidation with aqueous bromine led to the isolation of microcrystalline N-benzoyl-D-(−)-aspartic acid (XXIX), the IR spectrum and layer chromatographic behaviour of which were identical with that of the authentic sample [44]. These chemical data and results of mass spectrometric studies with XXV and XXVI permitted the assignment of the 3-amino-2,3,6-trideoxyhexopyranose structure (III) to ristosamine [41].

The stereochemistry of N-benzoyl-D-(−)-aspartic acid obtained by the sequence XXV→XXVII→XXVIII→XXIX determines the steric position of the C-3 amino group. Comparison of the molecular optical rotation value of ristosamine ($[M]_D = -62.97°$) with those of the α- and β equilibrium mixtures of the eight possible 2,6-dideoxy-hexoses showed that only the L-*ribo* compound (L-digitoxose, $[M]_D = -68°$) had comparable sign and magnitude of rotation [45] (Fig. 3).

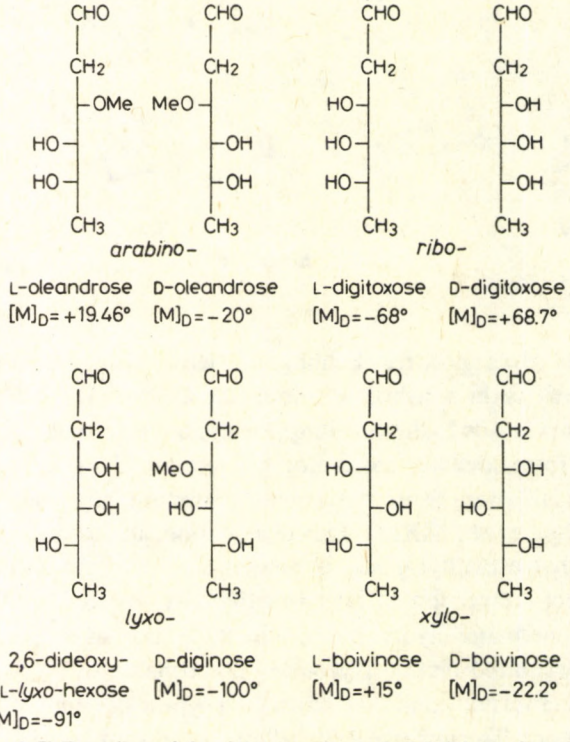


Fig. 3. $[M]_D$-values of the eight stereoisomeric 2,6-dideoxy-hexoses

The infrared spectroscopic investigations showed that the $\nu(C—OCH_3)_s$ band of the glycosidic linkage in compounds **XXV**, **XXVI** and **XXVII** appeared in the region characteristic of *axial* bonds [45]. According to the values of the ν(C—O) frequencies of the C—OH group, this bond was assigned to be *equatorial*. The value of the ν(OH) resonance at 3400–3450 cm^{-1} (measured in solution) did not change on dilution, indicating that the hydroxyl group was involved in intramolecular hydrogen bonding.

The ^{1}H-NMR spectrum of **XXVI** recorded at 220 MHz definitely confirmed the structure and configuration proposed [41, 42]. The values of the $J_{3,4}$ and $J_{4,5}$ coupling constants unequivocally showed the *cis* orientation of H-3 and H-4 and the *trans–diaxial* relation between H-4 and H-5. Such steric relation of these protons can only be attributed to an α-L-*ribo* (^{1}**C**$_4$ conformation) or β-D-*ribo* (^{4}**C**$_1$ conformation) configuration of **XXVI**. Since the molecular optical rotation of ristosamine excludes the β-D-*ribo* configuration, the structure of methyl N,O-diacetylristosaminide (**XXVI**) has been assigned as methyl 3-acetamido-4-O-acetyl-2,3,6-trideoxy-α-L-*ribo*-hexopyranoside, and its favoured conformation in both chloroform and benzene solution is ^{1}**C**$_4$. The amino sugar, isolated later from ristocetin, was found to be identical with L-ristosamine [28].

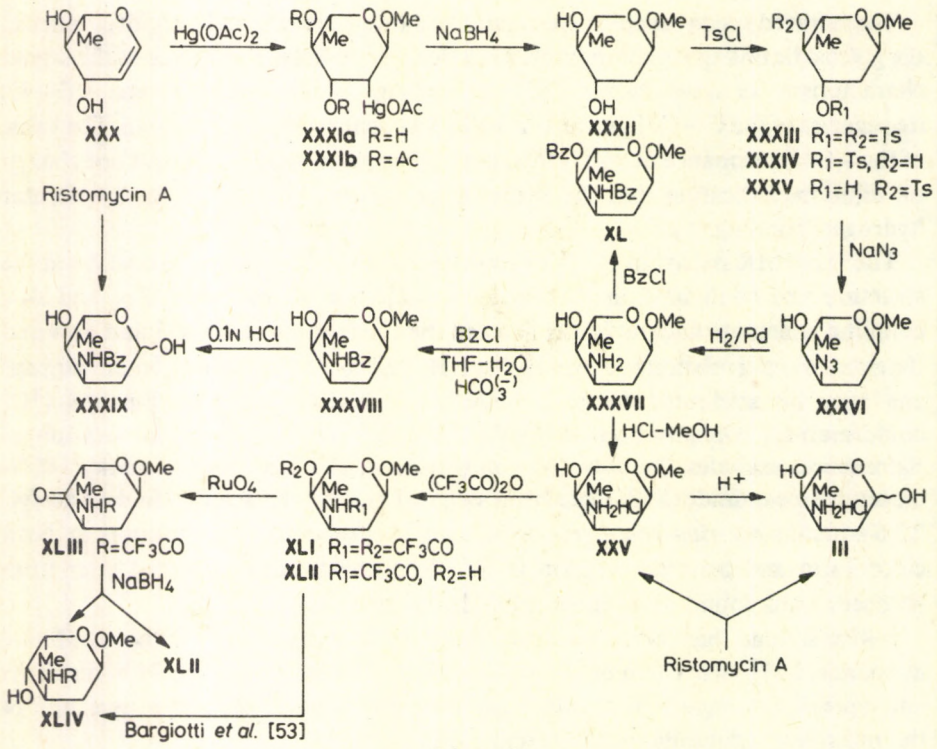
L-Ristosamine has been recently found as a constituent of the antibiotic avoparcin [48] (see Chapter 5). It is also to be noted that the N,N-dimethyl analogue of this sugar, megosamine, has been identified as an amino sugar unit of the macrolide antibiotic megalomycin [49].

6.3.2.3.1. SYNTHESIS OF L-RISTOSAMINE

Soon after the elucidation of the structure of L-ristosamine, the first definitive synthesis of this sugar was also reported by Sztaricskai *et al.* [50–52] (Scheme 26).

Methoxymercuration of L-rhamnal (**XXX**) gave **XXXIa**, which was also characterized in the form of the corresponding diacetyl derivative **XXXIb**. The high values (9.0–9.5 Hz) of the $J_{3,4}$ and $J_{4,5}$ coupling constants in the ^{1}H-NMR spectrum of both compounds indicated the ^{1}**C**$_4$ (L) conformation, and the low values of $J_{1,2}$ and $J_{2,3}$ showed the C-1 methoxyl- and C-2 acetoxy-mercury groups to be *trans–diaxial* in **XXXIa** and **XXXIb**.

Reductive demercuration of **XXXIa** with sodium borohydride gave methyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside (**XXXII**), which on treatment with *p*-toluenesulfonyl chloride in pyridine afforded 4–5% of **XXXIII** and methyl 2,6-dideoxy-3-O-*p*-toluenesulfonyl-α-L-*arabino*-hexopyranoside (**XXXIV**) as the main product. Although this latter sugar was contaminated by a small amount of **XXXV**, it could be converted, without purification, into crystalline methyl 3-azido-2,3,6-



Scheme 26

trideoxy-α-L-*ribo*-hexopyranoside (**XXXVI**) in 36% yield, upon treatment with sodium azide in N,N-dimethylformamide.

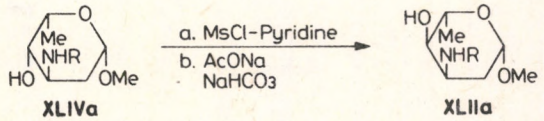
The presence of the azido group in **XXXVI** was clearly demonstrated by the elemental nitrogen content and also by the intensive ν_{C-N} band in the IR spectrum at 2100 cm^{-1}. The location of the azido group at position 3 was proved by mass spectrometry. Although no molecular ion was detected, the intensive loss of N_3 from the *m/e* 155 ion supported the allylic (C-3) position of the azido group to the double bond of the $[M—CH_3OH]^+$ ion. The high value (9 Hz) observed for $J_{4,5}$ in **XXXVI** indicated the $^1C_4$ (L) conformation of this derivative. Catalytic hydrogenation of **XXXVI** gave the extremely hygroscopic, crystalline methyl 3-amino-2,3,6-trideoxy-α-L-*ribo*-hexopyranoside (**XXXVII**; methyl α-L-ristosaminide). The mass spectrum of this compound was identical with that of methyl α-L-ristosaminide isolated from the antibiotic [41–43]. The hydrochloride of **XXXVII** was obtained by the addition of a calculated amount of hydrochloric acid in methanol, and the physical data of the product were identical with those of methyl α-L-ristosaminide hydrochloride (**XXV**), prepared by the methanolysis of ristomycin A.

Hydrolysis of **XXV** with hydrochloric acid gave L-ristosamine hydrochloride (**III**). Benzoylation of **XXXVII** using the Schotten–Baumann method and mild acid hydrolysis of the resulting **XXXVIII** yielded 3-benzamido-2,3,6-trideoxy-L-*ribo*-hexopyranose (**XXXIX**, N-benzoyl-L-ristosamine). Both **III** and **XXXIX** were found to be identical, in every respect, with the corresponding samples originating from the antibiotic [41, 50].

On the other hand, benzoylation of **XXXVII** in pyridine resulted in the hitherto unknown methyl N,O-dibenzoyl-L-ristosaminide (**XL**).

The availability of **XXV** by synthesis allowed the preparation of the L-*xylo* isomer (i.e. the C-4 epimer) of ristosamine *via* the reaction sequence **XXV**→**XLI**→**XLIII**→**XLIV** [51, 52]. Italian and Canadian researchers [53] prepared methyl 2,3,6-trideoxy-3-trifluoroacetamido-α-L-*xylo*-hexopyranoside (**XLIV**) by the inversion of configuration of the C-4 hydroxyl group of **XLII**.

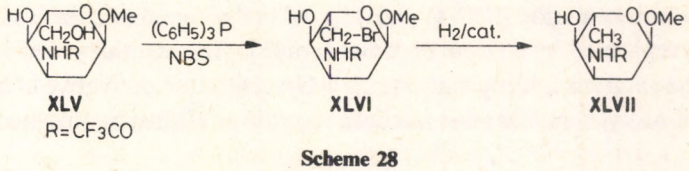
French chemists [59] synthesized the β-anomer of **XLII**, methyl 3-acetamido-2,3,6-trideoxy-β-L-*ribo*-hexopyranoside (**XLIIa**) in a similar, but reversed way, starting from methyl 3-acetamido-2,3,6-trideoxy-β-L-*xylo*-hexopyranoside (**XLIVa**) as shown in Scheme 27.



Scheme 27

Lee *et al.* [54] also prepared L-ristosamine using an essentially similar route to those detailed above in Scheme 26. For the synthesis of a new semisynthetic anticancer anthracycline glycoside antibiotic, L-ristosamine was also prepared by the Arcamone group [55]. Stereospecific glycosylation of daunomycinone with this sugar led to a novel antibiotic analogue, protected by a patent [56].

The key step of this method (Scheme 28) was the deoxygenation of methyl 2,3-dideoxy-3-trifluoroacetamido-α-L-*ribo*-hexopyranoside (**XLV**) at C-6 by N-bromosuccinimide-triphenylphosphine in N,N-dimethylformamide to obtain the 6-

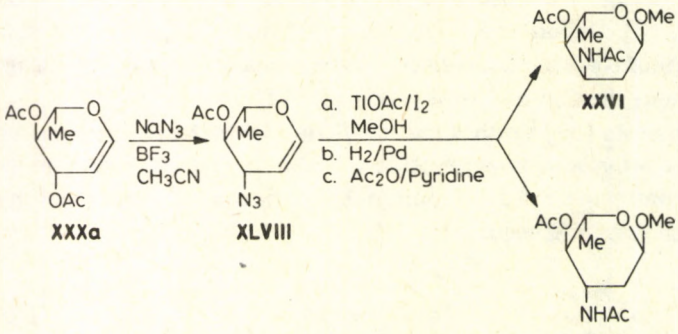


Scheme 28

bromo compound **XLVI**. The catalytic hydrogenation of this latter resulted in methyl N-trifluoroacetyl-α-L-ristosaminide (**XLVII**).

These works are considered important not only from the point of view of the structure investigation of ristomycin, but also because of the new promising possibilities of application of these sugars, e.g., for the preparation of antibiotic analogues, prompting the extension of synthetic research work in this field.

For the synthesis of L-ristosamine German authors [57] elaborated a method involving the generation of the C-3 amino and C-2 deoxy functions in one reaction step (Scheme 29). Boron trifluoride catalyzed addition of sodium azide to 3,4-di-O-



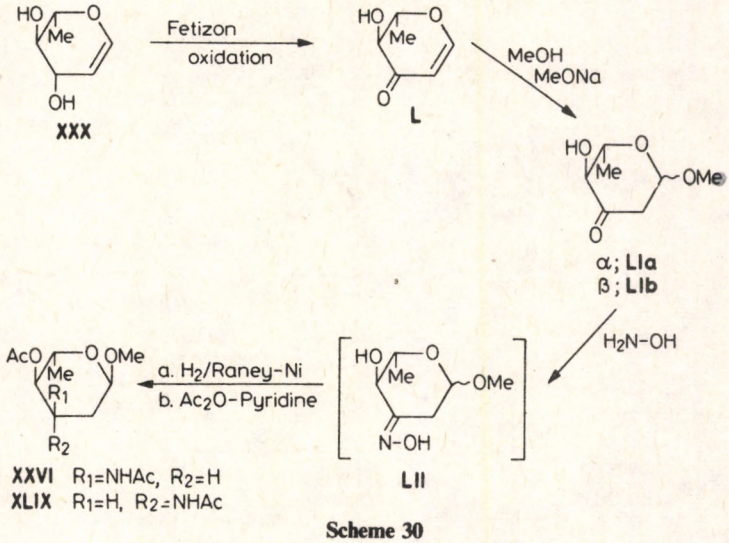
Scheme 29

acetyl-L-rhamnal (**XXXa**) gave — after allylic rearrangement — the epimeric mixture of the C-3 azides (**XLVIII**). Iodomethoxylation of this latter in the presence of thallium acetate, followed by reduction and acetylation afforded methyl N,O-diacetyl-α-L-ristosaminide (**XXVI**) and the corresponding L-acosamine derivative (**XLIX**) in 25 and 39% yield, respectively.

The most simple and convenient route for the synthesis of **XXVI** has been recently worked out by Pelyvás *et al.* [58] (Scheme 30).

Oxidation of L-rhamnal (**XXX**) with Fetizon reagent gave crystalline 1,5-anhydro-2,6-dideoxy-L-*erythro*-hex-1-en-3-ulose (**L**) which was subjected to Michael-type methanol addition to obtain a 15 : 1 mixture of methyl 2,6-dideoxy-α-L-*erythro*-hexopyranoside-3-ulose (**LIa**) and the corresponding β-anomer (**LIb**). This mixture was converted into the oxime **LII**, the reduction of which in the presence of Raney-nickel, followed by acetylation yielded 79% of methyl N,O-diacetyl-α-L-ristosaminide (**XXVI**) and 15% of the acosaminide derivative **XLIX**, which were separated by column chromatography. An essentially similar method, involving the catalytic hydrogenation of a 4-O-substituted derivative of α- **LII**, and leading to N-acetyl-L-ristosamine has been recently published by Brimacombe *et al.* [98].

In 1980 Fuganti and his group [59a] elaborated a non-carbohydrate based synthesis of N-benzoyl-L-ristosamine starting from D-tartaric acid. The most important steps of this method have been discussed in connection with the synthesis of L-acosamine (see pp. 130–131).



Scheme 30

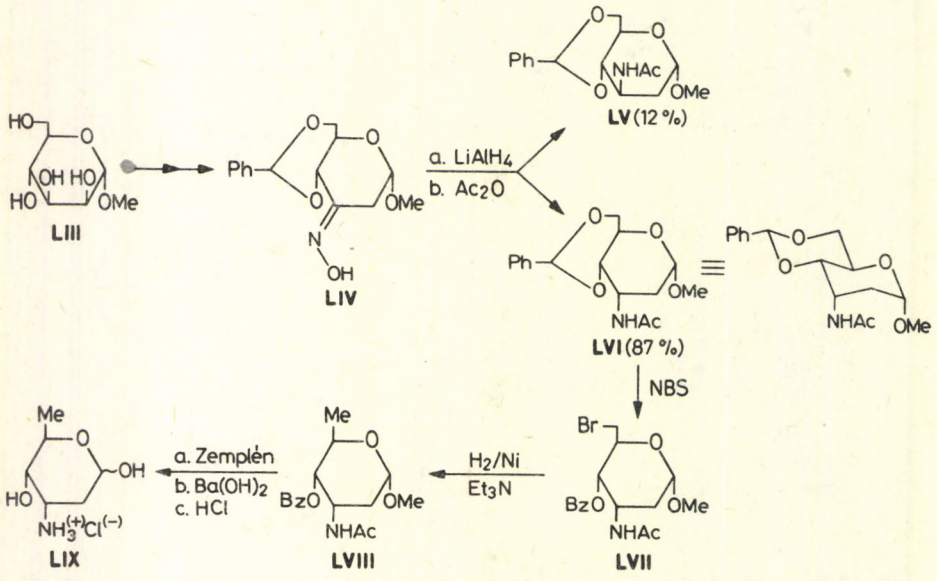
Owing to increasing interest in the application of deoxy amino sugars for the development of synthetic and semisynthetic medicines, additional methods are expected to be worked out for the preparation of L-ristosamine and its derivatives. Such efforts of carbohydrate chemists have resulted in the elaboration of methods for the production of enantiostereoisomers of these amino sugars.

6.3.2.3.2. SYNTHESIS OF D-RISTOSAMINE

The first synthesis of D-ristosamine was reported by Horton and Weckerle [60] in 1976. Methyl α-D-mannopyranoside (**LIII**) was converted (Scheme 31) in several known steps into methyl 4,6-O-benzylidene-2-deoxy-α-D-*erythro*-hexopyranoside-3-ulose-*syn*-oxime (**LIV**) which was reduced with lithium aluminium hydride to obtain, after acetylation, 87% of the D-*ribo* isomer **LVI** and 12% of the *arabino* compound **LV**. The high stereospecifity of this reaction can be explained by the inhibition of the attack of the hydride anion ($H:^-$) from the *axial* side of the pyranoside ring, caused by the *β–trans–axial* effect of the C-1 glycosidic group. Hence, the nucleophile attacks from the *equatorial* side, resulting in the formation of

axially oriented amino function. The intermediate **LVI** was used for the synthesis of both L-daunosamine [61] and D-ristosamine [60] by the Horton group.

Treatment of **LVI** with N-bromosuccinimide, according to the method worked out by Hanessian *et al.* [62] for the ring cleavage* of 4,6-O-benzylidenehexopyranosides, afforded the 6-bromo-6-deoxy sugar **LVII**. Hydrogenation of this



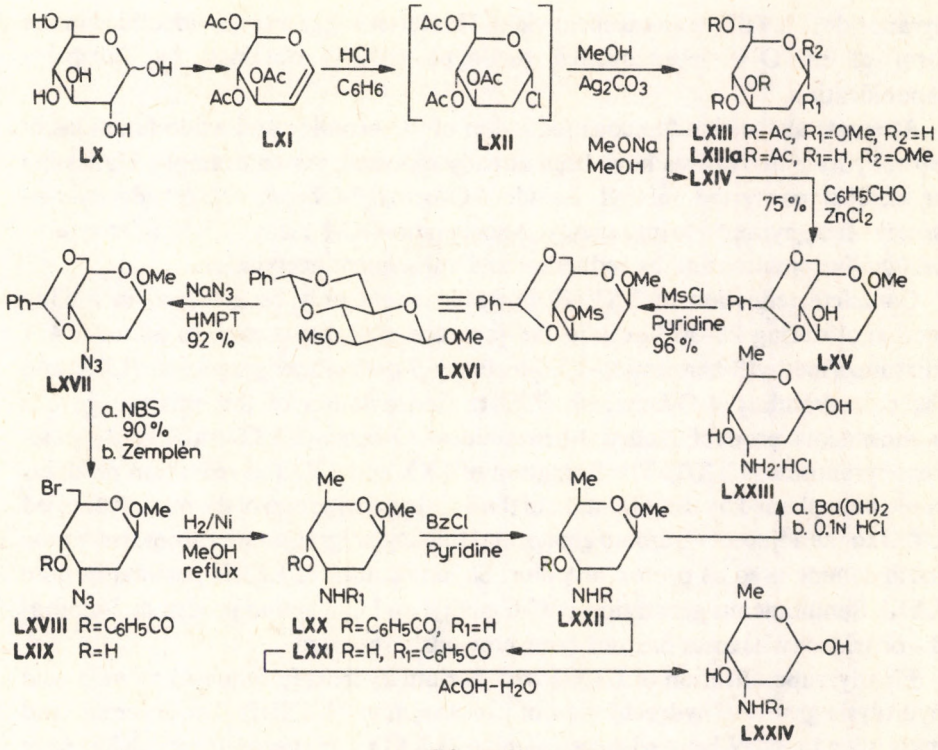
Scheme 31

latter under pressure in the presence of Raney-nickel and triethylamine, followed by Zemplén saponification, N-deacylation with barium hydroxide and mild acid hydrolysis led to the desired D-ristosamine (**LIX**).

In 1977 Pelyvás *et al.* [63, 64] and the Baer group [65] reported — independently of each other — a novel method for the synthesis of D-ristosamine (**LIX**) starting from the cheap sugar D-glucose (**LX**) (Scheme 32).

Addition of hydrochloric acid to 3,4,6-tri-O-acetyl-D-glucal (**LXI**), prepared from **LX**, in dry benzene, followed by glycosylation of the resulting chloride **LXII** with methanol in the presence of silver carbonate gave a 3:1 mixture of the 2-

* The bromine atom, formed from the reagent, attacks the benzylic hydrogen atom of the benzylidene acetal presumably in a reaction following radical mechanism. The resulting unstable bromoacetal dissociates into bromide and 2-phenyl-1,3-dioxolenium ions, and the attack of the latter at C-6 leads to the formation of **LVII**.



Scheme 32

deoxyglycosides **LXIII** and **LXIIIa**. The desired optically pure methyl 3,4,6-tri-O-acetyl-2-deoxy-β-D-*arabino*-hexopyranoside (**LXIII**) could be isolated in 41% yield, based on **LXI**. Zemplén saponification of **LXIII** afforded **LXIV** in nearly quantitative yield, which was converted into methyl 4,6-O-benzylidene-2-deoxy-β-D-*arabino*-hexopyranoside (**LXV**) by treatment with benzaldehyde and anhydrous zinc chloride. Mesylation of this latter with methanesulfonyl chloride yielded the key-intermediate 3-O-mesylate **LXVI** in excellent yield, and this was allowed to react with sodium azide in hexamethylphosphoric triamide to obtain 92% of methyl 3-azido-4,6-O-benzylidene-2,3-dideoxy-β-D-*ribo*-hexopyranoside (**LXVII**). Earlier Kovar *et al.* [66] reported that the same displacement reaction of the corresponding α-anomer could be performed only in 15–25% yield because of a *β–trans–axial* effect involving the *axial* glycosidic methoxyl group. Similar results were obtained by Overend *et al.* [67] with the respective α-glycoside containing O-*p*-bromobenzenesulfonyloxy function at position C-3.

Ring cleavage of the benzylidene acetal group of **LXVII** with N-bromosuccinimide gave methyl 3-azido-4-O-benzoyl-6-bromo-2,3,6-trideoxy-β-D-*ribo*-hexo-

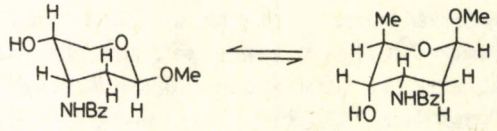
pyranoside (**LXVIII**) in an excellent yield. This latter sugar was also identified in the form of its O-4 debenzoylated analogue (**LXIX**) obtained by Zemplén-saponification.

Attempts at the simultaneous reduction of the bromine and azido functions of carbohydrate derivatives have been already reported. As an example, Hanessian *et al.* [68] converted methyl 2-azido-4-O-benzoyl-6-bromo-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside into methyl 2-acetamido-4-O-benzoyl-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside by reduction and subsequent acetylation.

Complete reduction of **LXVIII**, however, could only be achieved in boiling methanol during 20–30 hours in the presence of Raney-nickel, to afford a 4:1 mixture of methyl 3-benzamido-2,3,6-trideoxy-β-D-*ribo*-hexopyranoside (**LXX**) and the corresponding 4-O-benzoate (**LXXI**). Benzoylation of this mixture gave a homogeneous product: methyl 3-benzamido-4-O-benzoyl-2,3,6-trideoxy-β-D-*ribo*-hexopyranoside (**LXXII**). The formation of **LXX** and **LXXI** as reduction products could be explained by the migration of the 4-O-benzoyl group of the initially formed **LXXI** to the adjacent *cis* amino group, and this acyl migration was promoted by the triethylamine used as proton acceptor. Saponification of **LXXII** resulted in pure **LXXI**. Simultaneous generation of C-6 methyl and C-3 amino groups in 3-amino-di- or trideoxy-hexoses has not been previously reported.

Finally, saponification of **LXXII** with barium hydroxide, followed by mild acid hydrolysis gave the hydrochloride of D-ristosamine (**LXXIII**). The infrared- and mass spectra of N-benzoyl-D-ristosamine (**LXXIV**), prepared from **LXXI**, were identical with those reported for the corresponding enantiomer, obtained either from the antibiotic ristomycin A, or by synthesis.

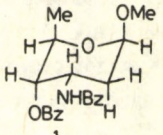
The values of the ^{1}H-NMR coupling constants observed for **LXX** demonstrated a $^4\mathbf{C}_1$ (D)$\rightleftharpoons{}^1\mathbf{C}_4$ (D) equilibrium strongly shifted towards the $^4\mathbf{C}_1$ (D) conformation in deuterochloroform:



$^4\mathbf{C}_1$ (D) $^1\mathbf{C}_4$ (D)

LXX

At the same time, the low value of $J_{4,5}$ and the *axial* orientation of H-3 clearly demonstrated that the $^1\mathbf{C}_4$ (D) conformation is strongly favoured for the N,O-dibenzoyl derivative **LXXII** in deuterochloroform:



$^1\mathbf{C}_4$ (D)

LXXII

For the synthesis of a configurational analogue of daunomycin containing D-ristosamine, in 1979 Horton *et al.* [69] stereospecifically glycosylated daunomycinone with a suitably protected glycal derivative prepared from D-ristosamine. However, the comparative biological tests showed that this analogue had less anticancer activity than adriamycin or daunomycin.

Although these latter results are not closely related to the structure elucidation of ristomycin, they well indicate the current trend where applied drug research is promoted by pure chemical studies of natural products.

6.3.3. AGLYCONE

6.3.3.1. ACID HYDROLYSIS, HYDROGENOLYSIS

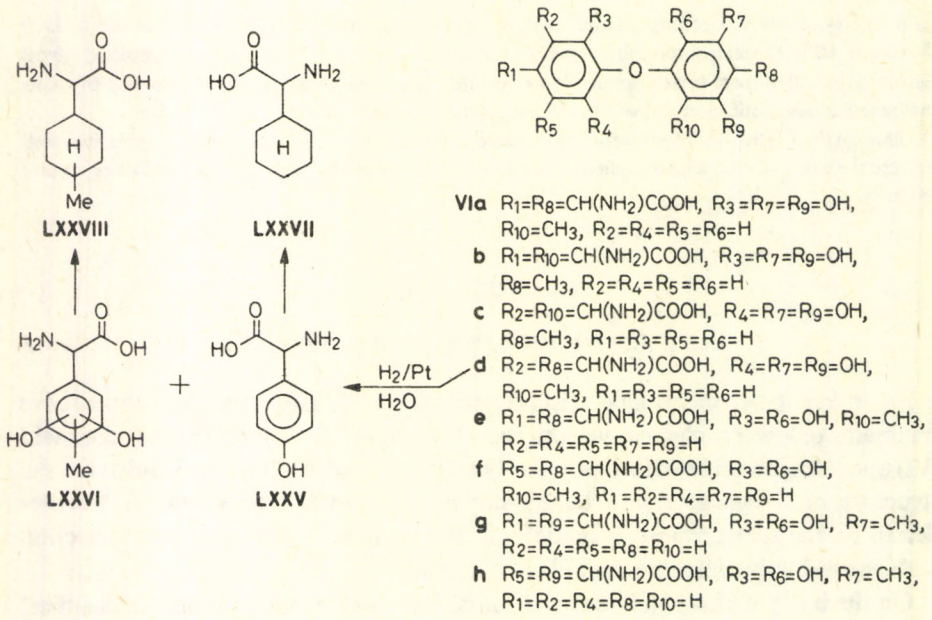
As it has been mentioned, ristomycin, ristocetin and their aglycones gave fragments of lower molecular weight, i.e. actinoidinic acid (**V**) and ristomycinic acid (**VI**) upon drastic acid hydrolysis. It has been mentioned in Chapters 3 and 4 that the structure of **V** was found to be 2,3′-diglycyl-4,6,6′-trihydroxydiphenyl. Further details on the structural investigation of this fragment will be given in connection with the reductive alkaline degradation studies.

On the basis of elemental analytical data, ^{1}H-NMR spectroscopic and chemical degradation studies, in 1968 Lomakina *et al.* [70] proposed four alternative structures [**VIa–d**] for the composition of ristomycinic acid. On hydrogenolysis over platinum catalyst ristomycinic acid decomposed into **LXXV** and **LXXVI**, which on further reduction afforded cyclohexylglycine (**LXXVII**) and methylcyclohexylglycine (**LXXVIII**), respectively (Scheme 33). These experimental results, and also the band observed at 1080 cm^{-1} in the infrared spectrum of ristomycinic acid led to the hypothesis that this compound was built up from **LXXV** and **LXXVI**, attached to each other by a diphenyl ether linkage. The presence of **LXXV** in the reaction mixture suggested that of the four alternatives structure **VId** could be the correct one [29].

Although later studies demonstrated that this structure was not entirely correct, the proposition was important, since it was the first to suggest the diphenyl ether structure of the molecule built from two substituted phenylglycine units. Four years later Fehlner *et al.* [71] concluded on the basis of mass and ^{1}H-NMR spectroscopic examinations that either of structures **VIe–f** — containing one oxygen atom less than **VId** — was more probable for the composition of the amino acid isolated from ristocetin.

Although no molecular ion could be detected in the mass spectra of ristomycinic acid derivatives, by means of exact determination of the fragment ions American chemists could give the correct formula of this amino acid as $C_{17}H_{18}N_2O_7$.

Contrary to earlier attempts, Harris *et al.* [72–74] made efforts to isolate the fully protected derivatives of the amino acid components of ristocetin (Scheme 34).



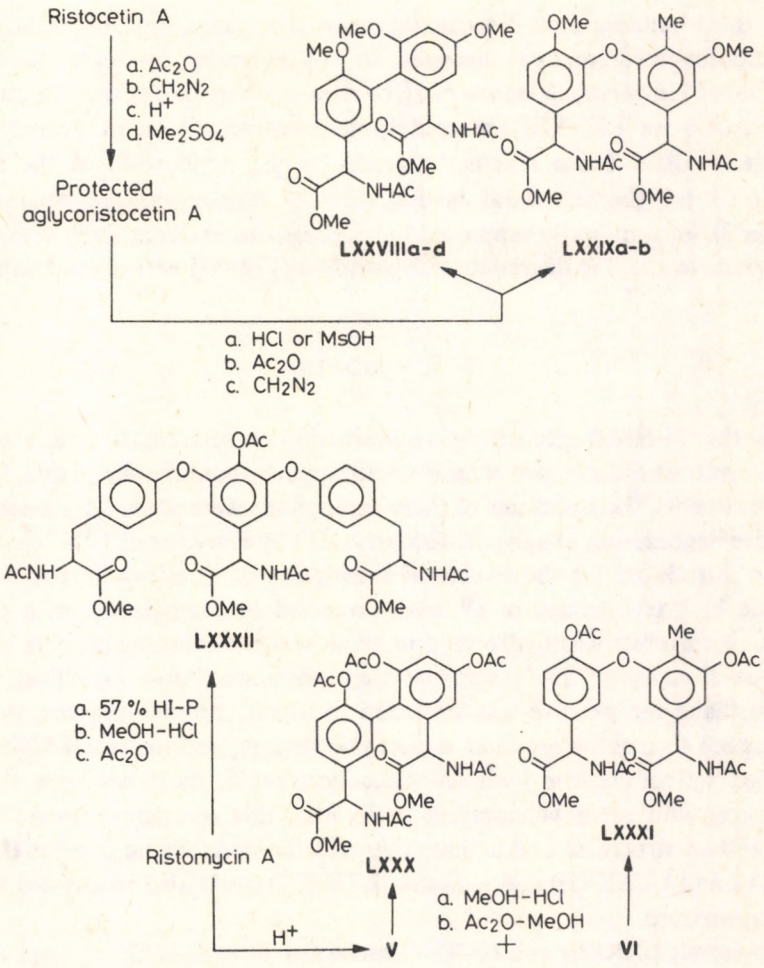
Scheme 33

Ristocetin A was first acetylated with acetic anhydride and then methylated with diazomethane. For the removal of the sugar components the partially protected antibiotic was hydrolyzed with 1 N hydrochloric acid and the phenolic hydroxyl groups of the resulting aglycone were blocked by methylation with dimethyl sulfate. The N-acetylated O-methylated aglycoristocetin was then hydrolyzed with 6 N hydrochloric acid or with 4 N methanesulfonic acid; these conditions were sufficient to racemize the majority of the amino acids. The resulting crude mixture of amino acids was acetylated again with acetic anhydride, esterified with diazomethane and separated by column and high pressure liquid chromatography.

The isolated stereoisomers **LXXVIIIa–d** could be readily distinguished from **LXXIXa–b** by ^{1}H-NMR measurements (on the basis of the C-CH_3 signal of ristomycinic acid), but not by mass spectrometry, since all products had the same composition $C_{25}H_{30}N_2O_9$. For the determination of the positions of the substituents in the aromatic rings, the examination of the products obtained by oxidative cleavage of the glycine residues was found to be more suitable.

No additional fragments could be isolated upon repeated acid hydrolysis of the above products.

Later Katrukha *et al.* [75] succeeded in the detection of molecular ions of low intensity in the mass spectra of the peracetyl-bis-methyl esters **LXXX** and **LXXXI** of



Scheme 34

actinoidinic acid (V) and ristomycinic acid (VI) isolated from the acid hydrolysate of ristomycin A. The composition of these molecular ions was in good accordance with the values calculated from the molecular formulae. At the same time, the appearance of ion $[M-COOCH_3-2CH_2{=}CO]^+$ in the spectrum of both derivatives indicated the common presence of an acetylated *p*-hydroxyphenylglycine unit in these molecules. At the same time the differences in fragmentation originating from other parts of the molecules clearly demonstrated the different substitution patterns as well as the diphenyl and diphenyl ether composition, respectively, of the two substances.

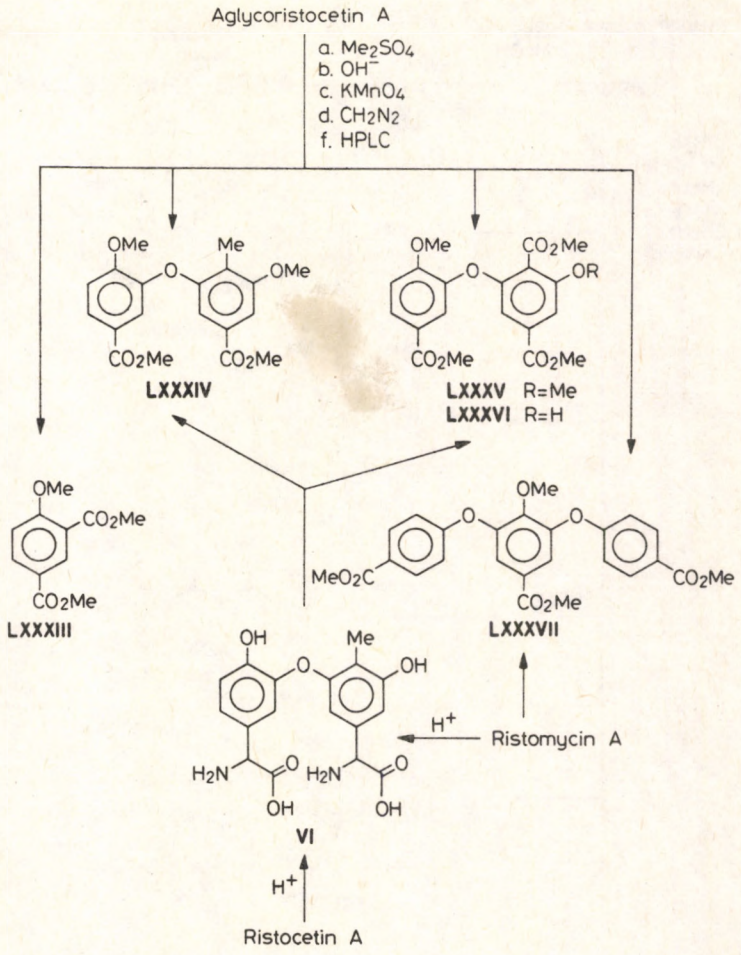
The third building unit, didechlorovancomycinic acid, unstable in both acidic and alkaline medium, was detected in the aglycone by researchers at the Lomonosov University, Moscow [76], by means of elucidating the structure of the deoxy derivative **LXXXII**, obtained from ristomycin A upon hydrolysis with hydrogen iodide. These results, achieved by the application of the methods introduced for the structural investigation of dideoxyvancomycinic acid (*cf.* Chapter 3), unequivocally supported the findings observed earlier in the oxidative and reductive alkaline degradation experiments [72–73] with these antibiotics.

6.3.3.2. OXIDATION

Since the ^{1}H-NMR spectroscopic examination of the aromatic amino acids and their derivatives did not give complete information about the site of attachment of the substituents, the structures of these compounds were studied by means of the oxidative degradation of aglycoristocetin A (**IV**) by Harris *et al.* [72–74]. First the method introduced for the structural elucidation of vancomycin was used. The phenolic hydroxyl groups of **IV** were protected by methylation with dimethyl sulfate, the product was hydrolyzed in alkali and the resulting mixture of amino acids oxidized with hot potassium permanganate. After esterification with diazomethane the mixture was subjected to liquid chromatographic separation (Scheme 35). One of the products, dimethyl 4-methoxyisophthalate (**LXXXIII**) was identified by comparison with an authentic sample. As it has been shown in connection with other vancomycin antibiotics, this compound arose from the trisubstituted structural unit actinoidinic acid. Three aromatic triesters (**LXXXV**, **LXXXVI** and **LXXXVII**) and a diester (**LXXXIV**) could also be isolated from the reaction mixture.

Compounds **LXXXIV** and **LXXXV** can be also obtained [72, 77] as products of the oxidative degradation of ristomycinic acid (**VI**) isolated either from ristomycin or ristocetin, and this finding explains the origin of these amino acids. Based on these results, the structure of ristomycinic acid was assigned as 3,3′-diglycyl-6-methyl-5,6′-dihydroxydiphenyl ether.

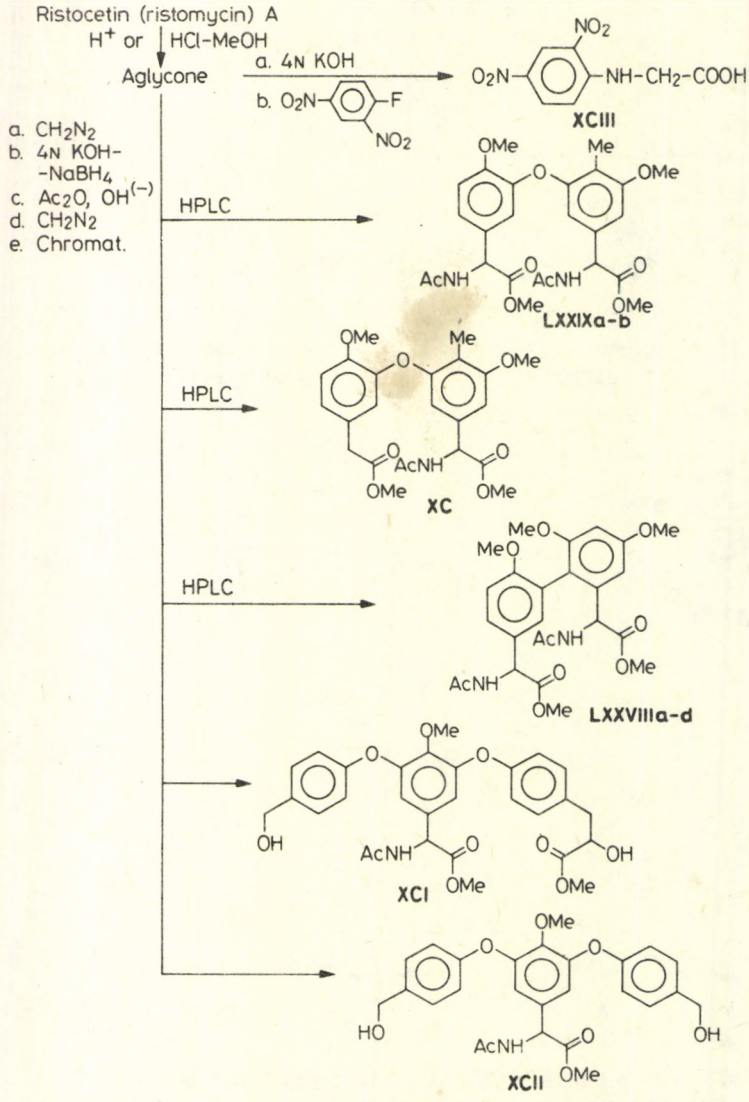
Later Berdnikova *et al.* [78] also isolated **LXXXVII** from ristomycin A by means of similar permanganate oxidation of the protected aglycone. The ^{1}H-NMR and mass spectroscopic investigations unequivocally demonstrated the above substitution pattern of the aromatic rings of these amino acids.



Scheme 35

6.3.3.3. REDUCTIVE ALKALINE DEGRADATION AND DECOMPOSITION WITH SODIUM HYPOCHLORITE

In recent years the methods used for the structural examination of vancomycin antibiotics have undergone considerable improvement. One of these procedures is the reductive alkaline degradation, followed by treatment with hypochlorite. This method has been introduced by the Harris group [72] for the structural investigation of ristocetin, and by Sztaricskai *et al.* [79] for similar studies on ristomycin.



Scheme 36

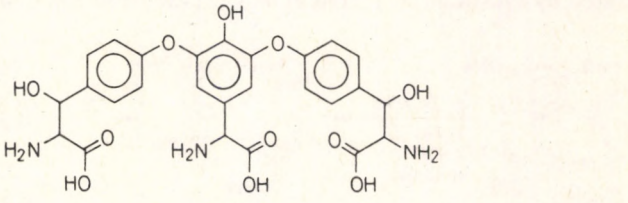
The aglycone, isolated after acid hydrolysis or methanolysis, was methylated with diazomethane, and the O-methylated product was hydrolyzed in 4 N potassium hydroxide in the presence of a large excess of sodium borohydride.

The compounds liberated were acetylated without isolation, extracted with a mixture of ethyl acetate and methanol, and methylated again with diazomethane.

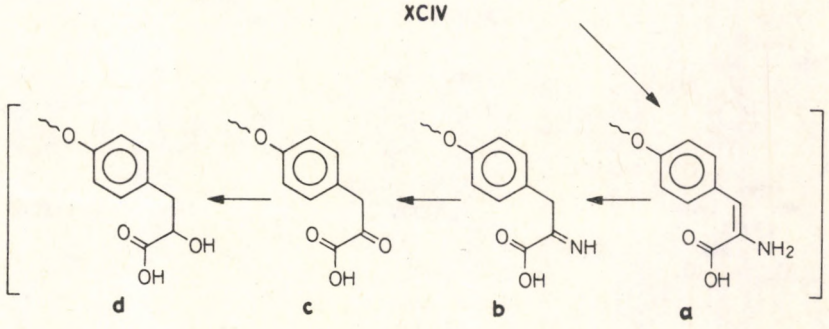
The components of the resulting mixture of the N-acetylated O-methylated amino acid esters were separated by column and high pressure liquid chromatography.

Compound **LXXIX**, derived from ristomycinic acid, was isolated in two diastereoisomeric forms. The more mobile one (**LXXXIXa**) originated from ristomycinic acid, and the other (**LXXIXb**) arose by epimerization during the alkaline hydrolysis. A deamino analogue of ristomycinic acid was also isolated and identified as **XC**. Later it was shown that this latter compound was not a component of the antibiotic, but a secondary product, formed by the loss of the free amino group of the aglycone.

Compound **LXXVIII**, derived from actinoidinic acid, was isolated in four diastereoisomeric forms (**a–d**) from ristocetin [72] and as two diastereoisomers (**a–b**) from ristomycin [79]. The free derivatives corresponding to **XCI** and **XCII** could not be detected earlier in the acid hydrolysate of ristocetin or ristomycin. The simplicity of the ^{1}H-NMR and mass spectra of **XCII** unambiguously indicated a symmetrical arrangement of the substituents in the system. This latter product, and



XCIV



Scheme 37

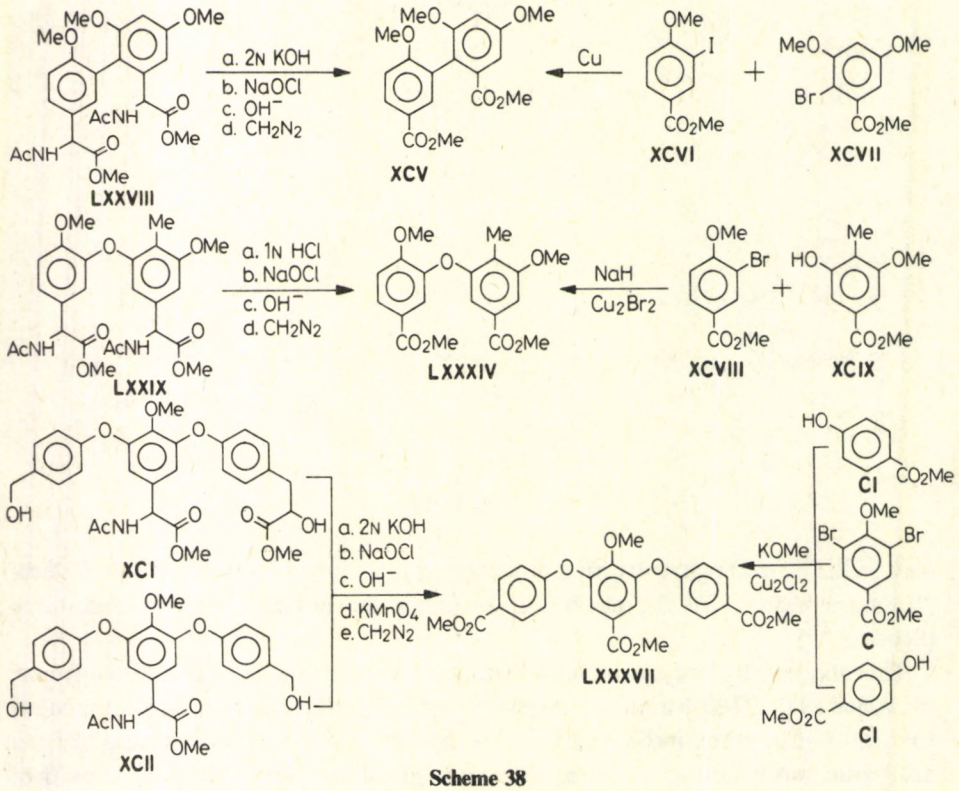
also **XCI**, formed apparently from didechlorovancomycinic acid (**XCIV**) — the new structural element of the antibiotic — under the applied reaction conditions (Scheme 37).

The alkaline hydrolysis of both ristocetin and ristomycin gave also glycine, identified as the N-(2,4-dinitrophenyl) derivative, **XCIII**. Glycine is never present in the acid hydrolysate, indicating that this amino acid is not a constituent of the antibiotic, but is formed by retro–aldol cleavage of the β-hydroxytyrosine units of

XCIV under the conditions of alkaline hydrolysis. The difference between the formation of **XCI** and **XCII** from **XCIV** is explained as follows: if the above process, resulting in glycine, occurs on both β-hydroxytyrosine units prior to the reduction, **XCII** is formed, but when it takes place only on one of these units, the product is **XCI**. The other β-hydroxytyrosine unit of didechlorovancomycinic acid was formed by dehydration, followed by hydrolytic loss of ammonia and reduction of the resulting phenylpyruvic acid moiety (**a**→**d**).

For further transformations of **LXXVIII**, **LXXIX**, **XCI** and **XCII** degradation with alkaline hypochlorite was used, a method useful for the structure investigation of amino acids, and this procedure was occasionally combined with potassium permanganate oxidation. The exceptional advantage of this method is that the indicated reaction steps (**a–d** and **a–e**) can be performed even with very small (15–20 mg) quantities, and only the final products, the diesters **LXXXIV**, **XCV** and the triester **LXXXVII** are to be isolated (Scheme 38).

The structures of the above products, obtained from ristocetin, were substantiated by synthesis by Harris *et al.* [72] (Scheme 38). Ullmann condensation of



Scheme 38

methyl 3-iodo-4-methoxybenzoate (**XCVI**) with methyl 2-bromo-3,5-dimethoxybenzoate (**XCVII**) in the presence of copper metal gave **XCV** in an excellent yield. The definitive synthesis of **LXXXIV** was achieved in a similar way using methyl 3-bromo-4-methoxybenzoate (**XCVIII**) and methyl 3-hydroxy-4-methyl-5-methoxybenzoate (**XCIX**). The structure of **LXXXVII**, formed from **XCI** and **XCII**, was also proved by condensation of one molecule of methyl 3,5-dibromo-4-methoxybenzoate (**C**) and two molecules of methyl 4-hydroxybenzoate (**CI**).

Sztaricskai *et al.* [79] made comparative studies with the above synthetic products and those isolated from ristomycin, the results demonstrate the identity of the aglycones of the two antibiotics to all probability.

The incorrect structures given earlier for actionidinic acid and ristomycinic acid (*cf.* Chapter 4, **VIIIa**, and Chapter 6, **VId**) have also been revised by Lomakina *et al.* [93]. In these studies the oxidation and degradation with alkaline hypochlorite was accomplished with O-methylated ristomycinic acid and actinoidinic acid, to obtain compounds **LXXXV**, **LXXXII** and **LXXXIV**, **XCV**.

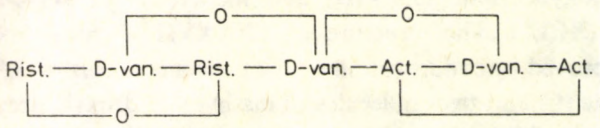
The above results unambiguously confirmed the structures of the isolated amino acids, determined by chemical degradation and spectroscopic methods. The stereochemistry of the glycine and serine residues, however, remained unknown at that stage of structural investigation.

6.3.3.4. THE STRUCTURE OF THE AGLYCONE MOIETY

Besides infrared spectroscopic examination, Soviet authors [21] also used potentiometric titration for the determination of the functional groups of ristomycins A and B. It was found that both variants contained two primary amino and five phenolic hydroxyl groups. Since one of the amino groups was included in the molecule of ristosamine, the other one must be located on the aglycone moiety. Later investigation showed that the number of the phenolic hydroxyl groups was four, and it was also demonstrated that — contrary to vancomycin and actinoidins — the ristomycin (ristocetin) variants did not contain free carboxyl groups. At the same time, the formation of methanol upon mild alkaline hydrolysis suggested the presence of a methoxycarbonyl group in the aglycones of ristomycins A and B [80].

In 1972 Lomakina and Tokareva [81] obtained four peptide derivatives by the partial acid hydrolysis of aglycoristomycins A and B which, on the basis of their electrophoretic behaviour, were identical with the products isolated from the ristocetin variants in the same way. These authors correctly assumed that in the initial step of the hydrolysis of the aglycone a carboxy-aglycone was formed by cleavage of the methyl ester group; on the other hand neither diketopiperazine nor another cyclic dipeptide could be isolated from ristomycinic acid and actinoidinic acid in later experiments.

Using the model given for the composition of vancomycin, in 1979 Harris *et al.* [72] made the first proposal for the sequential attachment of the amino acid components in aglycoristocetin A (Fig. 4). This model shows that the seven α-amino

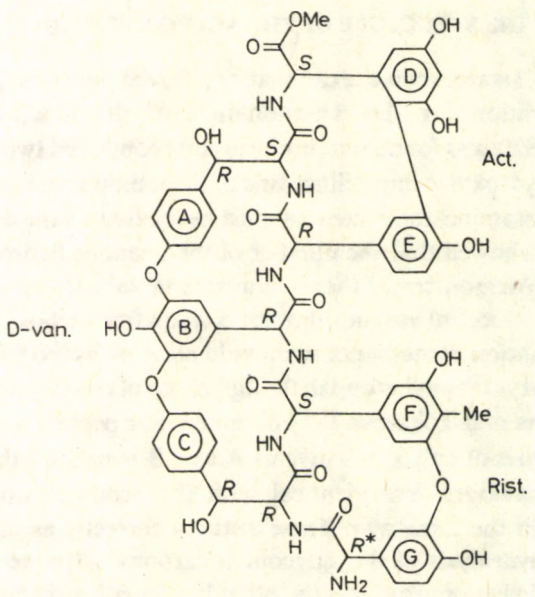


Act. = actinoidinic acid
Rist. = ristomycinic acid
D-van. = didechlorovancomycinic acid

Fig. 4. The sequence of attachment of the amino acid components of aglycoristocetin (aglycoristomycin), according to Harris *et al.* [72]

acid components of the three major structural units (Rist., Act., D-van) of the antibiotic constitute four cycles by cross-linkages along a linear heptapeptide chain.

At the end of the 1970's more and more information accumulated about the structural elements of ristocetin and ristomycin. Based on a careful and detailed analysis of these data, and also on their own experimental results, Williams *et al.* [2] have established that ristomycin and ristocetin are unambiguously identical, and structure **IV** has been suggested to represent the composition of the aglycone moiety.



IV

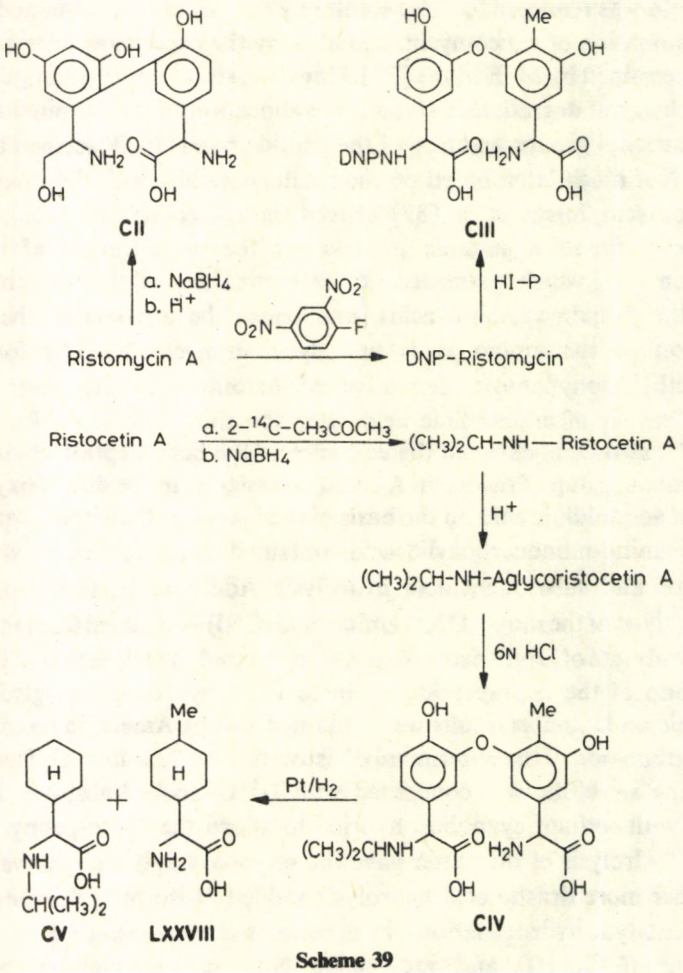
* Williams *et al.* [85, 91] proposed *S* absolute configuration for this asymmetric centre.

Great help was rendered to these studies by the experiences obtained during the NMR examination of vancomycin, and also, by the exact molecular weight (2063 dalton) determined by McFarlane [27]. However, since the above English chemists made no chemical degradation studies, the allocation of the terminal amino- and methoxycarbonyl groups and also of the peptide bonds in **IV** seemed to be rather arbitrary. Not much later, based on the results obtained with the investigation of aglycoristomycin, Silaev *et al.* [82] proved the correctness of structure **IV**. The Soviet group found a suitable process for the modification of the Edman degradation [83], which permitted the determination of the sequential attachment of the β-hydroxyamino acids, and hence the analysis of the sequential composition of the amino acids in aglycoristomycin A. The formation of the bismethylthiohydantoin derivative of ristomycinic acid and the monomethylhydantoin of actinoidinic acid after the third and sixth Edman cycles, respectively, also demonstrated the existence of the heptapeptide-chain structure. The C-terminal group of ristocetin A could be assigned to the dihydroxyphenylglycine unit of actinoidinic acid on the basis of the mass spectroscopic examination of the **CII** diamino-monocarboxylic acid, obtained upon reduction with sodium borohydride and subsequent acid hydrolysis. Additionally, the results of mass spectral analysis of the mono-DNP-amino acid (**CIII**) — isolated from the hydrogen iodide hydrolysate of DNP-ristomycin A — permitted the allocation of the terminal amino group of the heptapeptide chain to the *p*-hydroxyphenylglycine unit of ristomycinic acid. Similar results were obtained also by American researchers [84] in the determination of the N-terminal of ristocetin (and ristomycin). The free amino group of the antibiotic was condensed with 2-^{14}C-labelled acetone, followed by reduction with sodium cyanoborohydride to afford the N-iso-propyl derivative. Mild acid hydrolysis of this latter gave the respective aglycone derivative, which upon further more drastic acid hydrolysis yielded N-iso-propylristomycinic acid (**CIV**). On catalytic hydrogenation **CIV** decomposed into the known 4-methylcyclohexylglycine (**LXXVIII**) and radioactive N-iso-propylcyclohexylglycine (**CV**) (Scheme 39).

The appearance of this latter in the reaction mixture unambiguously demonstrates again that the terminal free amino group of the aglycone of the antibiotic belongs to the trisubstituted phenylglycine unit of ristomycinic acid.

In the knowledge of the above results, the formation of deaminoristomycinic acid [79], identified as **XC** from **IV** upon alkaline degradation is no longer surprising.

The aglycone (**IV**) of ristocetin A (ristomycin A) contains nine asymmetric centres. Based on the results of hydrogenolytic degradation experiments of ristomycinic acid (**VI**), Soviet and Hungarian authors [70] were the first to propose the D (*R*-absolute) configuration for the α-carbon atom of the *p*-hydroxyphenylglycine unit in ring G (*cf.* Chapter 6. p. 180). Later, in an ^{1}H-NMR spectroscopic analysis (negative

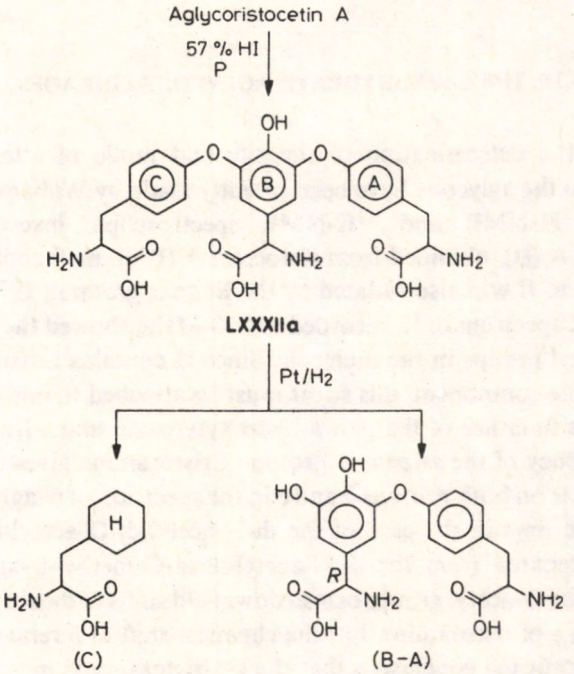


Scheme 39

nuclear Overhauser effects) Williams *et al.* [85, 91] assigned the *S* absolute configuration to both α-carbon atoms of ristomycinic acid (in rings G and F). The configurations of the other chiral centres were found to be the same as those of the respective structural units of vancomycin, determined by X-ray examination.

To solve these discrepancies, the Harris group [94] meticulously repeated the hydrogenolytic degradation of **VI**. The structure of the product, *R*-cyclohexylglycine (**LXXVII**) unequivocally proved that the first assignment [70] had been correct. At the same time, in agreement with the proposal of the English chemists, the *S*-amino acid **LXXVI** was obtained as the degradation product of the other part of the molecule (unit in ring F). Additionally, the ^{1}H-NMR investigation carried out

with the hydrolysis products of deuterium chloride treatment, proved that only one of the diastereoisomers of **VI** was formed from the heptapeptide. The examinations performed in the USA with actinoidinic acid (**V**) also supported the above assignation concerning the *R*- and *S* absolute configurations. Since less then 10% of deuterium was incorporated into the α-carbon of both amino acids, the extent of racemization was negligible during the hydrolysis. The opposite configurations of **V**



and **VI** could also be deduced from the negligible CD spectra and low values of specific optical rotation of the respective N-salicylidene derivatives.

Owing to the lability of the phenylserine fragments, didechlorovancomycinic acid (**XCIV**) cannot be isolated. Therefore — applying the well-proved method for ristomycin [76] — Harris *et al.* [94] prepared first the corresponding dideoxy derivative (**LXXXIIa**) by the treatment of aglycoristocetin with hydrogen iodide in the presence of red phosphorus. Hydrogenolysis of the product resulted in a new diphenyl ether derivative (A–B) and cyclohexylalanine (C).

A parallel hydrolysis experiment carried out with deuterium iodide revealed less than 10% D/H exchange at the phenylglycyl α-position of **LXXXIIa**, whereas the phenylalanyl α-positions did undergo D/H exchange to the extent of 70%.

As expected, both phenylalanyl β-positions incorporated one deuterium atom each. The value of specific optical rotation of **LXXXIIa** and the CD spectrum of its derivatives supported the *R* absolute configuration of the glycyl unit attached to ring B and a similar conclusion was derived from the investigation of the new diphenyl ether derivative obtained upon hydrogenolysis.

The exact determination of the configuration of the remaining asymmetric centres requires further experimental and instrumental studies.

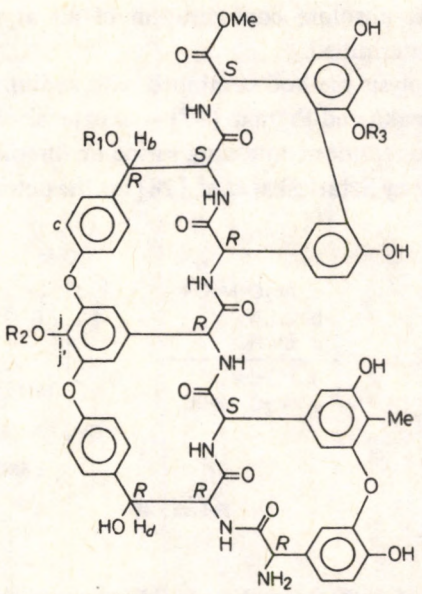
6.3.4. THE CARBOHYDRATE–AGLYCONE LINKAGES

Attempts at the determination of the site and mode of attachment of the carbohydrates to the aglycone have been recently made by Williams *et al.* [85, 86] by means of ^{1}H-NMR and ^{13}C-NMR spectroscopic investigation of ψ-aglycoristocetin A (**II**), obtained from ristocetin A (**I**) on methanolysis. As it was shown, compound **II** was also isolated by the Bognár group in 1977 (Scheme 40).

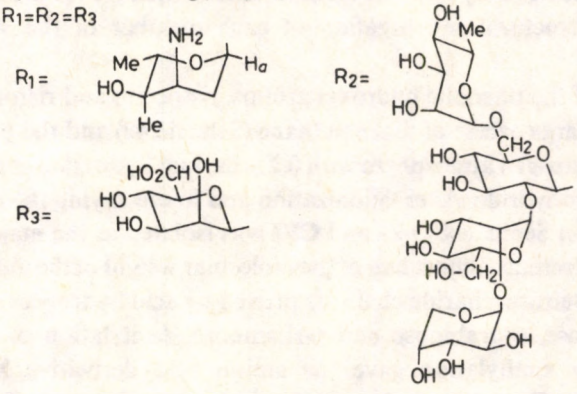
The ^{1}H-NMR spectrum of **II**, recorded at 270 MHz, showed the presence of six phenolic hydroxyl groups in the molecule. Since **II** contains L-ristosamine as the only carbohydrate component, this sugar must be attached to one of the aliphatic hydroxyl groups in either of the two β-hydroxytyrosine units. Irradiation at the resonance frequency of the anomeric proton of ristosamine gives rise to negative Overhauser effects on both protons *b* and *c* in the spectrum of ψ-aglycoristocetin A (**II**). At the same time in the case of the di-N-acetyl-di-O-acetyl-hexa-O-methyl derivative — prepared from the di-N-acetyl-hexa-O-methyl-ψ-aglycone — the introduction of the O-acetyl groups causes downfield shifts of the signals of proton *d* of **II** and proton *e* of ristosamine, but the chemical shift of *b* remains unchanged. These results permit the conclusion that the α-L-ristosaminyl moiety glycosylates the β-hydroxytyrosine unit which is adjacent to actinoidinic acid in the molecule.

When the product obtained from di-N-acetylristocetin A by methylation with diazomethane was subjected to drastic acid hydrolysis and the resulting amino acids were esterified and acetylated, compound **LXXIX** was isolated by English researchers, (Scheme 41) indicating that no sugars were attached to the ristomycinic acid unit.

The above findings suggest that the tetrasaccharide side chain (R_2) and the separate D-mannose unit (R_3) are linked either to the central ring of didechlorovancomycinic acid, or to one of the phenolic hydroxyl groups of actinoidinic acid. Since it was known that the disaccharide of vancomycin was linked to the phenolic hydroxyl group of vancomycinic acid, the changes in the ^{13}C-NMR shifts caused by the side chains (j,i,j′) were investigated. Comparison of these shift values in the



I R_1=α-L-ristosaminyl; R_2=β-ristotetrosyl; R_3=α-or β-D-mannopyranosyl
II R_1=α-L-ristosaminyl; R_2=R_3=H
IV R_1=R_2=R_3



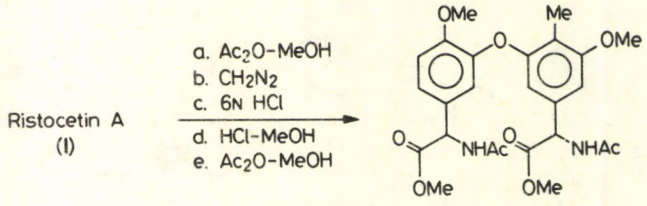
Scheme 40

spectra of aglycovancomycin and vancomycin with those of ψ-aglycoristocetin A (II) and ristocetin A (I) led to the unequivocal assignation of the tetrasaccharide as being linked at the position indicated by R_2, *via* a β-glycosidic bond.

The structure of the tetrasaccharide side chain was first given incorrectly by the Williams group based on results of acetolysis experiments [34]. Also, by application of the negative Overhauser effect, it was concluded [86] that the separate D-mannose unit was linked at the position represented by R_3, *via* and α- or β-glycosidic linkage. The exact assignment of the configuration of this band remained

unsuccessful, but the absolute configuration of all asymmetric centres of the aglycone **(IV)** was determined.

The alkaline hydrolysis method combined with sodium borohydride reduction — introduced by Tanaka and Pigman [87] — is extensively used for the structural investigation of glycopeptides containing serine or threonine. This procedure has been applied recently by Sztaricskai *et al.* [28] for the determination of the site and



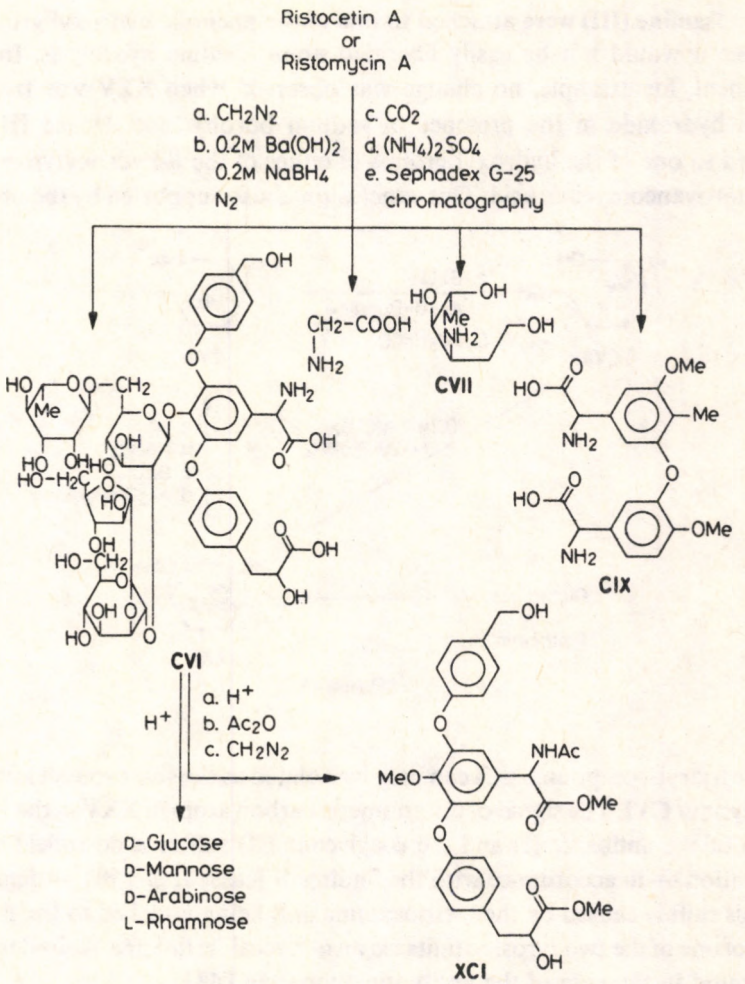
LXXIX

Scheme 41

mode of attachment of the tetrasaccharide side-chain to the aglycone of ristomycin A (ristocetin A). It seems likely that this method can be extended for use as a general technique in the structural investigation of each member of the vancomycin antibiotics.

For protection of the phenolic hydroxyl groups, ristocetin and ristomycin were methylated with a large excess of diazomethane (Scheme 42) and the product was hydrolyzed under nitrogen atmosphere with 0.2 M barium hydroxide containing 0.2 mole of sodium borohydride. After deionization and freeze-drying the residue was chromatographed on Sephadex G-25 and **CVI** was isolated as the major product. Compound **CVI** represents almost half of the molecular weight of the antibiotic and contains the intact tetrasaccharide chain, as proved by acid hydrolysis resulting in D-glucose, D-mannose, D-arabinose and L-rhamnose. Acetylation of the hydrolysate, followed by methylation gave the amino acid derivative **XCI** which, according to ^{1}H-NMR and mass spectroscopic examination, was found to be identical with the samples isolated earlier from ristocetin A [72] and ristomycin A [79].

Comparison of the ^{13}C-NMR spectra of pure crystalline ristomycin A, **CVI** and of methyl α-D-arabinofuranoside, used as model compound, clearly demonstrated [47] that D-arabinose is present at the end of the sugar chain in the form of α-arabinofuranoside, as supposed earlier [31]. The same result has also been published recently by Williams *et al.* [47a]. Therefore, the β-D-arabinopyranoside appearing in ristriose [34] must be formed as a secondary product under the conditions of the acid-catalyzed acetolysis. The NMR studies with phenyl α- and β-

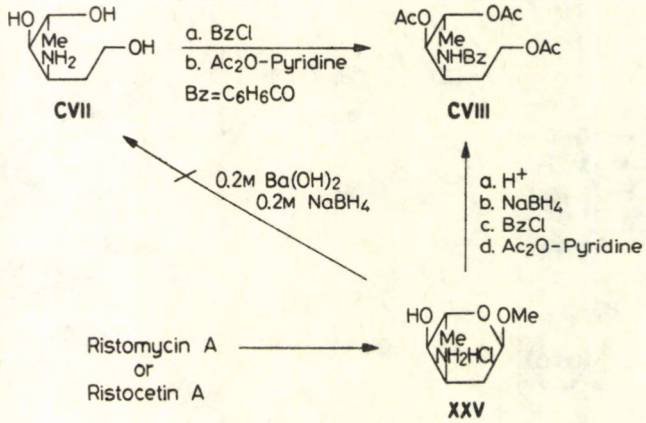


Scheme 42

D-glucopyranosides have demonstrated again that the glycosidic bond between D-glucose and the aglycone has β configuration.

The other product of the reductive alkaline hydrolysis was glycine, produced from didechlorovancomycinic acid. The third compound isolated was L-ristosaminitol (**CVII**) identified in the form of its N-benzoyl-tri-O-acetyl derivative (**CVIII**) after benzoylation and subsequent acetylation. The structure of **CVIII** was also established by synthesis from methyl α-L-ristosaminide (**XXV**) using an analogous reaction sequence (Scheme 43).

If ristosamine (**III**) were attached to one of the phenolic hydroxyl groups of the aglycone it would not be easily liberated upon alkaline hydrolysis. In a model experiment, for example, no change was observed when **XXV** was treated with barium hydroxide in the presence of sodium borohydride. Hence **III** must be attached to one of the hydroxyl groups of either of the β-hydroxytyrosyl units of didechlorovancomycinic acid. This conclusion is also supported by the observation

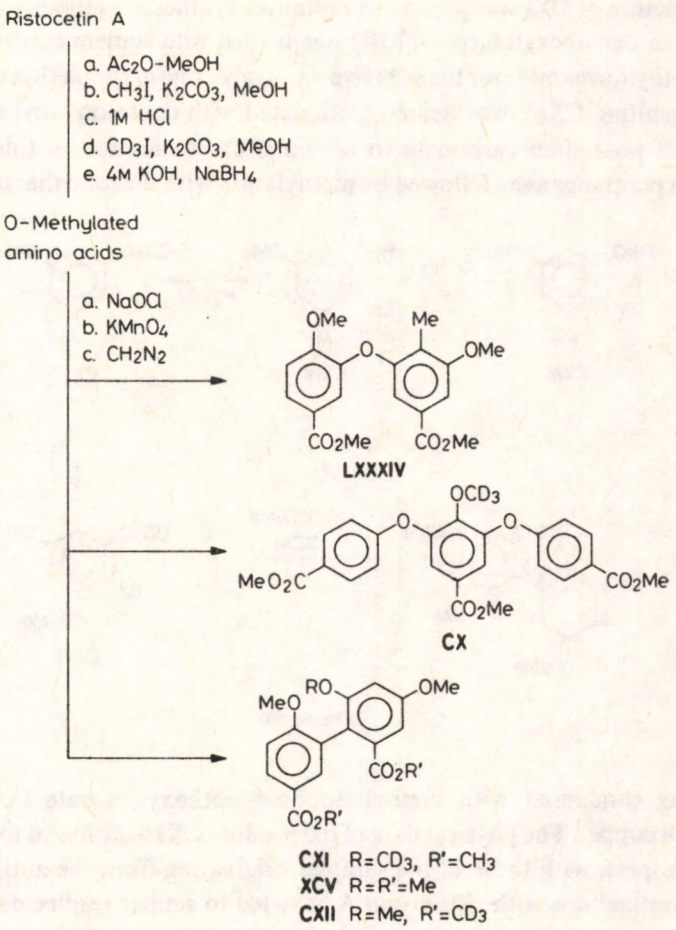


Scheme 43

that the triaryl-compound **XCI** can only be isolated as the sole product after the acid hydrolysis of **CVI**. The signal of the anomeric carbon atom of **XXV** in the ^{13}C-NMR spectra of the antibiotic (**I**) and the ψ-aglycone (**II**) suffers a downfield shift. This observation — in accordance with the finding of Kasai *et al.* [88] — demonstrates that this shift is caused by the L-ristosamine unit being attached to the β-hydroxyl group of one of the two tyrosine units *via* an α-glycosidic linkage. A similar effect has been found in the case of the antibiotic avoparcin [48].

Only traces of the aglycone fragment containing D-mannose could be detected in the reductive alkaline hydrolysis mixture. Therefore, for the determination of the place of attachment of this sugar the deuteromethylation technique — introduced for vancomycin and avoparcin — was applied by Hungarian and American researchers [28] (Scheme 44).

The N-acetylated antibiotic was methylated with methyl iodide in the presence of potassium carbonate, the sugars were removed by mild acid hydrolysis and the liberated hydroxyl groups of the resulting aglycone were methylated with deuteromethyl iodide. The product was hydrolyzed with potassium hydroxide in the presence of sodium borohydride and the O-methylated amino acids were subjected to degradation with hypochlorite, followed by oxidation with potassium

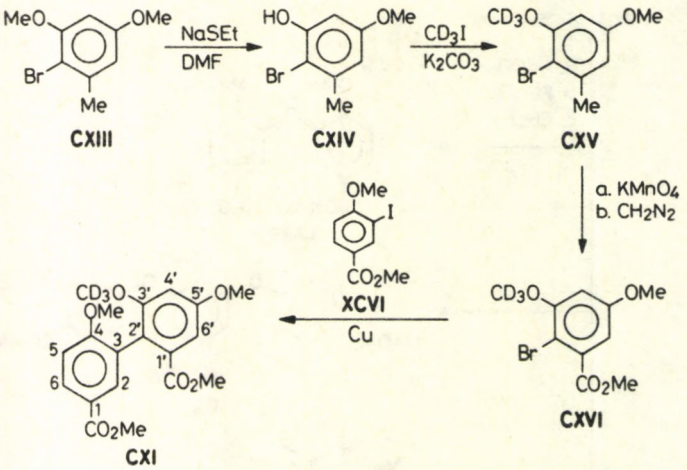


Scheme 44

permanganate and subsequent methylation with diazomethane to obtain the known **LXXXIV** and the deuteromethyl derivatives **CX–CXI**.

In accordance with the earlier statements, the diester **LXXXIV** formed from ristomycinic acid, whereas the triester **CX** — bearing a CD_3 group — derived from didechloro-vancomycinic acid. The diester **CXI**, originating from actinoidinic acid, was also found to contain a CD_3 group, the location of which was determined by both definitive synthesis and comparison of the ^{1}H-NMR spectrum of the unlabelled synthetic diester **XCV** with that of **CXII**, prepared from **XCV** by transesterification with deuteromethanol.

The structure of **CXI** was proved by definitive synthesis, as follows (Scheme 45). 2-Bromo-3,5-dimethoxytoluene (**CXIII**) was treated with sodium ethylmercaptide in N,N-dimethylformamide for the selective removal of one of the methyl ether groups, and the resulting **CXIV** was deuteromethylated with deuteromethyl iodide in the presence of potassium carbonate to obtain **CXV**. Oxidation of this latter with potassium permanganate, followed by methylation with diazomethane gave **CXVI**,



Scheme 45

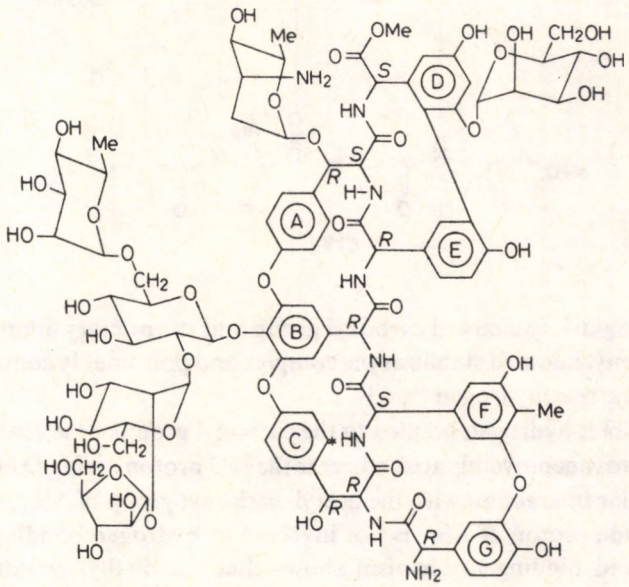
which was condensed with methyl 3-iodo-4-methoxybenzoate (**XCVI**) in the presence of copper. The physical data of the product **CXI** were found to be identical, in every respect, with those of the samples originating from the antibiotic.

The investigations with ristomycin A have led to similar results, demonstrating that the separate D-mannose unit is linked to the C-3′ phenolic hydroxyl group of actinoidinic acid.

Finally, comparative ^{13}C-NMR spectroscopic studies, accomplished by Sztaricskai *et al.* [47], using the antibiotic and phenyl α-D-mannopyranoside as model compound, have shown the mannose unit to be present in the α-D-mannopyranoside form. Applying *p*-nitrophenyl α-D-mannopyranoside, English chemists [47a] arrived at the same conclusion.

The configurations of the carbohydrate–aglycone linkages shown in structure **I**, was later also established by the Lomakina group [92]. Additionally, the experimental results reported for the macro-structure of ristomycin A by English [86] and Hungarian and American [28, 47, 79] chemists were supported in 1982 by the investigation of Soviet researchers [95]. Since with the exception of the absolute configuration of one chiral centre of aglycoristomycin A no data have been reported

concerning the stereochemistry of the additional asymmetric carbon atoms, if there is any structural difference between ristomycin and ristocetin this difference can arise exclusively from these stereochemical properties. However, according to the results so far obtained and summarized in this review, both antibiotics can be represented by the following common structure (**I**):

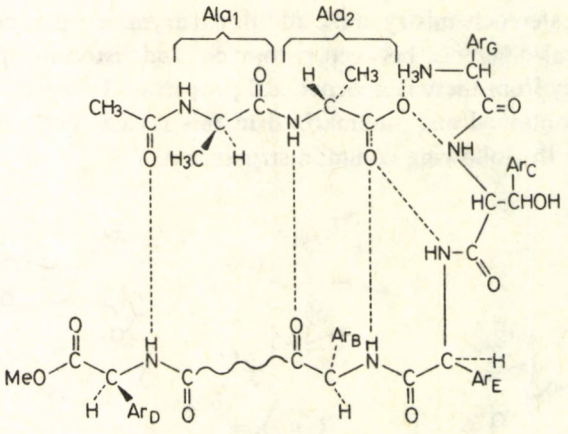


Ristomycin (ristocetin) A (I)

6.4. MECHANISM OF ANTIBIOTIC ACTION

Even before the recognition of the identity of ristocetin and ristomycin it was shown that both antibiotics formed complexes by binding to various bacterial cell-wall mucopeptide precursors, including the N-acetyl-D-Ala-D-Ala dipeptide [89, 90]. Similarly to the case of vancomycin, the mechanism of the formation of these complexes was studied by Williams *et al.* [2] by means of ^{1}H-NMR spectroscopy. It has been found that in the presence of ristocetin A, the methyl group of one of the alanine units of N-acetyl-D-Ala-D-Ala is remarkably shielded.

The binding of the dipeptide to ristocetin A occurs through hydrogen bonding involving five (or possibly six) sites. This binding is very similar to that proposed for vancomycin, but involves an additional binding of the carboxyl group of N-acetyl-D-Ala-D-Ala, as represented by structure **CXVII** [91]. The ionic interaction existing



CXVII

between the negatively charged carboxyl group and the primary amino function of ring G of ristomycinic acid stabilizes the complex and presumably contributes to the formation of further hydrogen bonds.

The Ala_2-NH is hydrogen bonded to the carbonyl group of the central ring (ring B) of didechlorovancomycinic acid, whereas the NH proton of ring D of actinoidinic acid is in similar interaction with the acetyl–carbonyl group of Ala_1. On the other hand, the amide proton of Ala_1 is not involved in hydrogen bonding.

The proposed binding mechanism shows that the methyl protons of the C-terminal alanine unit are fairly close to one of the protons of ring C of didechlorovancomycinic acid (marked by an asterisk in structure **I**), explaining the observed downfield shift of the foregoing methyl signal in the NMR spectrum.

The binding model also reveals that the side chain of the C-terminal D-alanine lies over the aromatic ring B, where there is more space for a larger group, since, contrary to vancomycin, the aromatic rings A and C, facing ring B, do not contain chlorine substituents. Additionally, the Ala_1-CH is in a very crowded environment, and evidently not even a methyl group can be fitted in that site. On the other hand, the Ala_2-CH is not near to any part of the aglycone of the antibiotic.

As it has been shown, Harris *et al.* [94] unequivocally proved the *R* absolute configuration of the α-CH attached to ring G of the aglycone **IV**, and this fact necessitates the change of the earlier proposal of the Williams group [85, 86] concerning the conformation of **IV**. This revised conformation can be depicted by a 180 °C rotation of the amide bond belonging to rings C and G. Although the N-terminal amino group is pointed away from the "molecular cleft" in this conformation, it still lies approximately 5 Å from the peptide carbonyl group, and may thus provide additional stabilization for the peptide–antibiotic complex.

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7. ANTIBIOTIC COMPLEX A-35512

The almost twenty five years which passed between the isolation of vancomycin — the first representative of the vancomycin group of antibiotics — and the discovery of antibiotic complex A-35512, clearly demonstrate that the scientific exploration of these glycopeptide antibiotics has not been completed as yet.

7.1. ISOLATION

The new antibiotic complex of the glycopeptide type was isolated by Michel and Higgens in 1978 [1, 2] from the submerged aerobic culture of *Streptomyces candidus NRRL 8156.*

The mixture of the antibiotic variants was first adsorbed on Amberlite XAD-4 resin and then eluted with 50% aqueous methanol. The resulting crude mixture of the active components could be separated by chromatography on polyaminde and aluminium oxide columns into five individual factors, A-35512 A, B, C, E and H. For the detection of the variants paper and reversed phase high pressure liquid chromatographic techniques were also applied [2]. According to biological activity tests, the major component of the antibiotic complex (about 80%) is A-35512 B. Variant A-35512 A constitutes about 10% of the complex, whereas the additional components are present in significantly smaller quantities (2–5% or less).

7.2. BIOLOGICAL ACTIVITY

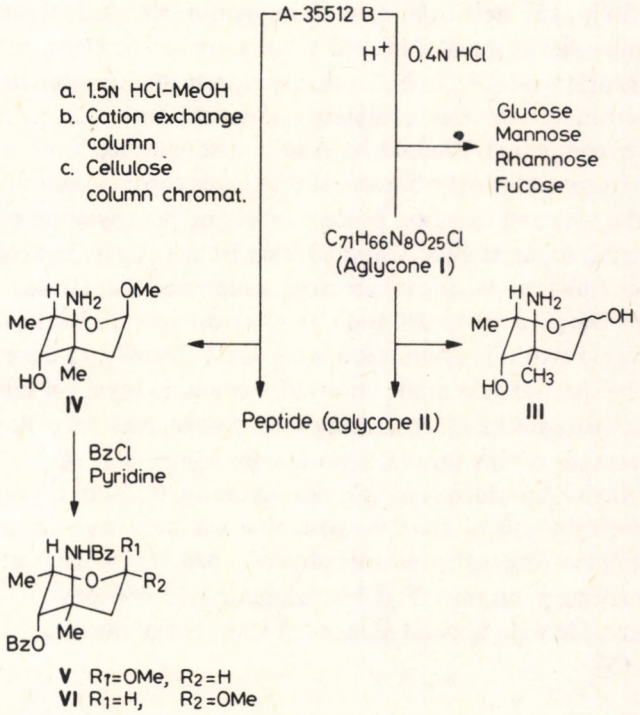
The antibiotic complex A-35512 has high *in vitro* and *in vivo* activity against pathogenic Gram-positive aerobic and anaerobic bacteria. It effectively promotes the growth and increases the feed utilization efficiency in animals [3]. Variant B is inhibitory *in vitro* against *Streptococcus faecalis* and *Streptococcus aureus* strains at 4 μg/ml, and against *Streptococcus pyogenes* and *Streptococcus pneumoniae* at 2 μg/ml or less. Subcutaneous doses of 5 mg/kg are effective in protecting mice from experimentally induced infections of the latter three strains of bacteria [2].

7.3. STRUCTURAL STUDIES

The most important physicochemical data of antibiotic complex A-35512 are shown in Tables I and II (see in Chapter 2). Components A–C form dihydrochloride, whereas factors E and H give monohydrochloride salts with hydrochloric acid. However, of the above salts only A-35512 B dihydrochloride could be obtained in crystalline form. The molecular weight and optical rotation of this latter variant are almost identical with those of ristocetin (ristomycin). Nevertheless, contrary to ristocetin and ristomycin, A-35512 B has been shown to contain one covalent chlorine atom and five potentiometrically detectable functional groups.

7.3.1. HYDROLYSIS

Vigorous acid hydrolysis (6 N hydrochloric acid) of each "antibiotic factor" resulted in the liberation of four amino acids, and one of these was identified as glycine [2, 4].



Scheme 46

The hydrolysis of A-35512 B with 0.4 N hydrochloric acid (reflux for 4 h) yielded a biologically active aglycone (**I**) with the composition $C_{71}H_{66}N_8O_{25}Cl$, and four neutral sugars, L-rhamnose, D-glucose, L-fucose and D-mannose (Scheme 46).

Surprisingly, the parent antibiotic and **I** had almost equal biological activities.

7.3.2. CARBOHYDRATES

Besides paper- and layer chromatographic examinations, the neutral carbohydrate units of the antibiotic were also identified in the form of trimethylsilyl derivatives by gas chromatography. Debono *et al.* [4] established that A-35512 B contained the above four sugars in equimolar quantities. Repeated acid hydrolysis of **I** resulted in a peptide (aglycone **II**) and a new branched-chain deoxy amino sugar (**III**). Compound **II** and the crystalline methyl glycoside (**IV**) of the sugar **III** were also isolated by means of the methanolysis of the antibiotic. Similarly to other vancomycin antibiotics, aglycone **II** retained only one-third of the biological activity of the parent variant B.

The isolation and elucidation of the structure of **III** were accomplished by Debono and Molloy [5]. Benzoylation of **IV** in pyridine afforded a mixture of **V** and **VI**, and the molecular formula of **IV** and **V** was found to be identical with that of methyl α-L-vancosaminide and its N,O-dibenzoyl derivative, respectively. Although the mass spectrum of **IV** was completely identical with that of methyl α-L-vancosaminide, compound **V** could be readily distinguished from methyl N,O-dibenzoyl-α-L-vancosaminide by means of thin layer chromatography. In experiments, using the reversed isolation process involving benzoylation of A-35512 B prior to methanolysis, crystalline **V** and **VI** were isolated again in excellent yields. Based on these results the American researchers supposed that **III** was an isomer of L-vancosamine. Detailed ^{1}H-NMR and CD spectroscopic studies have unequivocally demonstrated that this assumption is correct. Naturally, in the evaluation of these spectra the spectral data of the known deoxy amino sugars of similar type (L-vancosamine, L-ristosamine, L-acosamine) were of great assistance. Based on these results, the structure of this novel amino hexose component of A-35512 B is 3-amino-3-C-methyl-2,3,6-trideoxy-L-*xylo*-hexopyranose (C-3-epi-L-vancosamine).

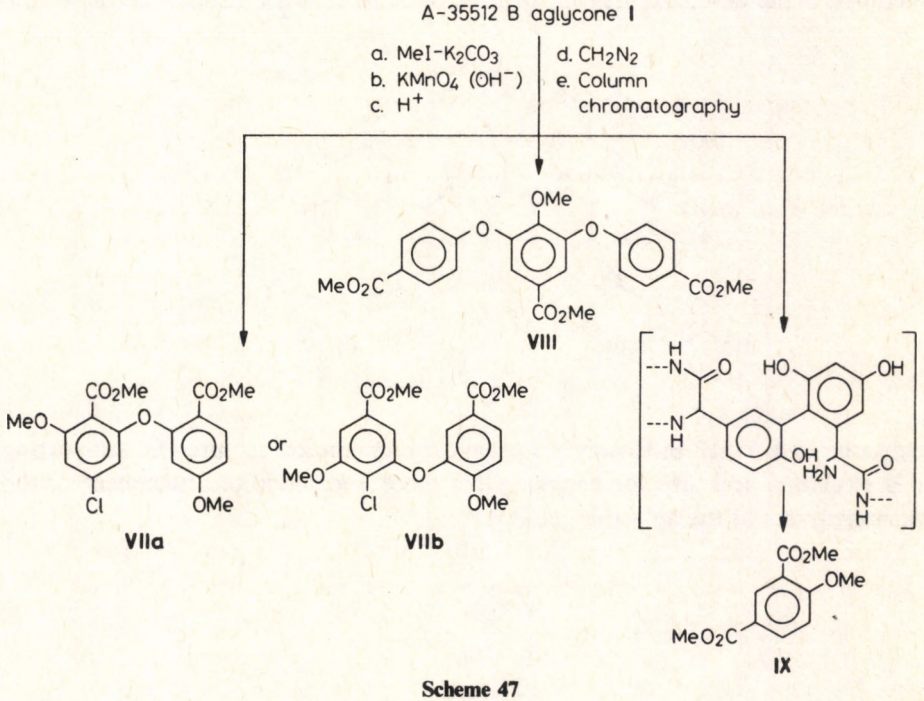
The definitive synthesis of epi-L-vancosamine has been recently published by Fronza *et al.* [9] starting from non-carbohydrate precursors. The specific optical rotation and melting point of methyl 3-benzamido-2,3,6-trideoxy-3-C-methyl-α-L-*xylo*-hexopyranoside were in good agreement with that of the sugar isolated from the antibiotic [5].

7.3.3. AGLYCONE

7.3.3.1. OXIDATIVE DEGRADATION

Since suitable methods for the structural elucidation of vancomycin antibiotics had been formerly developed, the researchers of the Eli Lilly Co. [4] applied these procedures, starting with the oxidative degradation method. Although the aromatic amino acids involved in the composition of the molecule suffer extensive decomposition under these conditions, the examination of the resulting products may give valuable information about the original structural elements.

The aglycone (**I**) of A-35512 B was first methylated with excess methyl iodide in methanol in the presence of potassium carbonate, and then oxidized with potassium permanganate. After acidification the liberated amino acids were esterified with excess diazomethane and the esters separated by silica gel column chromatography. Based on mass- and ^{1}H-NMR spectroscopic investigations one of the products was characterized as a chloro-substituted diphenyl ether derivative with structure **VIIa** or **VIIb**. In fact, according to biogenetic and model studies, structure **VIIb** was assumed to be more probable for this derivative (Scheme 47).



Scheme 47

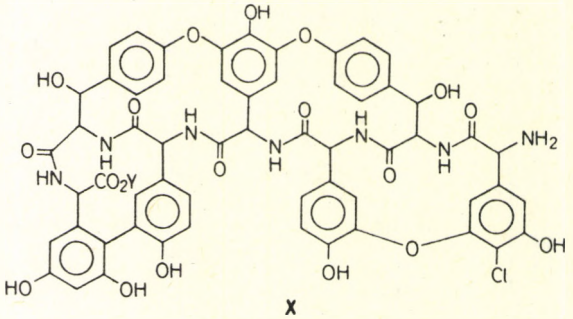
This structure is very similar to that of the diphenyl-carboxylic acid analogue, obtainable from ristomycinic acid in a similar way [6, 7] (*cf.* structure **LXXXIV** in Chapter 6), with the only difference that the methyl group of the tetrasubstituted ring of **LXXXIV** is replaced by chlorine atom in **VIIb**. Hence the aglycone of antibiotic A-35512 B contains 4′-chloro-4′-demethylristomycinic acid.

The formation of the bis-diphenyl ether derivative **VIII** refers to the presence of didechlorovancomycinic acid in the molecule of A-35512 B.

As it has been shown, actinoidinic acid is a common building unit of vancomycin antibiotics. The presence of this compound in the molecule of A-35512 B could be deduced also from oxidative degradation experiments with the aglycone of the antibiotic. Namely, in the course of these studies dimethyl 4-methoxyisophthalate (**IX**), originating from the trisubstituted aromatic ring of actinoidinic acid was isolated.

The results, summarized above, demonstrate that the peptide part of the antibiotic is built from 4′-chloro-4′-demethylristomycinic acid, actinoidinic acid and didechlorovancomycinic acid; glycine, isolated in the course of hydrolysis experiments, is formed by the cleavage of the hydroxytyrosine unit of the didechlorovancomycinic acid moiety.

Based on these data, and also on the common system of composition of the members of the vancomycin group of antibiotics, structure **X** is supposed [8] for the



X

aglycone of A-35512 B. However, further experiments are required for supporting this structure, and also for assigning the place and mode of attachment of the carbohydrates to the aglycone moiety.

7.4. MECHANISM OF ACTION

Hunt and Vernon [10] reported that the mechanism of the biological effect of antibiotic A-35512 B is very similar to that of vancomycin and ristocetin (ristomycin) A, involving complexation with the terminal D-alanyl-D-alanyl dipeptide part of the bacterial cell-wall mucopeptide. Model experiments have established that the affinity of antibiotic A-35512 B to N-Ac-D-Ala-D-Ala is higher than that of vancomycin. Consequently, this antibiotic is very similar to the other members of the vancomycin group of antibiotics, with respect both to its structural and biological properties.

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