STUDIES RELATED TO THE SYNTHESIS OF MONOMERIC AND DIMERIC VINCA ALKALOIDS

by

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ABSTRACT

The first part of this thesis describes a sequence which provides a total synthesis of cleavamine (23) and catharanthine (12). Dihydrocatharanthinol (76) obtained by lithium aluminum hydride reduction of dihydrocatharanthine (34) was converted to its tosylate derivative. The latter intermediate upon heating in benzene containing two equivalents of triethylamine underwent an interesting fragmentation reaction to provide a seco-diene (78) containing the cleavamine ring system. Reaction of this diene with osmium tetroxide provided a tetrol (96) which could be converted to cleavamine on the one hand and the C_4-functionalized cleavamine derivatives on the other. Thus treatment of the tetrol with sodium borohydride allowed the hydrogenolysis of the carbinol amine function and provided a triol (97). The vicinal diol present in 97 was cleaved by means of periodate and the resultant 2-acylindole chromophore was further converted by borohydride to a 4,18-dihydroxy dihydrocleavamine derivative (99). Reductive removal of the C_{18} hydroxyl function by means of lithium aluminum hydride provided isovelbanamine (100). Acid catalyzed dehydration of the latter yielded cleavamine (23) while isomerization under aqueous acidic conditions provided velbanamine (22).

To complete the total synthesis of catharanthine (12), cleavamine was reacted with t-butyl hypochlorite and the resultant chloroindolenine was then subjected to nucleophilic attack by cyanide ion to provide 18β-cyanocleavamine. Basic hydrolysis of the nitrile function followed by esterification provided 18β-carbomethoxycleavamine (60). This compound upon reaction with mercuric acetate was cyclized to catharanthine.

The second part of this thesis establishes the utility of both the chloroindolenine and the C_{18}-hydroxy analogues of the cleavamine systems to the synthesis of dimeric compounds structurally similar to the natural dimeric alkaloids. Treatment of either of these analogues with vindoline under mild acidic conditions yielded the appropriate dimers containing the indole and dihydroindole units present in vinca-leukoblastine.

The dimerization reaction was shown to be stereospecific and led in each case to the same stereochemistry at C_{18}, of the resulting dimers.

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ACKNOWLEDGEMENTS

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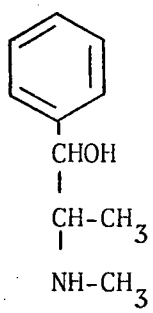
INTRODUCTION

The constant and continuing quest for new therapeutic agents and their development has been and remains to be one of the main objectives of the natural product chemist. Primarily his job in this regard has been the structural elucidation and synthesis of compounds of known therapeutic value. Knowledge of the structural details is of course essential to the understanding of the mode of action of these drugs. This, coupled with the synthetic capabilities of the chemist has led to the use of many synthetic analogues of the naturally occurring compounds in modern medicine.

It has been estimated that some fifteen percent or more of all vascular plants contain alkaloids. A very large number of different plant species have been investigated within the past two decades for alkaloidal constituents. Much of this work has been done by large pharmaceutical firms who are involved in a systematic screening of plants for specific pharmacological activity.[1] A more direct but limited approach has been the study of galenicals prescribed by folklore and primitive medicine. Although most alkaloids possess some degree of pharmacological activity, many are not medicinally useful either because their activity is too feeble or because their toxicity is too marked. Thus only a relatively small number among all the known

alkaloids are currently of importance from the therapeutic point of view.

A diversity of structure is exhibited by these compounds. The simplest are perhaps those of the phenethylamine group. The Ephedra alkaloid ephedrine (1) resembles the animal hormone epinephrine (2) both structurally and in its adrenergic properties. The Peyote alkaloid mescaline (3) is an hallucinogenic agent used to produce "model psychoses" in man for experimental purposes.



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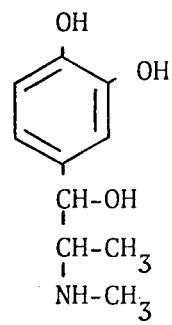

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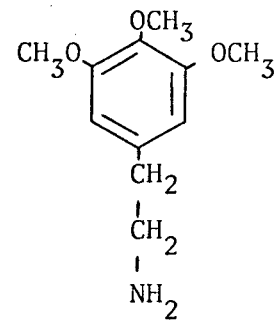
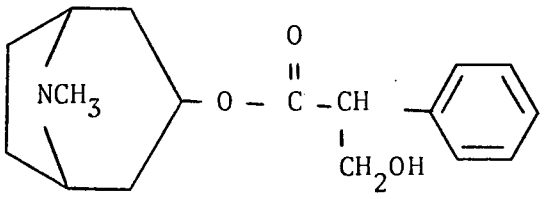


(3)

In part, the early biosynthetic postulates of Robinson[2] and many subsequent experiments[3] dealt with the formation of the slightly more complex tropane alkaloids. Important members of this family are the cholinergic blocking agent atropine (hyoscyamine) (4) and the local anaesthetic cocaine (5).

Morphine (6) and codeine (7) both depressants of the central nervous system are even more complex and have been the object of intense structural, synthetic[4] and biosynthetic investigation.[5]

A very large group of alkaloids numbering about 600, have as their basic structural unit, the indole moiety. The complexity of their



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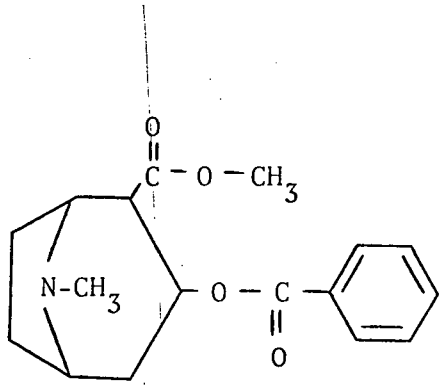

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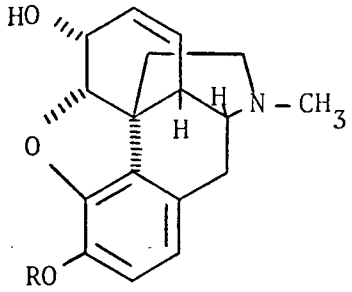
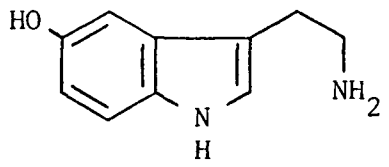


(6) R=H

(7) R=CH_3

structures range in a manner similar to those cited above, from the very simple, for example, the neurohormone serotonin (8) to the very complex such as the poison, strychnine (9), and the hypotensive agent, reserpine (10). The synthesis of compounds of intricate structure such as the latter two[6,7] represent major achievements in the field of synthetic organic chemistry.

Most of the indole alkaloids can be classified according to their



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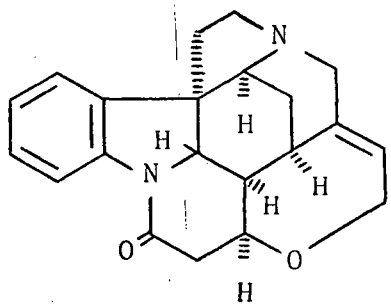

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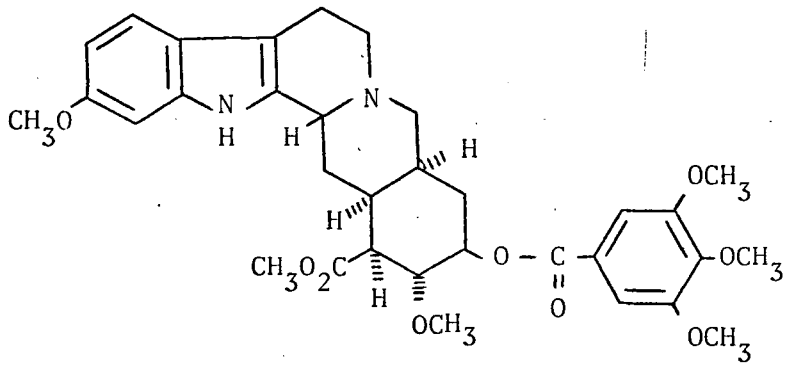
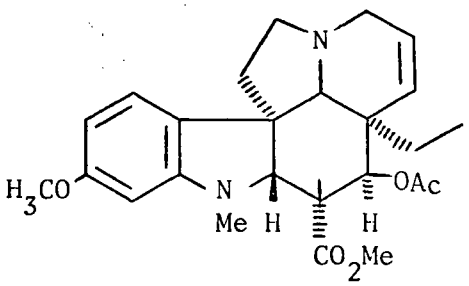


(10)

structural type into four groups; these are Corynanthe, Aspidosperma, Iboga and Strychnos. Examples of each of these respectively are reserpine (10), vindoline (11), catharanthine (12), and strychnine (9).



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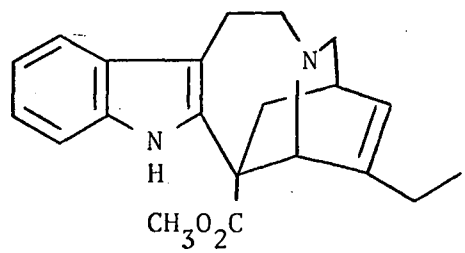


(12)

It has been established that biosynthetically these seemingly unrelated structures are in fact derived from a common intermediate. This intermediate, vincoside (13),[8] results from a condensation between tryptamine and a monoterpene unit, secologanin (15), derived from mevalonate through loganin (14) as shown in figure 1. The elucidation

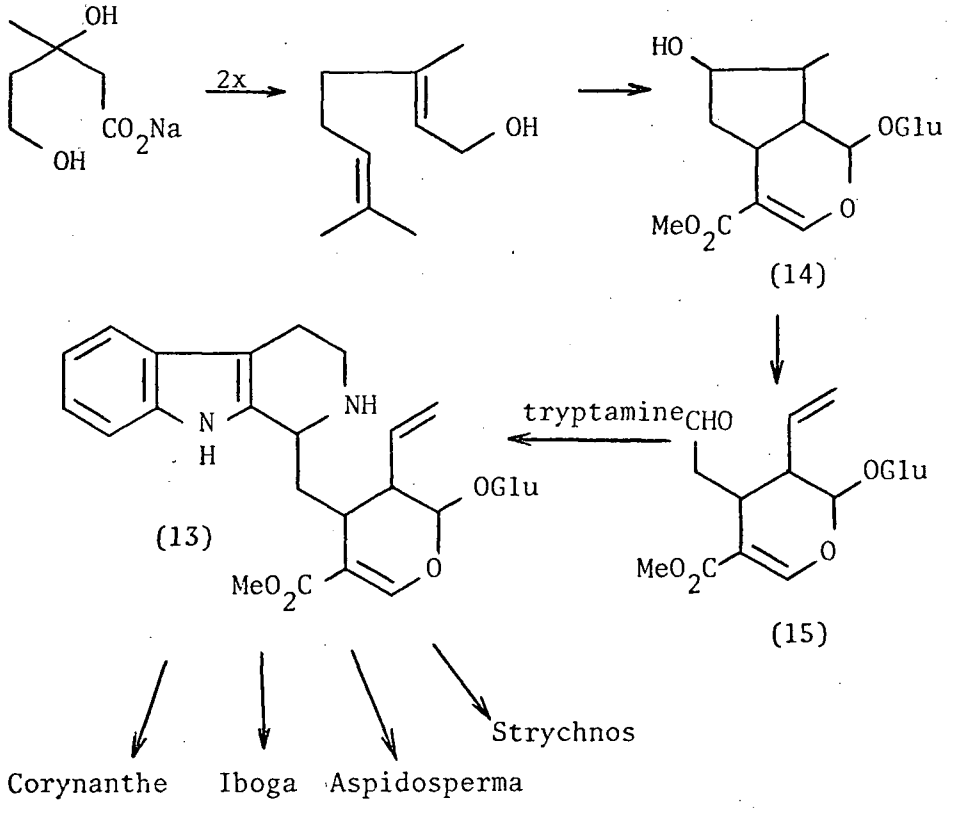


Figure 1. Summary of the pathway from mevalonate to indole alkaloids of tryptamine + C_{9-10} type

of the mechanism by which vincoside (13) is converted into each of the structural types remains an active area of research at present.

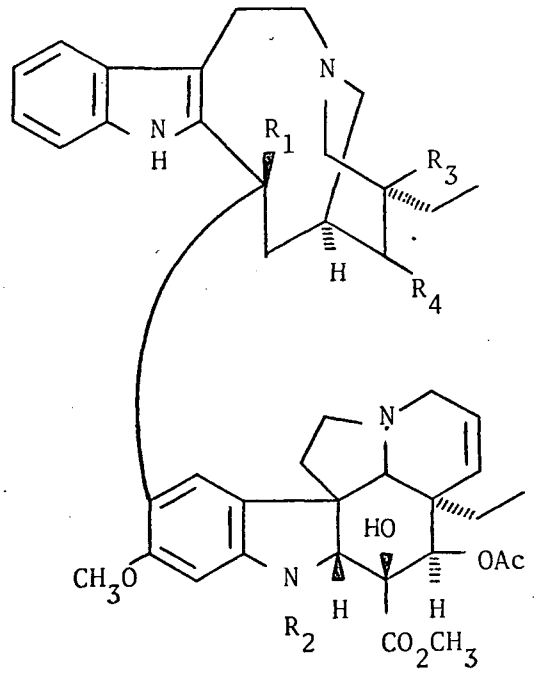
The basic structural unit of the alkaloids resulting from the above process, is made up of nineteen or twenty carbon atoms and two nitrogen atoms. However, a number of indole alkaloids have been isolated over the last fifteen years which possess a molecular formula made up of approximately two such units. These "dimeric alkaloids", in particular, those isolated from Catharanthus roseus G. Don, have provided a tremendous stimulus to all aspects of the field of indole alkaloid chemistry because of their proven oncolytic activity. The studies in our laboratory leading to the synthesis of these complex systems is the subject of this thesis.

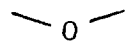
Catharanthus roseus G. Don, also referred to as Vinca rosea Linn, is a tropical member of the genus of plants commonly known as the periwinkles. The use of this species of plant throughout the history of folk-medicine has been varied, galenicals prepared from it have been used as an abortive agent, anti-diabetic anti-galactogogue, menstrual regulator, purgative and a number of other uses.[9] A recently published article entitled "Plants used against Cancer - A Survey"[10], is a literature survey embracing the recorded history of medicine, pharmacology, materia medica, medical botany, ethnobotany and folklore to 2800 B.C. It is interesting to note that of the more than 3000 different plant species reported to have been used against growths and tumors, no mentioned is made of C. roseus G. Don.

Interest in this species arose from its reported hypoglycemic activity. Noble and Beer could not substantiate this activity but observed instead peripheral granulocytopenia and bone marrow depression

in rats.[11] This led to the isolation of vinblastine (vincaleukoblastine, VLB) (16) which was found to produce severe leukopenia in rats.[12] Isolation of vinblastine and leurosine (17) and their activity against P-1534 leukemia in mice was reported shortly thereafter by Svoboda.[13]

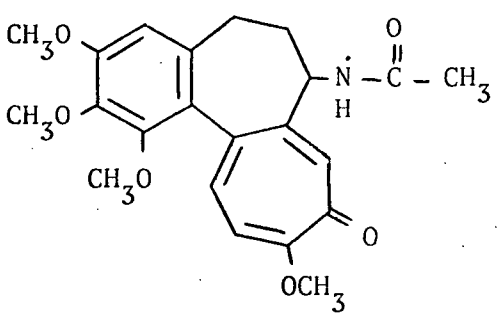
These preliminary investigations marked the beginning of a very active period of research in the Vinca alkaloids. A total of 61 alkaloids have been obtained from C. Roseus G. Don, 26 of them are dimeric but only 6 of these have oncolytic activity. These active compounds are all dimers of the indole-indoline type. The structures of four of them, vinblastine (16),[14] leurosine (17),[15] leurosidine (18)[16] and vincristine (leurocristine, VCR) (19),[14] have been established.



	R_1	R_2	R_3	R_4
(16)	CO_2CH_3	CH_3	OH	H
(17)	CO_2CH_3	CH_3		
(18)	CO_2CH_3	CH_3	H	OH
(19)	CO_2CH_3	CHO	OH	H

Vinblastine and vincristine are the most active and for this reason have been studied more extensively in their clinical use than the other alkaloids. Both have been shown to be effective in the treatment of lymphomas and various carcinomas .[17] Vincristine is also effective in the treatment of acute leukemia particularly in children. Their usefulness is limited, however, by their toxicity; vinblastine produces leukopenia while vincristine exhibits neuromuscular toxicity.

The mode of action of these drugs is not properly understood. A recent review of this subject[18] makes it clear that although the many experiments conducted so far have pointed out a number of physiological changes observed in a variety of tumor systems studied both in vitro and in vivo when subjected to these drugs, it has been difficult to correlate these results, and the biochemical mechanism of their action remains to be determined. It does appear that the Vinca alkaloids have a similar if not identical mechanism of action to that of the other mitotic poisons such as colchicine (20) and podophyllotoxin (21).



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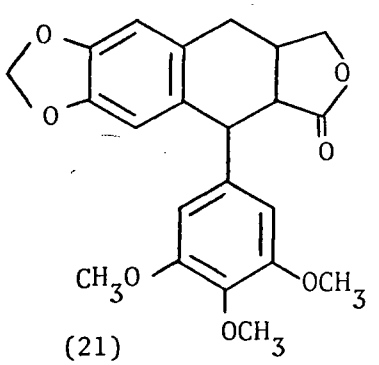


(21)

The following working hypothesis incorporating the observed phenomena has been presented by Armstrong at the conclusion of a recent symposium on vincritine.[19] The primary intracellular metabolic effect of the Vinca alkaloids seems to be the inhibition of transfer RNA synthesis. Because the function of transfer RNA is to carry amino acids from the cytoplasm into the ribosomes where proteins are formed, it is thus the decrease in transfer RNA synthesis which causes a decrease in protein synthesis. The Vinca alkaloids are permitted to enter the cells only between prophase and metaphase and thus intracellular protein production becomes decreased at the very time when protein should appear between the fibers of the spindle apparatus to spread them apart and support them in the fanned out position they normally adopt during metaphase. In the absence of this support the spindle fibers appear collapsed and tangled in cells arrested in metaphase by the alkaloid. When the spindle apparatus is in such a disarray. the chromosomes cannot migrate from the metaphase position. The inhibition of DNA synthesis observed, results from metaphase arrest.

Such an explanation provides an ordered sequence of events to account for the experimentally observed phenomena but provides no insight into the biochemical mode of action; for example what interaction with the alkaloids exists that inhibits the synthesis of transfer RNA. Having little or no knowledge of the actual reaction mechanism associated with the pharmacological properties exhibited by these compounds, the chemist is in a poor position to model a compound with the desired therapeutic properties and little or no toxicity. He need, of necessity, use a rather empirical approach, that is, start with a compound of known

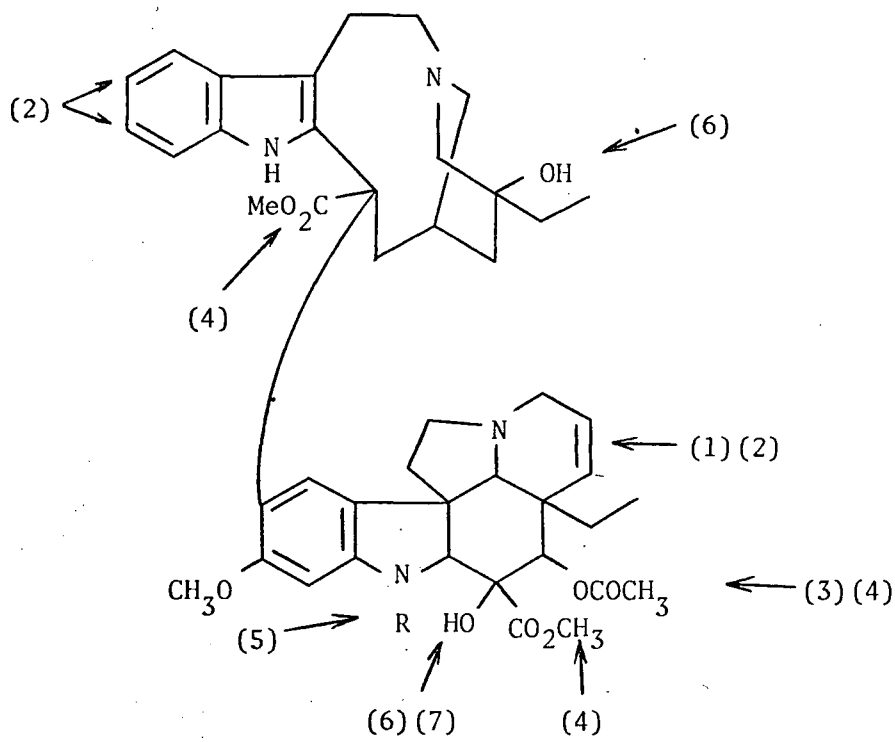
pharmacological properties and alter it stepwise noting simply the increase or decrease in activity associated with each structural modification. In such a way knowledge can be gained of the importance of structural units as related to activity. This method is fraught with two major difficulties. First, the chemist may have the misfortune of choosing the wrong compound as his basis for improvement and thereby put himself in the far from unique position of trying to do the impossible. Second, if he chooses as we have, a compound with a formula such as $C_{46}N_4O_9H_{58}$, he is faced with a near infinite number of choices of how best to modify or alter the structure. Unfortunately or fortunately he is limited in his choice by his synthetic capabilities and the amount of time available.

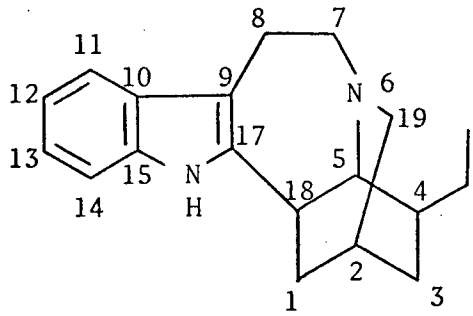
The very slight structural difference between vinblastine and vincristine and the relatively large difference in therapeutic properties and toxicity serves to indicate that perhaps only a minor change is necessary to effect the synthesis of an improved drug. An attempt to achieve this has been reported by Hargrove.[20] Vinblastine was converted directly into a number of analogues by reactions such as hydrolysis, hydrogenation and acetylation. The results are summarized in table I. The numbered arrows on the schematic indicate the position affected by the reaction.

As reference for further discussion, the numbering system commonly used for the Iboga and Aspidosperma alkaloids is given below.

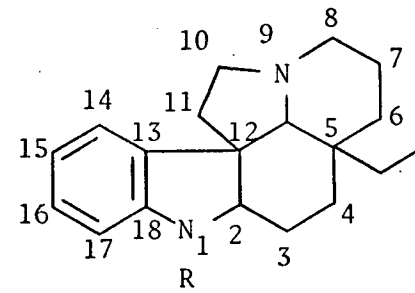
Table I. Structure-activity studies on vinblastine by Hargrove.[20]

	Modification	Activity relative to VLB	toxicity
1)	reduction of vindoline portion	1/3	less
2)	reduction (hexahydro)	none	--
3)	acid hydrolysis (desacetyl)	none	10X
4)	$LiAlH_4$ reduction	none	--
5)	acid hydrolysis of VCR (desformyl)	none	--
6)	acetylation using ketene (triacetate)	none	--
7)	acetylation using Ac_2O (diacetate)	none	--





Iboga



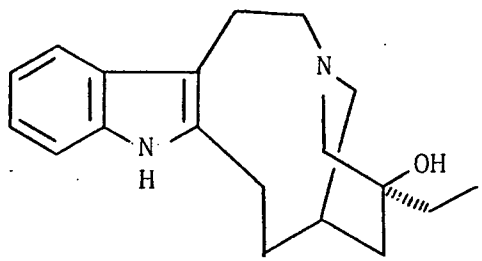
Aspidosperma

More promising results were obtained from the production of 4-acyl analogues readily prepared by the reaction of desacetyl VLB with a series of simple aliphatic acids. The best of these, vinglycine (desacetyl VLB 4-(N,N-dimethylaminoacetate)) produced from desacetyl VLB-4-chloroacetate and dimethylamine, equals vincristine in its action against P-1534 leukemia in mice. This compound proved to be much less toxic than vinblastine and could thus be tolerated at higher dose levels. Although at this point VLB has been transformed into an "improved" drug, this was no solution to the problem since its activity seemed to be similar to that of vincristine.

Changes other than in the functional groups on the molecule are much more difficult to introduce. Two possibilities present themselves: a) partially degrade vinblastine and build in structural modifications or b) attempt to synthesize analogues using the appropriate Iboga and Aspidosperma type units.

Some degradation of vinblastine has been reported and the work indicates that the dimer is readily cleaved into its monomeric units. It was the identification of these cleavage products which provided much

of the initial evidence for the structure of VLB. When VLB was treated under acidic reducing conditions, the products isolated were desacetyl vindoline and velbanamine (22). Prolonged treatment under the above conditions also produced some cleavamine (23).



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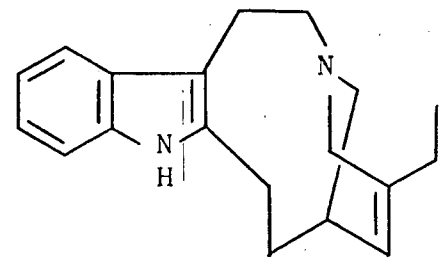
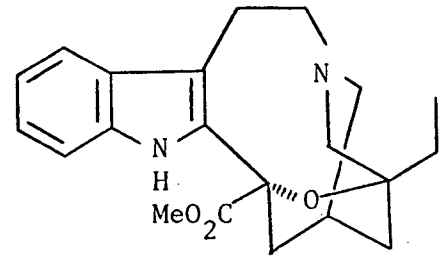


(23)

Desacetyl vindoline was readily identified by comparison with an authentic sample produced by mild hydrolysis of vindoline (11).[21] The structure of velbanamine was established via cleavamine, its dehydration product. Cleavamine had been isolated from a mixture of products obtained from catharanthine (12) treated under conditions identical to those used for the dimer cleavage.[22] Its structure had been correctly assigned based on mass spectral data[23] and was later substantiated by X-ray analysis of cleavamine methiodide.[24] Treatment of vinblastine under non-reducing acidic conditions also cleaved it; the product of this reaction was desacetyl vindoline and the ether (24).[14] This ether when subjected to reducing acidic conditions yielded velbanamine.

It is not surprising that the bond joining these two units in the dimeric system should be so labile, particularly under acidic conditions. The vindoline aromatic portion is essentially a meta-methoxyaniline

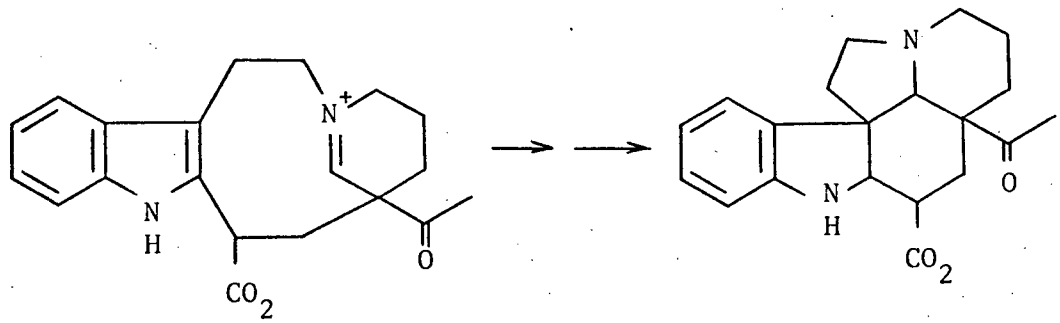


(24)

system which would be expected to undergo facile electrophilic substitution. In this case protonation at C_{15} would lead to a resonance-stabilized carbonium ion since both the ortho-methoxy and para-anilino nitrogen atom can make contribution to its stability. Bond fission is then merely a process which would lead to neutralization of the positive charge.

Theoretically, any reaction mechanism is reversible. Thus the problem in synthesizing the dimeric system from its two components is one of making it energetically favourable for the reverse of the above process to occur. Because Kutney and coworkers had developed a general synthetic route to both the Iboga and Aspidosperma types of alkaloid, the two halves of the dimer, this approach was adopted and is outlined in this thesis.

The key step in the synthesis of the Aspidosperma and Iboga alkaloids is a transannular cyclization which was postulated in 1962 by Wenkert[25] in a biosynthetic scheme leading to these compounds. The Aspidosperma system was proposed to result from the transannular cyclization of the appropriate biosynthetic precursor (25 → 26) and the Iboga could arise via a similar process (27 → 28).



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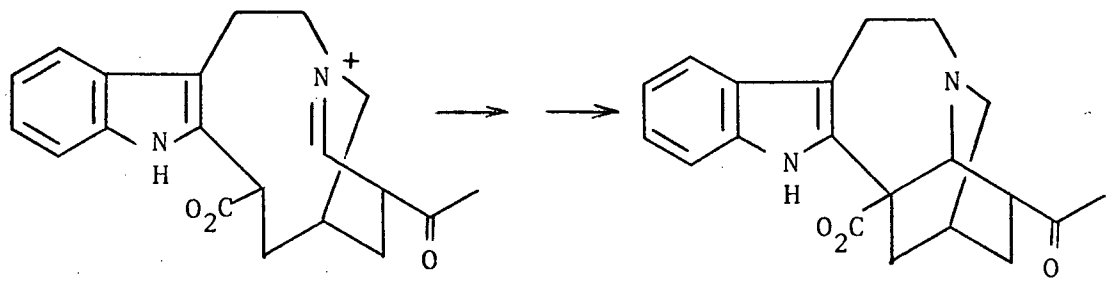
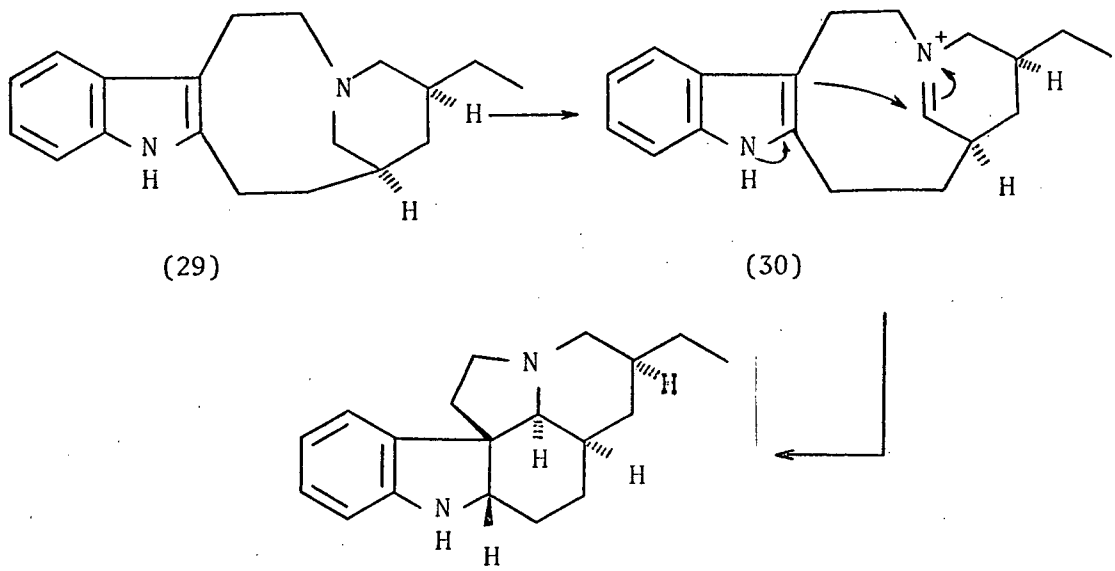


(27) (28)

The first experimental verification for this scheme was provided by Kutney.[26] Dihydrocleavamine (29) available from catharanthine (12),[22] provided a convenient model system. Oxidation with mercuric acetate gave the required iminium salt (30) which on ring closure followed by reduction resulted in the Aspidosperma skeleton (31).

When carbomethoxydihydrocleavamine (32) was oxidized under similar conditions pseudovincadifformine (33)[27] was isolated along with dihydrocatharanthine (34) and coronaridine (35).[28] Whereas the cyclization to the Aspidosperma skeleton in the dihydrocleavamine case must involve



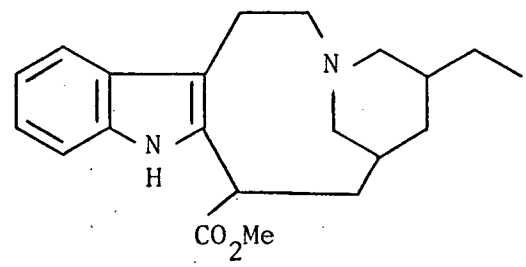
(29) (30)

(31)

the electrons inherent in the indole system as in the intermediate (30), in the carbomethoxydihydrocleavamine case, the driving force comes from the loss of a hydrogen alpha to the carbomethoxy group in the intermediate (36). The presence of both coronaridine and dihydrocatharanthine indicates that the iminium salt (37) is in equilibrium with its enamine isomer.

The Aspidosperma skeleton as synthesized in the experiments cited above is of an unnatural structure bearing the ethyl side chain at C_7 rather than at C_5. The correct structure was obtained by transannular-cyclization of quebrachamine (38), which led to the synthesis of aspidospermidine (39).

It is of interest to note the stereochemical details of this work. The Aspidosperma skeleton contains four asymmetric centers while the starting cleavamine or quebrachamine systems have only one. It has been



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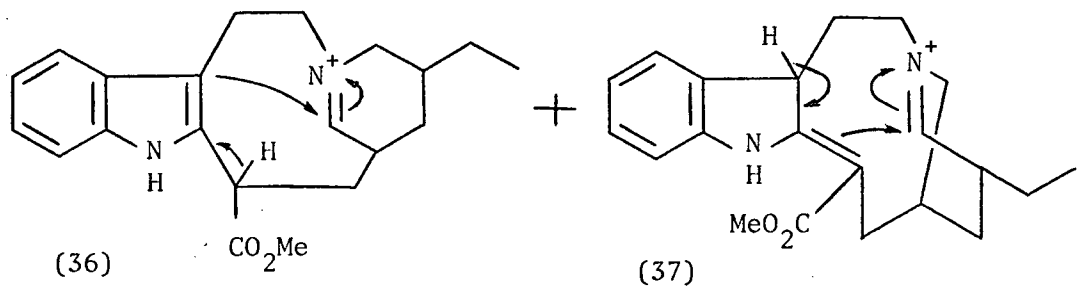

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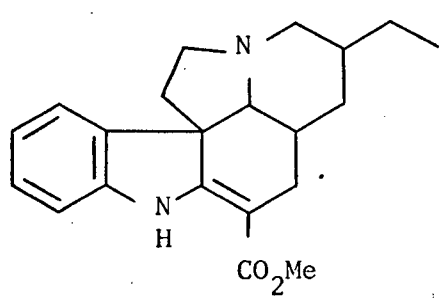

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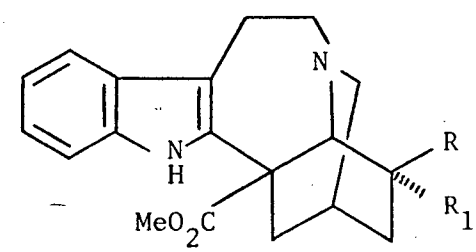
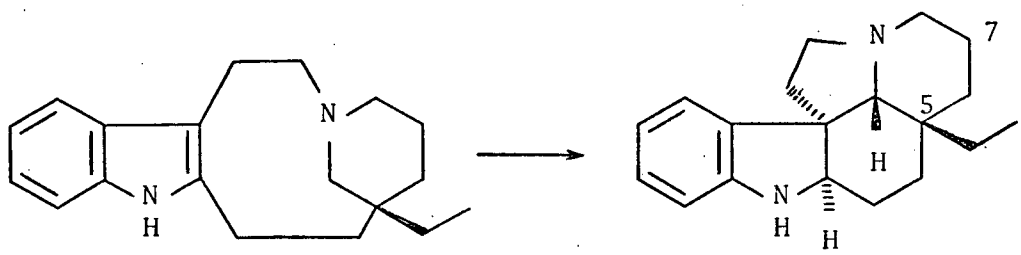


(34) R=H, $R_1=C_2H_5$

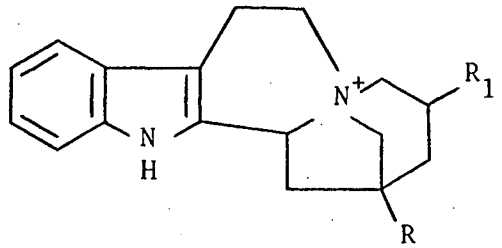
(35) $R=C_2H_5$, R_1=H



(38) (39)

established by chemical and X-ray studies that the lone asymmetric center in the starting material results in a stereospecific cyclization and determines the stereochemistry at the other three centers.[29] Thus both of the stereochemical series known in the natural Aspidosperma alkaloids can be obtained by the proper choice of starting material.

Having established the utility of the transannular cyclization approach to the synthesis of both the Aspidosperma and Iboga systems, a general approach to the synthesis of the appropriate nine membered ring intermediate was desirable. The difficulty of synthesizing intermediate size ring systems is well known. In this case the difficulty was obviated by advantageous use of the nitrogen atom present in the ring system. The key intermediate for this synthetic approach is the quaternary salt (40). Ring opening of this intermediate is readily achieved under reducing conditions to give dl-quebrachamine (38) (when R=C_2H_5 and R_1=H) and dl-dihydrocleavamine (29) (when R=H and R_1=C_2H_5).[30,31,32] The synthesis of the intermediate (40) was achieved in

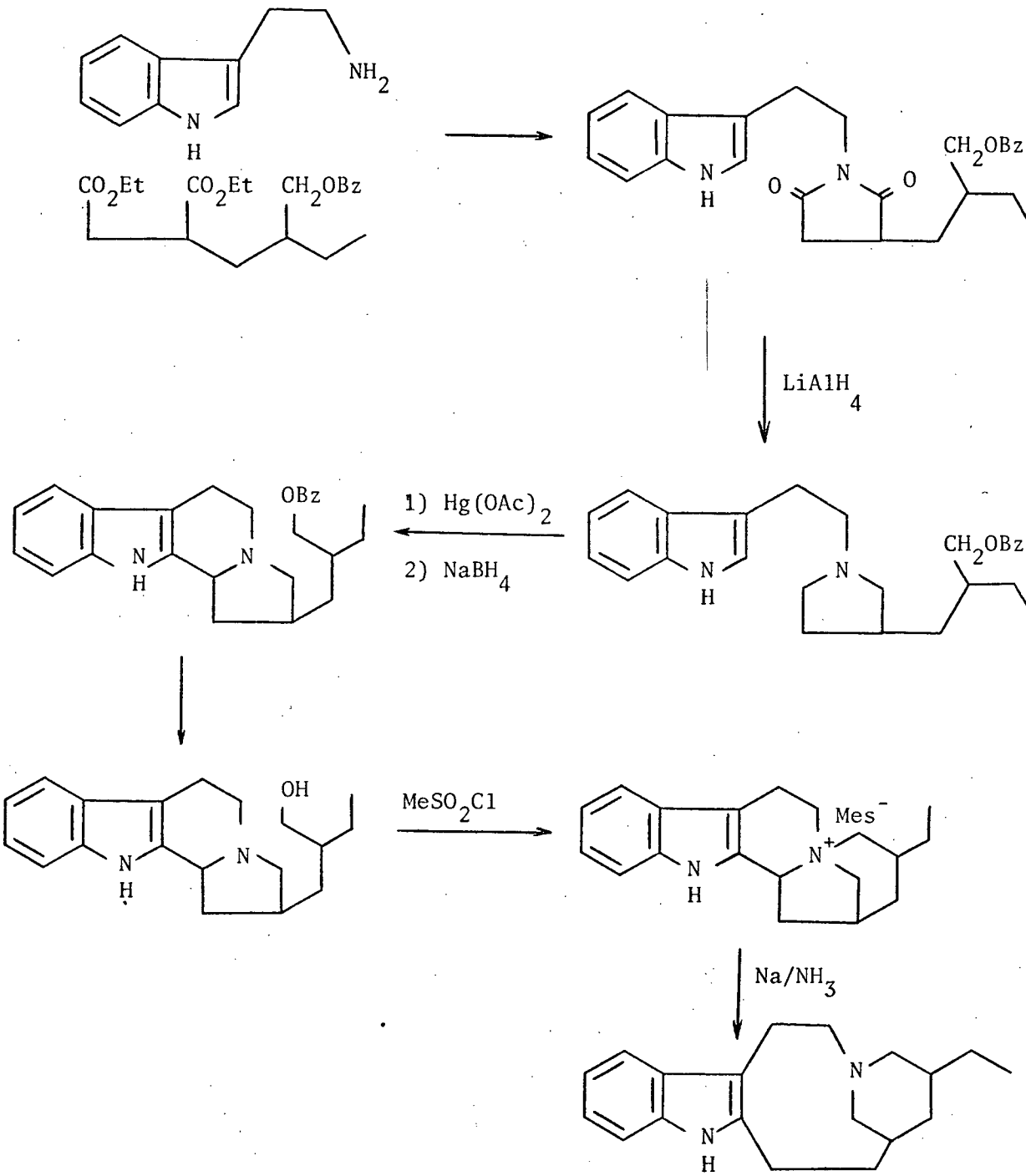


(40a) R=C_2H_5, R_1=H
(40b) R=H, R_1=C_2H_5

a straight forward manner as shown for the intermediate (40b) in figure 2.[31] Intermediate (40a) was obtained in a similar manner.[30]

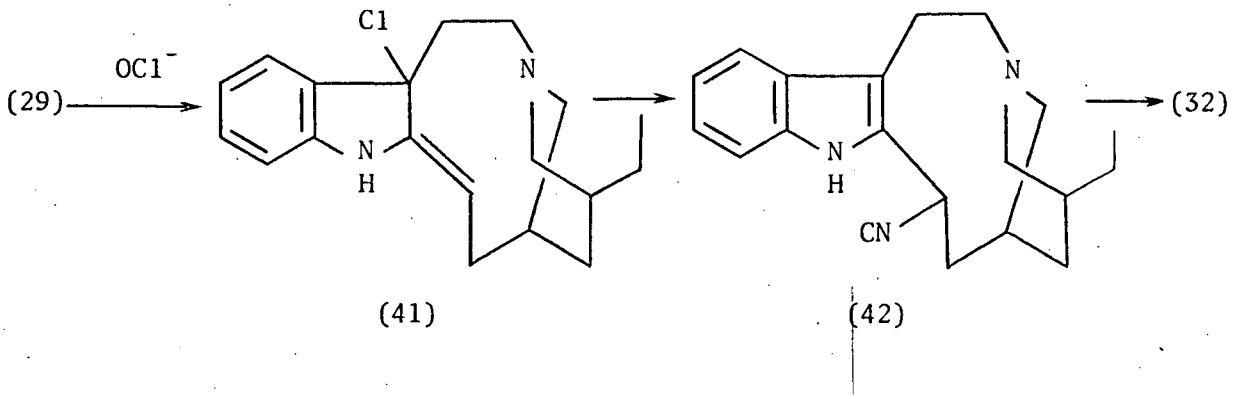
Having obtained dihydrocleavamine, it was then desirable to extend the synthetic sequence to a C_{18}-carbomethoxydihydrocleavamine derivative since this would then give a total synthesis of dl-dihydrocatharanthine and dl-coronaridine via the transannular cyclization already mentioned. Use was made of the tert-butylhypochlorite oxidation of indole systems to the corresponding chloroindolenine as employed by Buchi in his voacangine synthesis.[33] This reaction which serves an important role in the work for this thesis, will be discussed in detail when that work is presented. The chloroindolenine (41) formed from dihydrocleavamine was reacted with potassium cyanide to introduce the nitrile group at the C_{18} position. This, followed by treatment with methanolic hydrochloric acid produced the required 18-carbomethoxydihydrocleavamine (32).[31]

Subsequent work carried out to extend the total synthesis of dl-quebrachamine and aspidospermidine to the more complex members of



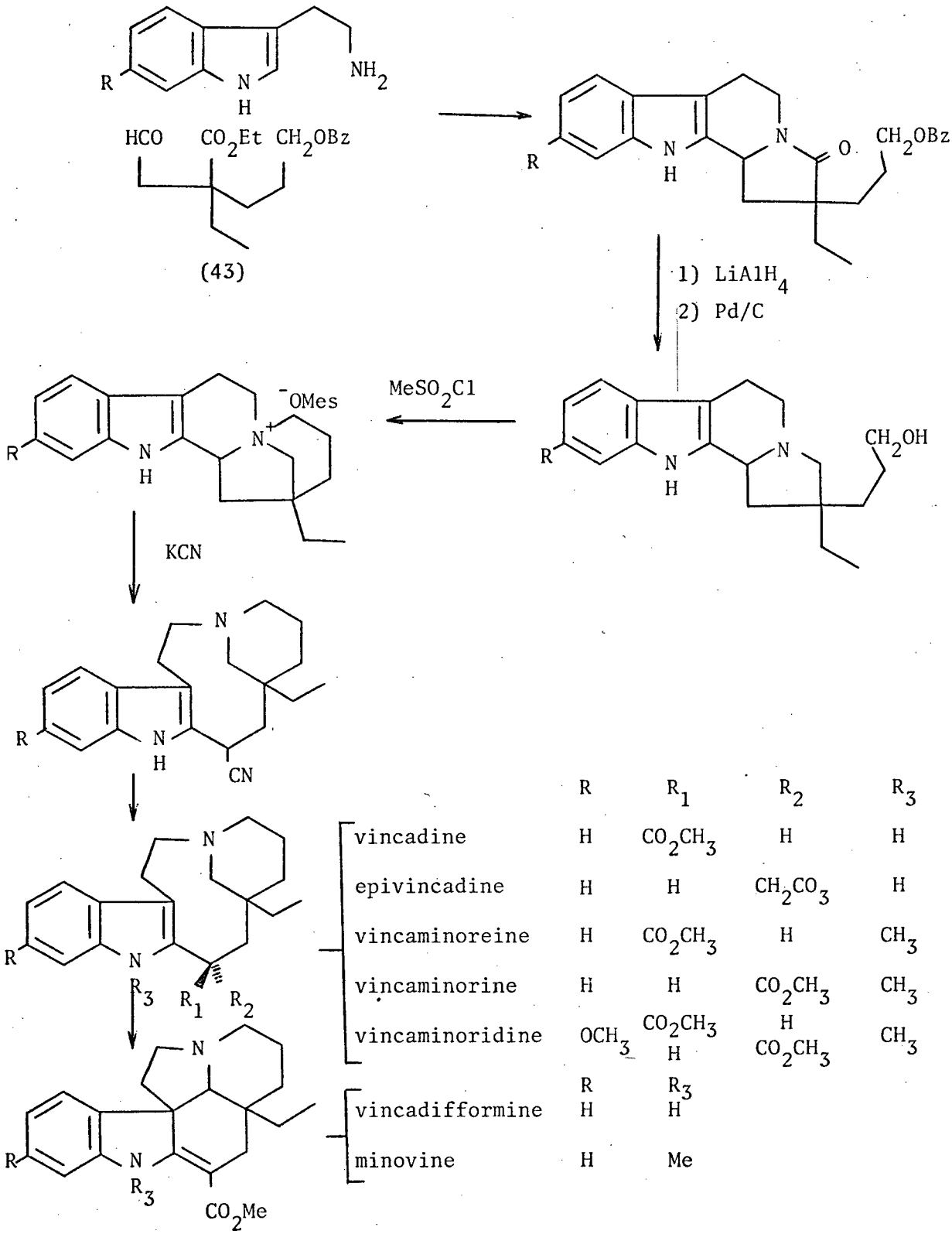
(29)

Figure 2. Kutney's total synthesis of dl-dihydrocleavamine (29).



the Aspidosperma alkaloids resulted in the improved sequence as outlined in figure 3.[32] Condensation of tryptamine with the aldehyde (43) provided the required tetracyclic intermediate directly thereby eliminating the low yielding oxidative step inherent in the first approach. This sequence led to the synthesis of the quaternary mesylate in a greatly improved over-all yield. Nucleophilic attack by cyanide on this intermediate effected both the ring opening and formation of the desired nitrile in a single step. Conversion of the nitrile to the carbomethoxy group produced dl-vincadine and dl-epivincadine, isomeric at C_{18}. This work also provided a total synthesis of dl-vincaminoreine and dl-vincaminorine, the corresponding indole N-methyl-analogues. Transannular cyclization of the above nine-membered ring natural products provided total syntheses of dl-vincadifformine and its N-methyl analogue, dl-minovine.

An extention of this sequence to encompass alkaloids bearing a methoxyl group in the aromatic ring was achieved by the use of 6-methoxytryptamine instead of tryptamine in the intial condensation. This has led to the total synthesis of the structure proposed for



	R	R_1	R_2	R_3
vincadine	H	CO_2CH_3	H	H
epivincadine	H	H	CH_2CO_3	H
vincaminoreine	H	CO_2CH_3	H	CH_3
vincaminorine	H	H	CO_2CH_3	CH_3
vincaminoridine	OCH_3	CO_2CH_3 H	H CO_2CH_3	CH_3

	R	R_3
vincadifformine	H	H
minovine	H	Me

Figure 3. Total synthesis of some Aspidosperma alkaloids.[32]

vincaminoridine. Ring closure provides a compound which has most of the structural features of vindoline.

From the summary of the experimental work provided, it is apparent that a general synthetic approach to the Aspidosperma and Iboga alkaloids has been developed. However, with respect to the synthesis of the dimeric systems, a comparison of the structures drawn for the natural dimers and the structures drawn in the synthetic schemes, indicate that although the correct gross structures have been synthesized, the synthetic materials lack some of the required functionality. It was therefore, necessary to develop a means by which the oxidation level of these compounds could be altered to that found in the natural dimeric systems. Once this had been accomplished, the means to couple the functionalized Iboga and Aspidosperma units to form the dimeric compounds would have to be developed.

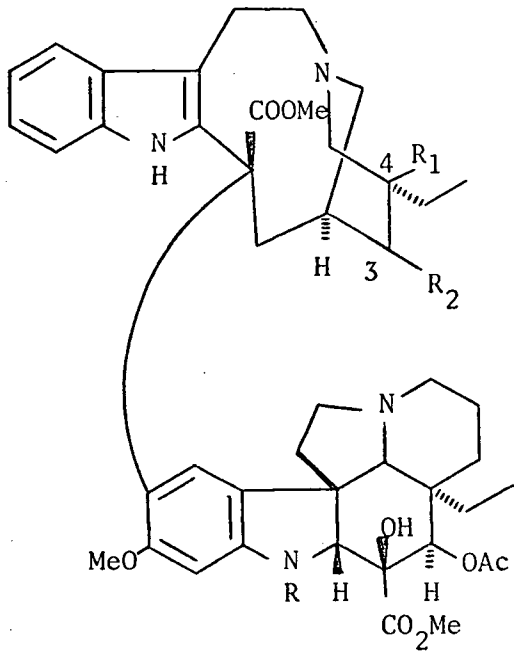
The synthesis of the appropriate Aspidosperma unit, specifically vindoline, is a demanding problem and is currently being studied by other workers in our laboratory. This thesis will deal with the work conducted towards achieving the correct oxidation level of the Iboga system and the modifications necessary to effect coupling with vindoline.

DISCUSSION

The aim of this work, as outlined in the introduction, is the synthesis of a series of compounds analogous to the natural dimeric Vinca alkaloids. The studies carried out to this end can be classified under two general headings: 1) the appropriate functionalization of the nine-membered ring present in the cleavamine-type system so as to make available units similar to those found in the natural alkaloids and 2), the coupling of these units with vindoline to give the appropriate dimeric molecules closely related in structure to the anti-tumor agents. For purposes of clarity and ease of presentation, these two parts of the work will be dealt with separately in this discussion.

Part I

The four dimeric Vinca alkaloids which have shown interesting anti-tumor activity possess a cleavamine type unit which is functionalized at the C_3 and/or C_4 positions. The nature of these functional groups, either hydroxyl or epoxide, suggest that the corresponding compound having a double bond between C_3 and C_4 would be a valuable intermediate in a general scheme for the synthesis of these functionalized systems. A number of methods could be utilized for the introduction of the necessary groups once the double bond is properly placed in the cleavamine system. At the time this study was initiated, compounds having the



	R	R_1	R_2
vinblastine	CH_3	OH	H
leurosine	CH_3	—O—	
leurosidine	CH_3	H	OH
leurocristine	CHO	OH	H

desired degree of unsaturation such as 18-carbomethoxycleavamine (60) on cleavamine (23) could only be obtained by degradation of the naturally occurring alkaloid, catharanthine (12).[22,37] However, the saturated analogues of these compounds, that is, dihydrocleavamine (29), 18-carbomethoxydihydrocleavamine (32) and dihydrocatharanthine (34) had been obtained in a totally synthetic manner in our laboratories.[27,31] It was thus of interest to develop a method by which the required double bond could be introduced into these compounds and thereby extend the synthetic work to encompass these members.

From another point of view it was also necessary to totally synthesize catharanthine (12). It became desirable in our synthetic studies to have a suitable relay material, one that would obviate the necessity of making large amounts of materials, necessary for further

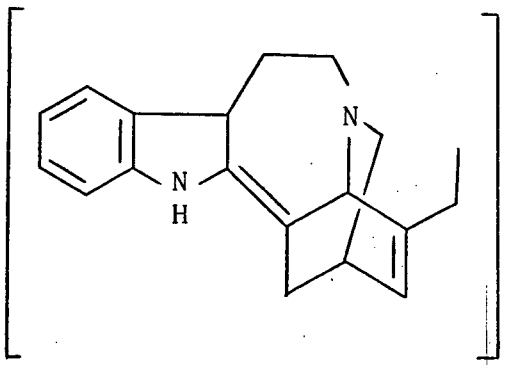
studies, via a lengthy synthetic sequence. Catharanthine, a major alkaloid isolated from the leaves of C. roseus, was the compound most suitable to our interest and one which was used extensively throughout this work. Therefore any studies which would achieve the introduction of the double bond into the dihydrocatharanthine system would be highly desirable.

Our approach to this problem was to attempt to synthesize cleavamine from one of the materials already available via the previous synthetic studies. Earlier in our laboratory, it had been shown that dihydrocleavamine could be converted to 18-carbomethoxydihydrocleavamine and the latter via oxidation to an iminium intermediate, would undergo transannular cyclization to dihydrocatharanthine.[27,31] It was clear that this sequence of reactions could be applied in the conversion of cleavamine to catharanthine via 18-carbomethoxycleavamine. On this basis a synthesis of cleavamine should also constitute a synthesis of the alkaloid, catharanthine.

Catharanthine can be converted into a number of cleavamine derivatives by known procedures. These compounds as well as the chemistry implied in the transformation of catharanthine to them, have been used throughout this thesis. It is instructive for this reason to summarize briefly the relevant portions of this work.

Dihydrocatharanthine, like its Iboga relatives, is readily decarboxylated under acidic conditions. In contrast, the decarboxylation of catharanthine occurs in poor yield only under forcing conditions, (refluxing concentrated hydrochloric acid). The accepted decarboxylation

mechanism for the Iboga alkaloids[34,35] requires the intermediacy of (61).



(61)

The highly strained nature of this intermediate has been cited[22,36] to account for the failure of this reaction under normal reaction conditions. Initial reports of this work indicate the isolation of cleavamine as well as some descarbomethoxycatharanthine.[22] Subsequent investigation of this work by Kutney and coworkers showed that 4-β and 4-α-dihydrocleavamine were also products of this reaction. These results were rationalized on the basis of the mechanism shown in figure 4.[36] Ring cleavage utilizing the lone pair of electrons on N_b would result in the conjugated iminium ion(44). Hydrolysis of the ester function and decarboxylation of the flexible ring opened system gives the intermediate (46). Ring closure would result in descarbomethoxycatharanthine (47) while a prototropic shift to restore the indole system leads to(48). Direct 1,2-reduction of the conjugated iminium salt leads to cleavamine (23) while 1,4-reduction would produce the enamine (49) which could be reduced, via the iminium salt (50) in equilibrium with it, to yield the epimeric 4α (51) and 4β (52) dihydrocleavamines. In the absence of added reducing agents these reactions still occur but in lower yields. Under these conditions, one merely visualizes an oxidation-reduction

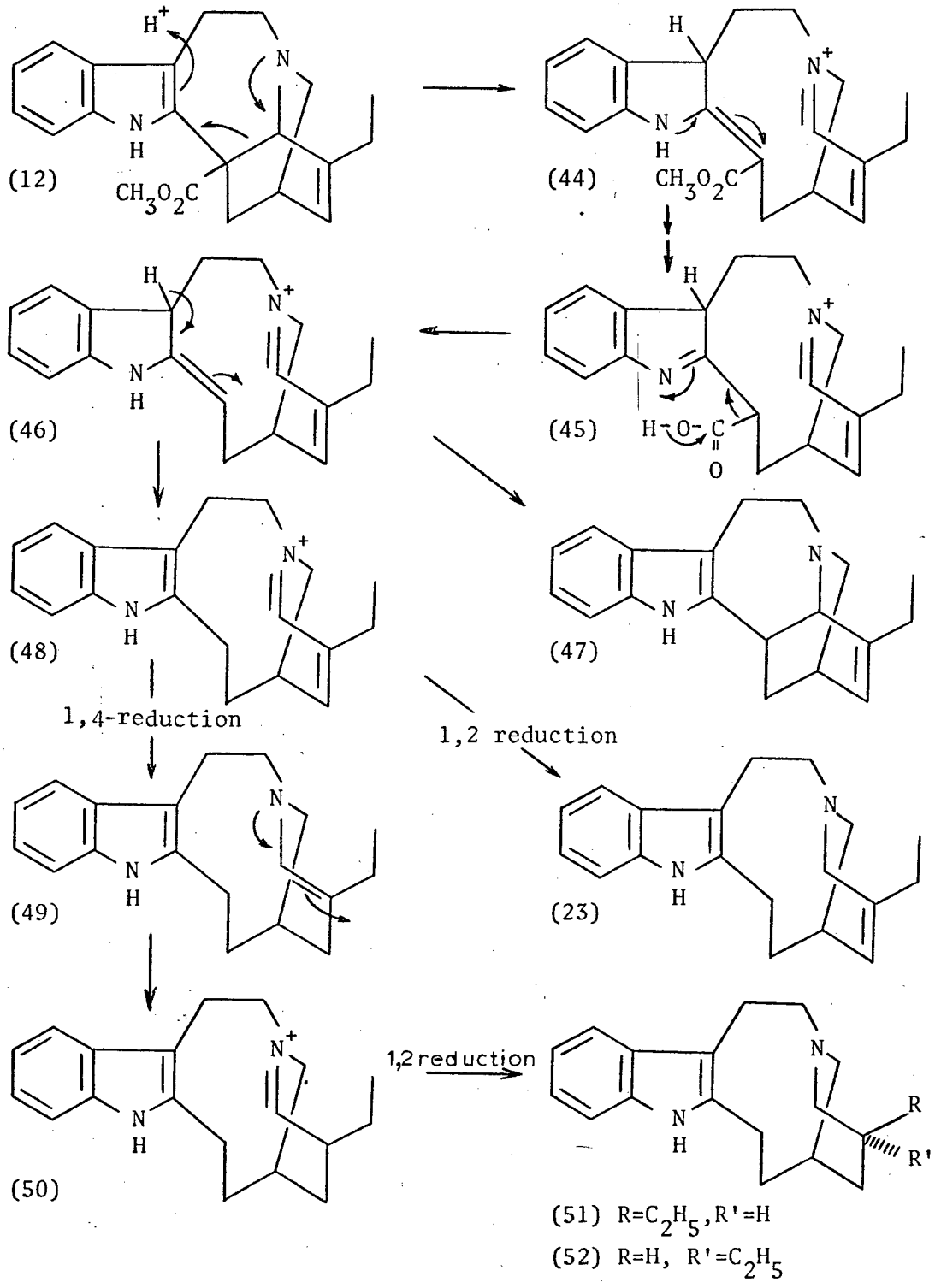
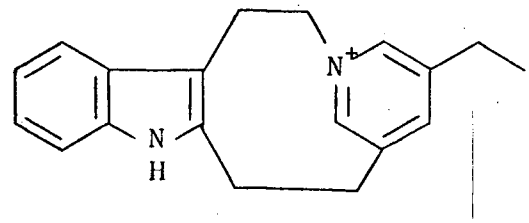


Figure 4. Mechanism proposed for the acid catalyzed rearrangement of catharanthine.[36]

process in which the dihydropyridinium intermediate (48) in its role as a reducing agent, is oxidized to the pyridinium salt (53) while converting 48 to the dihydrocleavamines.



(53)

In glacial acetic acid, decarboxylation is not observed for either dihydrocatharanthine or catharanthine. Dihydrocatharanthine (34) treated in this manner is recovered along with its C_4 epimer coronaridine (35). A mechanistic rationale of this interesting conversion reveals that the ring-opened species (54) is in equilibrium with the pentacyclic compounds via its enamine tautomer as shown in figure 5.[22]

Catharanthine in glacial acetic acid with zinc dust was reported to produce carbomethoxydihydrocleavamine.[14] A careful study of this reaction in our laboratories showed that four epimeric compounds (epimeric at C_{18} and C_4) were produced in this reaction.[37] These results can be readily explained by a reaction scheme (figure 6) similar to the one outlined in figure 4. The intermediate (44) instead of undergoing hydrolysis and decarboxylation as in the previous scheme, proceeds to restore the indole system (56) with resultant epimers at C_{18}. As before, 1,4-reduction followed by tautomerization etc. in the manner, 56 → 59 produces the four carbomethoxydihydrocleavamines (59).

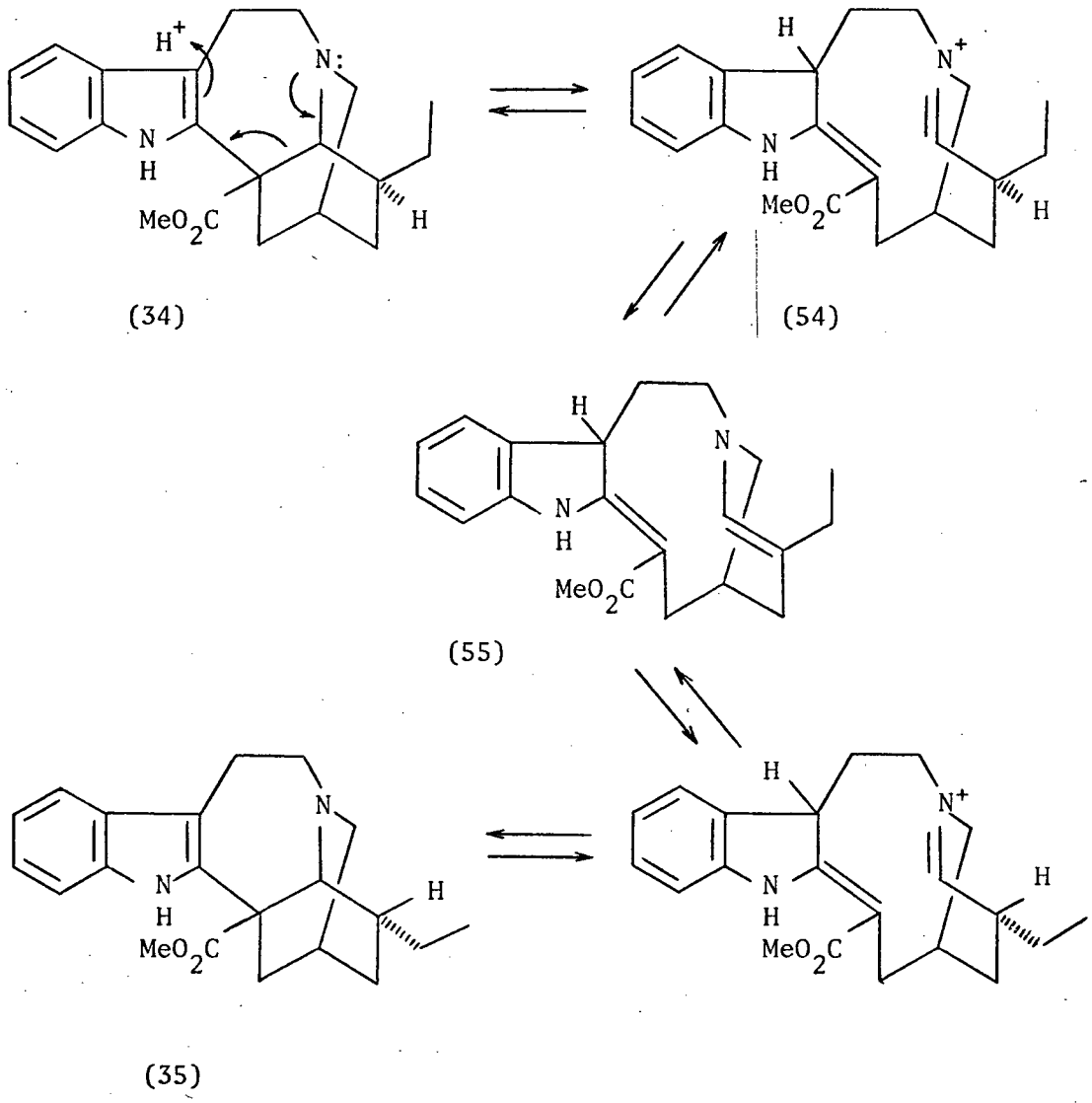


Figure 5. Acid catalyzed isomerisation of dihydrocatharanthine (34) to coronaridine (35).

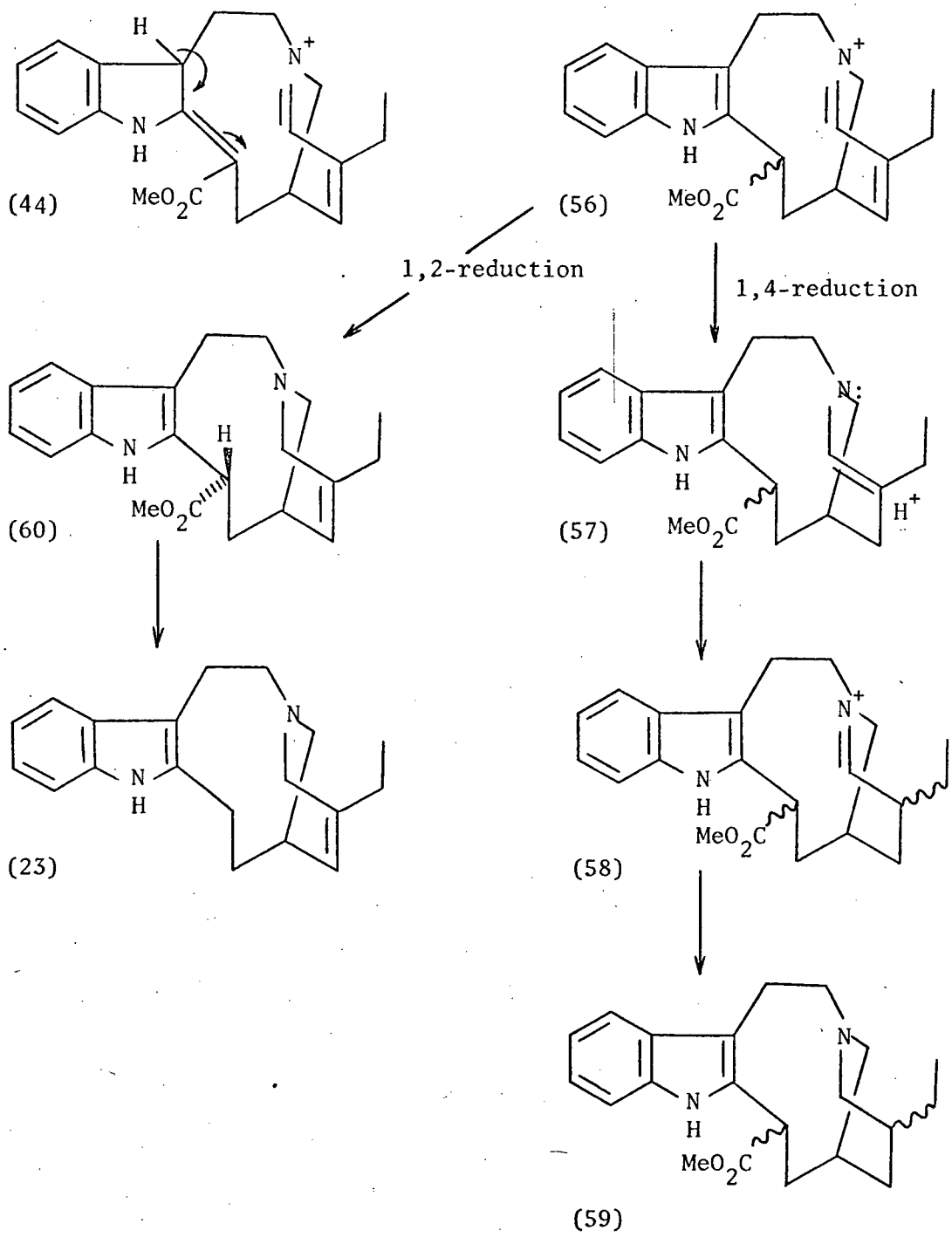
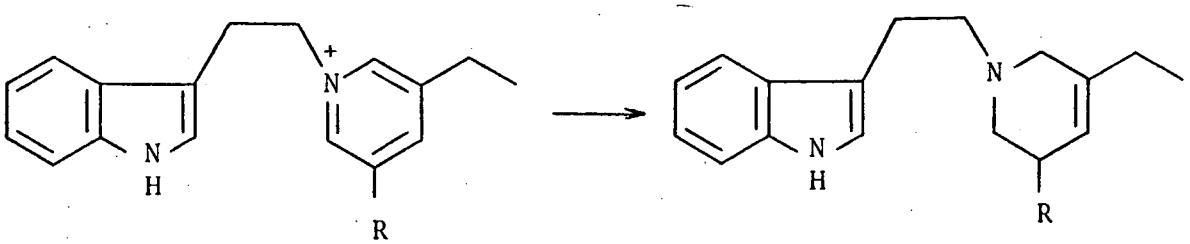


Figure 6. Reduction of catharanthine to the epimeric carbomethoxydihydrocleavamines and 18β-carbomethoxycleavamine.[37]

On the other hand, 1,2-reduction of the intermediate (56) would be expected to yield carbomethoxycleavamine. Indeed catharanthine on reaction with sodium borohydride in hot glacial acetic acid gave a good yield of the previously unknown 18β-carbomethoxycleavamine (60).[37] Because this latter compound can be easily decarboxylated, this procedure thereby made cleavamine (23) and dihydrocleavamine (29) readily available.

Previous to the work carried out for this thesis, attempts had been made by other workers in our laboratory to obtain the unsaturated cleavamine system from one of the totally synthetic compounds. Their approach made use of the acid catalyzed ring opening of dihydrocatharanthine (34) (figure 5). The resulting intermediate iminium salt (62) which may be in equilibrium with (54) was expected to provide a "handle" for subsequent facile dehydrogenation to an aromatic system. Thus the tetrahydropyridinium system (63) would lead to the pyridinium salt (64) as shown in figure 7. Sodium borohydride reduction of such pyridinium salts is known to provide the tetrahydropyridinium system (for example, 65 → 66)[38] and it is clear that the above conversion could lead to the desired carbomethoxycleavamine (60).



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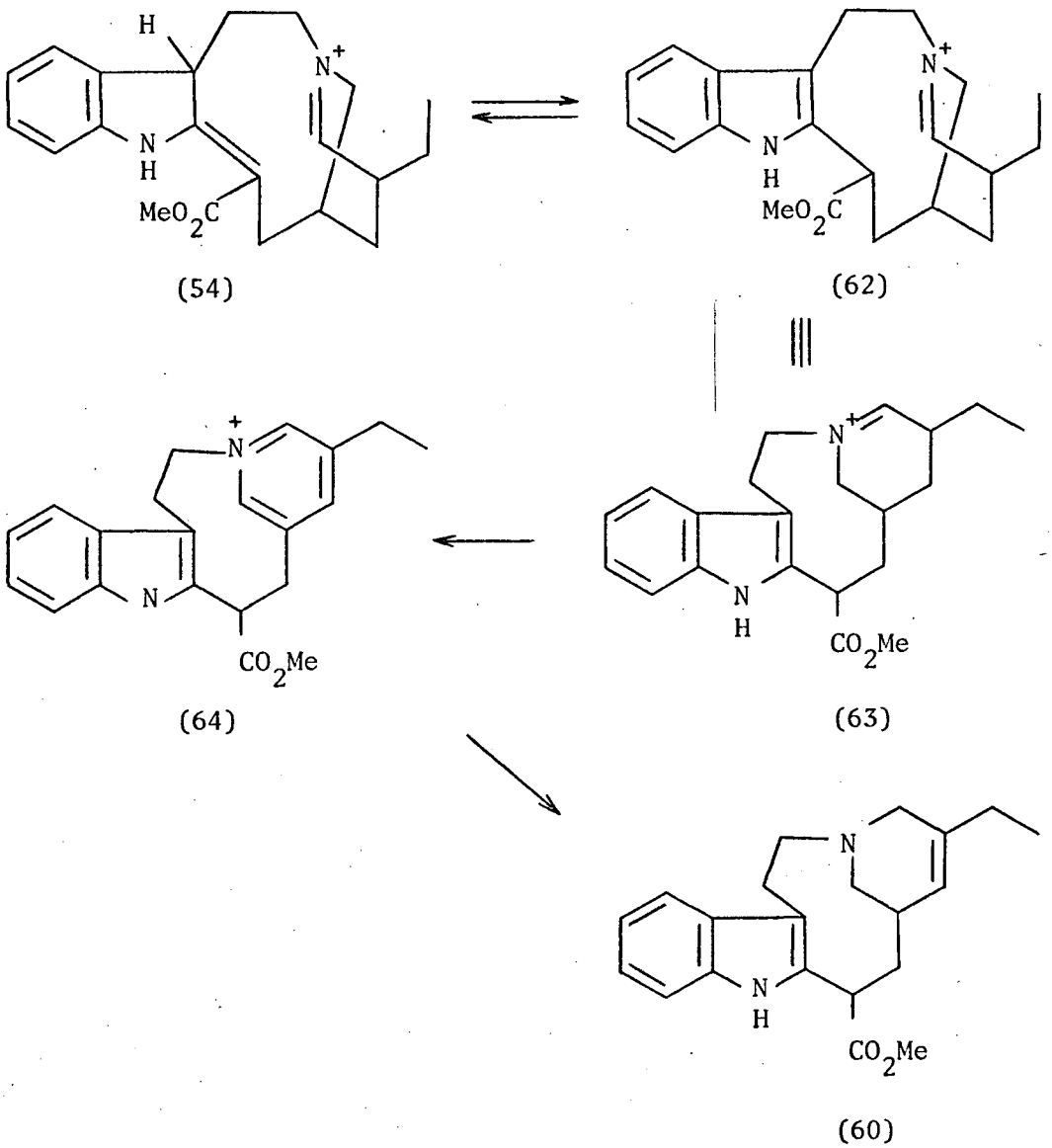
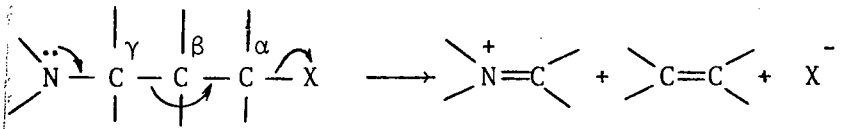


Figure 7. Proposed dehydrogenation-reduction sequence using the ring opened catharanthine intermediate (54).

Experimentally, this reaction scheme could not be realized. The acid catalyzed ring opening step could be readily achieved; evidence for this reaction was the presence of coronaridine in the product mixture

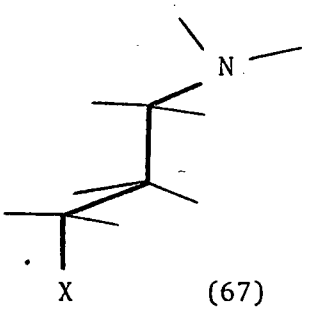
(see figure 5). The difficulty was encountered in the dehydrogenation step. Reagents used for this purpose were 2,3-dichloro-5,6-dicyano-benzoquinone (DDQ), palladium, mercuric acetate and lead tetracetate. A broad range of experimental conditions were tried. Under mild conditions no reaction was observed and dihydrocatharanthine was recovered along with some coronaridine. More forcing conditions consumed starting material but gave no identifiable products. Consideration of the mechanism for this oxidation[39,40] indicates that the iminium salt intermediate is incapable of being oxidized further without first undergoing isomerization to the corresponding enamine. Because the acidic conditions required for ring opening may not favour enamine formation, a number of experiments were also carried out in which the acidic reaction mixture containing the ring opened species was taken to dryness and then oxidized in a basic medium. No favorable results were obtained under these conditions. Attempts to obtain the enamine of 4β-dihydrocleavamine (29) and of 18β-carbomethoxydihydrocleavamine (32) by isomerization of the corresponding iminium salts resulting from mercuric acetate oxidation using basic treatment were also unsuccessful. Some of the transannular cyclization products (29 → 31 and 32 → 33) were obtained in each case indicating that oxidation was indeed occurring but isolation of the appropriate enamines (if formed) could not be achieved.

The approach adopted for this thesis involved a fragmentation reaction which, if successful, would generate the nine-membered ring system inherent in cleavamine as well as the enamine group. In a general way, this reaction can be represented as follows:



X = halogen, OTs, $-OH_2^+$ etc.

The desired heterolytic cleavage between the β and γ carbon atoms would be dependent on the ease with which the -C-X bond is broken and the satisfaction of the resultant electron deficiency at the γ carbon atom. The latter condition is well accommodated by the basic nitrogen while good leaving groups such as tosylate would satisfy the first requirement. A number of competing reactions are possible: 1) substitution; 2) 1,2-elimination and 3) inter- or intramolecular quaternization of the nitrogen atom (see for eq. 74 → 75). It is found, however, that when the involved centres are in a trans coplanar arrangement as shown in structure 67, it is favourable for the fragmentation to occur and this is the predominant mode of reaction observed.[44]



(67)

This reaction was originally employed in the alkaloid field in the degradation of ajmaline (68).[41] The isoquinuclidine system of the sarpagine (69) derived from ajmaline was converted to the corynanthe-type structure (70) as shown in figure 8.

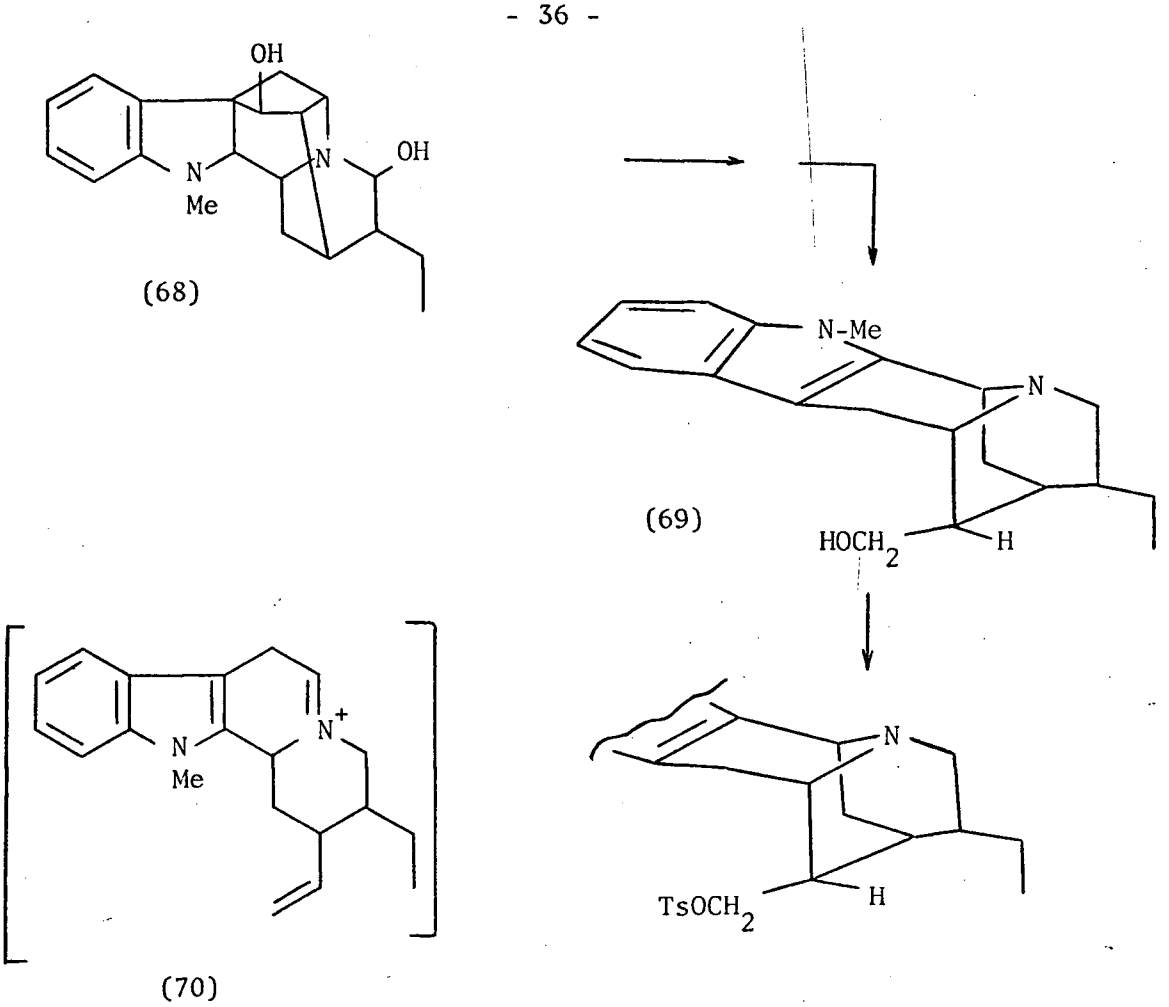
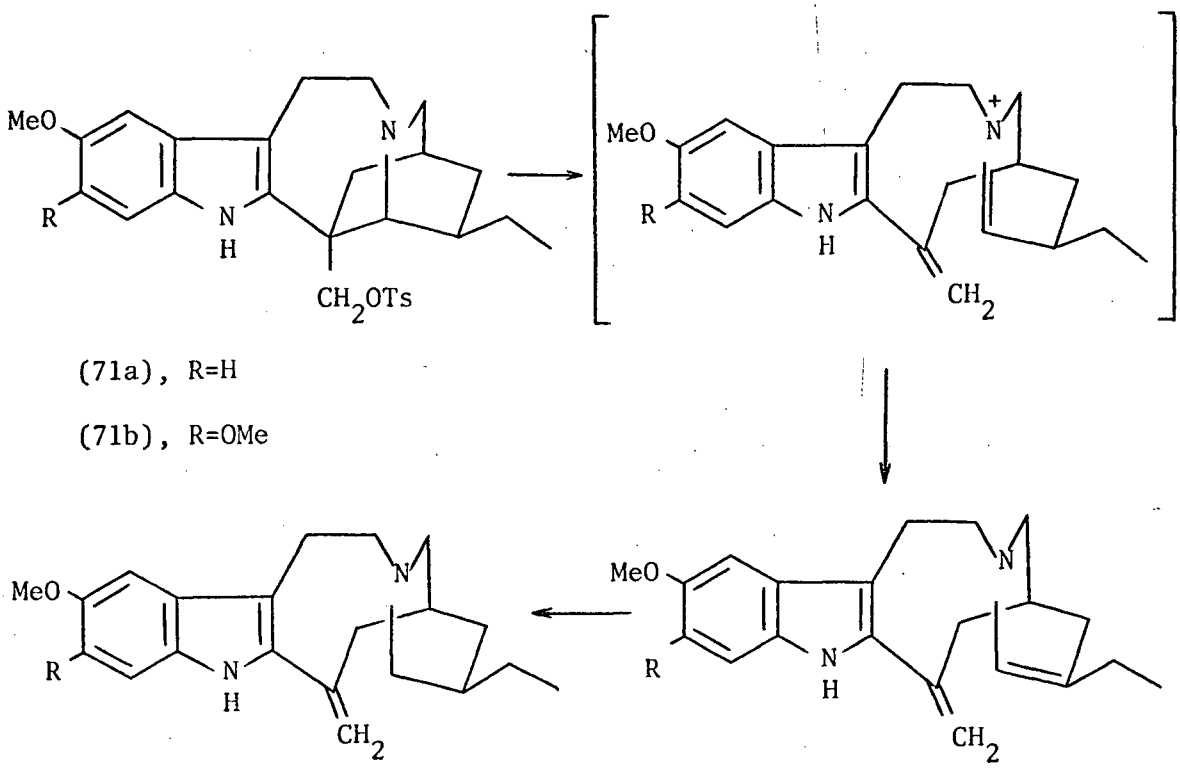


Figure 8. Partial scheme of the degradation of ajmaline (68).

This same reaction scheme was subsequently applied by Renner to the Iboga alkaloids.[42] Voacanginol-O-tosylate (71a) underwent fission to yield the 5,18-secodiene (72a) which was reduced by sodium borohydride to the dihydro compound (73a). Similarly conopharyngol-O-tosylate (71b) was converted to the corresponding fragmentation product (72b) and its dihydro derivative (73b). In contrast iboxygaine (74) which does not have the desired trans coplanar arrangement of the 1,3-aminoalcohol grouping fails to undergo ring cleavage on tosylation



(71a), R=H

(71b), R=OMe

(73a), R=H

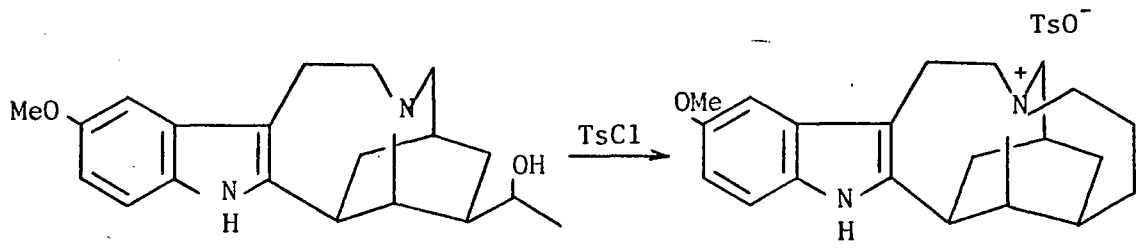
(73b), R=OMe

(72a), R=H

(72b), R=OMe

Figure 9. Fragmentation of voacaginol-O-tosylate (71a) and conopharyngol-O-tosylate (71b).

and gives instead the quaternary tosylate salt (75).[43]



(74) (75)

Renner's reaction scheme (figure 9) seemed particularly relevant to our problem. Catharanthine could be converted to dihydrocatharanthinol (76) using known procedures. Fragmentation of the corresponding tosylate (77) should then give the 5,18-seco-diene (78). Whereas in the acid catalyzed ring opening of dihydrocatharanthine (figure 5) it was necessary to trap the enamine (55) from an equilibrating system, in this case, the enamine would be the result of an irreversible fragmentation reaction. Once this enamine system were available, a number of methods might then be employed to effect a migration of this double bond to the desired cleavamine system. The exocyclic methylene in this intermediate would also be useful in that this function would provide a "handle" by which the carbomethoxy group could be reintroduced.

Catharanthine was readily converted to dihydrocatharanthine by catalytic hydrogenation using Adam's catalyst in ethanol. Lithium aluminum hydride reduction of this product gave dihydrocatharanthinol (76),[22] the starting material for our sequence. Treatment of dihydrocatharanthinol with an excess of tosyl chloride in dry pyridine gave the desired tosylate. This material failed to crystallize using the normal work up procedure.[45] Extraction from aqueous medium gave the crude tosylate in a pyridine solution. The pyridine had to be removed <u>in vacuo</u> at 0°C since the presence of this solvent could not be tolerated in the subsequent displacement step. Rapid decomposition of the tosylate occurred if the above operation was conducted at room temperature. The red-brown gum thus obtained could be induced to crystallize by adding a little benzene and letting the solution stand at 0°C overnight. The tosylate obtained as a light brown crystalline material appeared to be

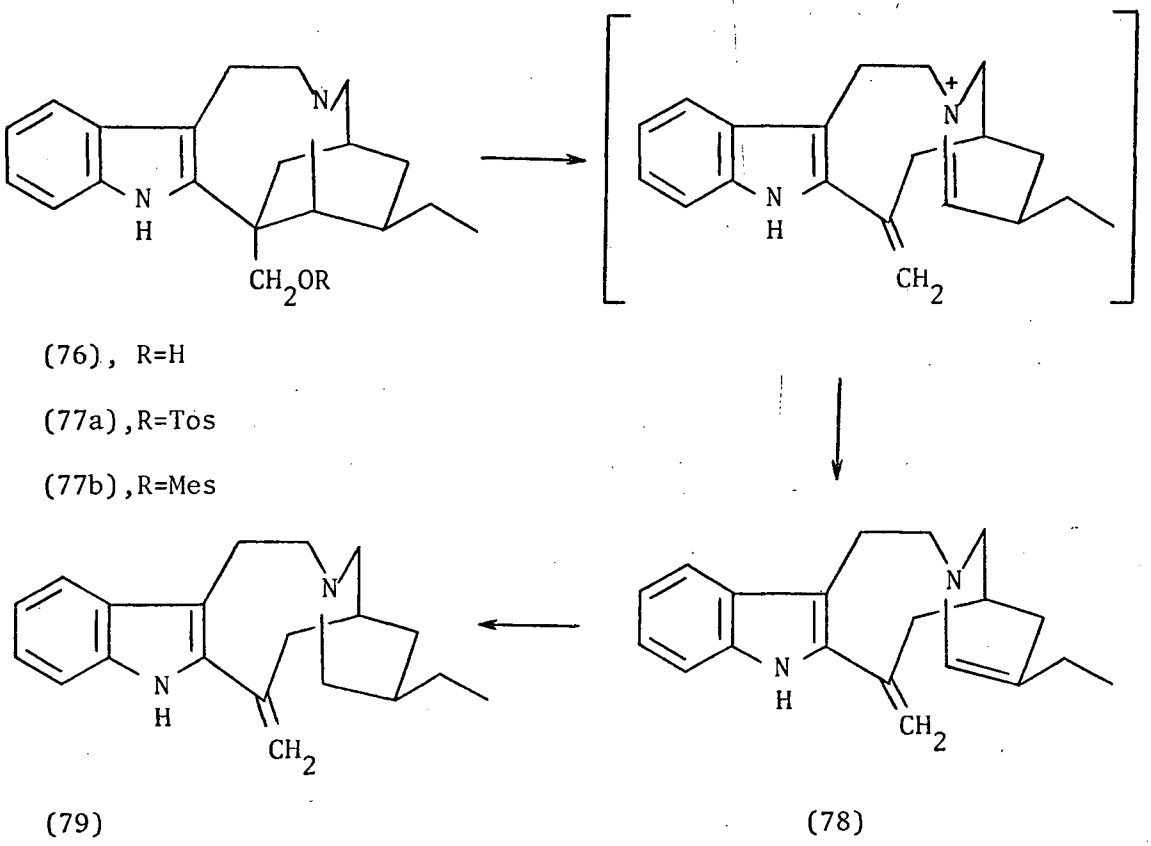


Figure 10. Proposed scheme for ring opening of catharanthine to the 5,18-seco-diene system (78).

stable at room temperature. All attempts to recrystallize this material failed. The ir and uv spectra of this crude crystalline material were in accord with the desired structure. The benzene mother liquors from the above crystallization could be freeze dried to give additional quantities of the tosylate as a brown amorphous powder. The tosylate could be used in the following step, however, as the crude gum provided

very little pyridine were present.

Displacement of the tosylate occurred rapidly in a warm solution of benzene containing some triethylamine. The change of chromophore from indole to a vinyl-indole system allowed this reaction to be conveniently monitored by uv spectroscopy. Optimum results were obtained by keeping the solution at 70°C for two hours under a dry nitrogen atmosphere. The 5,18-seco-diene was quite unstable in solution when exposed to the atmosphere. The work up procedure consisted of cooling the solution under the nitrogen atmosphere, flushing it under positive pressure through a very short column of deactivated alumina and then stripping off the solvent at room temperature under reduced pressure. In this manner, the dark brown reaction mixture was converted to a light yellow solution which crystallized readily on evaporation of solvent. This material (mp 129-135) was almost pure as determined by spectroscopic means and was obtained in 62% overall yield based on starting dihydrocatharanthinol.

Much of our earlier work in this sequence was frustrated by the apparently complex mixtures obtained from this reaction as evidenced by tlc (both alumina and silica) on the crude product as well as a multitude of products obtained by column chromatography. It turned out in fact that the 5,18-seco-diene obtained crystalline and pure by the above procedure also gave an extremely complex mixture on tlc (about eight spots on both alumina and silica) and it became obvious at this point that the material was very unstable to chromatographic procedures.

An analytically pure sample, obtained by sublimation, mp 136-136.5 was exposed to a detailed spectroscopic analysis. High resolution mass spectrometry gave the correct molecular formula, $C_{20}H_{24}N_2$, for the

compound. The enamine gave a strong ir absorption at 1657 cm^{-1} and the single enamine proton of this system appeared at τ 4.29 as a broad singlet in the nmr spectrum. The exocyclic olefin was apparent as part of the vinyl-indole chromophore in the uv (λ_{max} 306),[47] by the absorptions at 1408 and 880 cm^{-1} in the ir, and two singlets at τ 4.75 and 4.89 in the nmr. Finally spectroscopic comparison with an authentic sample of the 5,18-seco-diene system derived from voacanginol-O-tosylate (figure 8) which differs from our compound only in the presence of the C_{12}-methoxyl, established with certainty that our material possessed the desired structure (78).[46]

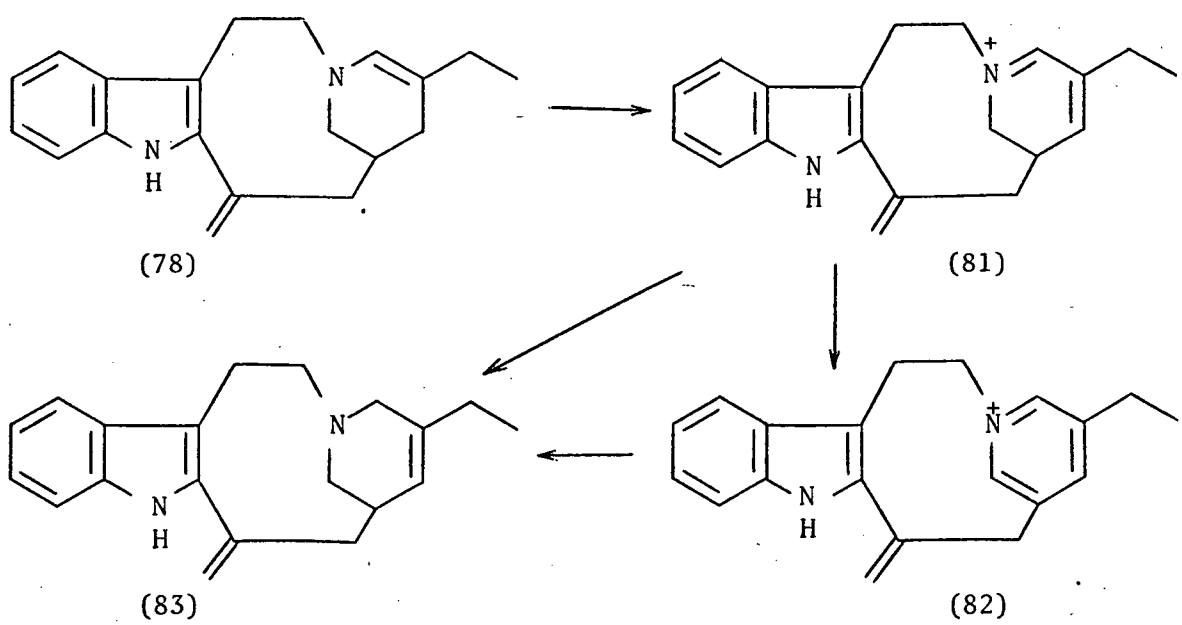
Reduction of this compound with sodium borohydride in methanol produced the expected exocyclic olefin (79). The nmr of this material still showed the protons attributed to the exocyclic olefinic protons as observed in the starting material but the single proton singlet attributed to the enamine system had disappeared. The ultraviolet spectrum also showed the same vinyl indole chromophore. The yield of the overall sequence from dihydrocatharanthinol to the exocyclic olefin (79) was optimum (72%) when the intermediate seco-diene (78) was not isolated but immediately reduced directly in the reaction mixture.

The analogous reaction sequence using the mesylate instead of tosylate was also studied. The mesylate of dihydrocatharanthinol (77b) formed readily by reacting the starting material with methanesulphonyl chloride in pyridine at 0°C. This material could be purified by chromatography although with considerable loss and thus the best yields were obtained by the use of the crude material in the subsequent displacement reaction. Displacement of the mesylate in this case was

carried out using potassium tert-butoxide in tert-butanol. The intermediate (78) could not be isolated from this reaction mixture. Sodium borohydride reduction of this mixture gave the exocyclic olefin identical to that from the tosylate sequence but in much lower yields (20%).

The above studies had now provided, in good yield, an enamine grouping in the nine-membered ring cleavamine-type system. It was now necessary to effect an isomerization of this double bond to the C_3-C_4 position.

Our first approach to this problem was to attempt to dehydrogenate this tetrahydropyridine system to either the dihydropyridine or the fully aromatic pyridinium salt. Selective reduction on either of these intermediates in a manner parallel to that attempted on the acid catalyzed ring opened species derived from dihydrocatharanthine (cf. figure 7) could provide an entry to the cleavamine system bearing a C_3-C_4



double bond. Although aromatization would be the driving force thereby favouring the formation of the intermediate pyridinium salt (82), an examination of the molecular models of this intermediate showed it to be severely strained and it was thought that dehydrogenation would not occur to this extent. However, the dihydropyridinium salt (81) which retains an sp^3 hybridized carbon at the bridged position is not strained and would be expected to form. Reduction of such a system had been accomplished with sodium borohydride[37] and would lead to the desired diene system (cf. figure 6, 56 → 60).

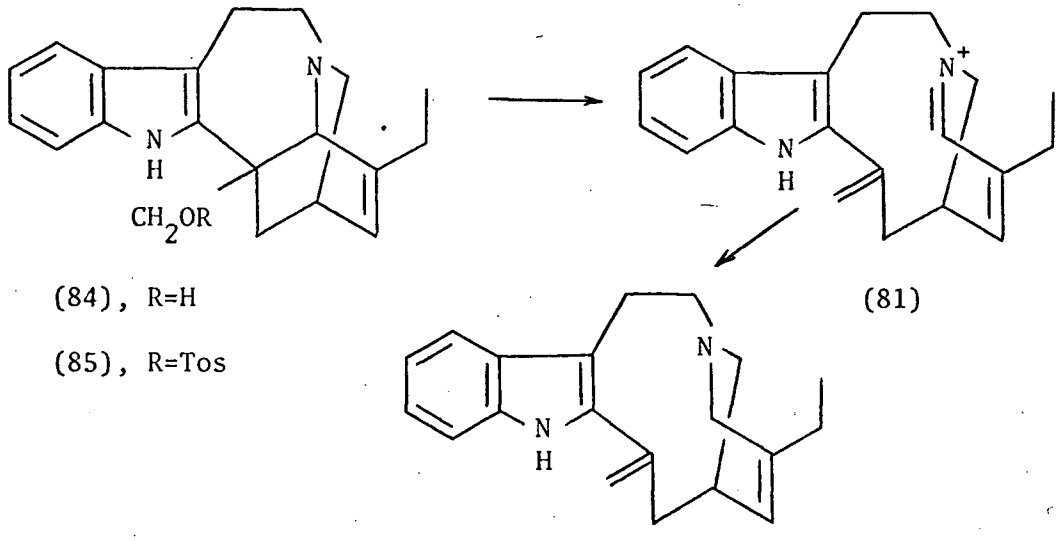
Reaction of the seco-diene (78) with DDQ in benzene at room temperature led to the immediate formation of a dark green precipitate. This material on reduction with sodium borohydride in methanol led to a number of products, a major one being the exocyclic olefin (79). The remaining products showed indole absorption in the uv spectra instead of the vinyl-indole system which was necessary for our further studies. Dehydrogenation with mercuric acetate led to a very complex mixture of products. The uv spectra of fractions from the chromatography of this mixture also indicated an indole chromophore.

An examination of the model of the desired diene product (83) indicated that it is more difficult for this structure than it is for the olefin (79), to exist in a conformation which would allow the exocyclic double bond to be in the plane of the indole system and hence in conjugation with it. Thus although the olefin (79) exhibits the characteristic vinyl-indole absorption, the diene which could conceivably have the exocyclic double bond in a plane orthogonal to that of the indole moiety, might in such a case exhibit a simple indole absorption.[48] The multitude of products obtained from the dehydrogenation reactions

could not therefore be ignored as being undesired materials just because they exhibited an indole absorption in the uv spectrum.

From these initial experiments it became clear that isolation of the diene (83) in reasonable yield from this dehydrogenation-reduction approach might be difficult. It was thought that this task could be simplified considerably if this compound were obtained first via a pathway starting with a model substance already containing the desired C_3-C_4 double bond. If the model sequence could provide the diene, its stability, spectral properties and particularly procedures for its isolation could be studied. It would then be an easier matter to determine whether the dehydrogenation-reduction approach was actually capable of producing this compound and if so, then to set about optimizing the reaction conditions.

The model substance which appeared ideal for this study was the alkaloid, catharanthine. Ring opening of catharanthinol-O-tosylate (85) would give the same intermediate (81) desired in the dehydrogenation reaction. Reduction of this compound would then give the diene (83).



(84), R=H

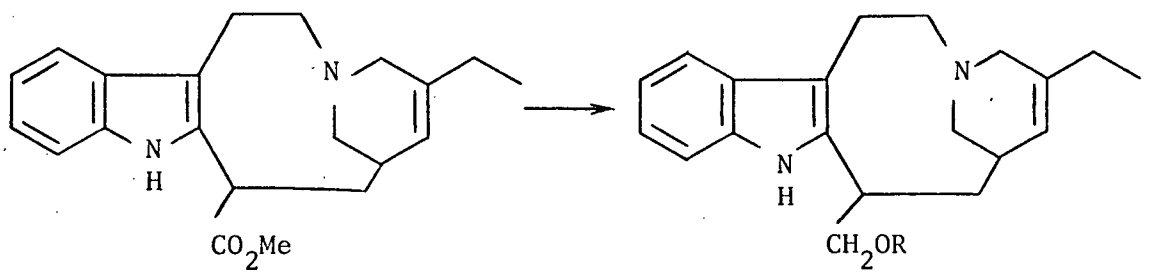
(85), R=Tos

(81)

(83)

Catharanthinol (84) obtained by lithium aluminum hydride reduction of catharanthine was tosylated in the usual manner. This compound was subjected to conditions identical to those successfully employed in the case of dihydrocatharanthinol-O-tosylate. The product obtained was not isolated but subjected immediately to sodium borohydride reduction. A complex mixture of products was obtained. Attempts to isolate these materials by chromatography led to an even worse product mixture than had been observed in the previous studies.

It was now clear that this procedure would provide no simple solution to our problem and we turned to an alternative method for the preparation of the desired diene (83). Carbomethoxycleavamine (60) had



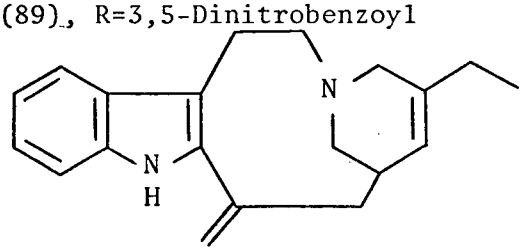
(60)

(86), R=H

(87), R=Tosyl

(88), R=Acetyl

(89), R=3,5-Dinitrobenzoyl

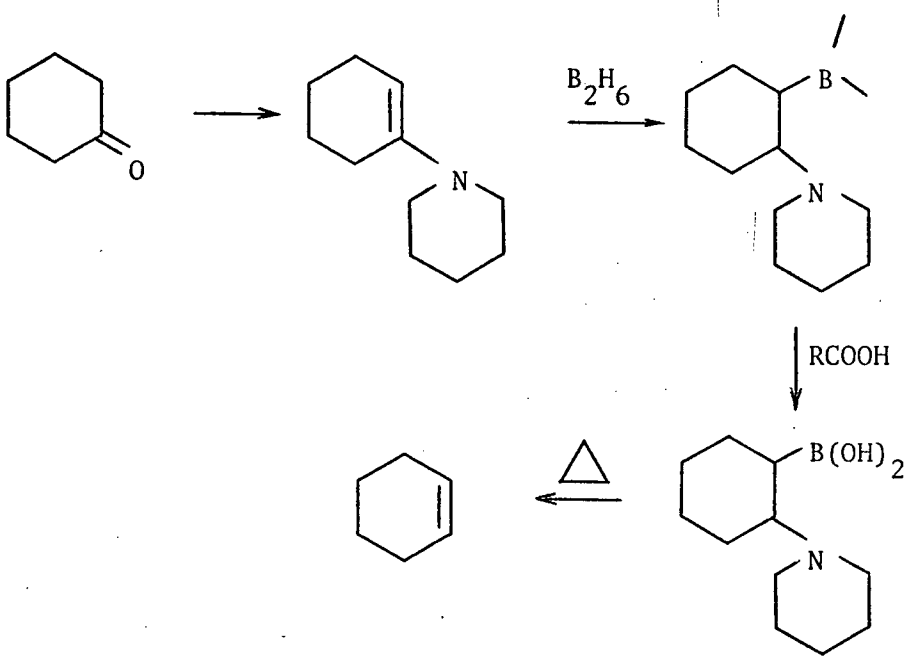


(83)

most of the required structural features and a conversion of the carbomethoxy function to a methylene group would provide the diene (83). The carbomethoxy group in (60) was readily reduced by lithium aluminum hydride to 18-hydroxymethyl cleavamine (86). Dehydration using a variety of conditions gave no encouraging results. The corresponding tosylate (87) decomposed rapidly to give a mixture of products. The acetate (88) was considerably more stable and treatment with strong base gave mainly unchanged starting material. Pyrolysis, however, gave a product mixture which on tlc showed a continuum of spots. The 3,5-dinitrobenzoate (89) was prepared in an attempt to achieve an intermediate between the unstable tosylate and stable acetate. Treatment of this derivative with a number of bases produced a mixture of the unreacted benzoate, the corresponding alcohol and some very polar materials which because of their polar nature could be disregarded as not being the desired diene. This collection of negative results discouraged further work in this direction and thus an alternate study to effect the migration of the double bond in the seco-diene system (78) was initiated.

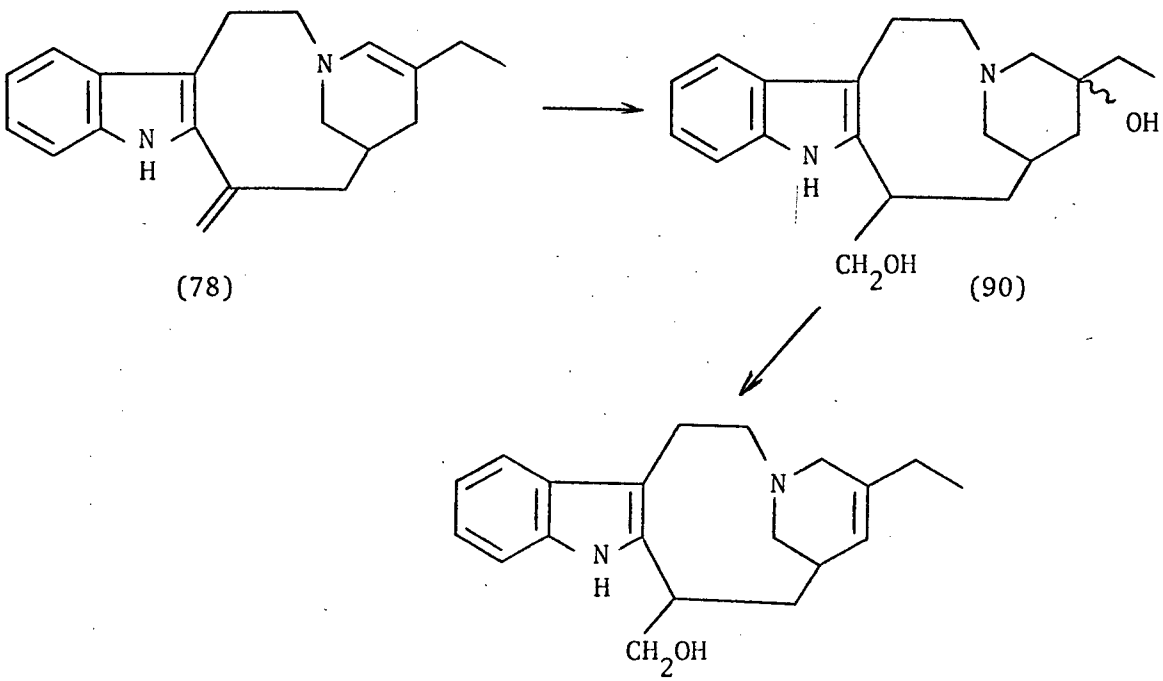
Enamines are subject to electrophilic substitution and addition reactions; both the nitrogen and β-carbon are capable of reacting with electrophiles.[49] In our case the β-position of the enamine is a tertiary carbon and steric factors might in fact favour attack at the nitrogen. However, if reaction did occur at the tertiary β-position, the substituent thus introduced should be easily eliminated and thereby reintroduce a double bond into the system.

The reaction of choice in our case was hydroboration. Hydroboration of enamines has been used in the conversion of ketones to alkenes via an intermediate enamine.[50] In our system, hydroboration followed by



oxidation would be expected to introduce an hydroxyl function at the C_4 position. This is the same position which bears the hydroxyl in the natural dimers vinblastine (16) and vincristine (19). The alcohol obtained from this reaction would therefore be a valuable intermediate for subsequent dimerization reactions. Furthermore tertiary alcohols readily dehydrate to the corresponding olefins. It had already been shown that velbanamine (22), the monomeric unit derived from the degradation of vinblastine, dehydrated to give cleavamine (23) under acidic conditions.[21] This approach could therefore also be expected to yield the unsaturated compound (86) having the double bond in the desired C_3-C_4 position. It was expected that the exocyclic double bond would

also be hydrated in this process. Oxidation of the primary alcohol thus formed, followed by esterification of the acid would provide a convenient method for introducing the carbomethoxy function.



(78) (90)

(86)

In the course of this work and in subsequent hydroboration experiments, it was found that the tertiary nitrogen in these compounds could complex with the diborane to form amine-borane adducts. These adducts were stable to chromatographic separation and could be isolated in this manner as crystalline materials. As a class of compounds these amine-borane complexes are easily identified by a medium to strong, fairly broad absorption bands in the ir spectrum in the region 2200-2400 cm^{-1} usually accompanied by a sharp absorption at about 2300 cm^{-1} which is an overtone of the BH_3 asymmetric deformation seen at about 1150 cm^{-1}.[51]

A convenient method was found to convert these amine-borane complexes to the free bases. This procedure simply involved exchange of the borine from one amine base to a stronger amine base. Thus, the complex when refluxed in tetrahydrofuran in the presence of triethylamine, provided the free base and triethylamine-borane. Some of the complexes obtained were found to undergo only partial exchange using this procedure; an equilibrium mixture was obtained. In these cases, complete exchange could be achieved by using triethylamine as solvent at refluxing temperature.

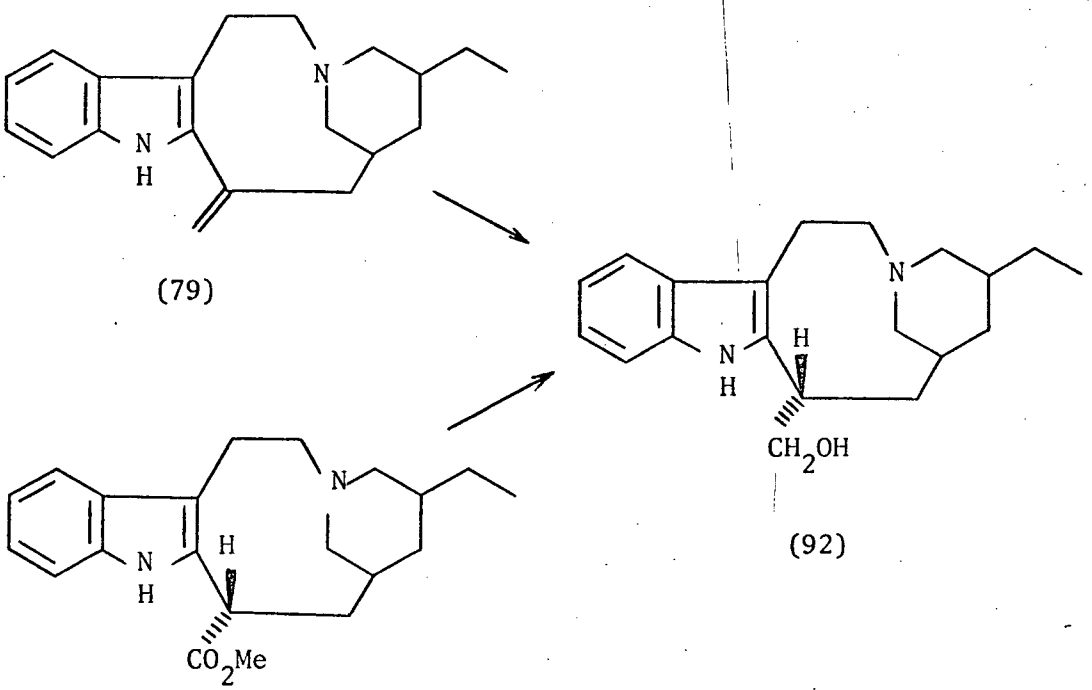
The hydroboration of the seco-diene system (78) in tetrahydrofuran using an excess of diborane, led to a mixture of products. One of these was shown to be the amine-borane complex of the major decomposition product observed previously in other reactions of the enamine system. Three other materials of interest were obtained. The ir spectra of these compounds indicated that one was the alcohol and the other two were amine-boranes. Both of these compounds gave on exchange a compound which was identical with the alcohol obtained directly from the reaction mixture. The presence of two amine-boranes which yield on exchange the same compound indicates that these compounds are most likely epimeric at the nitrogen atom. It is well known that amines undergo facile inversion and it is reasonable to expect that each of the inversion epimers formed by tertiary amines could be trapped by complexing with the lone pair of electrons.

The alcohol obtained from this hydroboration reaction showed in the ir spectrum, an absorption at 3300 cm^{-1} (ν O-H) and at 1045 cm^{-1} (ν C-O for primary alcohols) while the uv gave a typical indole absorption.

Nmr showed a loss of both the enamine proton and the two protons of the exocyclic methylene. Two multiplets of interest appeared, a one proton quintuplet (two overlapping triplet, J = 9 and 5 Hz) at τ 5.81 and a two proton doublet (J = 5 Hz) at τ 6.24. In a decoupling experiment, irradiation at the frequency of the doublet, reduced the quintuplet to a broad doublet (J = 9 Hz). These data were in accord with the hydration of the exocyclic olefin which would give an hydroxymethyl grouping on a tertiary carbon. No evidence for the hydration of the enamine part of this molecule was obtained although it was clear from the ir and nmr, that the enamine was no longer present in the product.

The mass spectrum gave the first indication that this alcohol was not the desired diol (90). The molecular ion was seen at m/e 312 instead of the expected value of 326, and showed a fragmentation pattern indicating reduction of the enamine rather than hydration. A pure sample of the alcohol obtained by sublimation (mp 146.5-147.5) gave the correct analysis for the reduced enamine alcohol.

To establish the identity of this compound, the olefin (79) was hydroborated. As in the previous case, two amine-borane complexes were isolated along with the free alcohol. Exchange of these complexes with triethylamine produced the same free alcohol. This alcohol (92) was identical with that obtained from the seco-diene as shown by comparison of spectral data, tlc, and melting points.



(79)

(92)

(32)

Reduction of 18β-carbomethoxy-4β-dihydrocleavamine (93) with lithium aluminum hydride gave the same alcohol (92) and established without doubt that the structure of the alcohol obtained from both the olefin (79) and secodiene (78) by hydroboration was 18β-hydroxymethyl-4β-dihydrocleavamine.

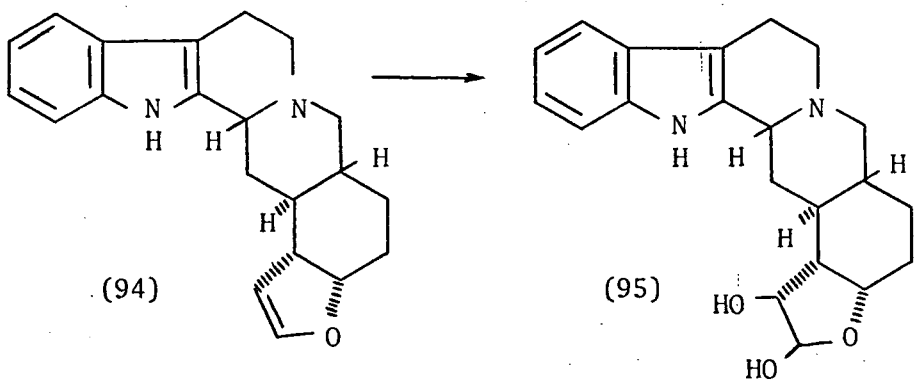
In an attempt to limit the reduction of the enamine system, a number of hydroboration experiments were run using decreasing amounts of diborane. In the above hydroboration of the secodiene system a ten-fold excess of diborane had been employed. When this excess decreased to 0.5 moles based on mono-alkylboranes being formed rather than di- or tri-alkylboranes, the olefin (79) and its amine-borane were isolated from the reaction mixture in addition to the products noted

before. Reduction of the enamine system, therefore, was occurring before hydroboration of the exocyclic double bond. When the excess of diborane was further decreased, carbonyl containing products were obtained indicating hydolysis of unreacted enamine presumably in the work up.

These results show that the desired hydration of this enamine system could not be achieved by hydroboration and thus an alternate route had to be devised. A number of reagents have been used to oxygenate enamines.[52] The reaction generally gives a β-oxygenated iminium salt intermediate which normally is then hydrolyzed to give the α-oxygenated carbonyl system. In our case hydrolysis was not permissible and the intermediate imminium salt would have to be reduced to give the β-oxygenated amine. Oxidation in the presence of an indole moiety, however, generally presents a problem because the indole group is itself rapidly oxidized under mild conditions.[53] The use of the conventional reagents for enamine oxidations, such as peracids, was therefore not suitable.

Our choice of reagent for this reaction was osmium tetroxide. Although there have been no previous reports of this reagent having been used for the hydroxylation of enamines, examples of its use in other systems indicated that it would probably serve well in carrying out the required transformation. For example, β-allylindole had been oxidized to 3,3'-indolylpropan-1,2-diol in high yield using osmium tetroxide at low temperature.[54] Under these conditions, the indole system was unaffected and reaction was specific to the double bond. Use of this reaction was made subsequently by van Tamelen in his

yohimbine syntheses to achieve hydroxylation of the cyclic enol (94) to the diol (95).[55] In both of the above instances, the double bond



(94) (95)

which is oxidized is activated to electrophilic attack. The enamine double bond would also be expected to undergo facile attack and it seemed reasonable that hydroxylation should occur under these conditions.

The reaction scheme envisaged for the conversion of the secodiene (78) to cleavamine and/or velbanamine is presented in figure 11. The tetrol (96) resulting from the osmylation of 78 would be expected to undergo selective reduction to the triol (97) and the latter, via periodate cleavage and reduction should provide the diol (99). Hydrogenolysis of the "benzylic" alcohol in this compound would then give (100) which should be either velbanamine (22) or its epimer. Dehydration of this compound would give cleavamine (23).

The osmylation reaction was carried out at dry-ice-acetone temperature using exactly two equivalents of osmium tetroxide in a tetrahydrofuran-pyridine solvent system. The major product obtained in 35-40% yield gave spectral indications of being the correct tetrol.

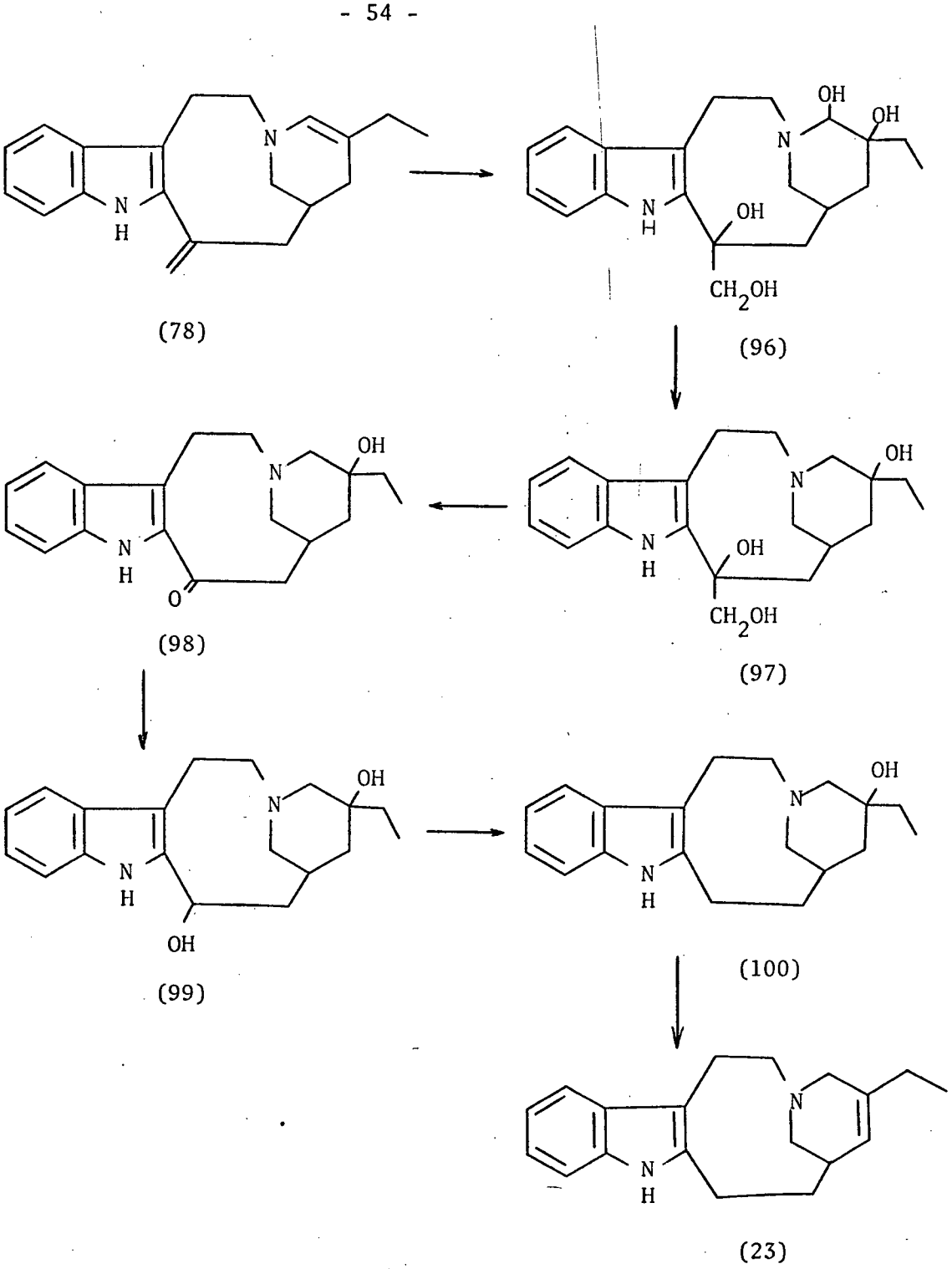


Figure 11. Synthesis of cleavamine (23) from the secodiene (83) via osmium tetroxide oxidation.

The uv spectrum showed a typical indole absorption while the ir spectrum confirmed the presence of an alcohol with three broad overlapping bands in the region, 3300-3500 cm^{-1}. No molecular ion could be seen in the mass spectrum, (figure 12) the highest peak appearing at m/e 342 (M-18). High resolution mass measurement established the formula $C_{20}H_{26}N_2O_3$, corresponding not unexpectedly, to a loss of water from the molecular formula $C_{20}H_{28}N_2O_4$. The nmr spectrum of this compound (figure 14) was particularly instructive. A one-proton singlet at τ 5.42 was in the expected position for a carbinol-amine proton.[58] A somewhat broadened singlet at τ 6.36, integrating for two protons and which showed a dramatic sharpening on deuterium exchange, could be assigned to the hydroxymethyl function. Deuterium exchange resulted in the loss of at least three protons, other than the indole N-H, as shown by comparison of the integral before and after exchange. Two of these hydroxyl protons appeared as surprisingly sharp singlets at τ 6.58 and τ 7.51 and this result indicates that these protons are probably experiencing strong intramolecular hydrogen bonding. The relatively non-polar nature of this compound as shown on tlc and column chromatography undoubtedly also reflects this same phenomenon.

It was expected that the carbinol-amine hydroxyl function would readily undergo hydrogenolysis with a metal hydride reducing agent. The complex normally obtained from the reaction of an alcoholic function and the hydride would eliminate readily to form an iminium salt intermediate. This intermediate in the presence of additional metal hydride would rapidly form the saturated amine.

The tetrol was, therefore, treated at room temperature with sodium

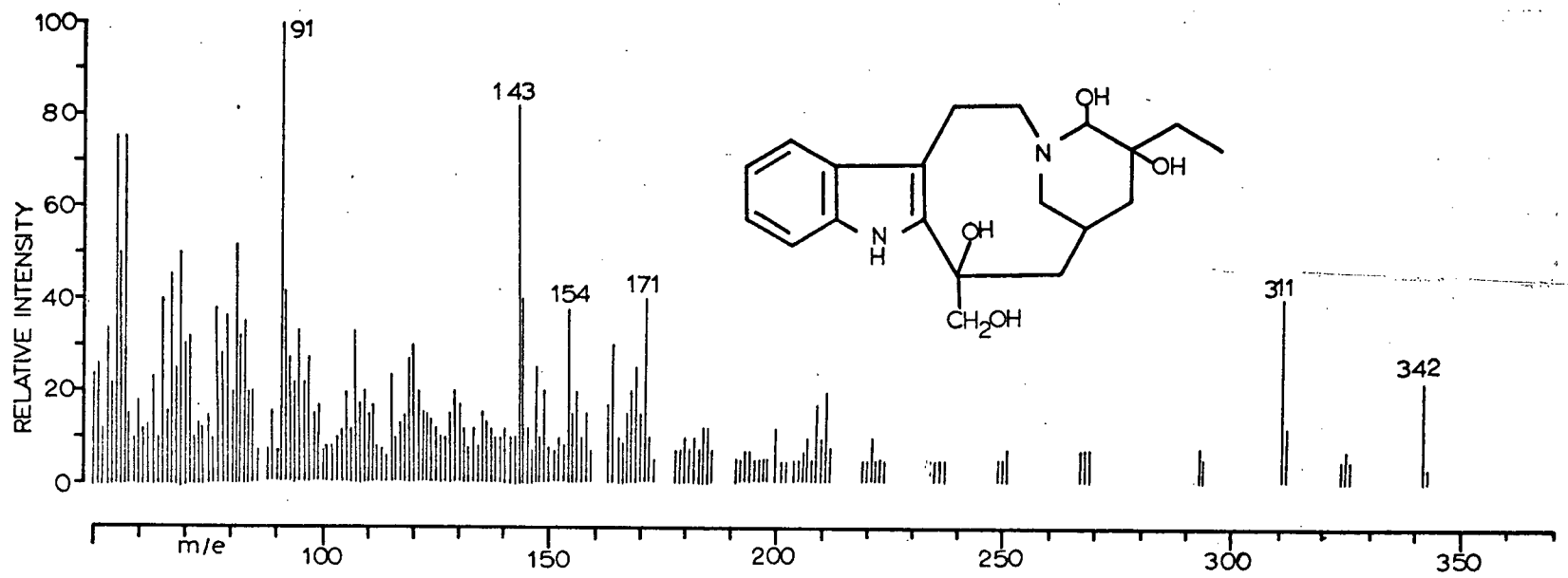


Figure 12. Mass spectrum of the tetrol (96).

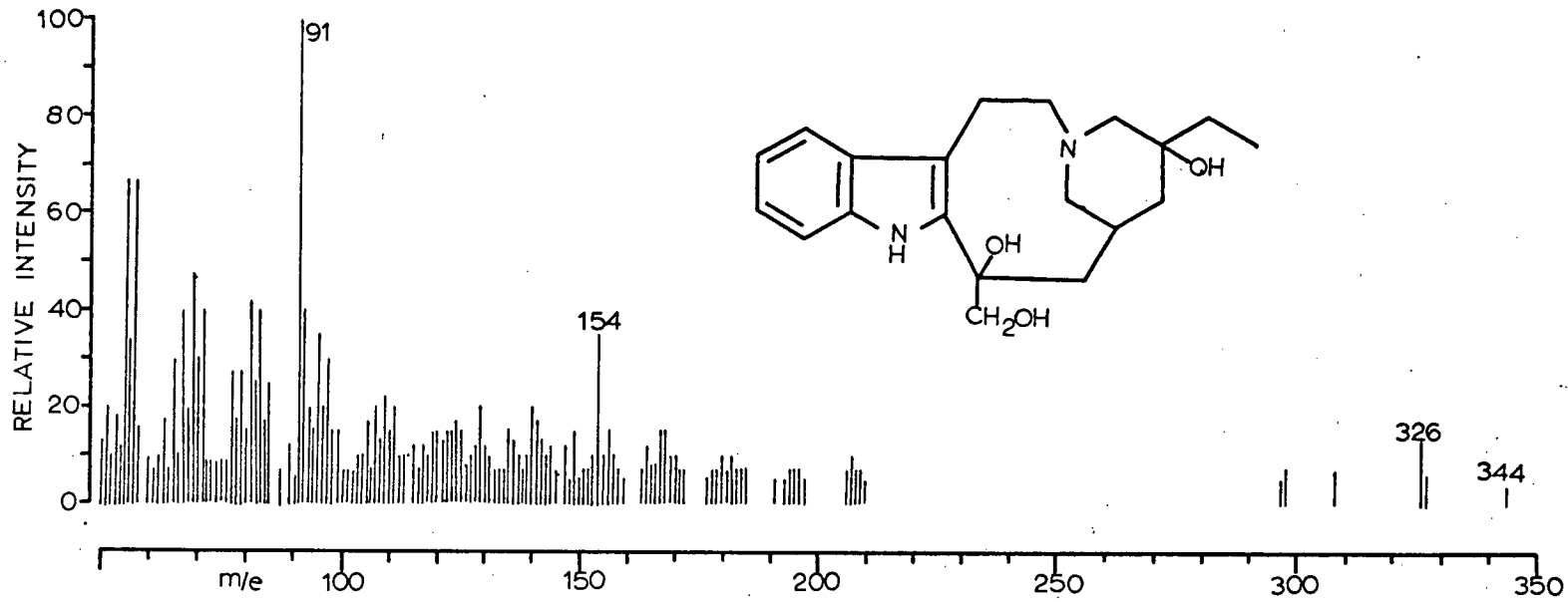


Figure 13. Mass spectrum of the triol (97).

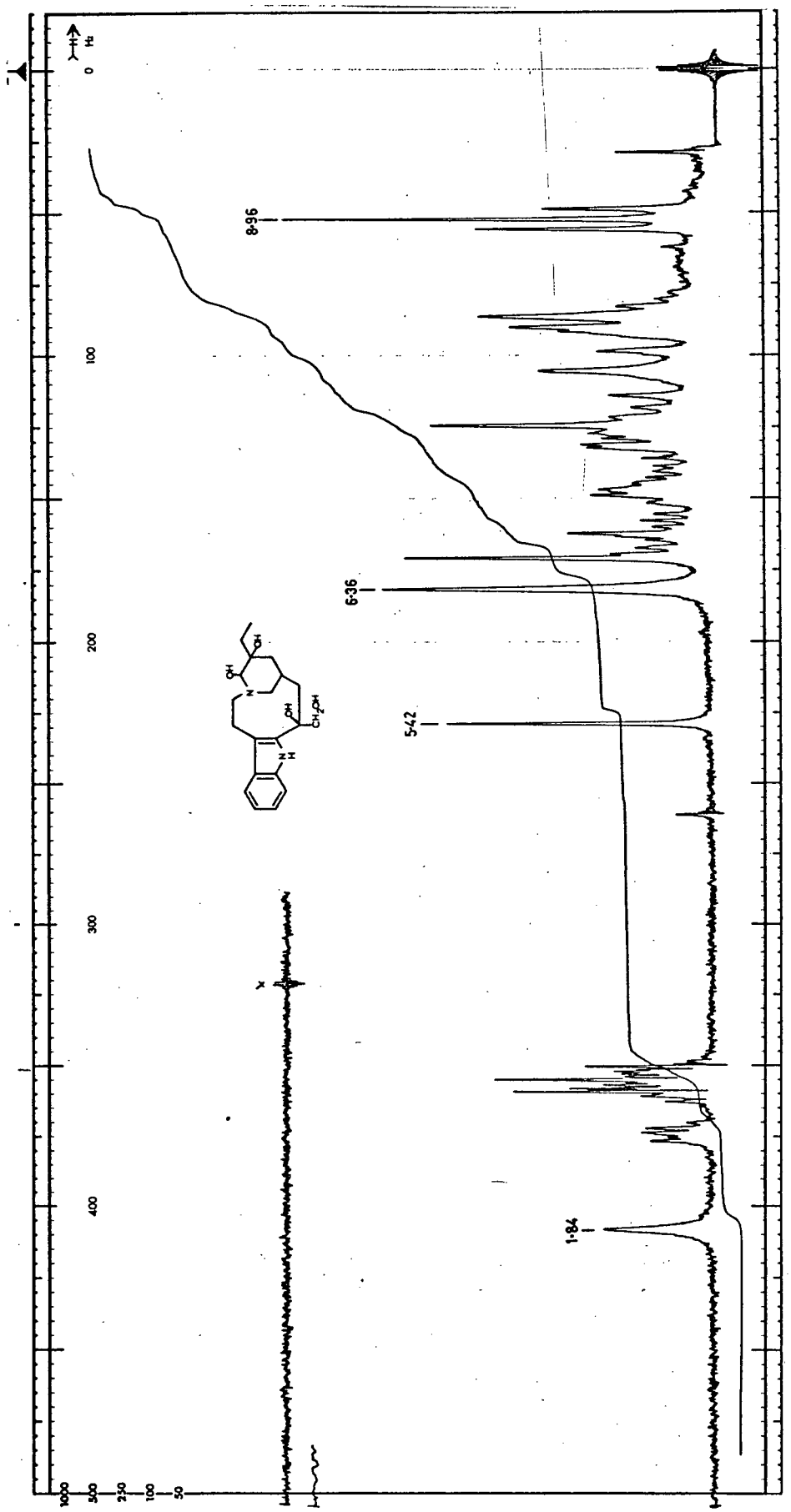
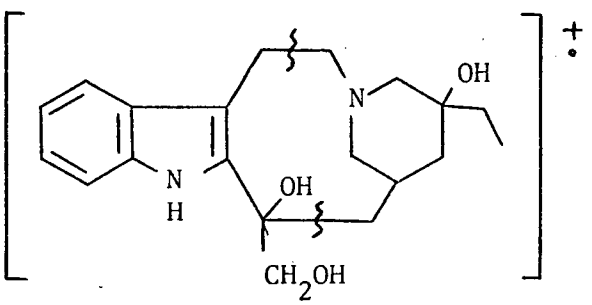


Figure 14. Nmr spectrum of the tetrol (96).

borohydride in methanol. The resultant compound formed in essentially quantitative yield had all the properties expected for the triol (97). The mass spectrum (figure 13) gave a parent ion at m/e 344 and high resolution of this peak established the formula, $C_{20}H_{28}N_2O_3$. One of the important features of this spectrum is the fragment ion at m/e 154. By analogy with the mass spectral work done on velbanamine, this ion can be considered to result from the fragmentation shown in structure 101.[14] It therefore presented good evidence for the presence



(101)

of an hydroxyl function in the piperidine portion of the compound. The ir spectrum showed three broad overlapping absorptions in the N-H, O-H stretching region. Comparison of the nmr (figure 15) of this compound with that of the tetrol showed the loss of the singlet attributed to the carbinol amine proton. The two-proton singlet of the hydroxymethyl function was still present, appearing now at τ 6.22. Deuterium exchange indicated a loss of three protons other than the indole N-H; only one of these hydroxyl protons can be seen as a broad singlet at τ 0.29. Loss of the other two protons is shown by the integration after deuterium exchange

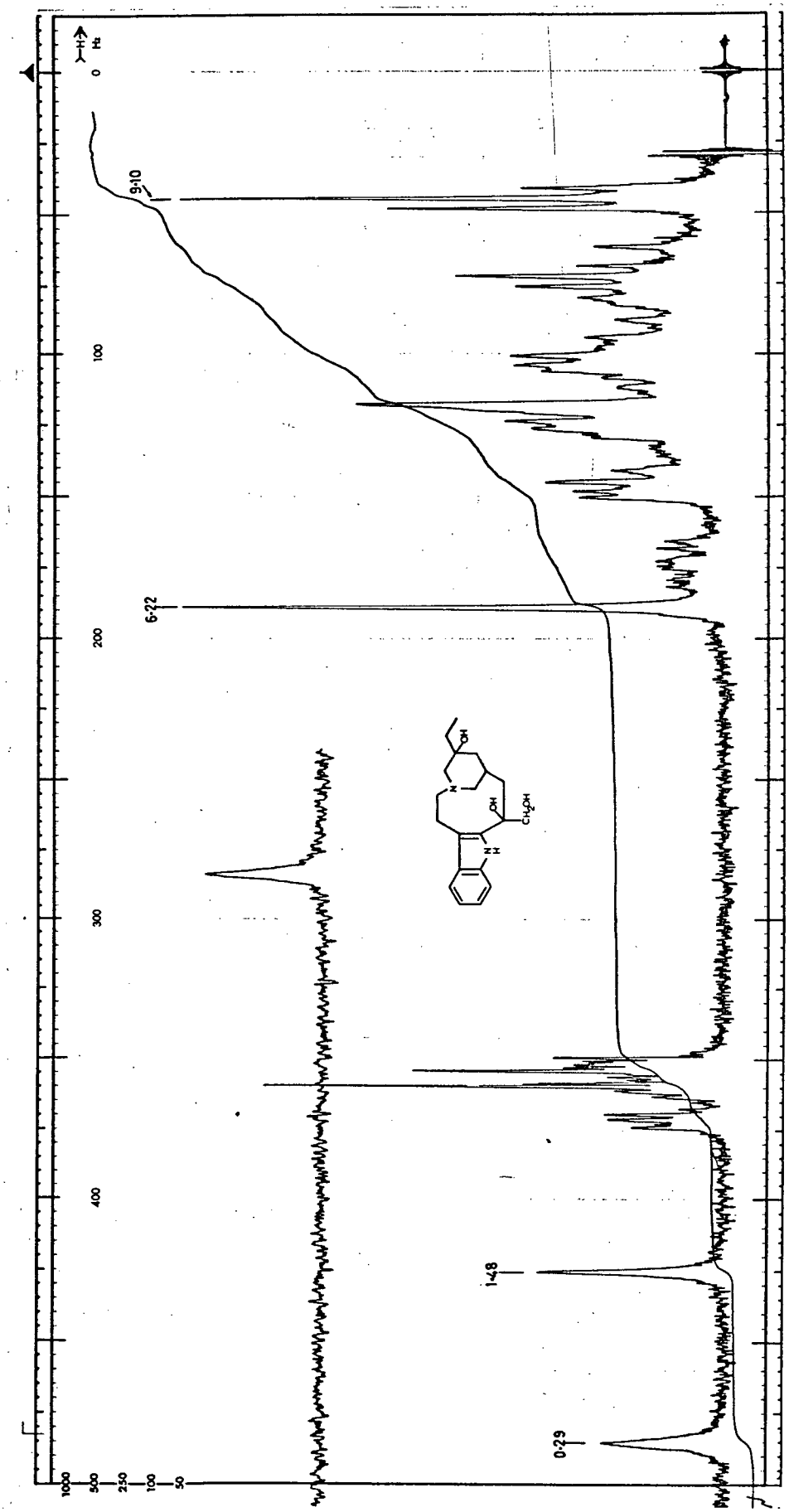
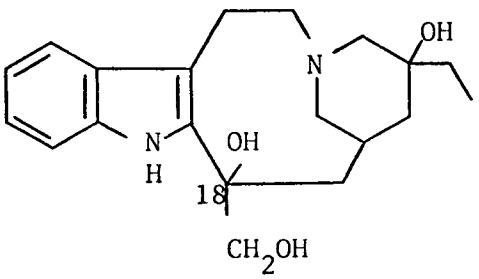
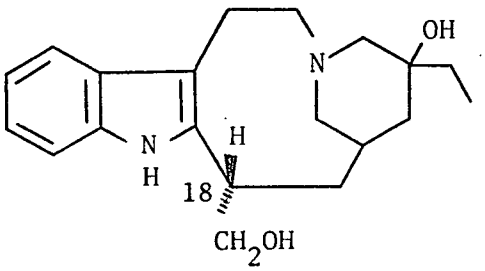


Figure 15. Nmr spectrum of the triol (97).

Further hydrogenolysis of the triol could be achieved when it was treated with lithium aluminum hydride in refluxing tetrahydrofuran. Under these conditions, the "benzylic" hydroxyl at C_{18} (see 102) was lost. Although this reaction was not on the main synthetic pathway, the production of this diol (102) was useful because its nmr spectrum proved conclusively the osmylation of the exocyclic olefin portion of the starting secodiene. The nmr spectrum of 102 possessed a



(97)



(102)

quintuplet at τ 5.90 (J = 5 Hz) and a doublet at τ 6.28 (J = 5 Hz), virtually identical to the multiplets observed for 18β-hydroxymethyl-cleavamine (86) and 18β-hydroxymethyldihydrocleavamine (92) (figure 16). It was thus certain that the compound obtained from reduction of the triol 97 was represented by 102.

For the purpose of completing the cleavamine and functionalized cleavamine syntheses it was necessary to remove the C_{18}-hydroxymethyl function and reduce this C_{18} position to its saturated state. The first step in this process was the periodate cleavage of the vicinal glycol portion of the triol. The best yield, (about 60% based on triol consumed) was obtained when this reaction was carried out in an

	Chemical shift in τ of C_{18}-proton A	methylene protons, B
18β-hydroxymethyl-cleavamine	5.74	6.32
18β-hydroxymethyl-4β-dihydrocleavamine	5.84	6.27
diol (102)	5.90	6.28

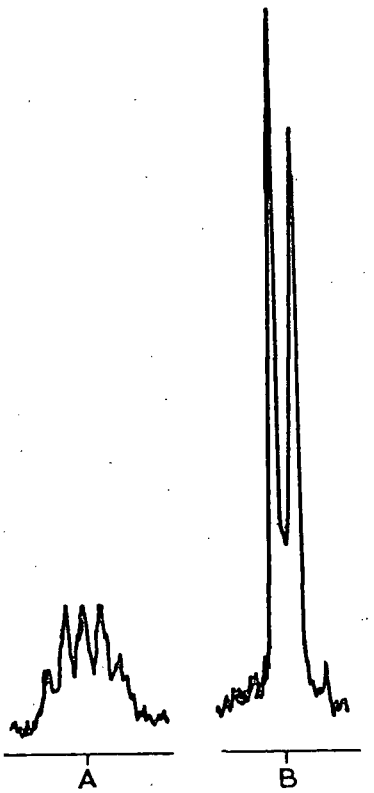
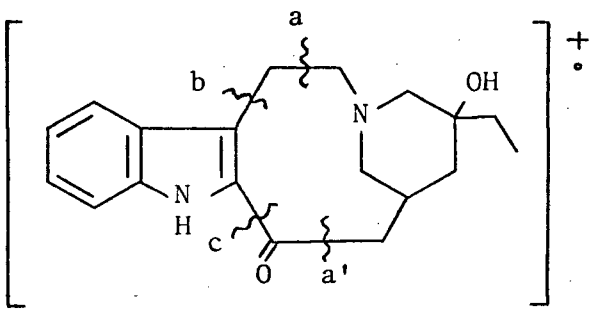


Figure 16. Partial nmr spectrum illustrating the pertinent signal patterns corresponding to the 18β-hydroxymethyl function.

acetone-water solution at 0°C. The spectral data of the product were in excellent agreement with that expected for the ketol (98). This molecule has one remaining hydroxyl function and this feature was evident in the ir spectrum by a broad O-H stretching absorption at 3100 cm^{-1} and in the nmr by a broad one-proton singlet at τ 7.82 which disappeared on deuterium exchange. The extended conjugation produced by the C_{18} carbonyl function affects the aromatic absorption in the uv and the carbonyl absorption in the ir regions. A typical 2-acylindole absorption[56] (λ_{max}^{MeOH} (log ε): 317 (4.25), and 238 (4.16)) is

observed in the uv and the carbonyl stretching absorption in the ir is observed at 1615 cm^{-1} which is also normal for this system.[57] Further strong evidence for this structure came from the mass spectrum (figure 17). High resolution on the parent ion gave a molecular composition corresponding to the formula of the ketol. Examination of the spectrum (figure 17) showed a prominent fragment ion at m/e 154. A fragmentation analogous to the one shown for the triol, namely fission a and a' in 103 would be expected to provide this ion. Loss of a 14 mass units (probably CH_2) from this ion would



(103)

result in the peak at m/e 140. The other half of the molecule resulting from the fission a, a', could provide the ion at m/e 157. The alternate fragmentations shown as b, a' could account for fragments at m/e 143 and 144, while fission a, c would produce the ion observed at m/e 130.

Reduction of the ketol to the diol (99) could be readily achieved by the action of sodium borohydride in methanol at room temperature. This reductive process regenerated the indole system and this was apparent from the uv spectrum. Once again the mass spectrum (figure 18) supported the correct structure. The main fragment ion appeared at m/e 154 and a high resolution mass measurement on the strong parent peak

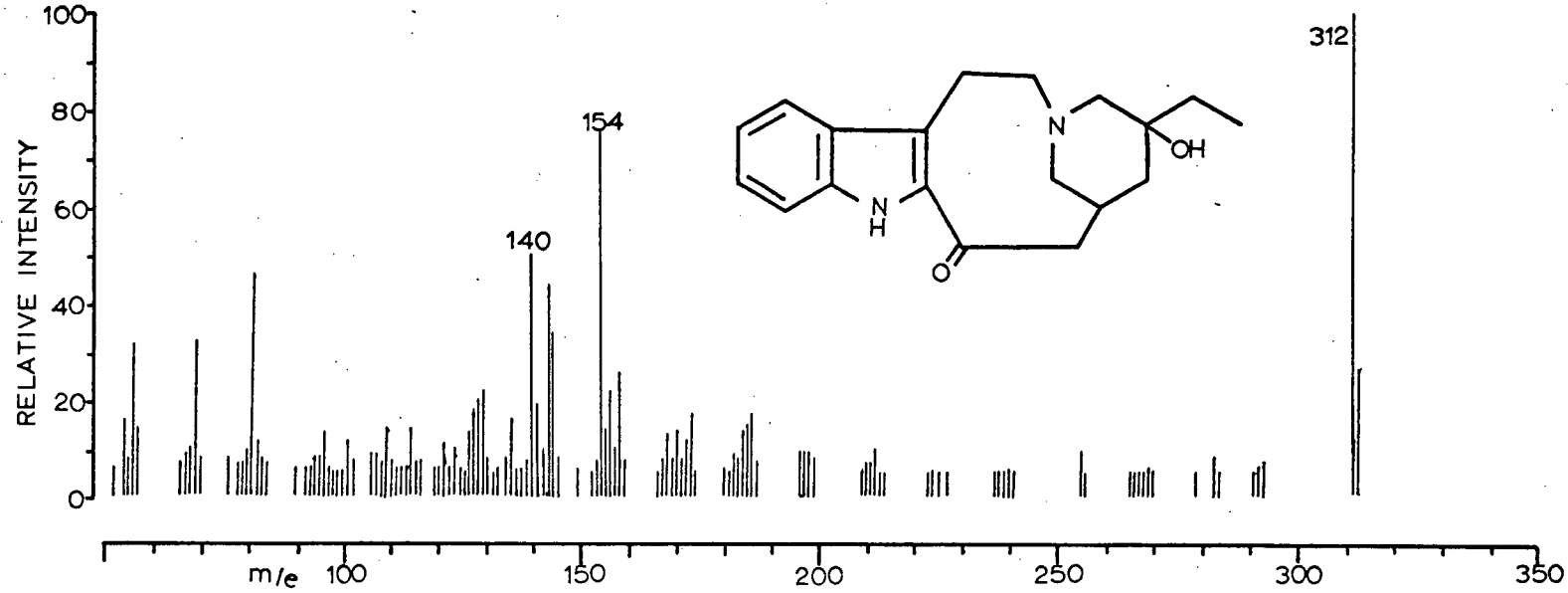


Figure 17. Mass spectrum of ketol (98).

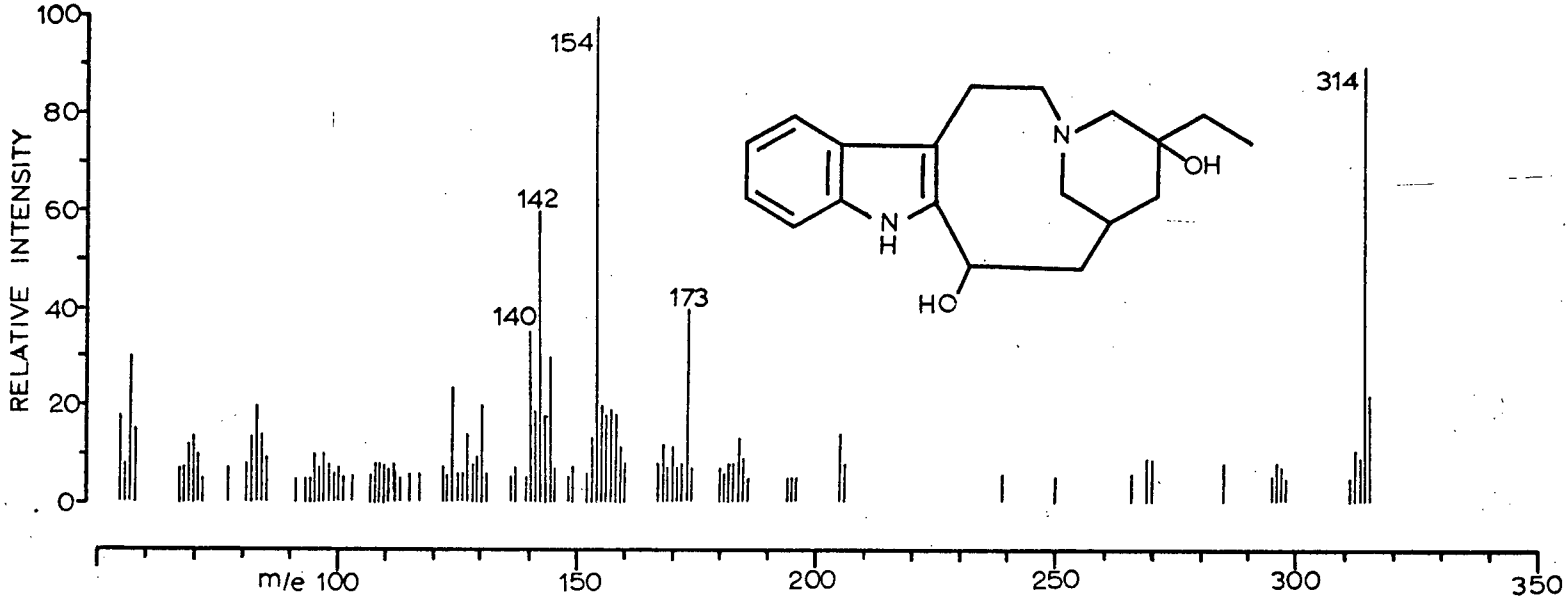
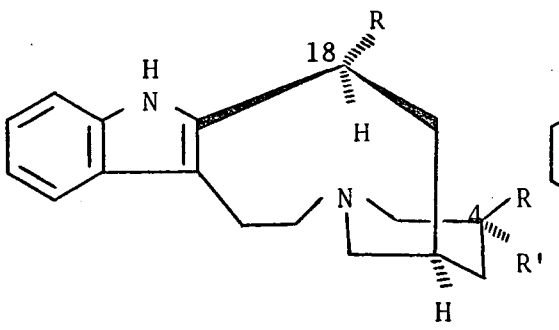


Figure 18. Mass spectrum of the diol (99).

gave the molecular composition corresponding to the formula for the diol.

On the basis of the nmr spectrum (figure 19), the stereochemistry at C_{18}, one of the three asymmetric centres in this molecule, could be assigned. In the course of earlier studies in our laboratory on the acid catalyzed ring opening of catharanthine[37] and in the total synthesis of dihydrocatharanthine[59] alluded to earlier, a series of dihydrocleavamine derivatives possessing a variety of substituents (carbomethoxyl, methoxyl, hydroxyl and nitrile) at C_{18} had been obtained. It was found that these compounds could be classed into two groups, those with the C_{18}-proton absorbing in the region τ 4.5-5.0, and those absorbing in the region τ 6.0-6.2. These compounds by virtue of these chemical shifts, could be assigned to the 18β-substituted or 18α-substituted series respectively. The reason for this dramatic dependence of the chemical shift of this proton to its stereochemistry can be appreciated by considering the conformational structures which are possible in these two series.[37,60] In the 18β-substituted compounds



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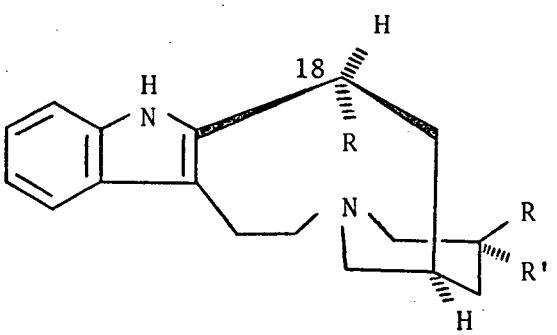

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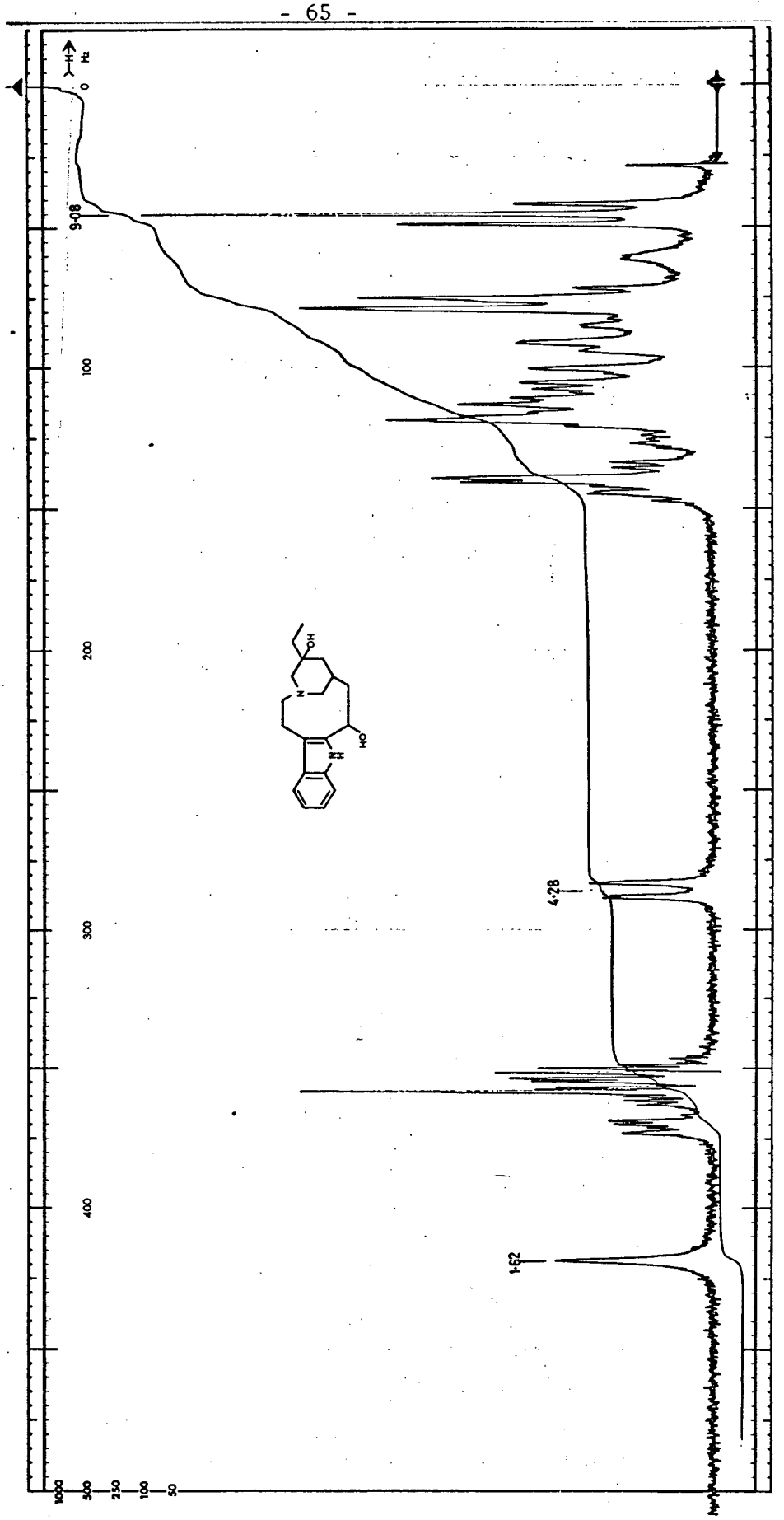


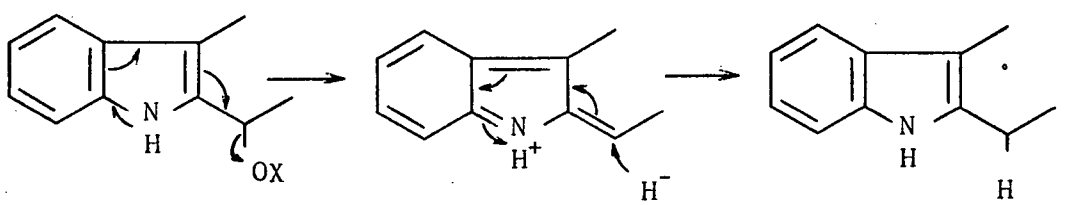
Figure 19. Nmr spectrum of the diol (99).

(104) the C_{18}-proton is in close proximity to the basic nitrogen atom of the piperidine moiety and it would be expected to absorb at lower frequency. Such a situation does not prevail for the 18α-substituted compounds (105) and a more normal resonance frequency would be anticipated for the C_{18}-proton.

The chemical shift observed for the C_{18}-proton in the diol (99) is τ 4.28. This is clearly in the region for the 18β-substituted compounds although in this case it is shifted to slightly lower field than in the above mentioned compounds. This enhanced deshielding effect must be attributed to the C_4 alcohol substituents which can be expected to affect the conformation of the piperidine ring and hence, alter the spatial relation between the nitrogen and C_{18}-proton. This same effect can be seen by a comparison of the C_{18}-proton chemical shift for 18β-carbomethoxy-4β-dihydrocleavamine and 18β-carbomethoxy-4β-dihydrocleavamine in which the ethyl group would affect the conformation depending on its orientation relative to the nitrogen atom. The 4α-compound absorbs at τ 4.53 while the 4β absorbs at τ 4.98.

Hydrogenolysis of the diol (99) to the monohydroxy derivative (100) could be achieved using the conditions prescribed by Dolby and coworkers.[61] These workers found that 2-indolecarbinol derivatives were hydrogenolyzed using lithium aluminum hydride in ether solvents. When ethyl ether or tetrahydrofuran was used as solvent, a mixture of the alcohol and the hydrogenolyzed material was usually obtained. The proportion of hydrogenolyzed material could be raised by carrying out the reaction in refluxing N-methylmorpholine or in dioxane. Thus,

1-hydroxytetrahydrocarbazole was converted almost quantitatively to tetrahydrocarbazole using boiling dioxane. N-methyl-1-hydroxytetrahydrocarbazole, however, gave only 21% of the hydrogenolyzed product. This inhibition of hydrogenolysis by methylation of the indole nitrogen was observed in a series of compounds and suggested that the reaction involved an elimination-addition sequence involving an imine intermediate such as (106). Such a mechanism is not possible when the



(106)

indole nitrogen is methylated and it was suggested that under drastic conditions some simple nucleophilic displacement takes place to give the low yield of the hydrogenolyzed product observed.

In our instance, the desired derivative (100) could be obtained from the diol in 44% yield using lithium aluminum hydride in refluxing N-methylmorpholine. The nmr spectrum of the product (figure 20) shows that the peak assigned to the C_{18}-proton in the diol has moved considerably upfield to τ 6.56 and now appears as a complex multiplet. This upfield shift was expected on loss of the geminal hydroxyl function. The remaining O-H gives a broad, one-proton singlet at τ 8.77 which disappears on deuterium exchange. High resolution mass

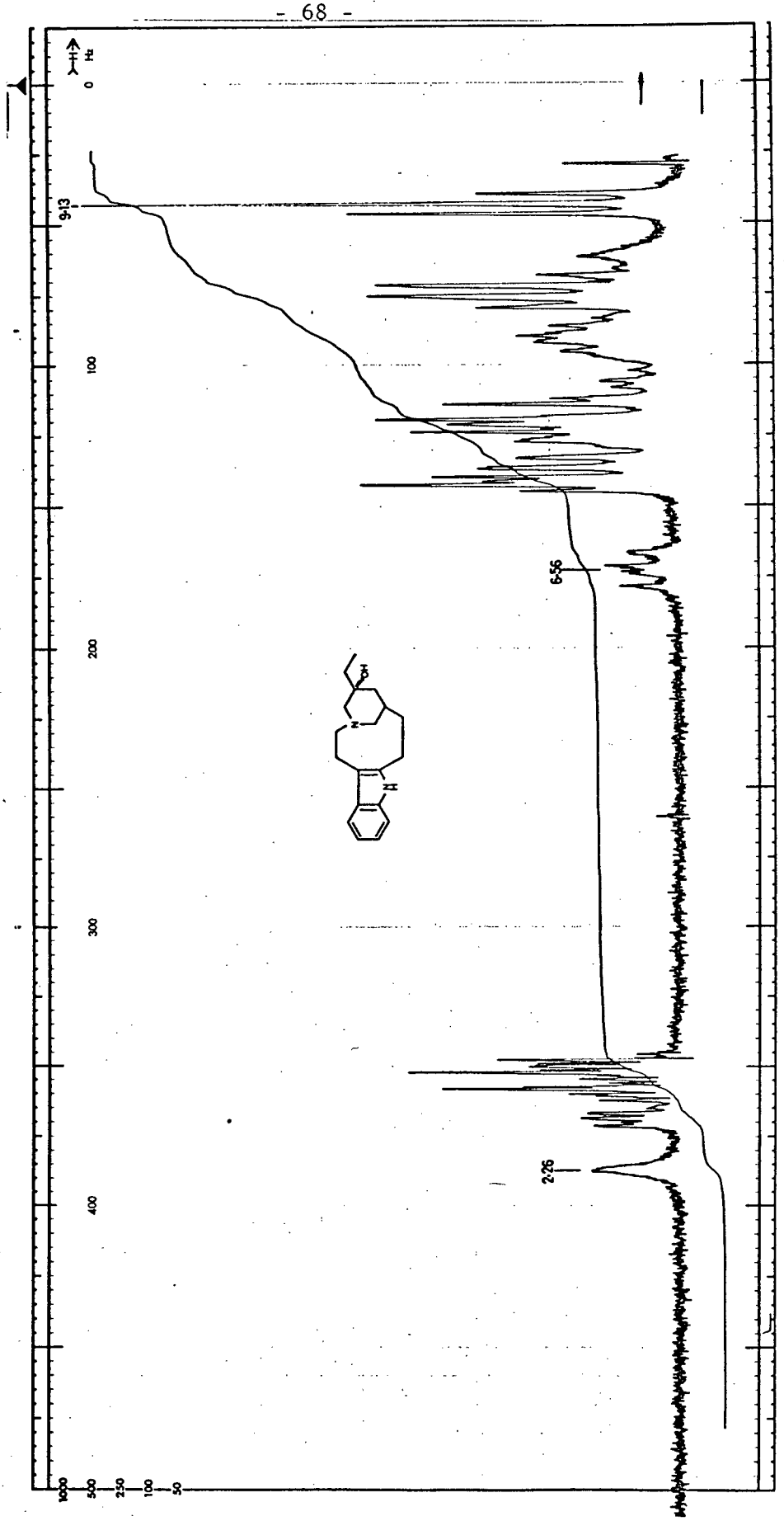


Figure 20. Nmr of isovelbanamine (100).

analysis of the parent ion in the mass spectrum established a molecular composition consistent with the formula for the mono-ol. This compound had to be either velbanamine (22), the monomeric degradation product of vinblastine and vincristine or the epimeric alcohol. Thin layer chromatographic comparison of our compound with an authentic sample of velbanamine obtained by the established procedure from vinblastine,[21] showed that these compounds were not the same. The mass spectrum of our compound which has been named isovelbanamine (figure 21) was, however, virtually superimposable with the spectrum obtained for velbanamine run under the same conditions and agrees also very well with the published mass spectrum for velbanamine.[62] This result provided strong evidence for the fact that these compounds were epimeric and this situation was proved by the next series of reactions.

It was known that velbanamine undergoes some dehydration to give cleavamine (23) under the acidic condition used to cleave vinblastine.[21] Because the hydroxyl group eliminated is that of a tertiary alcohol, an E_1 type elimination which need not have a particular stereochemical requirement, would be expected. Therefore isovelbanamine should dehydrate under the same conditions as velbanamine.

Treatment of isovelbanamine with concentrated sulphuric acid at 0°C for 2 hours gave approximately a 30% yield of cleavamine as identified by a comparison with an authentic sample. Apart from establishing the structure of 100, this reaction provided a total synthesis of cleavamine since the starting material for this sequence dihydrocatharanthine, had already been synthesized in our laboratory.

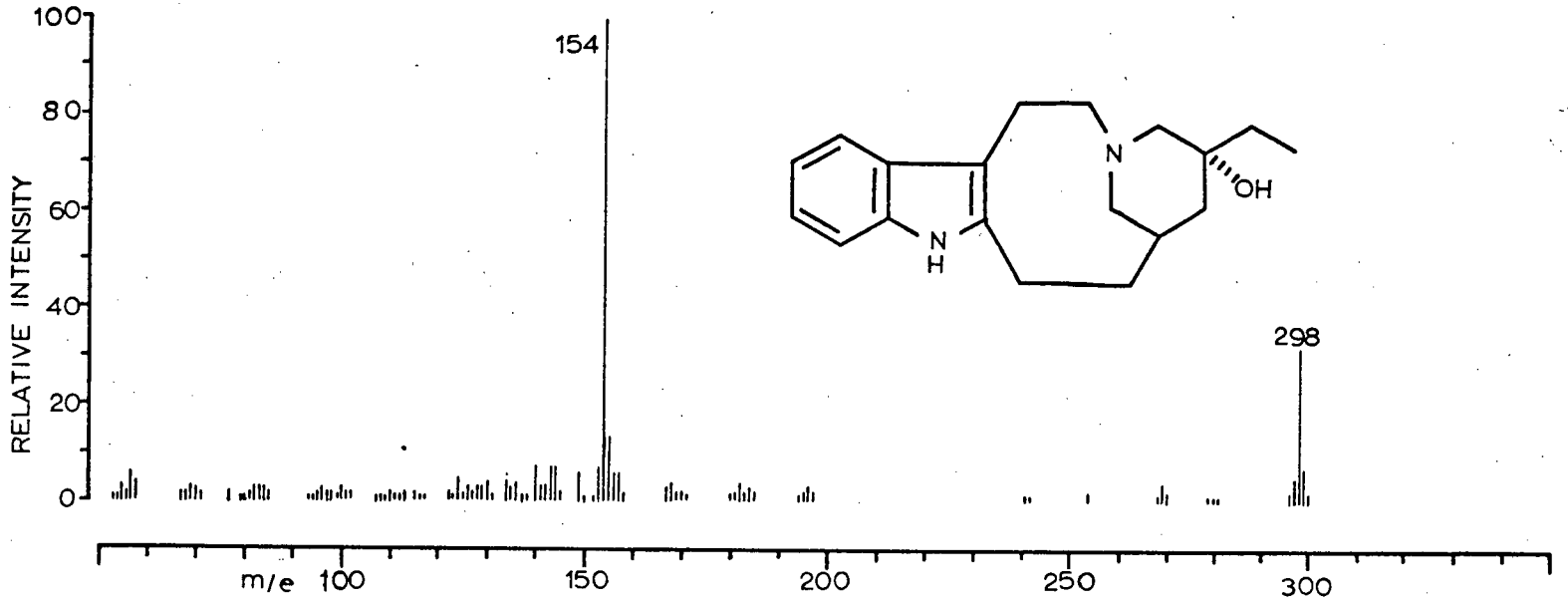


Figure 21. Mass spectrum of isovelbanamine (100).

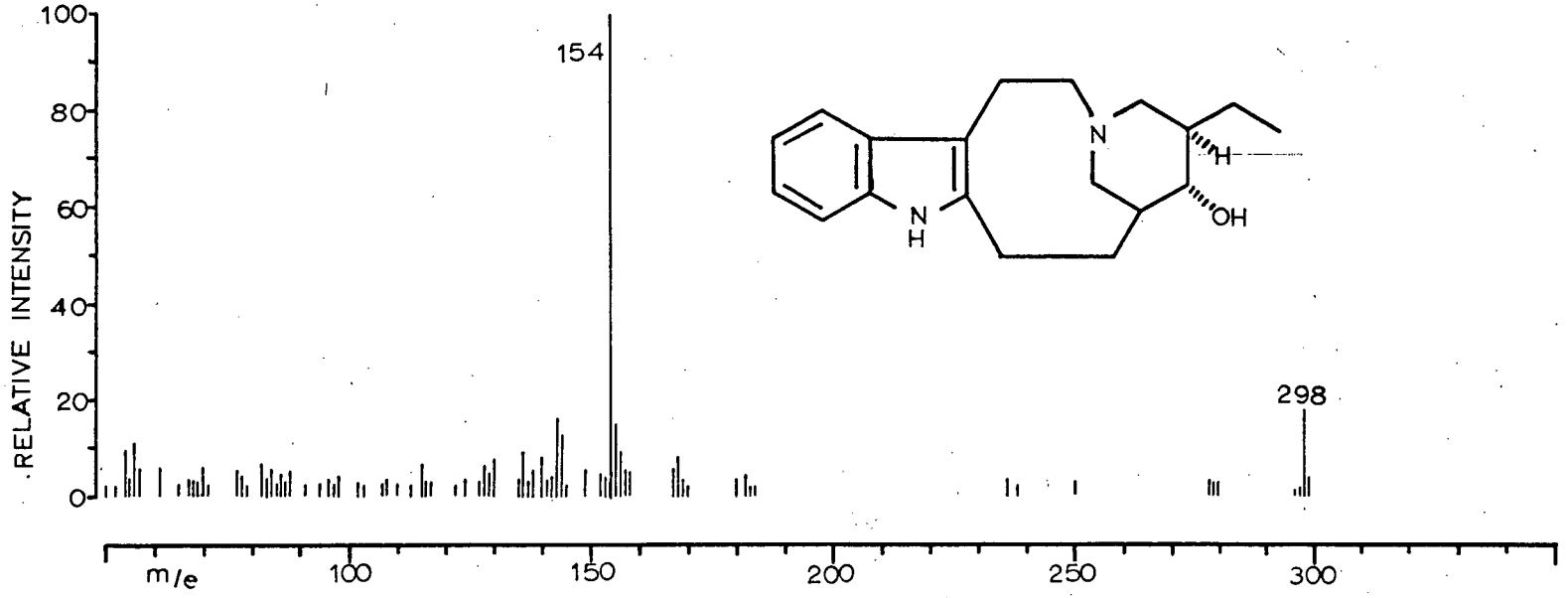


Figure 22. Mass spectrum of 3α-hydroxy-4β-dihydrocleavamine.

It was now of interest to extend this sequence further to the synthesis of catharanthine since the transannular cyclization reaction discussed previously should allow conversion of a carbomethoxy-cleavamine to this alkaloid. The problem of introducing a C_{18} carbomethoxy function into the cleavamine molecule was now at hand.

The first part of the requisite reaction sequence (23 → 109, figure 23) is a special case of a general type of reaction undergone by the indole system. This was first recognized by Taylor who proposed the scheme shown in figure 24 to explain a number of transformations present in the literature.[63] Electrophilic attack at the reactive β-position of the indole system produces the indolenines (111a) and (111b) which are in equilibrium with each other. The indolenine (111b) is activated towards nucleophilic attack at the carbon adjacent to the α-position of the original indole system and such a reaction leads to the functionalization at this carbon. Buchi's conversion of ibogaine (112) to voacangine (113),[33] presented an analogy to the transformation desired in our sequence.

The studies in our laboratory[59] on dihydrocleavamine indicated, however, that the nitrile introduction at the 18-position using the chloroindolenine directly was a very poor yielding process. A substantial improvement was achieved by first converting the chloroindolenine of 4β-dihydrocleavamine (41) to its quaternary ammonium salt (115) and then treating this salt with cyanide to obtained the nitrile (42). The formation of this salt, was readily achieved by the reaction of the chloroindolenine with acetate ion in glacial acetic acid, giving the 18-acetoxydihydrocleavamine (114) as

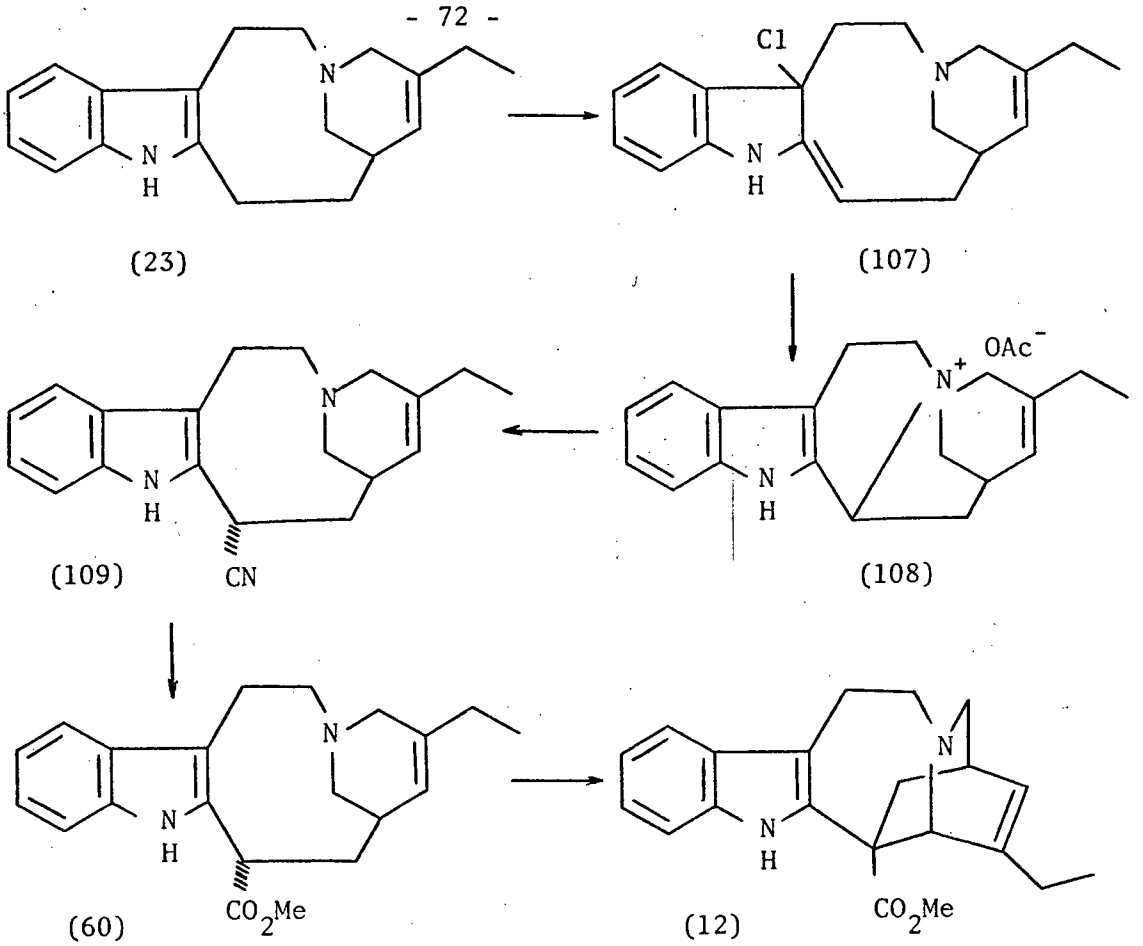


Figure 23. Conversion of cleavamine (23) to catharanthine.

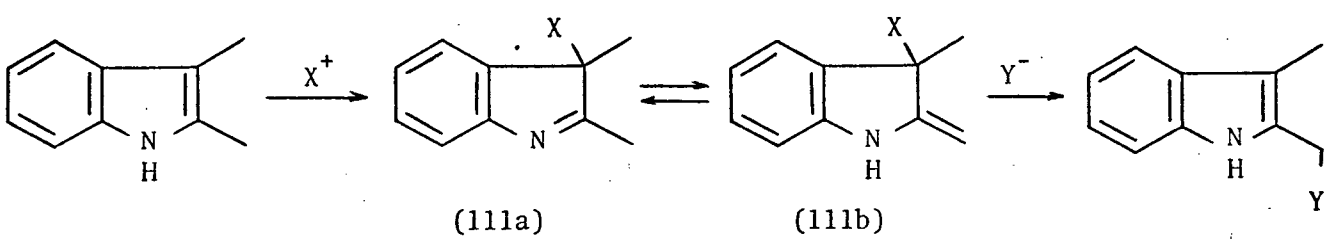
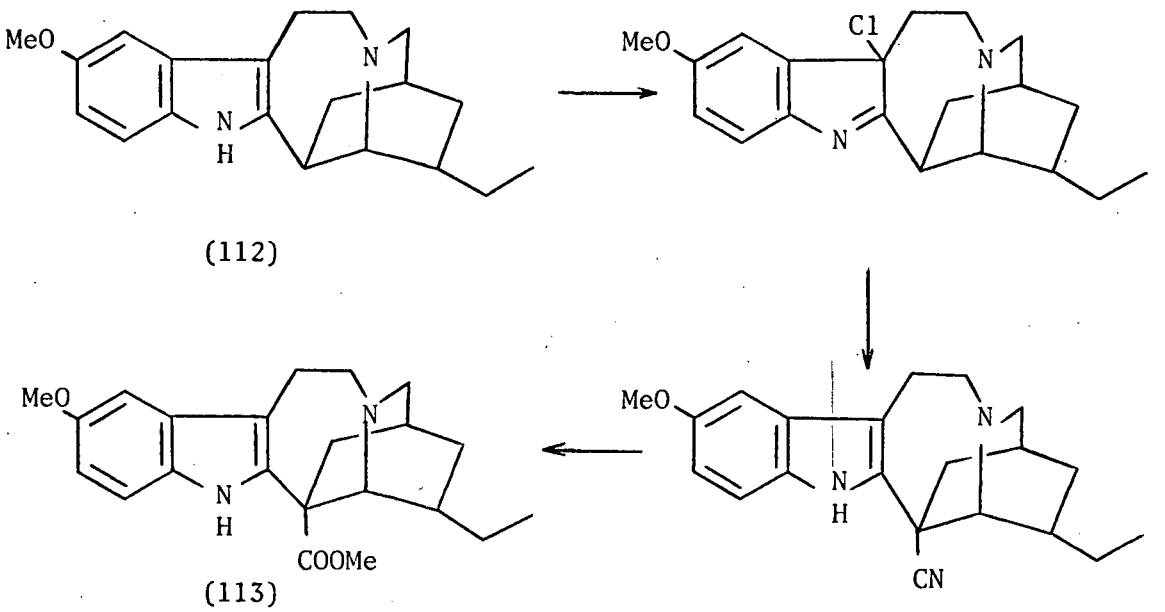


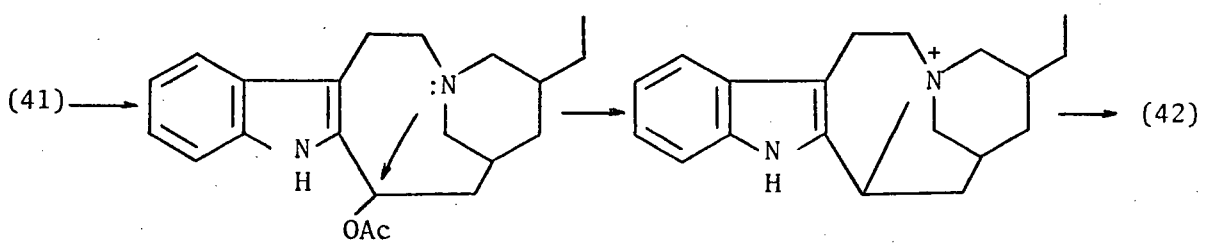
Figure 24. Taylor's reaction scheme for functionalization using indolenine intermediates.



(112)

(113)

an intermediate. This compound under the conditions of the reaction, undergoes intramolecular displacement of the acetoxy function resulting in quaternization. Thus the unstable chloroindolenine was



(114) (115)

converted to a much more stable compound, the salt (115). Nucleophilic attack on this salt at the 18-position by the cyanide could be achieved and produced the desired nitrile.

Because of the experience available from the work in the dihydrocleavamine series, it was a relatively simple matter to convert cleavamine to 18β-cyanocleavamine. The oxidation with tert-butyl hypochlorite was carried out at -15°C to form the chloroindolenine (107). The chloroindolenines are in general reactive intermediates and no attempt was made to isolate this material. Instead, it was treated immediately with fused sodium acetate in a solution of glacial acetic acid and acetic anhydride to form the quaternary ammonium salt (108). Rigorous precautions were taken to prevent the presence of water in the system during the preparation of the salt and the subsequent nitrile introduction. The very polar nature of the quaternary ammonium salt made it difficult to work with this material and it was found convenient to immediately treat the salt with a large excess of potassium cyanide in refluxing dimethylformamide. The desired 18β-cyanocleavamine (109) was obtained in 30% yield based on starting cleavamine. This compound exhibited the expected spectral properties, in particular, the characteristic nitrile stretching bond was observed in the ir region at 2240 cm^{-1}. High resolution mass spectrometry established the correct molecular composition for the strong parent peak observed. The nmr spectrum of this compound (figure 25), showed a one-proton doublet of doublets at τ 4.48 (J = 2 and 10 Hz). This multiplet corresponded very closely to one observed in the spectrum of 18β-cyano-4β-dihydrocleavamine (τ 4.55, J = 2 and 10 Hz) and was assigned to the C_{18}-proton.

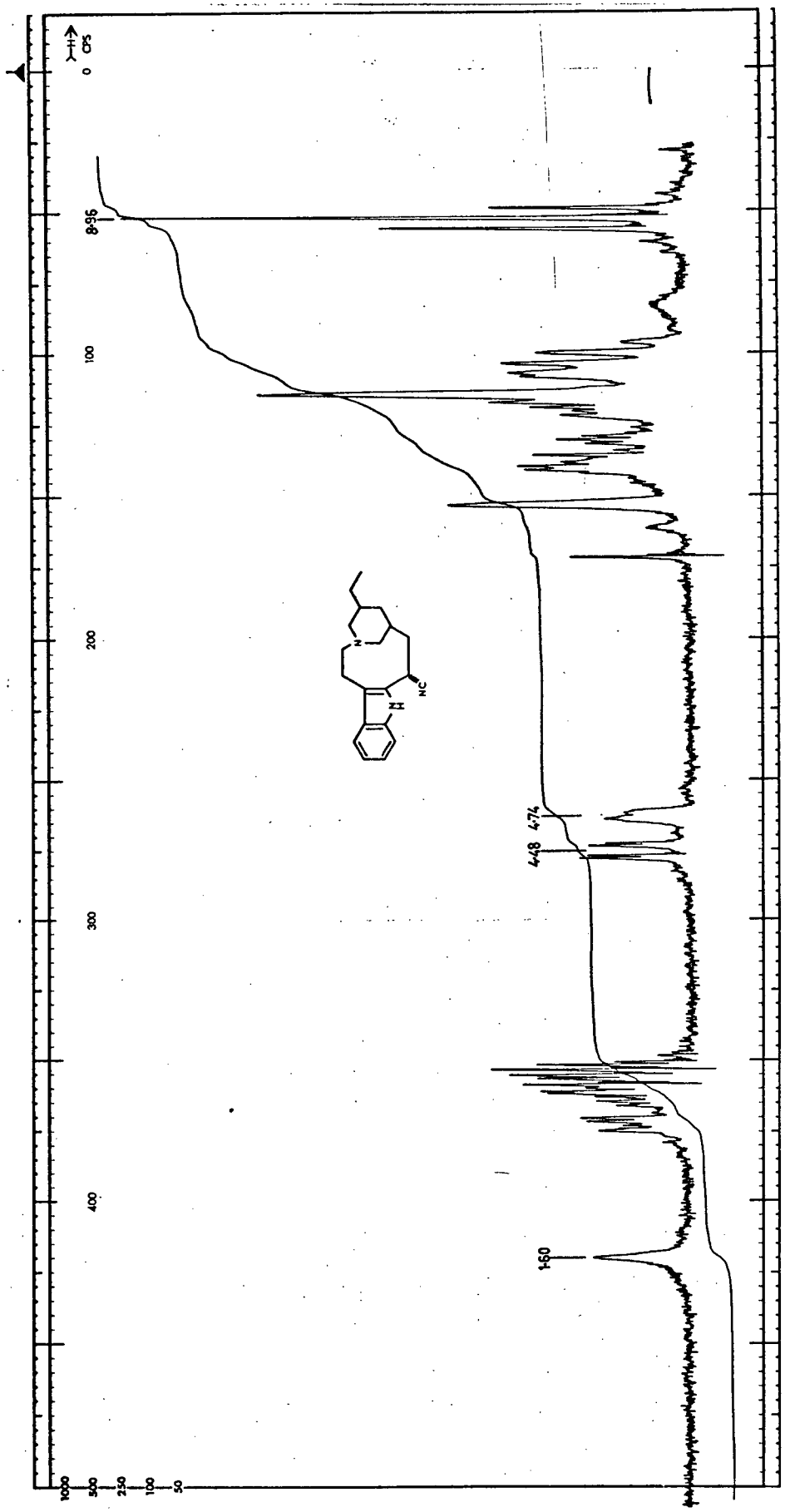


Figure 25. Nmr spectrum of 18β-cyanocleavamine (109).

On the basis of the chemical shift of this multiplet, the stereochemistry of the C_{18} substituent could be assigned as having the β-configuration. The major difference observed between this spectrum and the one observed for the dihydro analogue could be explained by the additional double bond in the molecule. A broad one-proton doublet at τ 4.74 was observed for the vinyl proton and the three proton methyl triplet at τ 8.96 was slightly deshielded relative to its normal position (τ 9.1-9.3) observed in the saturated analogues possessing the cleavamine skeleton.

The hydrolysis of the nitrile could be achieved only under fairly drastic conditions (20% potassium hydroxide in diethylene glycol at 150°C for nine hours). Esterification of the acid with diazomethane gave the desired 18β-carbomethoxycleavamine (60) in about 50% yield based on starting nitrile. The product was identified by comparison with an authentic sample of 18β-carbomethoxycleavamine. The synthetic material had the same melting point, identical tlc properties and gave an ir spectrum which was superimposable with that of the authentic sample.

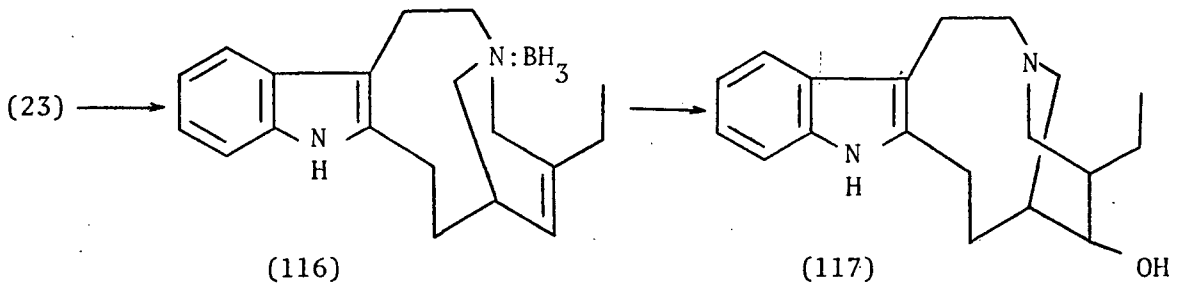
The transannular cyclization of 18β-carbomethoxycleavamine to catharanthine had already been accomplished in our laboratory[64] and thus the total synthesis of 18β-carbomethoxycleavamine also completed the total synthesis of the alkaloid, catharanthine (12).

Our first main objective, the total synthesis of catharanthine and cleavamine had therefore been achieved. Hence all further studies in this series which used compounds derived from catharanthine as starting

materials, could be considered totally synthetic. Our next consideration was the introduction of the appropriate functionalities at the C_3 and C_4 positions of the cleavamine systems. As explained earlier, these compounds were important to us as intermediates for subsequent dimerization work. The reaction sequence which was outlined above already gave us a series of compounds having the C_4 hydroxyl function. The stereochemistry at this centre,as established in isovelbanamine (100), differs from that at the corresponding centre in the natural dimers. These compounds would be valuable in our dimerization work since they would lead to the synthesis of dimeric materials epimeric at C_4 and ultimately allow the evaluation of the importance of the stereochemistry at this centre to biological activity.

The syntheses of the isomeric alcohols, that is, velbanamine (22) and those having the hydroxyl function at C_3, were now desired. The least amount of difficulty was anticipated with the introduction of the hydroxyl function at the secondary carbon atom and this became our first consideration. Our approach to this was simply to hydroborate cleavamine (23). It has been well established that hydroboration is a sterically sensitive process[51] and it was expected the secondary alcohol would be obtained exclusively. It was difficult to predict whether a mono-alkyl or di-alkylborane would form in this reaction or whether complexing with the basic nitrogen would compete with attack at the double bond. The quantity of diborane needed in our initial experiments could not, therefore, be calculated and was gauged by the disappearance of the starting material, cleavamine, as seen by tlc. An experiment conducted in such a manner led to the isolation of a

crystalline material in about 80% yield. This material turned out to be an amine-borane which on treatment with triethylamine in tetrahydrofuran gave back the starting cleavamine. This amine-borane was thus simply the cleavamine N-borane adduct (116) and no hydroboration of the double bond had been achieved.



(116) (117)

An interesting experiment was carried out with this amine-borane. It had been reported that the amine-boranes of simple amines are useful as hydroborating agents.[65] An equilibrium is set up between the amine-borane and free amine at high temperatures and the borine thus

$$R_3N{:}BH_3 \rightleftharpoons R_3N + 1/2(BH_3)_2$$

$$3RCH{=}CH_2 + 1/2(BH_3)_2 \longrightarrow (RCH_2CH_2)_3B$$

liberated is available for the normal hydroboration reaction. In our case, we had in fact a source of borine built into our molecule and it was anticipated that at elevated temperature, an intra or intermolecular transfer of borine from the nitrogen to the double bond would take place.

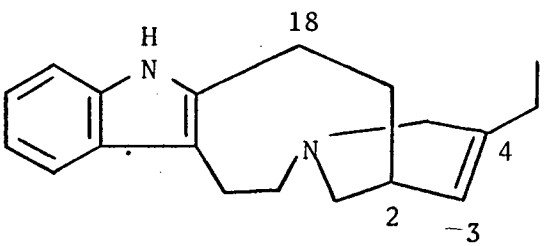
The experiment was conducted in diglyme at refluxing temperature and was followed by the oxidative work up normally employed in the hydroboration procedure. The main products obtained were starting material, cleavamine, and dihydrocleavamine (29). Two minor, more polar products were also obtained in insufficient quantity for characterization. In subsequent work, however, the secondary alcohol (117) was obtained. A comparison of these minor products with this alcohol showed in fact that one of these had the same tlc properties and ir spectrum. The other product had very similar tlc properties, being very slightly less polar and gave a somewhat similar ir spectrum. This material is possibly the epimeric alcohol. No further work was done in this direction because of the poor yields encountered. Instead we turned back to the normal hydroboration process.

The isolation of the cleavamine N-borane in the previous hydroboration experiment indicated that an insufficient amount of diborane was used in the reaction. When a large excess of diborane was utilized, the desired secondary alcohol was obtained in 77% yield. The spectral data for this compound were in complete agreement with the structure of the alcohol (117) and sublimation of the compound provided an analytical sample. A comparison of the mass spectrum of this compound (figure 22) with that of velbanamine and isovelbanamine (figure 21) showed, as expected, a very close resemblance between them. The nmr spectrum showed two protons in the region τ 6.4. One of these could be assigned to the C_{18}-proton, since this is its normal position in cleavamine-type compounds bearing no substituent at C_{18}.

The other proton with this chemical shift could be assigned to the C_3-proton which is geminal to the hydroxyl function. The nmr spectrum of the acetate of this alcohol (figure 26) showed a single proton as a complex multiplet at τ 6.4, (C_{18}-proton) and now showed the C_3-proton at lower field, τ 4.90, as a doublet of doublets (J = 6 and 10 Hz).

The stereochemistry of this alcohol (117) at the two asymmetric centres could be assigned by a combination of chemical and nmr evidence. The hydroboration process is known to result in the cis hydration of a double bond. That is, the hydroxyl function and the proton are introduced at adjacent carbons in a cis relationship toward each other and this is explained mechanistically by the intermediacy of a cyclic transition state.[51] From this information, the relative stereochemistry between C_3 and C_4 is established.

An examination of a model of cleavamine shows clearly that because of the steric effect of the bridge at C_2, approach is very



much favoured from the side of the piperidine ring opposite this bridge. This is indeed generally observed in other reactions of the cleavamine system. Hydrogenation of cleavamine therefore gives

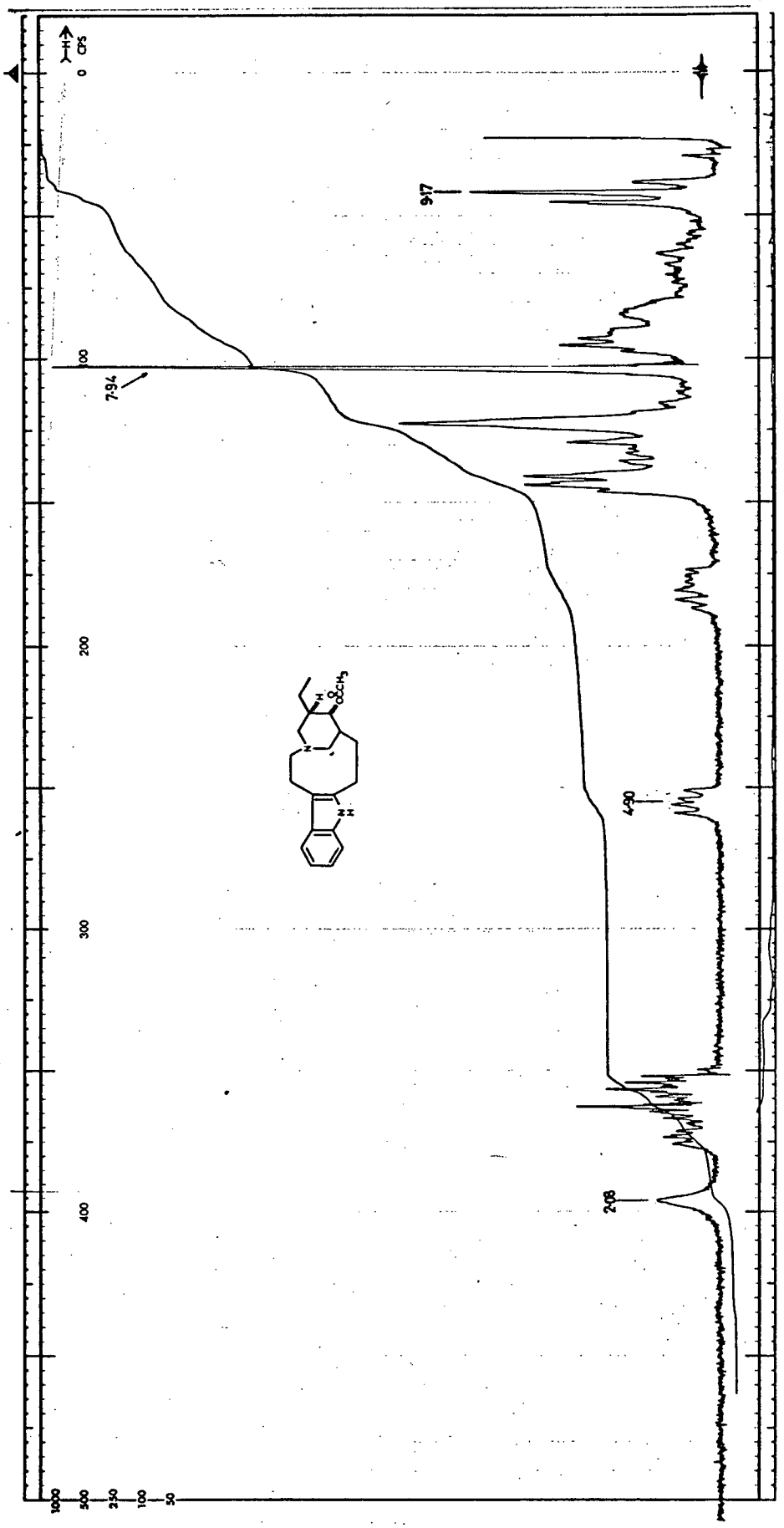
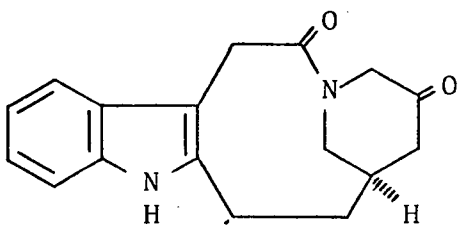


Figure 26. Nmr spectrum of 3α-acetoxy-4β-dihydrocleavamine.

exclusively 4β-dihydrocleavamine (29). The osymlation reaction on the enamine (83) reported earlier in this work was also stereoselective, resulting in the eventual formation of isovelbanamine (100). The stereochemistry at C_4 of isovelbanamine which has the ethyl group in a β-orientation shows that attack in this case is also favoured from the side opposite the bridge. Buchi's synthesis of velbanamine[66] makes use of this steric approach control. Thus treatment of (150) with ethylmagnesium bromide, followed by lithium aluminum hydride reduction



(150)

gave velbanamine (22).

On the basis of these results it would be expected that the hydroboration of the cleavamine double bond would follow the same steric course and lead to the 3α-hydroxy-4β-dihydrocleavamine. This suggestion was supported by nmr evidence. The C_3-proton in the acetate of this molecule is observed as a doublet of doublets J = 6 and 10 Hz. If attack is from the side opposite the bridge, the resulting compound would have the ethyl and hydroxyl groups in a trans-diaxial relationship assuming the chair conformation for the piperidine ring. Because of the conformational mobility in the cleavamine ring system it is difficult to be entirely definitive about

the conformational structure. We can, however, rule out the alternate mode of attack. Attack of the double bond from the same side as the bridge at C_2 would lead to a compound which has both the ethyl and hydroxyl groups in the equatorial position in the chair conformation. This situation represents the most stable conformation possible. In this case, the dihedral angles are well defined being 180° and 60° between the C_3-C_4 and C_2-C_3 protons respectively. From the Karplus relation,[67] these angles should lead to coupling constants of about 16 and 2 Hz. This clearly does not agree with the observed values of 10 and 6 Hz and therefore excludes this course of attack. Therefore, on the basis of models, chemical precedence and nmr data, the alcohol (117) can be assigned as 3α-hydroxy-4β-dihydrocleavamine.

It is of interest to compare this compound with the monomeric unit derived from leurosidine (18). Cleavage of this dimer leads to the isolation of a secondary alcohol, vinrosamine, which was assigned the structure of 3α-hydroxy-4α-dihydrocleavamine.[68] A comparison of our data with the physical constants reported for vinrosamine and vinrosamine acetate shows definitely that these compounds are different. It is reported that vinrosamine exhibits a mass spectrum in which the major fragment ion corresponds to a loss of water from the molecular ion (M^+ -18), and that the rest of the spectrum corresponds to that normally found for cleavamine type compounds. Our alcohol, despite the fact that it was epimeric to this material at only one position (C_4) behaved entirely differently and gave a spectrum which corresponded closely to that of velbanamine and isovelbanamine. This perhaps unexpected difference between the mass spectra of these two secondary

alcohols can be easily explained by a consideration of their structures. Vinrosamine in the chair conformation, has the ethyl group in an equatorial position and the C_3 hydroxyl and C_4 hydrogen in a trans-diaxial relationship. This spatial arrangement between the hydroxyl and adjacent hydrogen is very favorable for elimination and the facile dehydration to cleavamine as observed in the mass spectrum is not unexpected. In our alcohol (118), there are no hydrogen atoms which are trans to the hydroxyl group and thus only a cis elimination is possible. The major fragment ion in the mass spectrum of the alcohol (118) is m/e 154 indicating that fragmentation at carbon centers adjacent to the indole aromatic system is favoured [cf structure (101)] and the ion observed corresponds to the hydroxylated piperidine portion of the molecule.

Having achieved the functionalization at both C_3 and C_4 we now turned our attention to the formation of the epimeric systems. The compounds synthesized so far differed in stereochemistry at the asymmetric centres C_3 and/or C_4, from the monomeric units derived from the natural dimers and it was desirable to have available as synthetic materials, the compounds having the same stereochemistry at these centers. Of particular interest was the synthesis of velbanamine (22). It will become clear in the second part of the discussion that the sequence already outlining the total synthesis of isovelbanamine was important in the synthesis of dimeric systems. A means of allowing epimerization at the C_4 position of intermediates utilized in the present sequence would give us the synthetic capability of synthesizing vinblastine or isovinblastine epimeric at only C_{18}. This achievement

would provide the first real opportunity to evaluate the importance of this and other asymmetric centers in imparting anti-tumor activity in this series.

The epimerization of the tertiary C_4-hydroxyl function was anticipated to be a complex process. From our experience with the dehydration of isovelbanamine to cleavamine, which was readily achieved under acidic conditions, it seemed likely that any approach which involved the intermediate carbonium ion would suffer the undesired loss of a proton to give olefinic products. We thus turned our attention to S_N2 type reactions even though a tertiary center is not normally prone to such situations. Our first experiment involved the use of concentrated sodium hydroxide in a water-dimethyl sulphoxide solution. Dimethyl sulphoxide was used because it is known to greatly enhance the activity of anions and thus is an excellent solvent for nucleophilic displacement reactions.[69] The driving force for the reaction would be the attainment of a more stable isomer; isovelbanamine has the ethyl substituent in an axial position when the piperidine ring is in a chair conformation, whereas velbanamine has the ethyl group in an equatorial position and also the hydroxyl function in the β-axial position is favorably oriented for hydrogen bonding with the tertiary nitrogen. However, the experiment under a variety of conditions, failed to produce any velbanamine as evidenced by tlc.

The following approach was based on a known procedure for the cleavage of steroidal methyl ethers using boron trifluoride-etherate.[70] In these instances the combined reaction of BF_3-etherate and acetic anhydride in ether converted secondary methyl ethers to the corresponding

acetates. The product was reported to sometimes consist of a mixture of epimeric materials. It was felt that this reaction sequence should be applicable to our system.

Treatment of isovelbanamine under the prescribed conditions led to the disappearance of starting material as evidenced by tlc and one major product resulted. Treatment of this product with lithium aluminum hydride gave, however, isovelbanamine as a major product and no velbanamine. Thus isovelbanamine acetate was formed indicating attack by the acylium ion at the hydroxyl oxygen rather than displacement of the hydroxyl by the acetate anion.

These experiments confirmed our initial suspicions that steric factors were prohibitive to the required nucleophilic attack at this center. We were thus forced to examine the alternate approach, that is, reaction conditions which would be expected to generate carbonium ion intermediates. Under the conditions for the conversion of isovelbanamine to cleavamine (concentrated sulphuric acid at 0°C for 2 hours), the carbonium ion rapidly loses a proton to provide the olefinic systems. We required in this case non-dehydrating conditions such that the reaction at the carbonium ion would instead be nucleophilic attack. We thus treated isovelbanamine with dilute aqueous acid at 0°C up to three days and found under these conditions, the compound underwent no change. Also at room temperature no change was observed. However, refluxing this same solution, gave after four hours, the first indication on tlc of the presence of some velbanamine. The proportion of velbanamine seemed to increase with time and the reaction was allowed to continue for two days. Work up after this time gave

both velbanamine and starting isovelbanamine. The velbanamine obtained had the same melting point, identical tlc properties and superimposable ir spectrum with that of an authentic sample of velbanamine.[71]

The extension of our sequence to the total synthesis of velbanamine satisfied one of our main synthetic objectives with respect to the synthesis of the natural dimeric Vinca alkaloids and their closely related dimeric analogues. Now that synthesis of the indole (velbanamine) unit was in hand, two problems still remain to be solved. These are 1) the completion of the dihydroindole (vindoline) unit and, 2) the coupling of these systems to provide the dimeric alkaloids. While the vindoline synthesis is under study by several other workers in the group, the investigations concerning the dimerization reaction form Part II of this thesis.

Part II

The dimeric Vinca alkaloids contain a linkage between the C_{15} position of vindoline or its relatives and C_{18} of the nine-membered ring system characteristic of the cleavamine family. Although it would be satisfying to complete the total synthesis of the natural series, it was of utmost importance to develop, if possible, a versatile and general coupling reaction which would provide a new family of synthetic analogues for biological evaluation. In this way information concerning the relationship between chemical structure and anti-tumor activity, can be obtained.

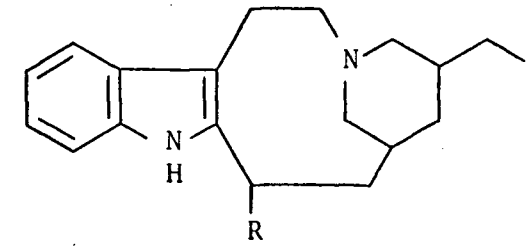
Our approach to this work was to employ a reaction with which we previously had considerable experience and which seemed attractive for

this purpose. It was pointed out earlier that indole systems can be converted by oxidative procedures to indolenines which are reactive to nucleophilic attack at centers adjacent to the α-position of the original indole system (see figure 23). Thus the formation of the chloroindolenines served as an intermediate step for the introduction of nucleophilic substituents at the 18 position of the Iboga systems (for example see figure 22). Vindoline on the other hand is activated toward electrophilic substitution. The aromatic portion of this molecule is a meta-methoxy-aniline system which because of the combined "electron donating" effect of the methoxy and anilino functions is activated at both the 15 and 17 positions toward electrophilic attack. The 15 position should in fact be more reactive than the 17 position when steric factors are taken into consideration. Thus it was predicted that a reaction between the chloroindolenine of the cleavamine type system and vindoline, would result in a dimerization involving the correct centres, $C_{18'}$ and C_{15}.

The numbering system that will be used in referring to the dimers, will be that of the corresponding monomeric units, that is, Aspidosperma alkaloid numbering for the dihydroindole unit while Iboga numbering for the velbanamine moiety. To distinguish the numbering systems, the numbers of the "Iboga half" will be primed (see 118).

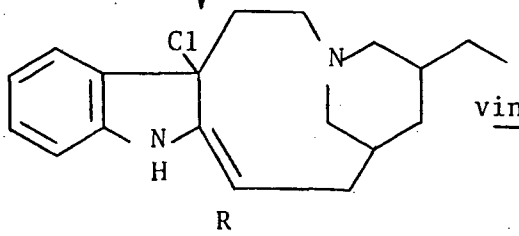
Our initial experiment was conducted using the least functionalized of the cleavamine systems, 4β-dihydrocleavamine (29) in the hope that the possibility of side reactions would be minimized. Also, the chloroindolenine of this compound had already been synthesized as an intermediate in the conversion of 4β-dihydrocleavamine to 18β-carbo-

methoxy-4β-dihydrocleavamine (32).[59]



(29) R=H

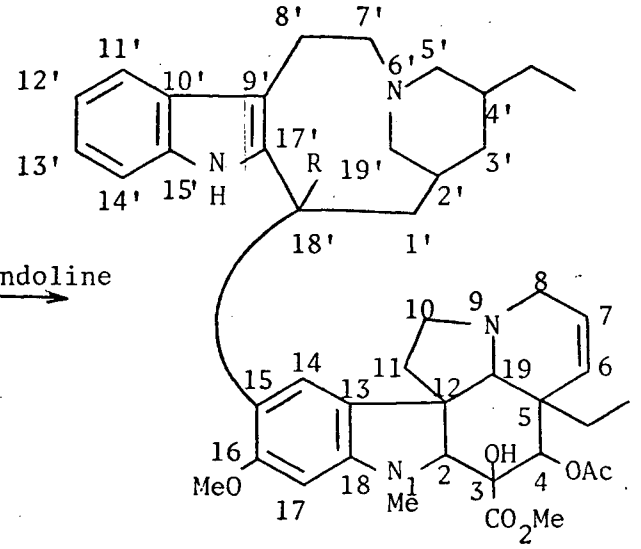
(32) R=CO_2Me



(41) R=H

(120) R=CO_2Me

vindoline



(118) R=H

(119) R=CO_2Me

The rather unstable chlorindolenine intermediate (41), formed from t-butyl hypochlorite oxidation of 29, was reacted with vindoline in an anhydrous methanolic 1% hydrochloric acid solution. The reaction seemed complete after two hours at reflux temperature. The major product of this reaction exhibited all the properties expected for the dimer (118). The uv spectrum appeared as a simple superimposition of the indole and indoline absorption and this agreed qualitatively with the spectrum obtained for vinblastine. The mass spectrum (figure 27) shows a number of fragment ions corresponding to the fragmentation pattern exhibited by dihydrocleavamine and vindoline. The prominent absorptions at m/e 124, 138 and less intense ones at 143, 144, 156, and

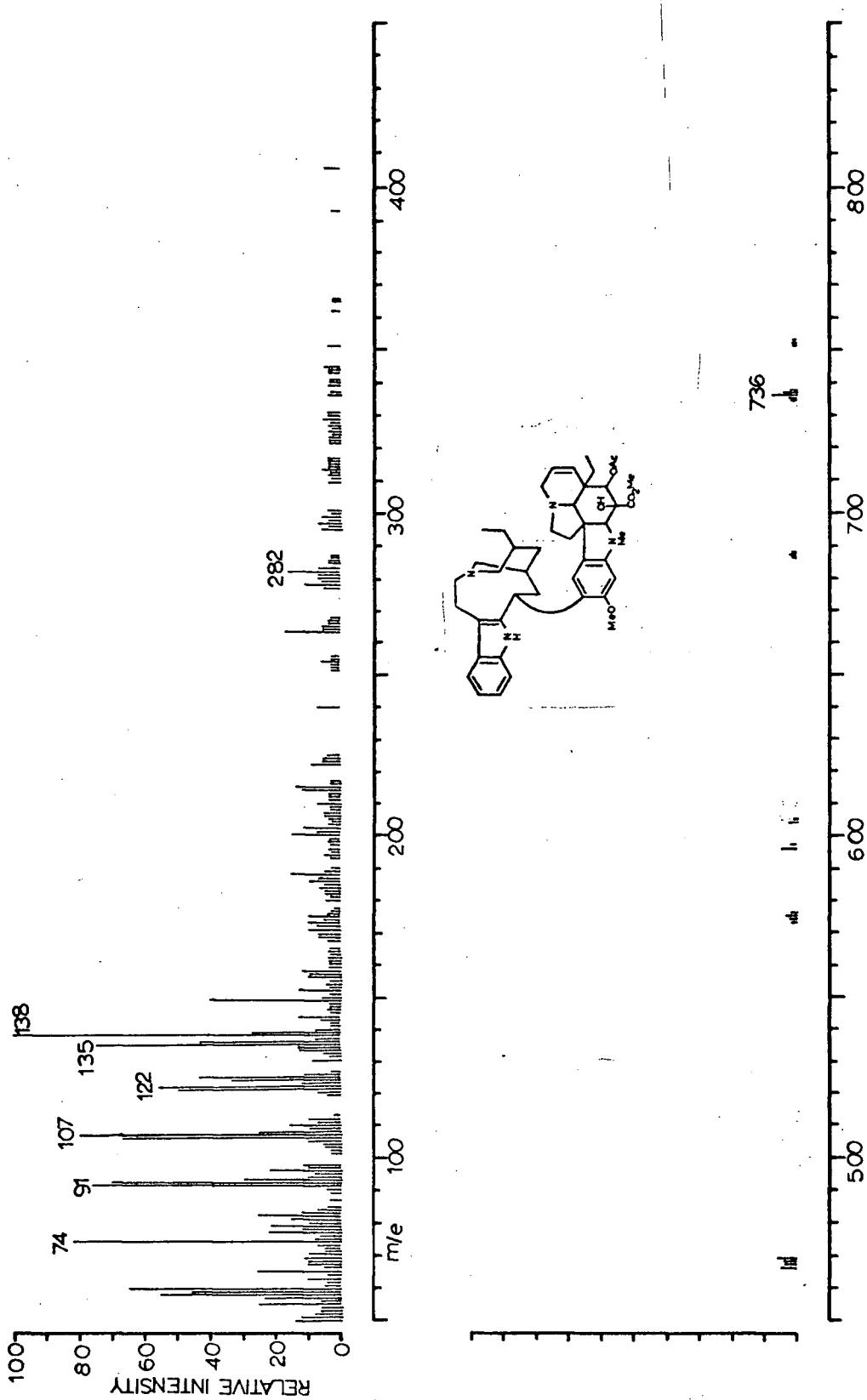


Figure 27. Mass spectrum of dimer (118).

282 are very characteristic of dihydrocleavamine.[22] Similarly the strong absorptions at m/e 121, 122, 135, and 149 with less intense ones at 174 and 188 define the vindoline portion of the molecule.[72] High resolution mass determination of the parent ion established a molecular formula in agreement with the structure of the desired compound.

From the evidence presented so far, there was no doubt that we had a dimeric system composed of a cleavamine and a vindoline portion. Strong evidence in favor of the dimer as well as the proof for the correct junction between the two units came from the nmr spectrum. In many respects, the nmr spectrum of the dimer (figure 28), appeared to be a combination of the spectra of the individual monomeric units (figure 29,30) but a few important changes were observed. The aromatic protons of vindoline appear as three discrete multiplets in the region τ 6-7; normal coupling, ortho and meta with no para coupling is observed. In the dimer, the signal corresponding to the C_{15} proton is absent as expected and the C_{14} and C_{17} protons now appear as singlets because of the small para coupling. The junction is therefore established to be at the C_{15} position of vindoline. The $C_{18'}$ proton is observed as the broad doublet characteristic for 18-substituted cleavamines. As discussed previously, the chemical shift of this proton has been used in the simple substituted cleavamine systems to assign the stereochemistry at this position. However, it was unlikely that the dimeric system, because of the vastly increased steric bulk of the substituent, the vindoline moiety, would fit into this general scheme and no stereochemical assignment for this junction could be made at

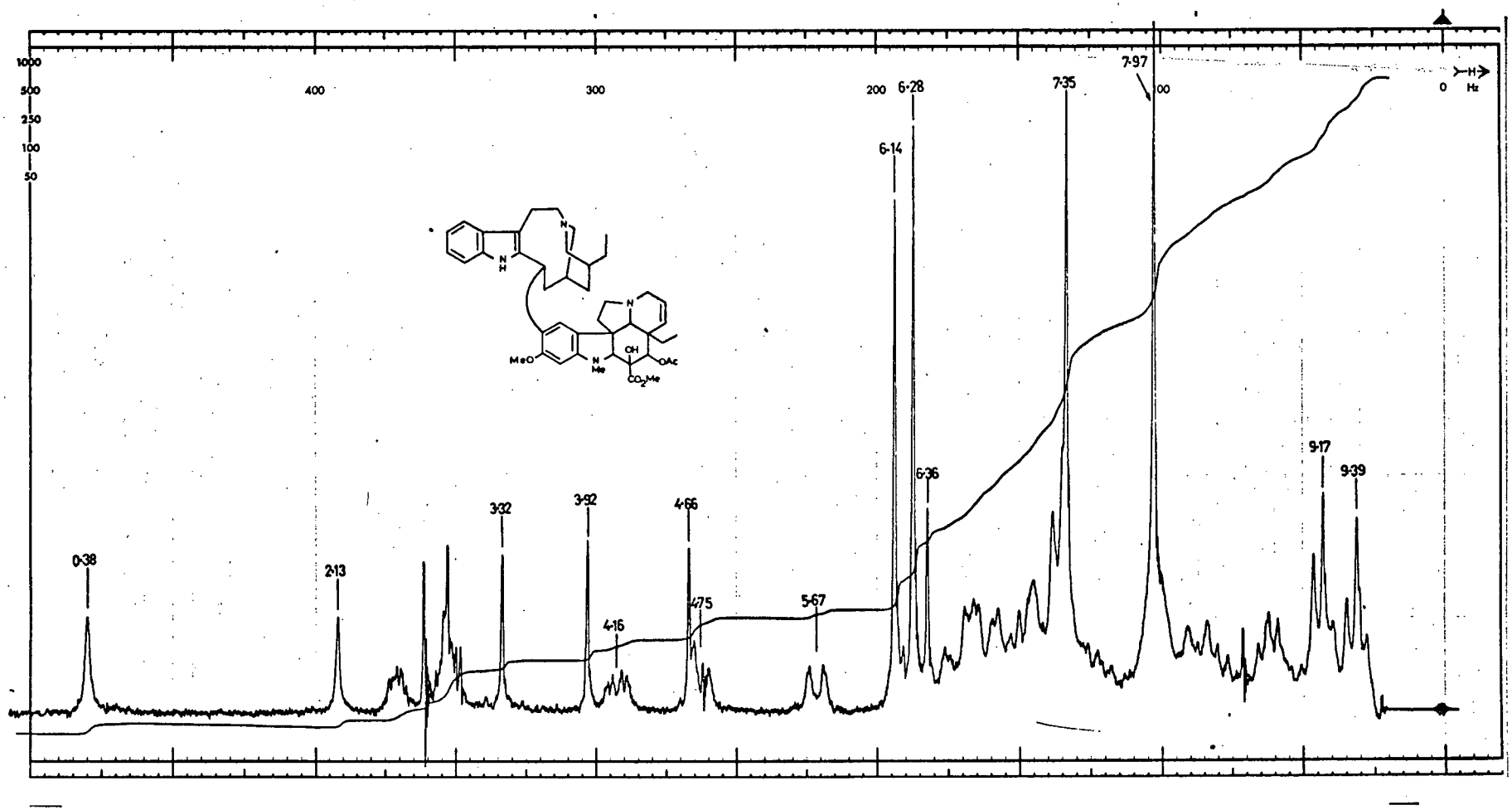


Figure 28. Nmr of dimer 118.

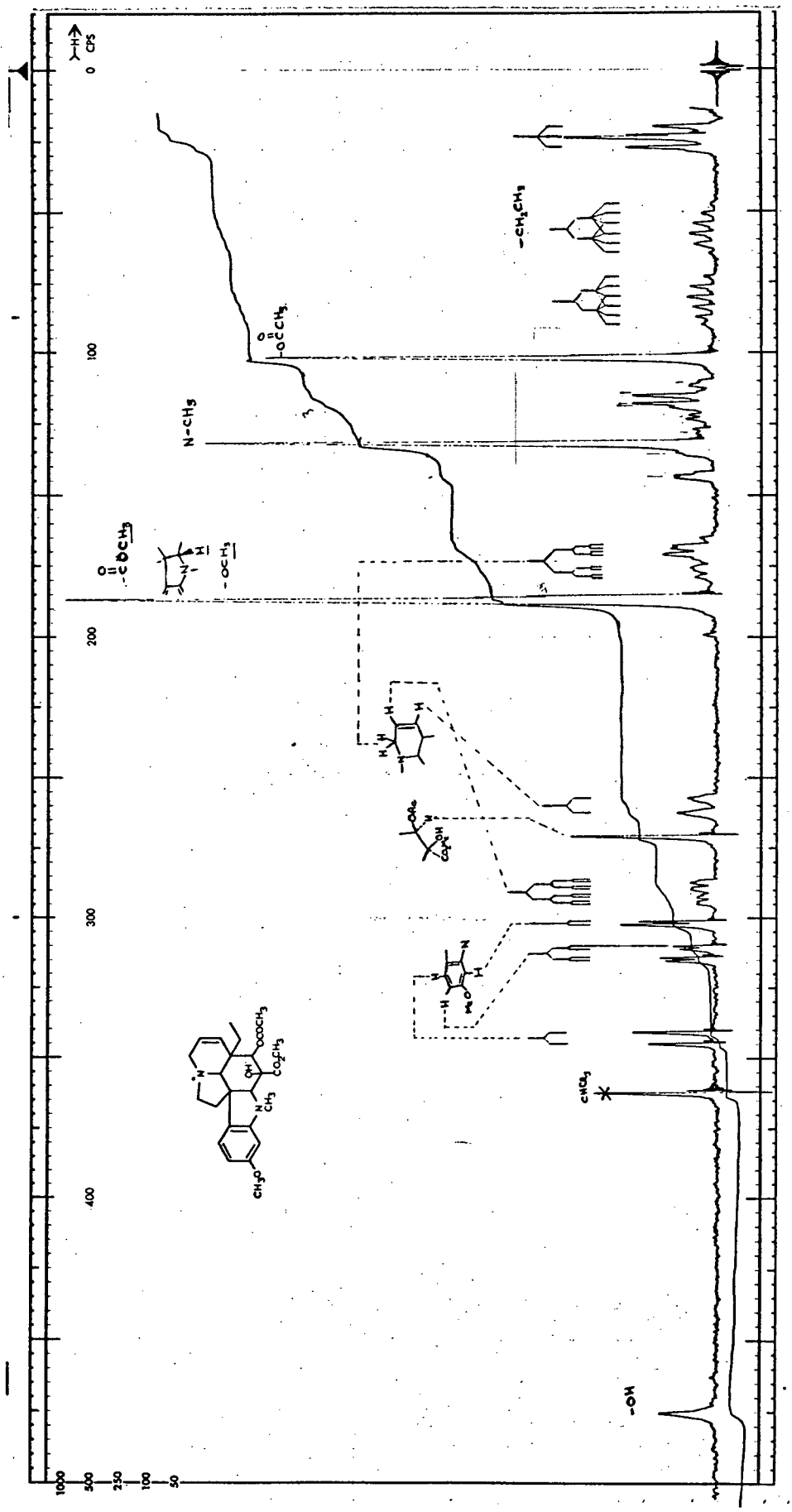


Figure 29. Nmr spectrum of vindoline (11).

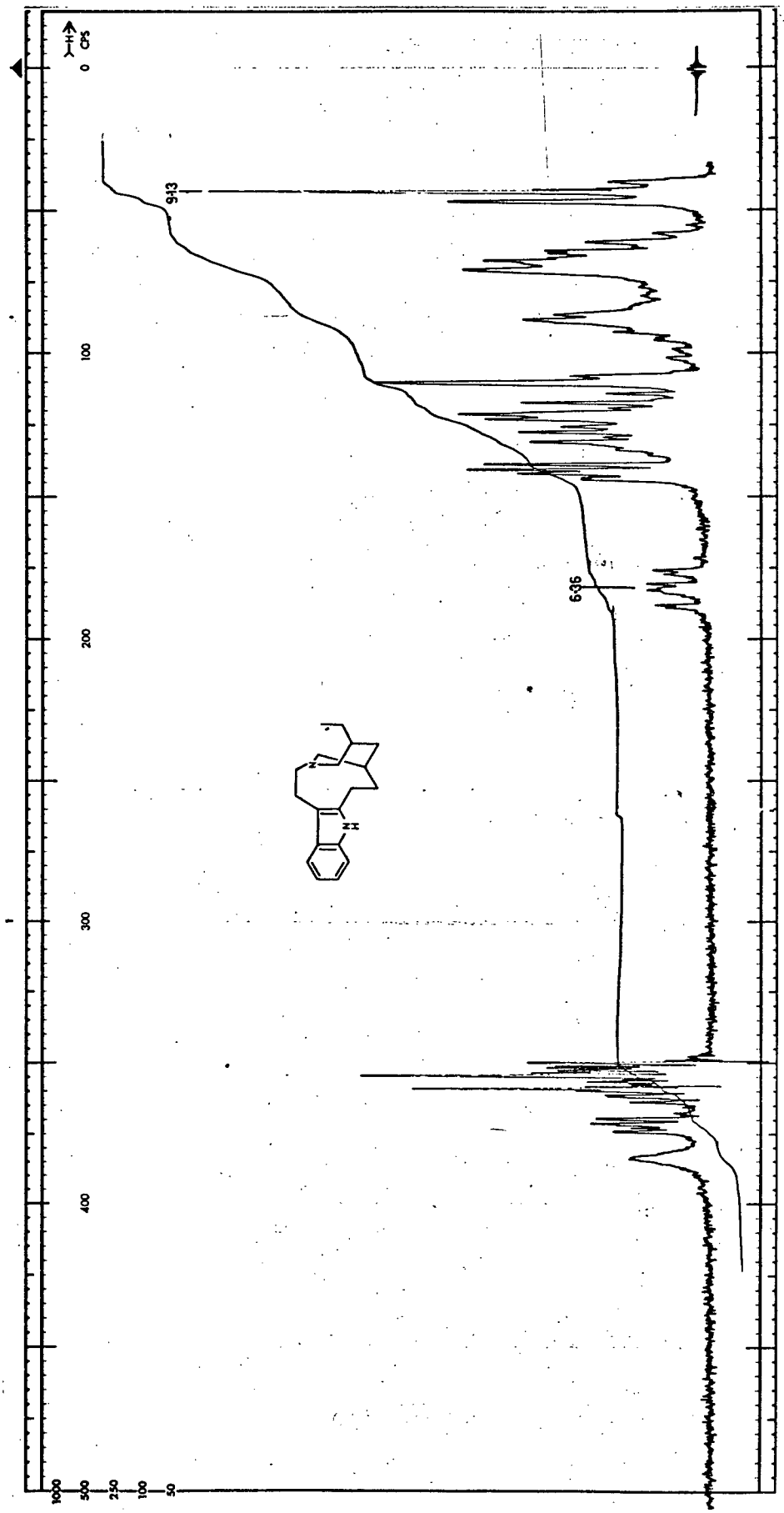


Figure 30. Nmr spectrum of 4β-dihydrocleavamine.

this point. One further observation could be made from this spectrum. The two methyl absorptions for the carbomethoxy and methoxy substituents have the same chemical shift in vindoline. However, in the dimer it can be seen that one of these methyls is shifted to higher field. It is probable that this signal represents the methoxyl methyl which, because it is adjacent to the junction, is now put in close proximity to the indole aromatic system and is thereby affected by the magnetic field associated with this system.

To establish beyond a doubt that this compound was indeed the dimer (118) and not for example a dimer composed of a rearrangement product, a sample of the material was cleaved. Treatment of this compound under acidic reducing conditions gave a mixture of products which were separated and identified as 4β-dihydrocleavamine, vindoline, desacetylvindoline and starting dimer. These isolated products accounted for 75% of the starting material. This experiment in conjunction with the spectral data, presented a rigorous proof of the structure of the dimer (118).

Our next experiment was designed to test the applicability of this approach to the synthesis of dimeric materials having a carbomethoxy function at $C_{18'}$. This function is present in all the natural dimers of known structure. It seemed preferable to couple the 18-carbomethoxycleavamine-type system with vindoline rather than to try and introduce the carbomethoxy group at a later stage. For this experiment we therefore started with 18β-carbomethoxycleavamine (32) which was converted to the chloroindolenine (120) in the usual fashion. Reaction of this chloroindolenine with vindoline gave one major product

which exhibited the spectral properties expected for the dimer (119). As before, the uv spectrum showed the presence of both chromophores and the mass spectrum (figure 31) gave strong evidence for the presence of both the indole and dihydroindole units. High resolution mass measurement on the parent ion established the molecular formula expected for the product. The nmr spectrum of this compound (figure 32) showed many of the features of the spectrum for the previous dimer but with a few important changes. The $C_{18'}$ proton seen at τ 5.6 for dimer (118) was missing in this spectrum and it was noted that the singlet attributed to the carbomethoxy methyl was enlarged and integrated for two methyl groups. A comparison of the chemical shifts for the vindoline aromatic protons showed in this spectrum a shielding effect for the C_{14} proton and a deshielding effect for the C_{17} proton relative to that observed in the descarbomethoxy dimer (118). These shifts could be attributed to either of two factors or a combination of them; 1) a difference in the spatial arrangement of the two halves of the dimer caused by the extra $C_{18'}$ substituent which could place these protons in a different proximity to the magnetic field associated with the indole aromatic system, 2) an additional anisotropic effect caused by the introduction of the carbonyl substituent at $C_{18'}$.

As with the previous dimer, this compound was subjected to cleaving conditions. The products isolated were identified by tlc and spectral comparison with authentic materials and were found to be: 18α-carbomethoxydihydrocleavamine, 18β-carbomethoxydihydrocleavamine, vindoline and desacetylvindoline. Thus the structure of the dimer (120) was proved.

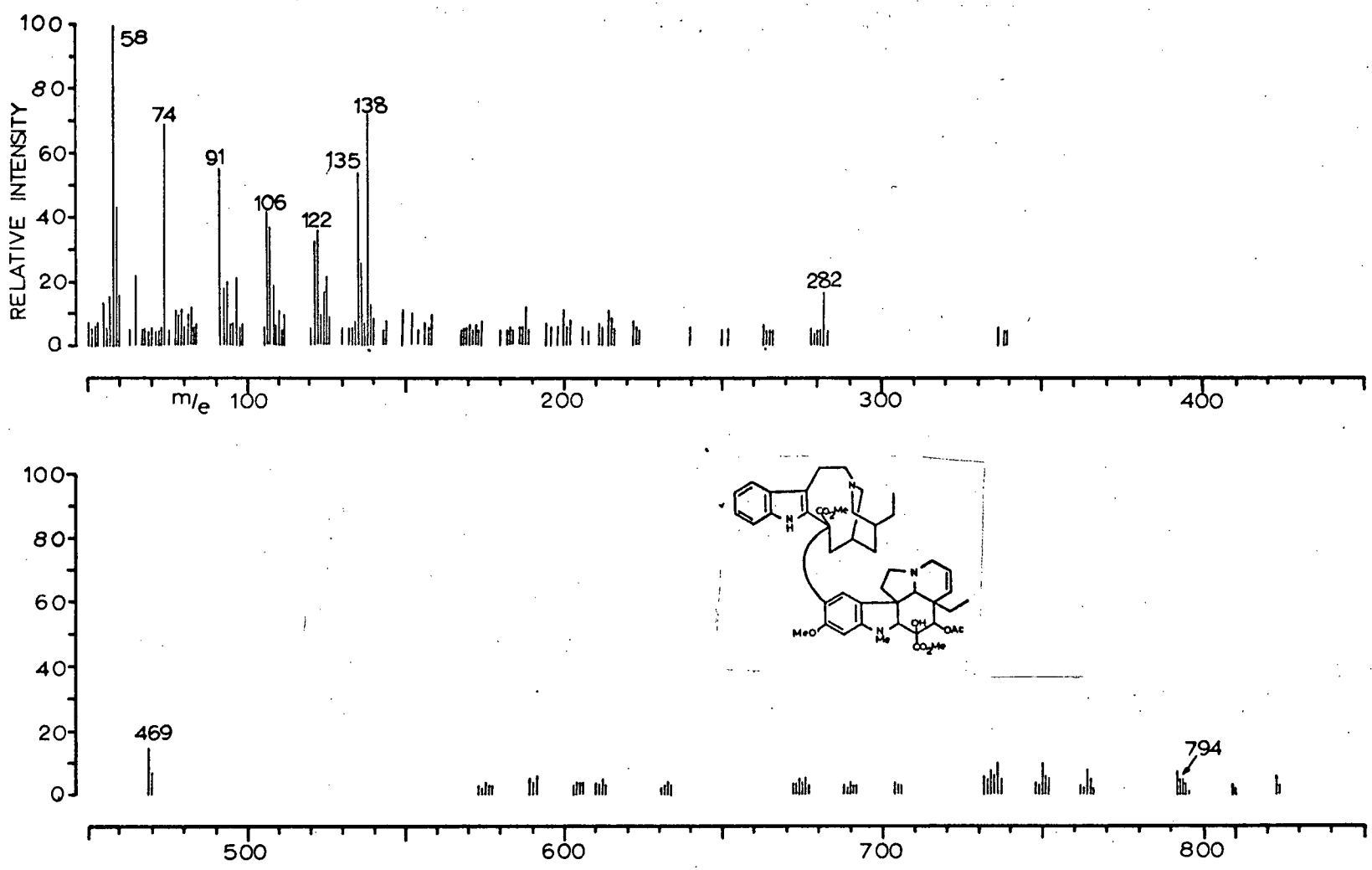


Figure 31. Mass spectrum of dimer (119).

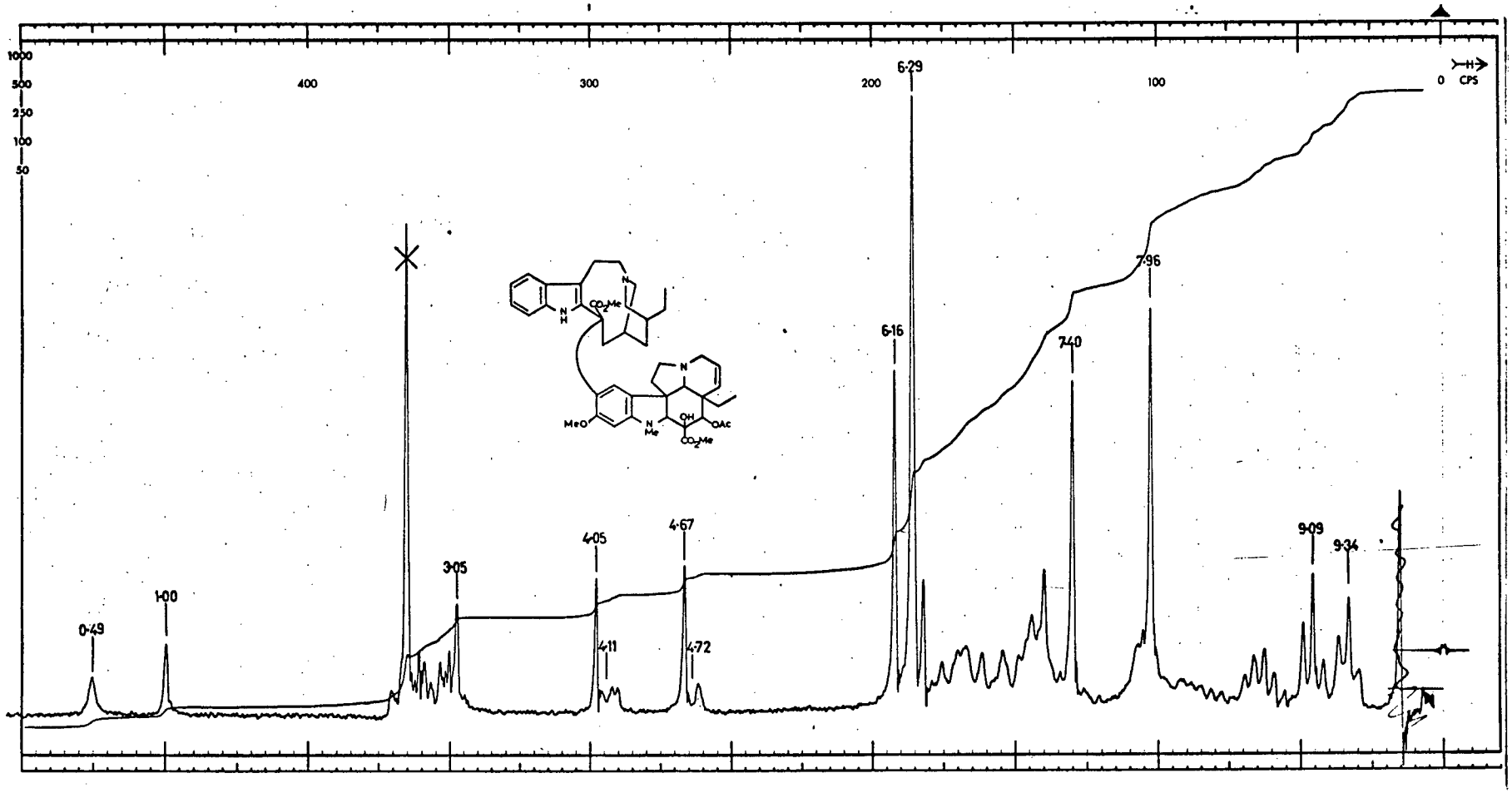
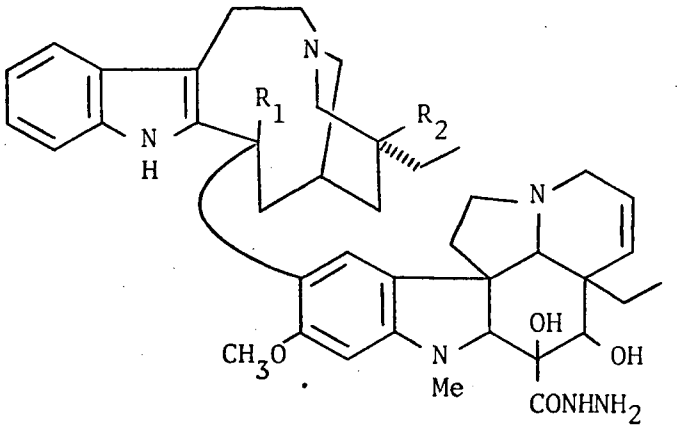


Figure 32. Nmr spectrum of dimer (119).

In the mass spectrum of both of these dimeric compounds, the molecular ion was not the highest molecular weight peak observed in the spectrum. Instead, M^+ +14 and M^+ +28 peaks were present although in each of the spectra obtained, they were extremely weak. These spurious peaks had been previously reported in the mass spectrum of vinblastine[62] and voacamine[74] and have been attributed to an intermolecular methyl transfer from a carbomethoxy group of one molecule to the nitrogen atom of another, followed by a thermal Hofmann-elimination. This process can be repeated twice since two basic nitrogen atoms are available for reaction and thus leads to the M^+ +14 and M^+ +28 ions observed. When vinblastine was treated with hydrazine, the hydrazide (121) obtained possessed no carbomethoxy functions and indeed, did not show these spurious peaks in its mass spectrum.



	R_1	R_2
(121)	H	OH
(122)	H	H
(123)	CO_2Me	H

Because it was anticipated that mass spectroscopy would play an important role in the structural proof of the synthetic dimeric compounds, a parallel series of dimerization experiments were carried out by other workers in our laboratory using vindoline hydrazide instead of vindoline as reported in this work. The dimerization of 14β-dihydrocleavamine with vindoline hydrazide gave dimer (122)

possessing no carbomethoxy function. Its mass spectrum had as expected, the highest m/e value as the molecular ion and thus supported the view that we had in fact observed this same transmethylation phenomenon in the spectra of our other dimers and were justified in not taking the highest m/e value as our molecular ion. Two other dimerizations were carried out, both of these used the chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (120). In one case it was coupled to vindoline hydrazide to give the dimer (123) and in the other case it was coupled with dihydrovindoline. The details of these three dimerizations, reported in full elsewhere,[76] were completely consistent with the results presented for the dimerizations in this work. It should also be pointed out, that the synthesis of the dimer of 4β-dihydrochleavamine with vindoline hydrazide using the chloroindolenine approach has also been reported recently by another group of workers.[75]

One further point which has been ignored so far, is the stereochemistry at the $C_{18'}$ end of the junction in the dimers synthesized using the chloroindolenine as intermediate. In an endeavour to establish the stereochemistry at this point, we chose to compare dimer (119) with vinblastine in which the stereochemistry has been established by X-ray analysis. Ignoring stereochemical detail, dimer (119) differs from vinblastine in only one feature, a lack of a hydroxyl function at $C_{4'}$. This point of difference is far removed from the $C_{18'}$ position and would not be expected to influence the spectral properties associated with the stereochemistry at this junction.

An inspection of the models of the dimers, indicated that a

difference in stereochemistry at $C_{18'}$ profoundly influenced the overall shape of the molecule. The spatial arrangement between the indole and indoline chromophores would be altered if a difference in stereochemistry existed between these compounds and although these two chromophoric systems are not in conjugation with each other, it was thought that such a change in spatial arrangement when these groups are in close proximity, would be reflected by a difference in the uv spectra of these compounds. A comparison of their uv spectra, figure 33, indicates a considerable difference in extinction coefficient and a small shift in the absorption maxima. These spectra were recorded using methanol as solvent and it is possible that the difference in solvation which undoubtedly exists and is caused by the $C_{4'}$ hydroxyl, can account for the difference in extinction coeficients. The shift in the absorption bands, although they are small could be indicative of a change in stereochemistry. A comparison of the nmr spectra of these compounds, however, showed pronounced changes which could not be explained by the presence or absence of the hydroxyl function at $C_{4'}$. The major differences observed between the spectrum of vinblastine (figure 34) and that of dimer (119) (figure 32) are listed in Table II. It can be seen from this table that the substituents on the aromatic portion of the vindoline half of the dimer show a considerable change in chemical shift. These protons are very near the junction between the halves of the dimer and the changes in chemical shift no doubt reflects a substantial change in their environment. These data suggest therefore that the stereochemistry at $C_{18'}$ differs in the synthetic dimer (119) from that of the natural series.

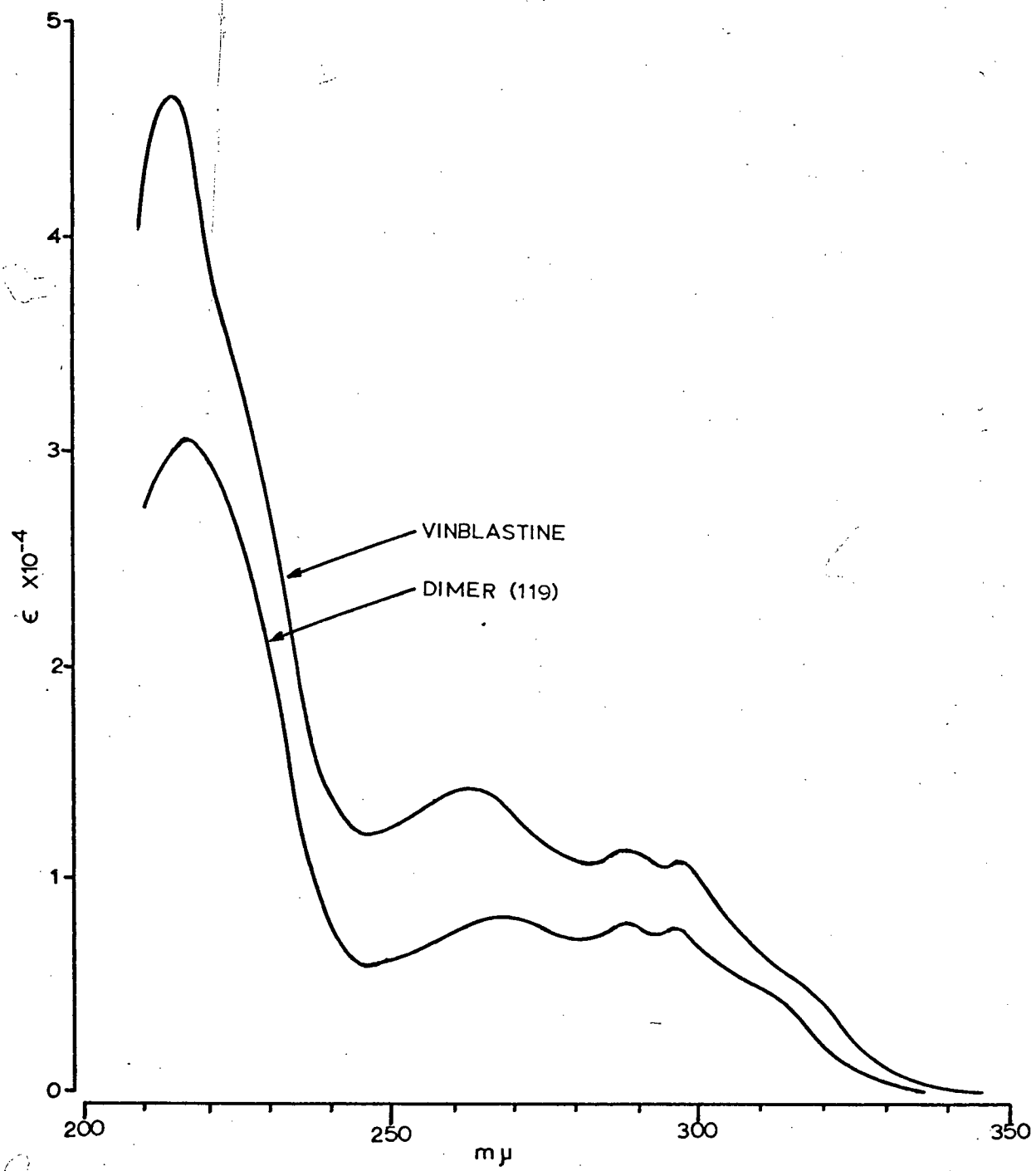


Figure 33. Uv spectrum of dimer (119) and vinblastine (16).

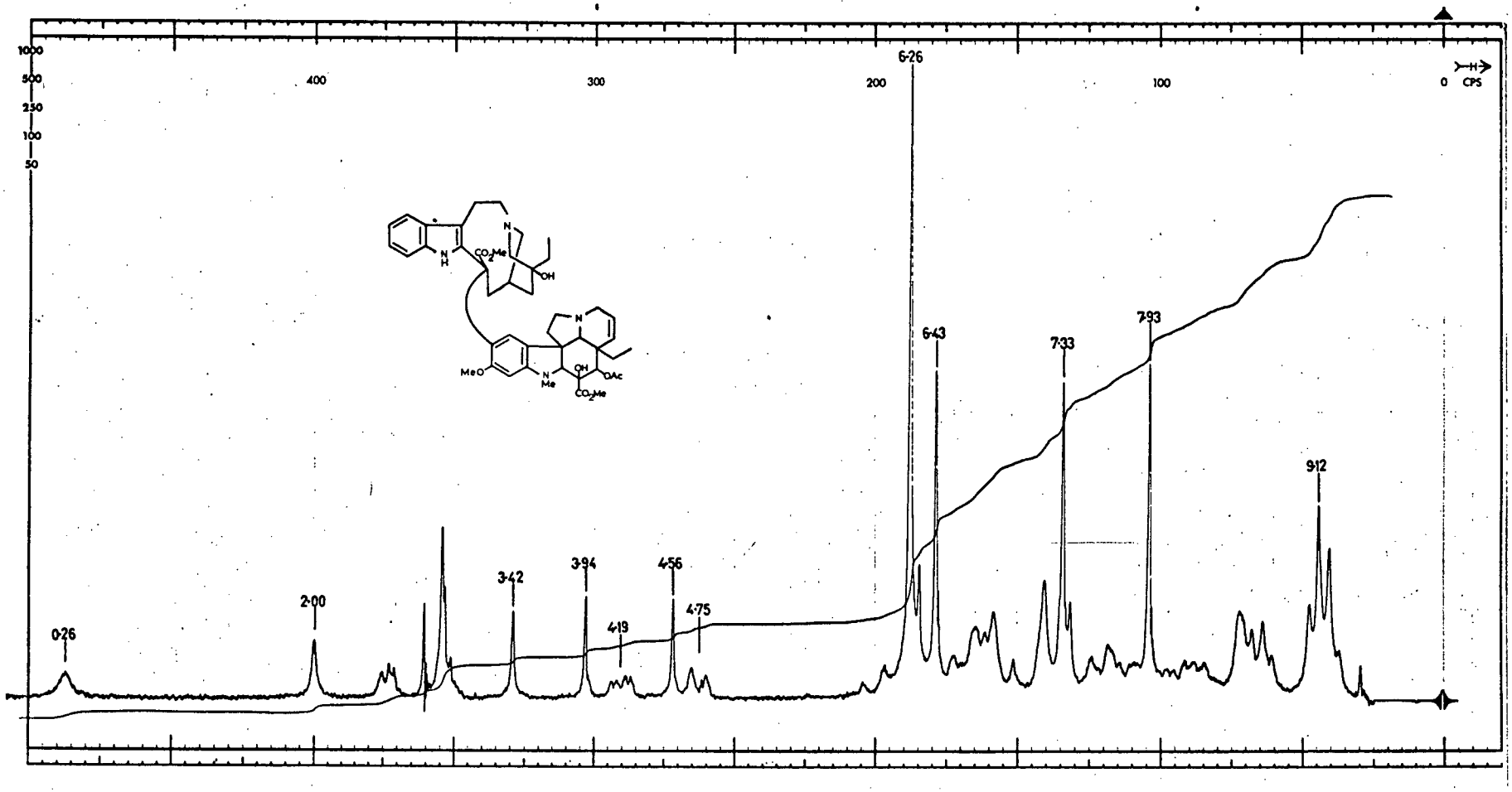


Figure 34. Nmr spectrum of vinblastine (16).

Table II. The major differences observed between the nmr spectra of vinblastine and dimer (119)

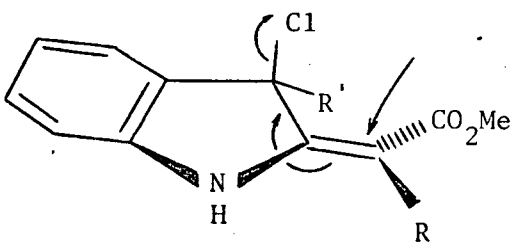
	Chemical Shift	
	vinblastine	dimer (119)
C_{14}-H	3.42	3.05
C_{17}-H	3.94	4.05
C_{16}-OCH_3	6.43	6.16
$C_5CH_2CH_3$	} 9.12	9.34
$C_4'CH_2CH_3$		9.09

Experimentally, only one isomer has been obtained from each of the dimerization reactions. Since the same reaction is involved for the synthesis of each dimer, it is reasonable to assume that these dimers all possess the same stereochemistry, and, on the basis of the above comparison, that this stereochemistry differs from that of the natural series.

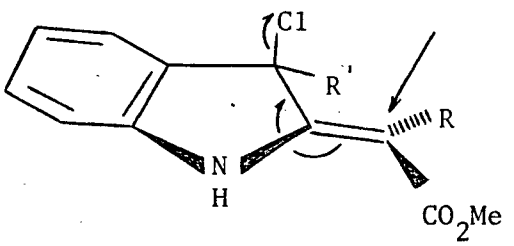
It is difficult to explain mechanistically the stereoselectivity of this reaction. The stereochemistry of the chloroindolenine intermediates have not been established. From tlc and spectral data, it is seen that they are single materials rather than epimeric mixtures. An inspection of molecular models show that the alicyclic portion of the molecule effectively blocks one side of the indole system from attack and for this reason these compounds must be β-chloroindolenines. Reaction of this compound with a nucleophile can occur in

two ways: 1) a concerted displacement of chloride by the nucleophile in an S_N2'-type reaction, or 2) initial loss of chloride with subsequent nucleophilic attack on the intermediate formed.

In the first case, attack by the nucleophile is on the chloroindolenine. For steric and electronic reasons, the direction of attack will be from the same side of the molecule as the chlorine. The chloroindolenine intermediate is, however, capable of existing in both the isomeric forms, indicated by structures (124) and (125) and these may be in equilibrium. This situation may result from the fact that a) the chloroindolenines as shown by structures (124) and (125) may be in equilibrium with the structure (126), allowing

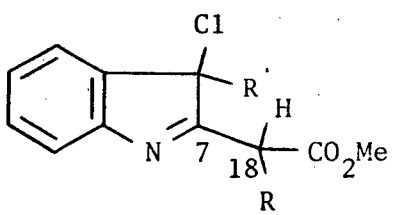


(124)



(125)

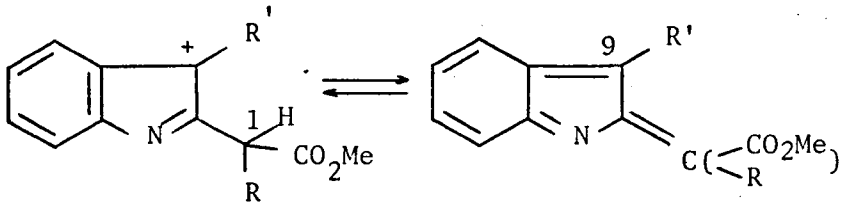
rotation about the C_{17}-C_{18} axis, and b) the nine-membered ring is



(126)

conformationally very mobile, particularly when inversion of the nitrogen is allowed. On this basis both double-bond isomers (124) and (125) could exist with no unfavourable interactions. Thus although attack by the nucleophile may occur from one direction, both epimers could in fact be formed.

The second reaction mode may involve an initial loss of chloride in a rate determining step to form the intermediate (127). This latter species may exist in tautomeric equilibrium with 128 which upon reaction with a nucleophile would be expected to yield a mixture of isomers.

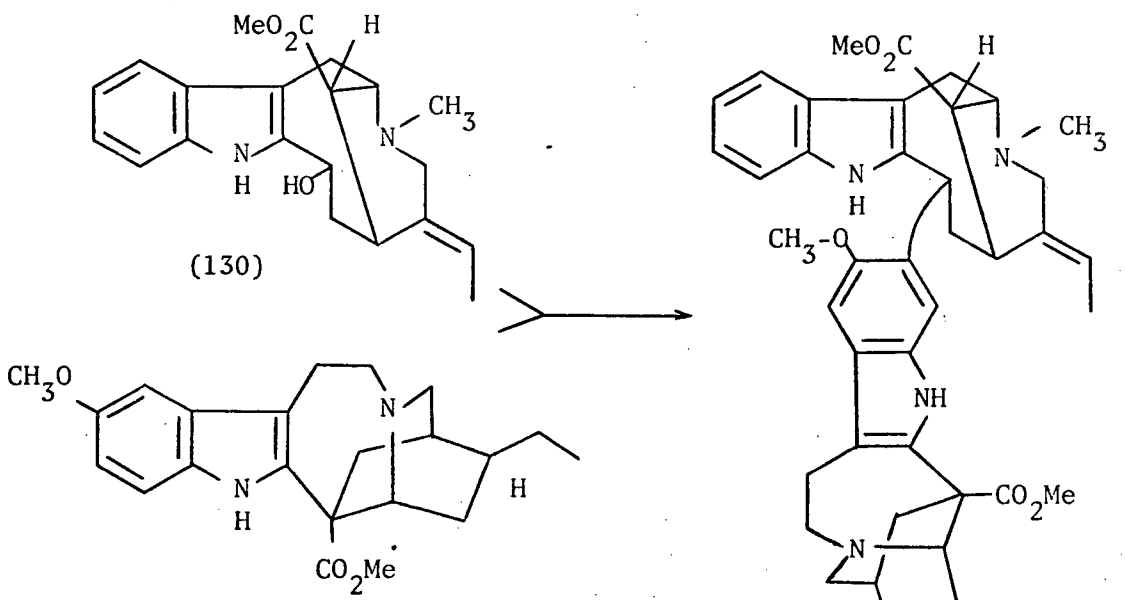


(127) (128)

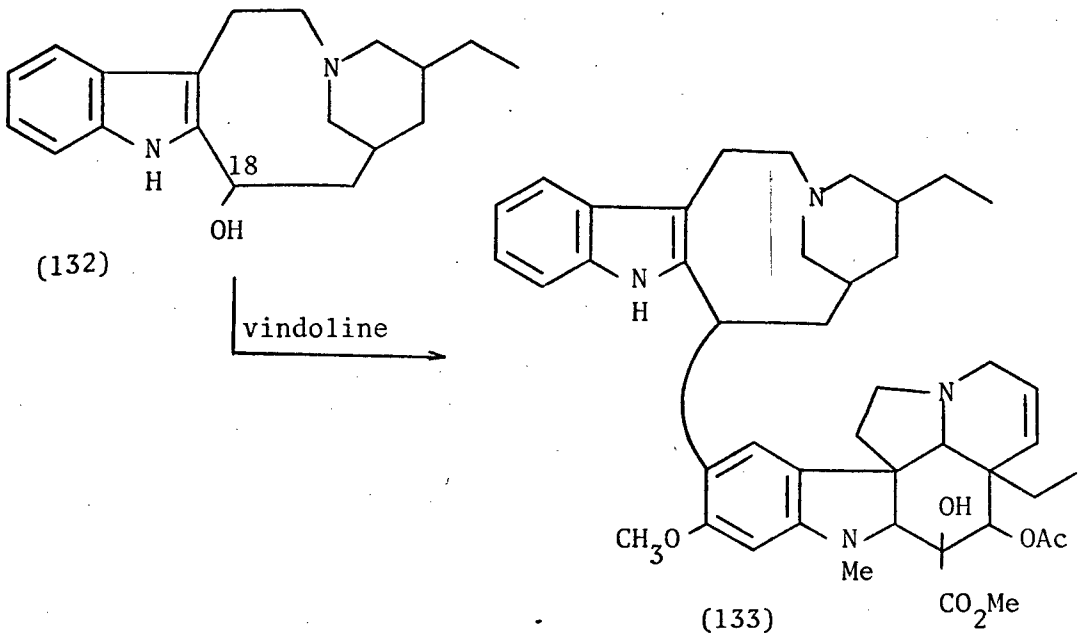
Although we had expected a mixture of isomers from this reaction, the experimental findings were that only one epimer was formed. From the data presented previously this dimer appeared to possess the incorrect stereochemistry at C_{18}'. It was desirable, however, to obtain dimers with the stereochemistry as found in the natural systems because it could be seen from molecular models that a change of stereochemistry at this C_{18}' position made a substantial difference to the overall shape of the molecule. Since molecular shape often plays an important role in determining the biological activity, an

unnatural stereochemistry at this centre may reveal a dramatic alteration in the desired anti-tumor action of these molecules. We therefore turned to an alternate approach for the coupling of the monomeric units.

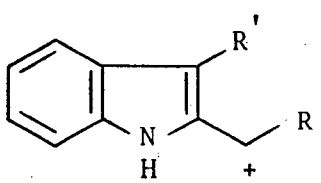
The second dimerization approach was based on a method developed by Buchi for the synthesis of voacamine (129).[74] He found that this compound could be synthesized by simply heating vobasinol (130) and voacanginol (131) together in a solution of methanolic hydrochloric acid. This method was subsequently used by Harley-Mason to condense



18-hydroxydihydrocleavamine (132) with vindoline to give the dimer (133).[77] The brief report on this work makes no mention of the stereochemical aspects of this reaction.



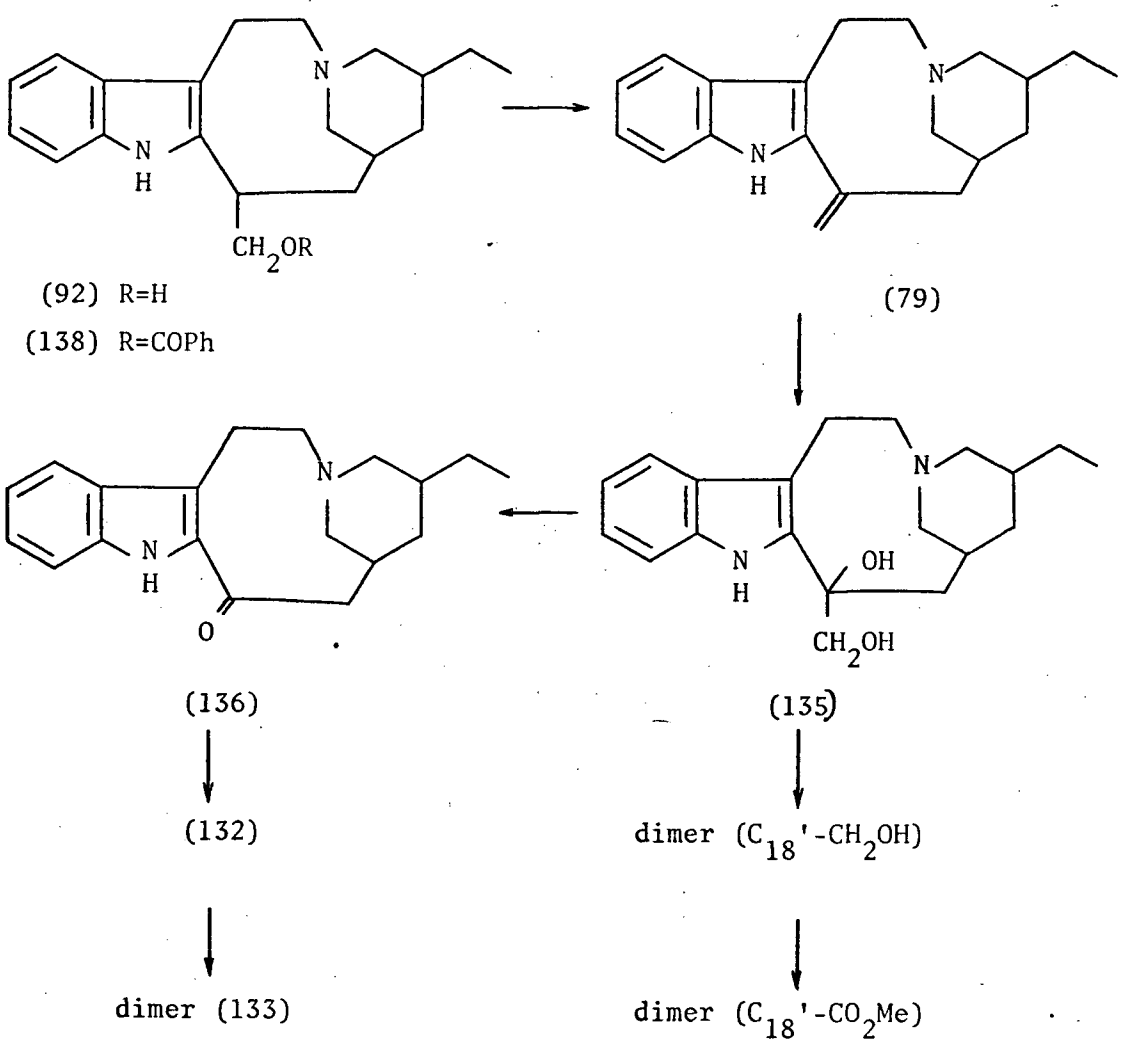
The mechanism of this reaction would be expected to involve a protonation of the C_{18} alcohol function to form an intermediate oxonium ion which upon loss of water would yield the carbonium ion (137). Again as in the mechanism involving the intermediate 127 and 128, the



(137)

formation of both epimers at the C_{18}' position would be expected.

In our initial investigations of this reaction, the sequence outlined in Part I of this discussion (figures 10 and 11) had not been developed. The reaction sequence proposed at that time involved the synthesis of the exocyclic olefin (79) from 18-hydroxymethyl-4β-dihydrocleavamine (92). Osmylation of this olefin would give the diol (135). This material could be used for dimerization and the product would contain the hydroxymethyl function at the C_{18}' position. This function would provide a convenient handle for the eventual production



of the C_{18}'-carbomethoxy group to give a dimer either identical to dimer (119) or its C_{18}' epimer. An alternate and perhaps more simple route for a comparison of the stereochemistry of this dimerization with the chloroindolenine approach would be the synthesis of dimer (133) via the acyl indole (136). The latter intermediate could be readily obtained fromthe diol (135) by periodate cleavage.

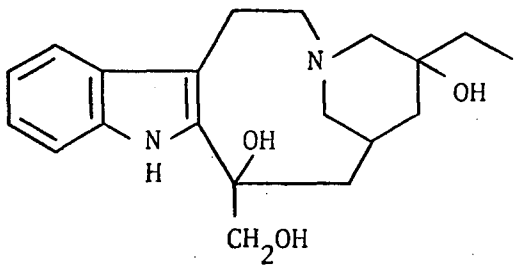
In order to evaluate this sequence 18β-hydroxymethyl-4β-dihydrocleavamine (92), readily available from catharanthine by ring opening to 18β-carbomethoxycleavamine (see figure 6), followed by catalytic and lithium aluminum hydride reduction, was considered as a starting material. The approach equivalent to dehydration involved simply the conversion of the alcohol to a good leaving group and then elimination either by use of heat or base. Our first attempt at this sequence was the preparation of the 3,5-dinitrobenzoate. The reaction was carried out at room temperature in pyridine and was followed by a weak aqueous base work up to remove the excess 3,5-dinitrobenzoyl chloride. One product other than starting material was obtained and this material gave spectral evidence indicating clearly that it was not the 3,5-dinitrobenzoate. Characterization of this material showed it to be instead, the elimination product, olefin (76), obtained in 40% yield based on starting material consumed. This material was shown to be identical to the olefin obtained subsequently by the reaction sequence already discussed (see figure 10).

It was thought that the yield of olefin could be increased by the preparation of a derivative which was stable enough to be isolated

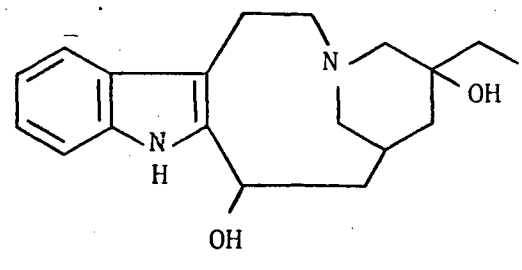
and would undergo elimination under controlled conditions rather than during the work-up. For this reason, the benzoate (138) was prepared. This material was stable and could be purified. Treatment of this compound with base consumed the benzoate but failed to give the olefin. Pyrolysis under high vacuum yielded benzoic acid but the residue contained none of the desired olefin. A parallel series of experiments were carried out on the acetate of the alcohol and a similar set of negative results were obtained. It was at this time, that the sequence of reactions outlined in figure 10 were developed and the olefin became available in better than 70% yield from dihydrocatharanthinol.

The osmylation, which was the next reaction in our proposed sequence could not be achieved. A variety of conditions were attempted including those found to be successful for the secodiene (78) (figure 11). In all cases, extremely complicated mixtures of products resulted.

The subsequent osmylation of the secodiene and ensuing reactions (figure 11) led to the synthesis of two compounds particularly well suited to this dimerization approach, the triol (97) and the diol (99).



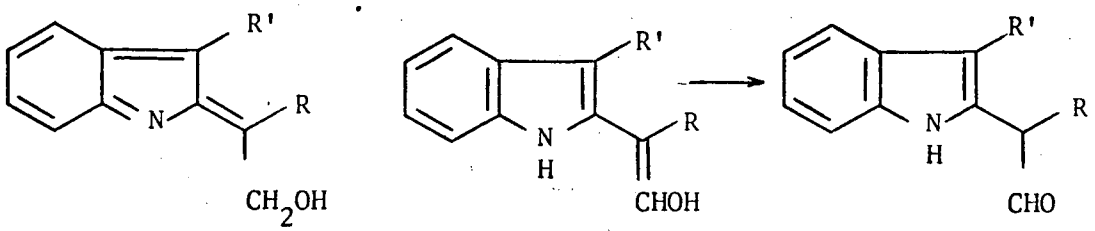
(97)



(99)

Coupling of these compounds with vindoline would give dimeric materials having the hydroxyl function at C_4' as is found in the natural dimers, although it was known from the work previously discussed, that the stereochemistry at this center was epimeric to that in the natural series. The triol dimerization should give a dimer with an hydroxymethyl group at the C_{18}' position and the conversion of this function to the ester would give a dimer possessing all the functionality of vinblastine and differing only in the stereochemistry at the C_4' and perhaps at the C_{18}' positions.

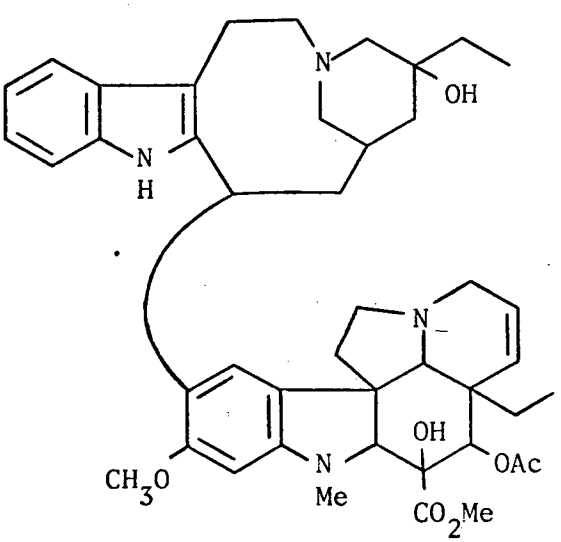
The coupling of the triol (97) with vindoline could, however, not be achieved. The reaction led to a total recovery of vindoline and loss of all the triol. The products obtained showed a typical indole absorption in their uv spectrum and showed a carbonyl stretching bond in the ir. That the triol should decompose under acidic conditions to give carbonyl containing compounds is not entirely unexpected. The tertiary alcohol at C_{18} could be visualized to undergo a loss of water to an intermediate such as (139) which would be susceptible to nucleophilic attack by vindoline. An alternative mode



(139) (140) (141)

of dehydration which does not involve the loss of aromaticity as entailed by structure (139), gives instead the conjugated enol (140). Simple tautomerization of this intermediate leads to an epimeric mixture of aldehydes (141).

The coupling of the diol (99) with vindoline was achieved under refluxing conditions in an anhydrous methanolic hydrochloric acid (1%) solution. The major product, obtained in 45% yield, gave spectral data consistent with the structure of the dimer (142). The dimeric nature was evident from the superimposition of the indole and the indoline chromophores in the uv spectrum. High resolution mass analysis on the molecular ion in the mass spectrum gave a molecular composition in perfect agreement with that expected for the dimer. A comparison of the nmr of this compound (figure 35) with that of dimer (118) (figure 28) prepared by the chloroindolenine approach confirms the similarity of



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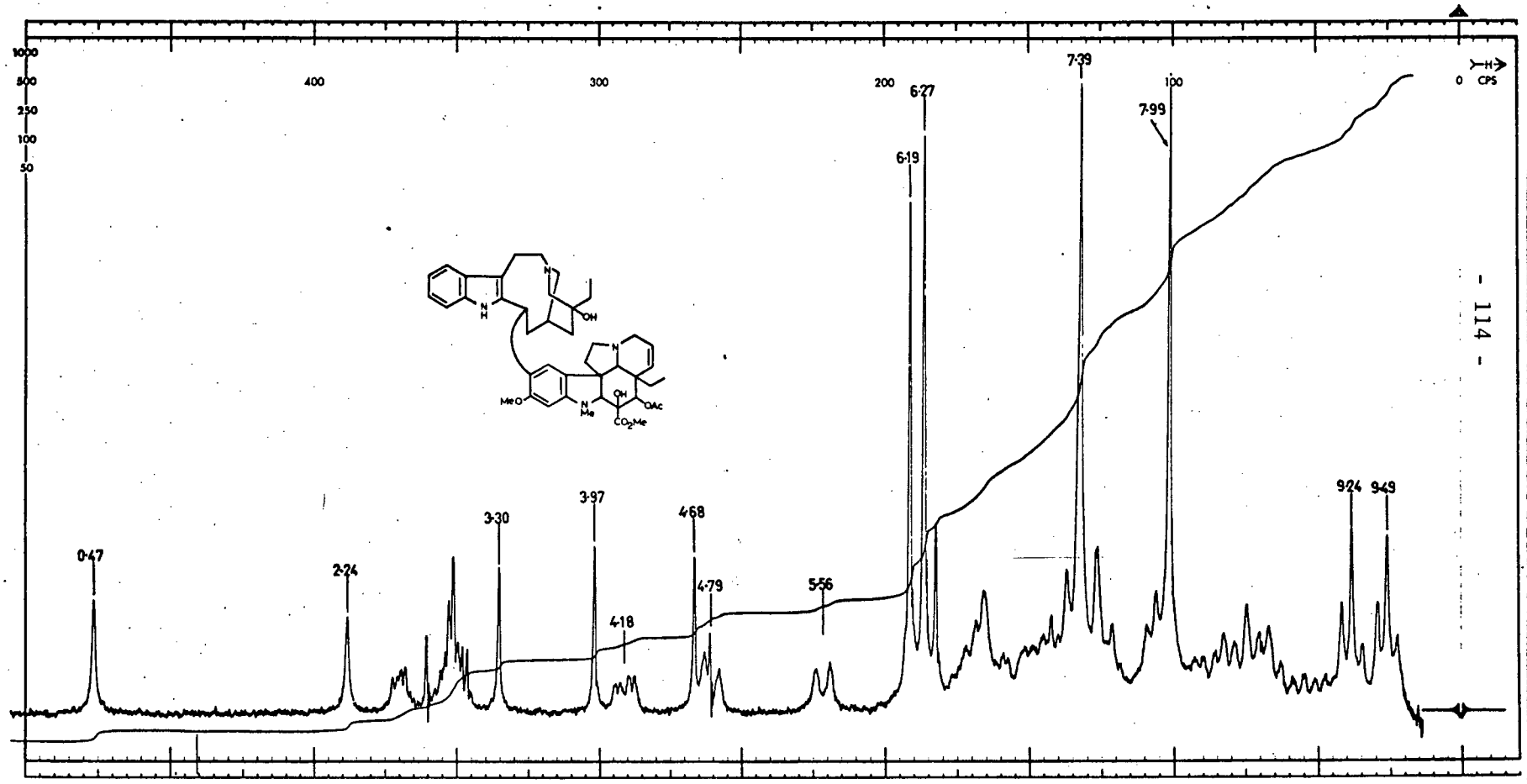


Figure 35. Nmr spectrum of dimer (142).

these two compounds. With respect to functionality, these compounds differ only in that the dimer (142) possesses a hydroxyl at C_4'. This leads to minor differences in the nmr spectrum particularly at higher field (> τ 6.5). At lower field, however, these spectra correspond very closely to each other. This area of the spectrum contains the protons associated with both aromatic systems and, as pointed out before, should be most affected by changes in stereochemistry at C_{18}'. The fact that these spectra are nearly identical in this region provides strong evidence for the same stereochemistry at this position in both these dimers. Cleavage of this dimer under the usual acidic reducing conditions gave a mixture of vindoline, desacetylvindoline, isovelbanamine, unchanged dimer and the desacetyl derivative of the dimer. This evidence in addition to the spectral data, established with certainty the structure of the dimer (142). One other dimeric material was obtained in very minor amounts from this dimerization reaction. An nmr of this compound revealed that it was the desacetyl analogue of the dimer (142). This material probably resulted from hydrolysis during the work up.

It appears from the evidence presented so far, that this dimerization approach has the same limitations as the dimerization via the chloroindolenines, that is, only one of the two possible isomers at C_{18}' is formed and this stereochemistry is probably not the one found in the natural systems. The proof for this statement is not conclusive because it is based on comparisons of spectral data for dissimilar compounds. To establish this with certainty we returned to the problem of synthesizing either of the dimers (118) or (119), which were

available from the chloroindolenine approach by this second coupling procedure.

For the synthesis of the simplest of these, dimer (118) or its epimer, we were required to synthesize 18-hydroxy-4β-dihydrocleavamine (132). This compound had been obtained in earlier work in our laboratory from studies involved with the introduction of cyanide at the C_{18} position in the chloroindolenine of 4β-dihydrocleavamine. As described earlier, it was advantageous to convert this chloroindolenine (41) to the quaternary ammonium salt (115). The quaternary salt resulted from an intramolecular displacement of the acetoxy function at C_{18} in the intermediate acetate (114). The 18-acetoxy-4β-dihydrocleavamine (114) could be obtained impure in low yield but attempts to purify it led to decomposition and only 18-hydroxy-4β-dihydrocleavamine could be identified from the products obtained. This material was obtained from the chloroindolenine in only 2-3% yield which is not good enough for preparative synthetic work. An alternate but very similar procedure was available from published work in the ibogaine series.[33] This sequence involved the introduction of an 18-methoxyl substitutuent by treatment of the chloroindolenine with methanol and acid and then to cleave this methyl ether to give the 18-hydroxy compound.

We chose to subject the chloroindolenine of 4β-dihydrocleavamine (41) to a solution of aqueous acetic acid. Either of two processes possible in this system would lead to the desired product. Initial attack by acetate at the C_{18} position would give the 18-acetoxy compound (114) which in this reaction medium would rapidly hydrolyze to

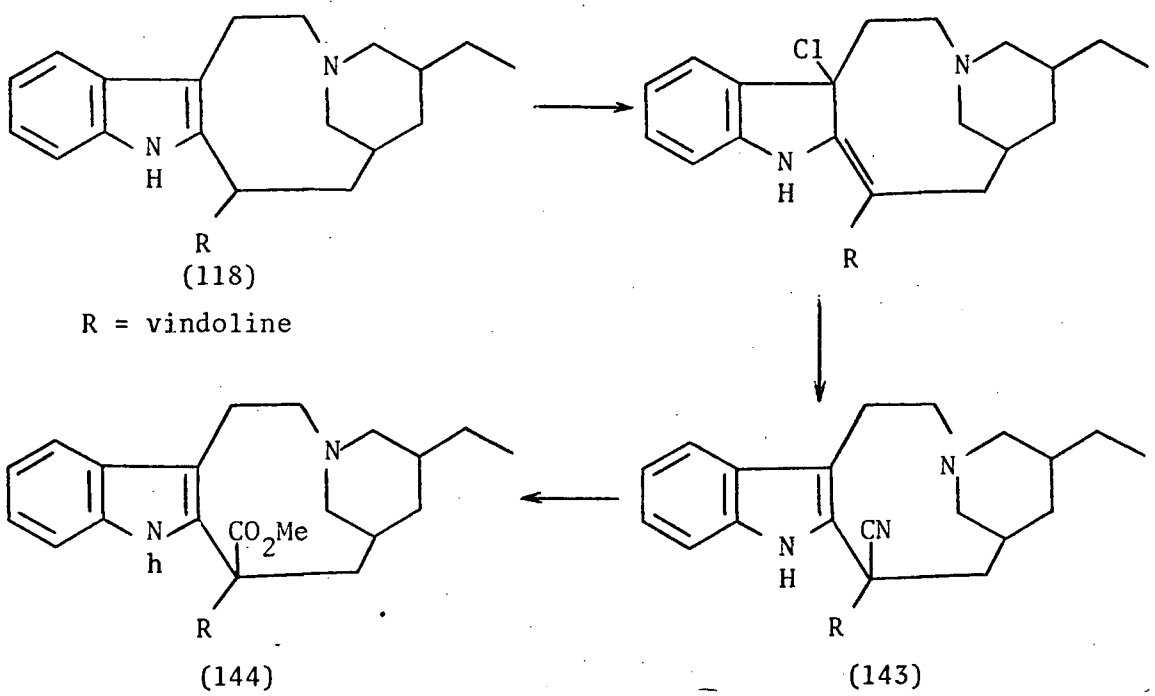
the 18-hydroxy analogue (132), or the 18-hydroxy compound could be formed directly by nucleophilic attack of water at the C_{18} position. The reaction was allowed to proceed at room temperature for 24 hours. After this time one major product was present along with some of the starting chloroindolenine. The product, obtained in 38% yield based on starting material consumed, had the same melting point, identical tlc properties and gave an ir superimposable with that of an authentic sample of 18β-hydroxy-4β-dihydrocleavamine.

The dimerization of this compound with vindoline was readily achieved in refluxing anhydrous methanolic 1% hydrochloric acid solution. The product of this reaction, isolated in 65% yield had identical tlc properties and gave superimposable ir and nmr spectra compared to the dimer (118) which was prepared using the chloro-indolenine-dimerization approach. The only other dimeric material obtained from this reaction was a small amount of the desacetyl derivative of dimer (118) present in about 5% yield.

A repeat of this reaction at a much lower temperature, 24 hours at -5°C, led to a product mixture containing much of the starting materials, some dimer (118) and one other product. The simple indole chromophore obtained for this product in the uv spectrum excluded the possibility of this material being a dimer. Although a good nmr spectrum was not obtained, the spectrum resembled very closely that of 18β-methoxy-4β-dihydrocleavamine and suggested reaction of the chloroindolenine with the solvent.

These experiments therefore, confirmed that this dimerization

followed the same stereochemical course as the chloroindolenine dimerization. Since it seemed unlikely at this time that the opposite stereochemistry could be obtained directly in the coupling process, work was initiated to alter the stereochemistry of the dimer once it was formed. Our approach to this would be to start with a dimer unsubstituted at the C_{18}' position, such as dimer (118) and to subject this compound to a tert-butyl hypochlorite oxidation in order to convert it to the chloroindolenine. Nucleophilic attack by cyanide would then form the 18'-cyano dimer (143). If attack by this nucleophile



(118)

R = vindoline

(143)

(144)

follows the same steric course as was the case with vindoline in the initial formation of the dimer (118), then the overall result would be an inversion of stereochemistry at the C_{18}' position. Conversion of the nitrile to the carbomethoxy function would then lead to an

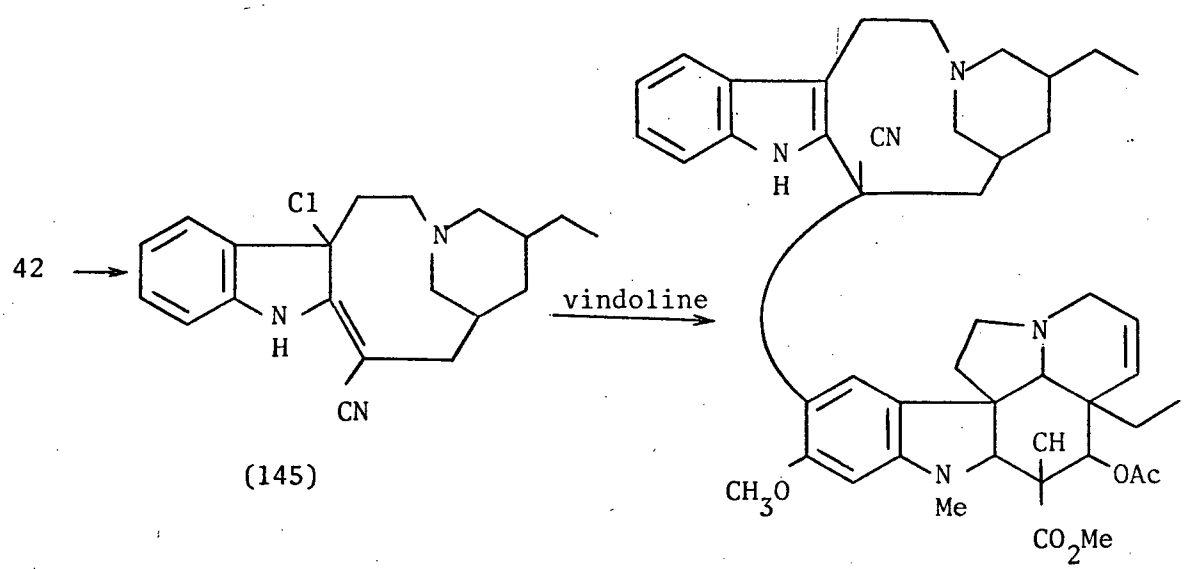
18-carbomethoxy dimer (144) having the opposite stereochemistry at C_{18}' to that of dimer (119).

Our experience with the formation of 18-cyanocleavamine and 18-cyanodihydrocleavamine by the reaction of the corresponding chloroindolenines with cyanide, suggested that this conversion in the dimeric series would be difficult to achieve. To facilitate the identification of the 18-cyano dimer (143), it was decided to also synthesize a cyano dimer by the coupling of 18-cyano-4β-dihydrocleavamine (42) with vindoline. The dimer thus obtained should in fact be the epimer of the cyano dimer (143) resulting from the inversion scheme, and would provide a further comparison of two dimers differing only in stereochemistry at C_{18}'.

The synthesis of 18β-cyano-4β-dihydrocleavamine (42) was developed earlier in our laboratory[59] and has already been discussed. Thus 4β-dihydrocleavamine was converted to its chloroindolenine (41) and this compound on treatment with sodium acetate in acetic acid gave the quaternary salt (115). Treatment of the salt with cyanide in refluxing dimethyl formamide provided 18β-cyano-4β-dihydrocleavamine. This compound was then treated with tert-butyl hypochlorite at -15°C to produce the chloroindolenine (145). This material was found to be unstable and was therefore treated immediately with vindoline under the usual dimerizing conditions (refluxing in anhydrous methanolic 1% hydrochloric acid solution) to give the cyano dimer (146).

The spectral data for this compound provided ample proof for the structure (145). Once again the dimeric nature of the material was evident from both the uv and mass spectra while a weak absorption in

the ir at 2250 cm^{-1} established the presence of the nitrile function. High resolution mass measurement provided a molecular composition agreeing with the molecular formula for this compound. The nmr spectrum (figure 36) can be seen on the basis of our previous experience to be typical for this class of dimers. One unusual feature was



(145) (146)

observed in this spectrum and that is the chemical shift of the C_{14}-proton signal. In the case of the dimers bearing no substituent at C_{18}' (cf figures 28 and 34) this signal is observed at τ 3.3. The dimer having a carbomethoxy function at C_{18}' shows this proton to be deshielded relative to this and is observed at τ 3.05. In this cyano dimer (146), however, the same proton is now strongly shielded and is observed at τ 3.79. This is not surprising since this proton and nitrile function are quite close to each other in one of the two possible rotomers and are arranged spatially such that the axes of their bonds are parallel. Since the shielding cone of the nitrile group

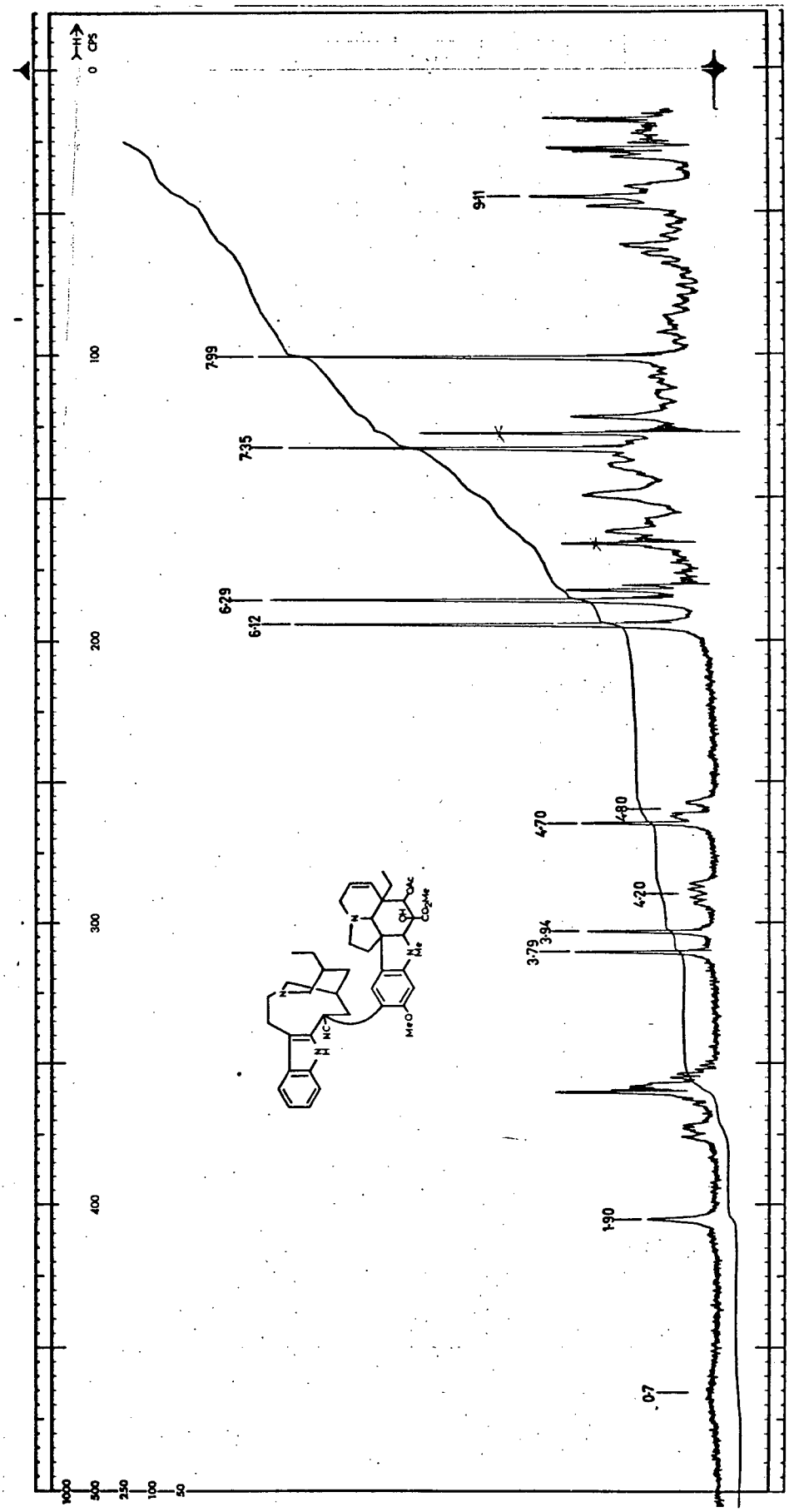


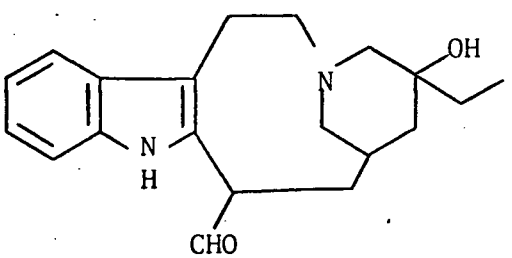
Figure 36. Nmr spectrum of dimer (146).

is perpendicular to the axis of the C≡N bond, the proton at C_{14} is put directly into this field.

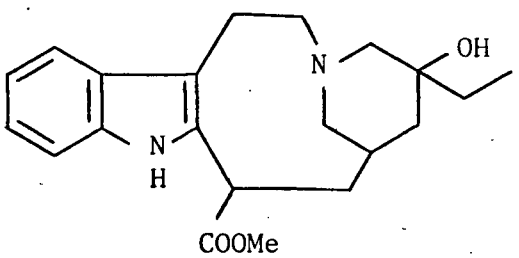
The approach to the cyano dimer (143) using the reaction of the chloroindolenine (118) with cyanide is currently being investigated by other workers in our laboratory. If this reaction can be developed, then the dimerization approaches using either the chloroindolenines or the C_{18}-alcohols will serve as a general synthetic scheme for both stereoisomeric series of dimers.

As yet, no synthetic materials are available which possess all the functionality of one of the natural dimers. Until such a compound has been synthesized, the question of whether or not these synthetic schemes provide the stereochemistry at C_{18}' corresponding to the natural systems, will not be properly settled.

This is a very important point and further studies in this direction are being initiated. For example, the work of this thesis now provides the means of synthesizing vinblastine or the stereoisomer at C_{18}'. On the basis of the successful isomerization of isovelbanamine to velbanamine and the results obtained from the attempts to dimerize the triol (97), it is anticipated that the triol will be converted to the compound (147) on acid treatment.



(147)



(148)

This compound now bears the hydroxyl function at C_4 with the correct stereochemistry. Oxidation of the 18-formyl substituent followed by esterification then gives 18-carbomethoxyvelbanamine (148). Dimerization will produce either vinblastine or its C_{18}-epimer and either result will determine with certainty the stereochemical course of the dimerization reaction. If the epimeric material is obtained the effect of this stereochemical difference on the biological activity can then be clearly ascertained. Once both these points have been settled, then a systematic study of the effects of further structural modification of the dimers on biological activity can being.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet spectra (uv) were recorded on a Cary 11 spectrophotometer using methanol as solvent unless otherwise specified. Infrared spectra (ir) were recorded on a Perkin-Elmer Model 21 and Model 137 spectrometers. Nuclear magnetic resonance (nmr) spectra were recorded in deuteriochloroform at 100 Hz on a Varian HA-100 instrument and the chemical shifts are given in the Tiers τ scale with reference to tetramethylsilane as the internal standard. Mass spectra were recorded on an Atlas CH-4 or an AE-MS-9 mass spectrometer. Analyses were carried out by Mr. P. Borda of the microanalytical laboratory, the University of British Columbia. Woelm neutral alumina and Silica Gel G (acc. to Stahl) containing 1% by weight of General Electric Retina p-1 Type 188-2-7 electronic phosphor were used for analytical thin-layer chromatography (tlc). Chromatoplates were developed using 1:1 carbontetrachloride-antimony pentachloride solution. Woelm neutral alumina (activity III) was used for column chromatography (unless otherwise indicated).

Dihydrocatharanthine (34)[22]

Catharanthine hydrochloride (20 g) was suspended in water (400 cc), the mixture cooled to 0°C and then basified with ice-cold ammonia.

The resulting suspension was then extracted with ether, the organic layer dried with anhydrous sodium sulphate and the solvent removed to give the free base as a white foam. This material was dissolved in 95% ethanol (1000 cc), Adam's catalyst (800 mg) was added and the mixture stirred under hydrogen at room temperature for 4 days. The catalyst was filtered off and the filtrate taken to dryness to give a white foam of substantially pure dihydrocatharanthine (17 g). Pure dihydrocatharanthine was obtained by chromatography using alumina (Woelm neutral, activity I, 600 g) and eluting with 5% diethyl ether in benzene (14.5 g, 80%). Crystallization from methanol gave plates mp 68-73° with recrystallization and subsequent mp 145-147° (lit. mp[22] i) 63-65°, ii) 150° decomp.)

Dihydrocatharanthinol (76)[22]

Lithium aluminum hydride (5.4 g) was added to a solution of dihydrocatharanthine (11.4 g) in dry tetrahydrofuran (500 cc) and the resulting mixture refluxed for 5 hours. It was then cooled in ice and water (35 cc) was added dropwise with stirring. The grey suspension was heated under reflux for 1/2 hour and the resulting white precipitate filtered off. The filtrate was taken to dryness to give a white foam (11.0 g) which was chromatographed on alumina (320 g). Pure dihydrocatharanthinol was eluted with chloroform (10.3 g). This material crystallized from chloroform-light petroleum ether as rhombs mp 142-143° (7.34 g) (lit.[22] mp 132°).

Dihydrocatharanthinol O-p-toluenesulfonate (77a)

Dihydrocatharanthinol (1.58 g) was dissolved in dry pyridine (30 cc) and p-toluenesulfonyl chloride (4.7 g) was added to this solution. The reaction was allowed to proceed for 10 hours at room temperature. The reaction mixture was then cooled to 0°C, ice-cold methylene chloride (50 cc) was added and this solution was washed with 3% sodium bicarbonate solution (3 x 50 cc), keeping the solution at 0°C at all times. The methylene chloride-pyridine solution was then taken to dryness using first waterpump pressure and finally high vacuum. Again the solution had to be kept below 0°C throughout this procedure. The resulting red gum still containing traces of pyridine, was dissolved in dry benzene (5 cc) and left to crystallize overnight in the refrigerator. The crude tosylate was filtered off to give 1.64 g of light brown crystalline material. The mother liquors were taken up in benzene (10 cc) and freeze-dried to give a further 0.65 g of substantially pure tosylate as a reddish amorphous powder. Attempts to recrystallize the crystalline material failed and generally led to less pure tosylate because of decomposition in solution. The crystalline material gave the following data: ν_{max}^{KBr} 1355 cm^{-1} (ν_{as} SO_2) and 1173 cm^{-1} (ν_s SO_2); λ_{max} 292, 285, 276 (sh).

5,18-Seco-diene (78)

Dihydrocatharanthinol o-tosylate (400 mgs; 0.86 mmole) was dissolved in a solution of dry benzene (15 cc) and triethylamine (0.24 ml; 1.73 mmole). This solution was stirred under a nitrogen atmosphere for 2 hours at 70°C to effect the displacement. The solution was then

cooled to room temperature and was flushed rapidly through alumina (basic Woelm III, 10 g set up as a 1 inch high column) using positive pressure. A further portion of benzene (100 cc) was used to wash the column. The combined solutions on removal of solvent under reduced pressure and at room temperature gave a light yellow gum which crystallized on standing to give virtually pure product (155 mg, mp 129-135°C). This material could be recrystallized from cold benzene mp 130-132. Sublimation gave an analytically pure sample mp 136-136.5. ν_{max}^{KBr} 3445 cm^{-1} (indole N-H stretch), 1657 cm^{-1} (ν C=C, enamine), 1408, 880 (exocyclic methylene); λ_{max} 306, 235 (sh), 340 (sh) (log ε 4.16, 4.34, 340 respectively); NMR: τ 2.05 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 4.29 (broad singlet, 1H, R_3N-C<u>H</u>=CR_2), 4.75 (singlet, 1H, R_2C=C<u>H_2</u>), 4.89 (singlet, 1H, R_2C=C<u>H_2</u>), 9.05 (triplet, 3H, -CH_2C<u>H_3</u>); mass spectrum: main peaks, m/e 292, 185, 168, 135, 122, 121, 107.

Anal. Calcd. for $C_{20}N_2H_{24}$: C, 82.19; H, 8.22; N, 9.59; M.W. 292.194. Found: C, 82.26; H, 8.27; N, 9.72; M.W. 292.191 (high resolution mass spectrometry).

<u>18-Methylene-4β-dihydrocleavamine (79); concerted sequence from dihydrocatharanthinol O-tosylate (77a)</u>

Dihydrocatharanthinol O-p-toluenesulfonate (400 mg, 0.86 mmole) was dissolved in a solution of dry benzene (15 cc) and triethylamine (0.24 cc, 1.73 mmole). This solution was stirred under a nitrogen for 2 hours at 70°C to effect the displacement. Solvent was removed under reduced pressure and the brown residue dissolved in methanol.

Sodium borohydride (200 mg) was added immediately and the reaction mixture stirred for 0.5 hours after which, the effervescence had ceased. This solution was stripped of solvent and the residue was partitioned between dichloromethane (100 cc) and water (100 cc). The aqueous layer was extracted with additional dichloromethane (2 x 50 cc) and the combined organic extract was dried over anhydrous sodium sulfate and the solvent was removed to give a yellow gum. This material was chromatographed on alumina (100 g). Benzene elution gave the desired 18-methylene-4β-dihydrocleavamine (182 mg); crystallized from methanol-water mp 90-94°C. λ_{max} 306, 315 (sh), 218 (log ε 3.97, 3.92, 4.16, respectively); NMR: τ 1.90 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 4.79 and 4.93 (two singlets, 1H each, $C=C\underline{H}_2$), 9.13 (triplet, 3H, $-CH_2-C\underline{H}_3$); Mass spectrum: main peaks, m/e 294, 292, 207, 139, 124.

Anal. Calcd. for $C_{20}N_2H_{26}$: M.W. 294.209. Found: M.W. 294.209.

Dihydrocatharanthinol O-methanesulfonate (77b)

Dihydrocatharanthinol (1.0 g) was dissolved in dry pyridine (10 cc) and to this solution cooled to -5°C was added freshly distilled methanesulfonyl chloride (6 cc). The resulting red solution was stirred at 0°C for 20 hours. Ice-cold chloroform (100 cc) was then added to the reaction mixture and the resulting solution extracted with water (3 x 50 cc). The cold chloroform solution was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a red gum. Rapid percolation of this material using dichloromethane as eluent, through alumina (IV, 30 g) removed most of the very polar

material and gave the crude methanesulfonate ester as a yellow oil. Chromatography on alumina (IV, neutral, 60 g) yielded non-polar impurities (15 mg) in the benzene fractions followed by the desired dihydrocatharanthinol O-methanesulfonate as a yellow unstable foam. λ_{max} 276, 283, 292. The methanesulfonate was unstable and was used immediately in the subsequent elimination reaction.

18-Methylene-4β-dihydrocleavamine (79); from dihydrocatharanthinol-O-methanesulfonate (77b)

A solution of potassium tert-butoxide was prepared by dissolving clean potassium (1.2 g) in refluxing tert-butanol (50 cc, freshly distilled from sodium). Dihydrocatharanthinol-O-methanesulfonate (350 mg) was dissolved in this solution and the resulting solution refluxed under a nitrogen atmosphere for 50 min. The uv of this solution showed a maximum at 304 mμ with no indole absorption evident. This solution was made just acidic with glacial acetic acid (2 cc) and an excess of sodium borohydride (600 mg) was added. This solution was refluxed for 3 hours. Solvent was removed under reduced pressure, the residue was dissolved in cold water (100 cc) and this mixture was extracted with dichloromethane (3 x 50 cc). The combined organic extracts were dried over anhydrous potassium carbonate and concentrated to give a yellow gum (170 mg) which was chromatographed on alumina (IV, neutral, 6 g) using benzene as eluent. The early fractions gave the pure exocyclic olefin (79) as a colourless glass (29 mg). This material had identical tlc and spectral properties to the exocyclic olefin obtained from the reduction of 5,18-seco-diene (78).

18-Methylene-4β-dihydrocleavamine (79); concerted sequence from dihydrocatharanthinol via methanesulfonate ester

Dihydrocatharanthinol (1 g) in anhydrous pyridine (9 cc) was treated at -5°C with methanesulfonyl chloride (3 cc). The resulting red solution was kept at 0°C for 3 hours. The mixture was poured into ice-cold dichloromethane (75 cc) and extracted with water (2 x 50 cc). The organic phase was concentrated to a red crystalline paste. This material was percolated rapidly through alumina (IV, neutral, 30 g) using dichloromethane and the solution on removal of solvent gave a yellow gum. This was dissolved in a solution of potassium tert-butoxide in tert-butanol (3.5 g of potassium in 200 cc dry butanol) and refluxed for 20 min. Solvent was removed under reduced pressure and the residue partitioned between ether and water. The ether extract was dried over anhydrous sodium sulfate and concentrated. The resulting yellow oil was then dissolved in isopropanol (70 cc) and acetic acid (1 cc) and excess sodium borohydride (2 g) was added. When the effervescence had subsided, solvent was removed, the residue partitioned between ether and water and the organic layer, after drying, concentrated to a yellow gum. This material was chromatographed on alumina (50 g). The early benzene fractions gave pure exocyclic olefin (76) (180 mg) identical on tlc and nmr with the material obtained from the reduction of 5,18-seco-diene (78).

18β-Hydroxymethylcleavamine (86)

18β-Carbomethoxycleavamine (100 mg) was dissolved in dry tetrahydrofuran and lithium aluminum hydride (50 mg) was added. This

mixture was refluxed for 2 hours under nitrogen. The reaction product was cooled in an ice-bath and saturated sodium sulfate (10 cc) was added dropwise. Water (50 cc) was then added and the mixture extracted with dichloromethane (4 x 25 cc). The combined organic extracts were dried over anhydrous sodium sulfate and the solvent was removed. The alcohol obtained gave the following data; $\nu_{max}^{CHCl_3}$: 3450 cm^{-1} (ν O-H), 1030 cm^{-1} (ν C-O); NMR: τ 1.50 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 4.66 (poorly defined multiplet, 1H, C=C<u>H</u>-), 5.74 (quintuplet, 1H, C_{18}-proton), 6.32 (doublet, 2H, CH-C<u>H_2</u>OH), 8.98 (triplet, 3H, -CH_2C<u>H_3</u>). Mass spectrum: main peaks, m/e 310, 187, 136, 135, 124.

<u>18β-Hydroxymethylcleavamine acetate (88)</u>

18β-Hydroxymethylcleavamine (92 mg) was dissolved in a solution of acetic anhydride in pyridine (10% v/v) and the resulting solution stirred at room temperature for 1 day under a nitrogen atmosphere. The reaction mixture was cooled to 0°C and was then poured onto ice (crushed ∿ 30 g). On stirring, the acetate crystallized from the mixture (83 mg) mp 123-127°C; ν_{max}^{KBr}: 1705 cm^{-1} (ν C=O), 1260 cm^{-1} (ν C-O); NMR:τ 1.87 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 4.75 (broad singlet, 1H, C=C<u>H</u>-), 5.5 (broad triplet, 1H, C_{18}-H), 5.70 and 5.98 (two doublet of doublets, 2H, CH-C<u>H_2</u>OAc), 7.98 (singlet, 3H, -OAc), 8.96 (triplet, 3H, -CH_2C<u>H_3</u>). Mass spectrum: main peaks, m/e 352, 293, 229, 136, 135, 124, 122.

18β-Hydroxymethylcleavamine 3,5-dinitrobenzoate (89)

18β-Hydroxymethylcleavamine (470 mg) was dissolved in dry pyridine (25 cc). This solution was cooled to 0°C and 3,5-dinitrobenzoyl chloride was added in portions over a 10 minute interval. The resulting red solution was stirred for 1 hour at 0°C and was then poured onto crushed ice (∿ 300 g). On stirring a precipitate formed which was filtered off to give the crude product (714 mg). Chromatography on alumina (200 g) gave the pure 3,5-dinitrobenzoate (89) (406 mg) with benzene elution. The material crystallized from methanol as bright orange needles; mp 155-157°C; λ_{max}: 293, 285, 228; ν_{max}^{KBr}: 3400 cm^{-1} (ν N-H), 1710 cm^{-1} (ν C=O), 1545 cm^{-1} (ν_{as}-NO_2), 1340 cm^{-1} (ν_s NO_2), 1280 cm^{-1} (ν C-O); NMR: τ 9.09 (triplet, 1H, benzoate p-proton), 1.03 (doublet, 2H, benzoate o-protons), 2.10 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 4.70 (broad doublet, 1H, C=C<u>H</u>-), 5.16 (quintriplet, 1H, C_{18}-H), 5.41 and 5.63 (two doublet of doublets, 2H, CH-C<u>H_2</u>OR), 8.99 (triplet, 3H, -CH_2C<u>H_3</u>).

18β-Hydromethyl-4β-dihydrocleavamine (92) from the hydroboration of the 5,18-seco-diene (78)

The 5,18-seco-diene (78), was prepared from dihydrocatharanthinol O-tosylate (300 mg) and was used as the crude reaction product. On forming, it was immediately dissolved in a diborane-tetrahydrofuran solution (diborane produced from sodium borohydride (200 mg) and boron trifluoride-etherate (1 cc) in diglyme (25 cc) and bubled into tetrahydrofuran (25 cc)). This solution was stirred for 1 hour at room temperature. 2 M aqueous potassium hydroxide solution was added

until the effervescence had ceased and then hydrogen peroxide (0.5 cc, 30%) was added. The solution was stirred for 10 min. and was then partitioned between dichloromethane (100 cc) and water (100 cc). Further extraction with dichloromethane (2 x 50 cc) and removal of solvent from the combined organic extracts gave the crude product as a yellow gum. This material was chromatographed on alumina (20 g). Elution with benzene-20% ethyl acetate gave the less polar decomposition products in the first three fractions. The following ten fractions contained a mixture of the alcohol and the amine boranes of the alcohol. These fractions combined (85 mg) was dissolved in a solution of tetrahydrofuran (25 cc) and triethylamine (1 cc) and refluxed under nitrogen for 2 hours. The solvent was removed under reduced pressure and the crude product chromatographed using alumina (III, 10 g). Benzene-20% ethylacetate elution gave 18β-hydroxymethyl-4β-dihydrocleavamine (47 mg) which crystallized from methanol-water. Sublimed sample mp 146.5-147.5°C; λ_{max} 293, 285, 275(sh), 221 (log ε 3.76, 3.84, 3.72, 4.44, respectively); ν_{max}^{KBr}: 3510 cm^{-1} (ν N-N), 3300 cm^{-1} (ν O-H), 1045 cm^{-1} (ν C-O); NMR: τ 1.54 (broad singlet, 1H, NH), 2.5-3.0 (diffuse, 4H, aromatic), 5.84 (quintuplet, 1H, C_{18}-H), 6.27 (doublet, 2H, CH-$\underline{CH_2}$OH), 9.16 (triplet, 3H, -$CH_2\underline{CH_3}$). Mass spectrum: main peak, m/e 312, 207, 138, 124.

Anal. Calcd. for $C_{20}H_{28}N_2O$: C, 76.91; H, 8.98; N, 8.98; O, 5.13. Found: C, 77.17; H, 8.88; N, 8.79.

18β-Hydromethyl-4β-dihydrocleavamine acetate mp 156-160°C, ν_{max}^{KBr}: 1700 cm^{-1} (ν C=O), 1265 cm^{-1} (ν C-O); NMR: τ 1.92 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 5.4-6.0 (diffuse, 3H, $-CH_2OAc$, C_{18}-H), 7.97 (singlet, 3H, OAc), 9.13 (triplet, 3H, $-CH_2CH_3$).

18β-Hydroxymethyl-4β-dihydrocleavamine (92), from hydroboration of 18-methylene-4β-dihydrocleavamine

Dihydrocatharanthinol (200 mg) was converted to 18-methylene-4β-dihydrocleavamine (79) using the procedure already outlined. The crude product was dissolved in anhydrous tetrahydrofuran (25 cc) and the solution was cooled to 0°C. Diborane in tetrahydrofuran (3.0 cc, 2.0 M) was added dropwise over a 1 hour period. The reaction mixture was then allowed to come to room temperature and stirred for an additional 0.5 hour. The excess diborane and solvent were removed using water-pump pressure. The residue was taken up in tetrahydrofuran (50 cc), aqueous sodium hydroxide (10 dp, 3M) was added followed by hydrogen peroxide (0.10 cc, 30%) and the resulting solution stirred for 15 min. The reaction mixture was partitioned between water (150 cc) and dichloromethane (100 cc). Further extraction with dichloromethane (2 x 50 cc) and removal of solvent from the combined organic extracts gave the crude product as a yellow gum. This material was chromatographed on alumina (70 g) using ethylacetate as eluting solvent. The main products were; the desired 18β-hydroxymethyl-4β-dihydrocleavamine (92) (22 mg), identical with the material obtained from hydroboration of 5,18-seco-diene; amine-borane, A, (R_f 0.7, alumina tlc, ethylacetate elution) (61 mg) and a second amine-borane, B,

(R_f 0.6, 14 mg).

Amine-borane A was dissolved in anhydrous tetrahydrofuran (10 cc) containing triethylamine (0.1 cc) and the solution refluxed for 2 hours under nitrogen. Pure 18β-hydroxymethyl-4β-dihydrocleavamine was obtained on taking the reaction solution to dryness and crystallizing the residue from methanol-water.

Amine-borane B, was dissolved in anhydrous tetrahydrofuran (2 cc) containing triethylamine (0.05 cc) and the solution was refluxed for 2 hours under nitrogen. A mixture of starting amine-borane B and the alcohol was obtained. Separation by preparative tlc gave pure 18β-hydroxymethyl-4β-dihydrocleavamine.

18β-Hydroxymethyl-4β-dihydrocleavamine (92) from reduction of 18β-carbomethoxy-4β-dihydrocleavamine (32)

18β-Carbomethoxy-4β-dihydrocleavamine (100 mg) was added to a solution of lithium aluminum hydride (70 mg) in tetrahydrofuran (15 cc) and the reaction mixture was refluxed for 2 hours. The mixture was cooled in an ice bath and saturated aqueous sodium sulfate solution (0.5 cc) was added dropwise. Water (100 cc) was then added and this mixture was extracted with dichloromethane (5 x 25 cc). The combined extract was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The slightly yellow gum could be crystallized from methanol-water (64 mg, mp 140-144). Sublimation gave a white crystalline sample mp 146.5-147.5°C identical to the material obtained from the hydroboration of both 5,18-seco-diene and 18-methylene-4β-dihydrocleavamine.

Tetrol (96)

Pure 5,18-seco-diene (78) (300 mg) was dissolved in a solution of anhydrous tetrahydrofuran (18 cc) and dry pyridine (1.8 cc). The reaction was carried out in a long-necked flask and this flask containing the solution was immersed in a dry ice-acetone bath such that 3 or 4 inches of the neck of the flask was also immersed. A solution of osmium tetroxide (522 mg) in anhydrous tetrahydrafuran (5 cc) was then added dropwise over a 1 hour period and in such a manner that it ran down the cooled neck of the flask. In this way it was assured that the osmic acid solution was cooled to the bath temperature when it reached the reaction mixture. Care was taken to keep the system closed to the atmosphere while adding the osmic acid solution to prevent condensation of water into the reaction solution. The reaction mixture was stirred for an additional 6 hours and was then allowed to come to room temperature over a 1/2 hour period. It was then poured into a solution of ethanol-dichloromethane (1:1, 50 cc) and hydrogen sulfide was bubbled through this solution with rapid stirring for 10 min. This mixture was filtered through celite and the black residue was washed with an additional amount of ethanol-dichloromethane (1:1, 100 cc). The residue was then suspended in triethylamine (20 cc) and stirred for about 15 hours. This mixture was filtered and washed as before. The combined filtrate was taken to dryness and chromatographed on alumina (50 g). Dichloromethane-1% methanol solution eluted the desired tetrol which crystallized on taking the eluent to dryness (187 mg). This material recrystallized from methanol-water; mp 120-123°C; λ_{max} 293, 285, 275, 228 (log ε 3.85, 3.89, 3.82, 4.52,

respectively); λ_{max}^{nujol}: 3360 cm^{-1}, 3510 cm^{-1}(sh), 3350 cm^{-1} (ν OH and NH); NMR: τ 1.84 (broad singlet, 1H, N-H), 2.4-3.0 (diffuse, 4H, aromatic), 5.42 (broad singlet, 1H, $\geq$N-C<u>H</u>OH), 6.36 (broad singlet, sharpened on D_2O exchange, 2H, -C<u>H_2</u>OH), 8.96 (triplet, 3H, -CH_2C<u>H_3</u>). Mass spectrum: main peaks, m/e 342, 311, 143, 91, no parent ion.

<u>Anal</u>: for M^+ - H_2O(18). Calcd. for $C_{20}N_2O_3H_{26}$: 342.194. Found: 342.192 (high resolution mass spectrometry).

<u>Triol (97)</u>

The tetrol (96) (330 mg) was dissolved in methanol (50 cc) and sodium borohydride (200 mg) was added. The solution was stirred at room temperature for 1 hour. The solvent was removed and the residue was partitioned between dichloromethane (100 cc) and water (100 cc). The aqueous phase was extracted with an additional quantity of dichloromethane (2 x 50 cc) and the combined extracts after drying over anhydrous sodium sulfate, was stripped of solvent. The residue crystallized on trituration with methanol to give pure triol (97) (325 mg). This material could be recrystallized from methanol-water; mp 230-235 (decomp.); λ_{max}: 293, 285, 277(sh), 227, (log ε 3.87, 3.91, 3.87, 4.53, respectively); λ_{max}^{nujol}: 3540, 3430 and 3200 cm^{-1} (ν O-H and N-H); NMR: τ 0.29 (broad singlet, lost on deuterium exchange, 1H, O-H), 1.48 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 6.22 (singlet, 2H, -C<u>H_2</u>OH), 9.10 (triplet, 3H, -CH_2C<u>H_3</u>). Mass spectrum: main peaks, m/e 344, 326, 154, 95, 92, 91.

<u>Anal</u>. Calcd. for $C_{20}H_{28}N_2O_3$: 344.206. Found: 344.210 (high resolution mass spectrometry).

Ketol (98)

The triol (97) (150 mg) was dissolved in acetone (15 cc), and after dissolution, water (5 cc) was added. This solution was cooled in an ice-water bath and to it was added dropwise an aqueous solution of sodium periodate (90 mg in 10 cc). The solution was stirred for 1.5 hours at 0°C. The reaction solution was then poured into ice-water (70 cc) and extracted with dichloromethane (3 x 50 cc). The combined extracts were dried over anhydrous sodium sulfate and then stripped of solvent to give a yellow gum. This material was chromatographed on alumina (20 g); dichloromethane-1% methanol elution brought down the desired ketol (98) (75 mg). Further elution using dichloromethane-2% methanol gave some of the starting triol (15 mg). The ketol could be crystallized from methanol-water to give yellow plates; mp 105-109 (dec.); λ_{max}: 317, 238, (log ε 4.25, 4.16, respectively); λ_{max}^{nujol}: 3430 cm^{-1} (ν N-H), 3100 cm^{-1} (ν O-H), 1615 cm^{-1} (ν C=O); NMR: τ 0.73 (broad singlet, 1H, N-H), 2.4-3.0 (diffuse, 4H, aromatic), 7.82 (broad singlet, lost on deuterium exchange, 1H, O-H), 9.03 (triplet, 3H, $-CH_2\underline{CH_3}$). Mass spectrum: main peaks, m/e 312, 154, 144, 143, 140.

Anal. Calcd. for $C_{19}H_{24}O_2N_2$: 312.184. Found: 312.183 (high resolution mass spectrometry).

Diol (99)

The ketol (98) (93 mg) was dissolved in methanol (20 cc) and the solution cooled in an ice-bath. Sodium borohydride (100 mg) was added; the reaction mixture was allowed to come to room temperature and was then stirred for two hours. The solution was taken to dryness and

partitioned between dichloromethane (50 cc) and water (50 cc). The aqueous phase was further extracted with dichloromethane and then the combined organic extracts, after drying over anhydrous sodium sulfate, was taken to dryness. The product crystallized readily from dichloromethane to give the pure diol (99) (86 mg) mp 195-200°C (dec.); λ_{max}: 294, 286, 279(sh), 227 (log ε 3.83, 3.86, 3.82, 4.50, respectively); ν_{max}^{nujol}: 3250 cm^{-1} and 3360 cm^{-1}(sh) (ν O-H and ν N-H); NMR: τ 1.62 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 4.28 (doublet of doublets, J=2,10 Hz, 1H, C_{18}-H), 9.08 (triplet, 3H, $-CH_2\underline{CH_3}$). Mass spectrum: main peaks, m/e 314, 173, 154, 144, 142, 140, 130, 124.

Anal. Calcd. for $C_{19}H_{26}O_2N_2$: 314.199. Found: 314.199 (high resolution mass spectrometry).

Isovelbanamine (100)

The diol (99) (100 mg) was dissolved in anhydrous N-methylmorpholine (20 cc), lithium aluminum hydride (100 mg) was added and the resulting mixture was refluxed for 10 hours. After this time, it was cooled in an ice-bath and a saturated aqueous sodium sulfate solution (0.5 cc) was added dropwise. Water (60 cc) was added to this mixture and this was then extracted with ethyl acetate (5 x 20 cc). The combined extracts were taken to dryness and the residue chromatographed on alumina (10 g). Dichloromethane elution gave isovelbanamine (100) which crystallized from this solvent (42 mg), mp 190-194°; λ_{max}: 293, 286, 276(sh), 229 (log ε 3.88, 3.90, 3.82, 4.54, respectively); ν_{max}^{nujol}: 3250 cm^{-1} (ν O-H), 3500 cm^{-1} (ν N-H); NMR: τ 2.26 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 6.56 (complex multiplet,

1H, C_{18}-H), 8.77 (broad singlet, disappears on deuterium exchange, 1H, O-H), 9.13 (triplet, 3H, $-CH_2\underline{CH_3}$). Mass spectrum: main peaks, m/e 298, 154.

Anal. Calcd. for $C_{19}H_{26}ON_2$: 298.205. Found: 298.205 (high resolution mass spectrometry).

Cleavamine (23); dehydration of isovelbanamine (100)

Concentrated sulfuric acid (0.5 cc, 36 N) was cooled in an ice-water bath and to this cold acid was added isovelbanamine (100)(10 mg). The compound dissolved slowly and the resulting solution was stirred under a dry nitrogen atmosphere for 2 hours. This solution was then added dropwise to an ice-cold ammonium hydroxide solution (10 cc, 2N) and the resulting suspension was extracted with dichloromethane (3 x 5 cc). The combined extracts were flushed through alumina (1 g) and the eluted material concentrated to give a pale yellow gum (9 mg). This material was chromatographed on alumina (1 g) eluting with benzene to give the desired cleavamine contaminated with some slightly more polar material. Rechromatography on alumina (1 g) eluting with petroleum ether-benzene (1:1) gave pure cleavamine which crystallized from methanol (2.6 mg), mp 113-117°C (Lit[22,36] mp 117-119°). This material had tlc properties identical to an authentic sample of cleavamine and the ir spectrum (nujol) was superimposable to that of the authentic sample.

18β-Cyanocleavamine (109)

A solution of cleavamine (250 mg) in dichloromethane (30 cc) and

triethylamine (0.15 cc) was cooled in an ice-acetone bath and to it was added dropwise a solution of tert-butyl hypochlorite in carbon tetrachloride (48 cc of 0.38 M), over the period of 1 hour. This reaction solution was then taken to dryness and the residue dissolved in a solution of fused sodium acetate (250 mg) in glacial acetic acid (22.5 cc) and acetic anhydride (2.5 cc). This solution was stirred at room temperature for 1 hour and then for 2 hours at 60°C. The solvent was removed under vacuum and the residue dissolved in ethanol and flushed through a short column of alumina (20 g as a 1" column). The eluted material on removal of solvent gave the crude quaternary ammonium salt as a pale yellow foam. This crude material was dried for 3 hours under high vacuum at about 70°C. Potassium cyanide (250 mg) dried in a similar manner was added to this residue and dimethylformamide (15 cc) was distilled from over barium oxide into the reaction flask. This reaction mixture was refluxed under a nitrogen atmosphere for 1 3/4 hours. The solvent was removed under reduced pressure and the residue obtained was chromatographed using alumina (III, 15 g). Benzene elution gave the desired product, 18β-cyanocleavamine (109), which crystallized readily from methanol-water (81.3 mg); mp 87-90°; λ_{max}: 293, 284, 277, 225, (log ε 3.89, 3.96, 3.93, 4.57, respectively); ν_{max}^{nujol}: 2240 cm^{-1} (ν C≡N); NMR: τ 1.60 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 4.48 (doublet of doublets, J = 2 Hz and 10 Hz, 1H, C_{18}-proton), 4.74 (poorly defined doublet, 1H, $\underline{CH}$=C), 8.96 (triplet, 3H, $-CH_2\underline{CH_3}$). Mass spectrum: main peaks, m/e 305, 136, 124.

Anal. Calcd. for $C_{20}H_{23}N_3$: 305.189. Found: 305.187 (high resolution mass spectrometry).

18β-Carbomethoxycleavamine (60), from 18β-cyanocleavamine (109)

18β-Cyanocleavamine (109) (81 mg) was dissolved in diethylene glycol (2.5 cc) and potassium hydroxide (0.5 g) was added. This mixture was kept at 150° for 9 hours. The solution was then cooled in an ice-bath, made slightly acidic with a solution of methanol saturated with hydrochloric acid. A large excess of diazomethane in diethylether was added and the reaction mixture was stirred vigorously for 0.5 hour. The solution was allowed to come to room temperature and the excess diazomethane was blown off using a stream of nitrogen. Water (150 cc) was then added and the solution was extracted with diethylether (4 x 50 cc). The combined extracts were washed with water (2 x 25 cc), dried over anhydrous sodium sulfate and taken to dryness. The residue was chromatographed on alumina (10 g) and pure 18β-carbomethoxycleavamine (45 mg) was obtained on elution with petroleum ether-benzene (1:1). This material crystallized from methanol, mp 122-126°C (Lit. mp 122-123°C); it had identical properties compared with an authentic sample of 18β-carbomethoxycleavamine on tlc, and gave a superimposable infrared spectrum.

Cleavamine N-borane (116)

Cleavamine (50 mg) was dissolved in anhydrous tetrahydrofuran (5 cc) and the solution cooled to 0°C. Diborane produced externally (by the reaction of a solution of sodium borohydride (17 mg) in anhydrous

diglyme (5 cc) with a solution of borontrifluoride-etherate (0.07 cc) in anhydrous diglyme (2 cc)) was passed into the reaction solution over the period of 1 hour. The reaction mixture was allowed to come to room temperature and stirred for an additional 1/2 hour. It was then partitioned between dichloromethane (50 cc) and water (50 cc); the aqueous phase was extracted with additional dichloromethane (2 x 25 cc) and the combined organic extracts taken to dryness. The product crystallized readily from benzene (43 mg). λ_{max}: 293, 285, 275(sh); ν_{max}^{CHCl} 3450 cm^{-1} (sharp, ν N-H), 2375 cm^{-1} (ν B-H), 1170 cm^{-1} with 2260 cm^{-1} overtone (δ B-H).

The amine-borane (25 mg) was dissolved in anhydrous tetrahydrofuran (1.0 cc), triethylamine (0.2 cc) was added and this solution was refluxed for 2 hours under a nitrogen atmosphere. The reaction mixture was partitioned between dichloromethane (20 cc) and water (20 cc) and the organic phase was taken to dryness. Chromatography on alumina (III, 10 g) gave on elution with petroleum ether-benzene (1:1) pure cleavamine (14 mg), identified by tlc and ir.

Hydroboration of cleavamine using cleavamine N-borane (116)

Cleavamine N-borane (40 mg) was dissolved in anhydrous diglyme (2 cc) and this solution was refluxed under a nitrogen atmosphere for 1.5 hour. The reaction solution was then allowed to come to room temperature, aqueous potassium hydroxide solution (1 dp, 2 M) was added followed by hydrogen peroxide (60 µl, 30%) and the resulting solution was stirred for 15 min. The reaction mixture was partitioned between ether (20 cc) and water (20 cc), and the organic phase taken

to dryness. The product mixture was separated into its components by preparative tlc. The initial separation on alumina with benzene-20% ethylacetate elution gave the non-polar materials (18 mg) and two more polar materials, A with R_f 0.2 (2.0 mg) andB with R_f 0.15 (2.5 mg). B was identical in tlc and ir with 3α-hydroxy-4β-dihydrocleavamine obtained by hydroboration in subsequent work and A on the basis of similar tlc and ir is probably the epimeric alcohol. A second preparative tlc system (alumina, benzene elution) was used to separate the less polar materials. Cleavamine (8 mg) and 4β-dihydrocleavamine (5.5 mg) were obtained and were identified by tlc and nmr comparison with authentic samples.

3α-Hydroxy-4β-dihydrocleavamine (117); hydroboration of cleavamine (23)

To a solution of cleavamine (23) (450 mg) in anhydrous tetrahydrofuran cooled in an ice-bath, was added to 10 molar excess of diborane (8 cc of 2 M diborane in tetrahydrofuran) dropwise over a 1 hour period. The reaction solution was then allowed to come to room temperature and stirred for an additional 0.5 hour. The solvent and excess diborane were removed under water-pump pressure to give a slightly yellow gum. This residue was dissolved in tetrahydrofuran (50 cc) and aqueous sodium hydroxide (0.5 cc, 3 M) was added followed by hydrogen peroxide solution (0.7 ml, 30%). This solution was stirred for 15 min. at room temperature and reaction was then quenched by partitioning it between dichloromethane (100 cc) and water (100 cc). The aqueous phase was further extracted with dichloromethane (2 x 50 cc) and the combined extracts were taken to dryness to give the crude

amine-borane (634 mg). This material was dissolved in tetrahydrofuran (50 cc), triethylamine (0.7 cc) was added and the solution was refluxed under a nitrogen atmosphere for 2 hours. The solvent was removed and the residue was chromatographed on alumina (200 g). Elution with dichloromethane gave the desired 3α-hydroxy-4β-dihydrocleavamine which crystallized on concentration of the eluent; mp 131-139°C, (342 mg). This material could be sublimed to give an analytical sample; mp 140-156°C; λ_{max}: 293, 286, 277(sh), 229 (log ε 3.87, 3.90, 3.85, 4.54, respectively); $\nu_{max}^{CHCl_3}$: 3440 cm^{-1} (ν N-H), 3250 cm^{-1} (ν O-H); NMR: τ 2.14 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 6.4 (complex multiplet, 2H, $C_3\underline{H}$-OH and C_{18}-H), 8.5 (broad, 1H, disappears on deuterium exchange, O-H), 9.10 (triplet, 3H, $-CH_2-\underline{CH_3}$). Mass spectrum: main peaks, m/e 298, 154, 144, 143.

Anal. Calcd. for $C_{19}H_{26}N_2O$: C, 76.51; H, 8.72; N, 9.40; M.W. 298.205. Found: C, 76.35; H, 8.57; N, 9.25; M.W., 298.203 (high resolution mass spectrometry).

3α-Acetoxy-4β-dihydrocleavamine

3α-Acetoxy-4β-dihydrocleavamine was obtained from 3α-hydroxy-4β-dihydrocleavamine by a normal acetic anhydride-pyridine acetylation. The compound crystallized from methanol-acetone, mp 212-215°C; ν_{max}^{nujol}: 1720 cm^{-1} (ν C=O), 3370 cm^{-1} (ν N-H); NMR: τ 2.08 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, aromatic), 4.90 (doublet of doublets, J = 6, 10 Hz, 1H, $C_3\underline{H}$OAc), 6.4 (complex multiplet, 1H, C_{18}-H), 9.17 (triplet, 3H, $-CH_2\underline{CH_3}$). Mass spectrum: main peaks, m/e 340, 280, 196, 144, 143, 138, 136.

Anal. Calcd. for $C_{21}H_{28}N_2O_2$: 340.215. Found: 340.213 (high resolution mass spectrometry).

Velbanamine (22)

Isovelbanamine (100) (65 mg) was dissolved in an aqueous sulfuric acid solution (10 cc, 10% v/v) and this solution was refluxed under a nitrogen atmosphere for 2 days. The solution was then cooled to 0°C and added dropwise to an ice-cold aqueous ammonium hydoxide solution (20 cc, 5 N). The resulting suspension was extracted using dichloromethane (3 x 25 cc) and the combined organic extract was taken to dryness. The residue was chromatographed on alumina (20 g). Dichloromethane elution gave velbanamine (7.9 mg) in fraction 4 and 5 (15 cc fractions) and isovelbanamine (21 mg) in fractions 7 to 10. The velbanamine crystallized from methanol-water, mp 144-146° (authentic velbanamine[71], mp 117-134°, recrystallized from methanol-water, mp 143-146°). The synthetic velbanamine exhibited identical tlc properties and had an ir spectrum (nujol) superimposable to that of the authentic sample.

18β-Carbomethoxycleavamine (60)

To a 1 litre, three-necked round bottomed flask, fitted with a reflux condenser and mechanical stirrer, was added glacial acetic acid (300 cc). The acid was heated to 100°C and then catharanthine hydrochloride (11.0 g) with a further portion of acetic acid (100 cc) was added with rapid stirring. Immediately a portion of sodium borohydride was added; 42 g were added over about a 1 hour period at

a rate which maintained the temperature at 90-105°C, taking caution to keep the rate of hydrogen evolution under control. After the addition was complete, the reaction mixture was cooled in an ice-bath and the resulting viscous mass was poured into aqueous ammonium hydroxide solution (500 cc, 9 N). The resulting suspension was extracted with dichloromethane (3 x 400 cc) and the combined organic extracted after drying over anhydrous sodium sulfate, was taken to dryness to give a white foam (10.85 g). Crystallisation from methanol gave pure 18β-carbomethoxycleavamine, first crop 4.4 g, mp 121-123°C (authentic sample[37] mp 122-123°C).

18β-Carbomethoxy-4β-dihydrocleavamine (32)

18β-Carbomethoxycleavamine (4.4 g) was hydrogenated at room temperature and atmospheric pressure over Adam's catalyst (350 mg) in ethylacetate (50 cc). Hydrogen uptake ceased after 2 hours. The catalyst was filtered off and the solvent removed to give a white foam. This material crystallized from methanol to give pure 4β-dihydrocleavamine, first crop 4.1 g, mp 143-145°C (authentic sample[37] mp 146-148°C).

4β-Dihydrocleavamine (29)

A solution of 18β-carbomethoxy-4β-dihydrocleavamine (3.0 g) in aqueous hydrochloric acid (190 cc, 5 N) was heated to 90°C under a nitrogen atmosphere and the temperature was maintained for 7 hours. The solution was cooled in an ice-water bath and made just basic by

the addition of ammonium hydroxide (15 N). The suspension was extracted with dichloromethane (3 x 125 cc), the organic extracts dried over anhydrous sodium sulfate and the solvent removed. The residue crystallized from methanol to give 4β-dihydrocleavamine (2.4 g, mp 134-138°) (authentic sample[37] mp 136-138°C).

Chloroindolenine of 4β-dihydrocleavamine (41)

A solution of tert-butyl hypochlorite in carbon tetrachloride (7.1 cc, 0.050 M) was added over a 0.5 hour period to a solution of 4β-dihydrocleavamine (100 mg) in dichloromethane (13.3 cc) and triethylamine (0.07 cc) which was cooled in an ice-acetone bath. After the addition was complete, the solution was stirred for an additional 15 min. at the bath temperature. The orange-coloured solution was then diluted with an equal volume of benzene and rapidly percolated through a column of alumina (1.5 g). Solvent was removed under reduced pressure, the last traces under high vacuum, to provide the chloroindolenine as a pale yellow oil (101 mg); $\lambda_{max}^{isooctane}$: 227, 260, 303 (log ε 4.31, 3.55, 3.42 respectively); $\nu_{max}^{CHCl_3}$: 2776 cm^{-1} (Bohlman bands), 1600 and 1560 cm^{-1} (indolenine C=N); NMR: τ 2.5-3.0 (diffuse, 4H, aromatic), 8.79 (quartet, 2H, $-\underline{CH_2}CH_3$), 9.14 (triplet, 3H, $-CH_2\underline{CH_3}$); Mass spectrum: main peaks, m/e 316, 281, 138, 124.

Anal. Calcd. for $C_{19}H_{25}N_2Cl$: 316.171. Found: 316.172 (high resolution mass spectrometry).

Dimer (118), from chloroindolenine of 4β-dihydrocleavamine (41) plus vindoline (11)

The chloroindolenine of 4β-dihydrocleavamine (557 mg) and vindoline

(523 mg) were dissolved in anhydrous methanolic 1.5% hydrochloric acid solution. This solution was refluxed for 2.5 hours under a dry nitrogen atmosphere. The reaction mixture was diluted with water (87 cc) and the resulting solution was made just basic with potassium carbonate. Extraction with dichloromethane (5 x 50 cc) and removal of the solvent after drying the extracts over anydrous sodium sulfate gave the crude dimer as a yellow glass-like material (1.006 g). Chromatography on alumina (100 g) gave the pure dimer (118) on elution with benzene-ethylether (1:1) as a colourless glass (656 mg). Crystallisation from methanol gave a sample, mp 205-206°; $\nu_{max}^{CHCl_3}$: 3430 cm^{-1} (ν N-H), 1733 cm^{-1} (ν C=O for -OAc, $-CO_2Me$), 1630 cm^{-1} (ν C=C for vindoline); λ_{max}: 214, 257, 287, 293, 310(sh), (log ε 4.63, 4.18, 4.06, 4.07, 3.88 respectively); NMR: τ 0.38 (broad singlet, 1H, O-H), 2.13 (broad singlet, 1H, indole N-H), 2.5-3.0 (diffuse, 4H, indole aromatic), 3.32 (singlet, 1H, indoline, C_{14}-H), 3.92 (singlet, 1H, indoline C_{17}-H), 4.16 (broad doublet of doublets, 1H, C_6=C_7HR), 4.66 (singlet, 1H, C_4HOAc), 4.76 (broad doublet, 1H, C_7=C_6HR), 5.57 (broad doublet, 1H, C'_{18}-H), 6.14 (singlet, 3H, C_{16}-OCH_3), 6.28 (singlet, 3H, $-\overset{O}{\overset{\|}{C}}OCH_3$), 6.36 (singlet, 1H, C_2-H), 7.35 (singlet, 3H, N-CH_3), 7.97 (singlet, 3H, OAc), 9.17 (triplet, 3H, $C'_4CH_2CH_3$), 9.93 (triplet, 3H, $C_5CH_2CH_3$). Mass spectrum: main peaks, m/e 58, 60, 74, 91, 92, 106, 107, 121, 122, 135, 138, 149.

Anal. Calcd. for $C_{44}H_{56}O_6N_4$: 736.420. Found: 736.420. Calcd. for M^+ + 1H, ($C_{44}H_{57}O_6N_4$); 737.428. Found: 737.425. Calcd. for M^+ - 1H ($C_{44}H_{55}O_6N_4$); 735.412. Found: 735.408.

Cleavage of dimer (118)

The dimer (118) as the hydrochloride (30 mg) was dissolved in anhydrous methanolic 7% hydrochloric acid solution (5 cc). To this solution was added tin (50 mg) and stannous chloride (50 mg) and the reaction mixture was refluxed for 2 hours under a nitrogen atmosphere. After this time, acetylchloride (1 cc) and an additional amount of tin (50 mg) was added and the mixture refluxed for another 1 hour. This solution was then made basic with ammonium hydroxide solution and extracted with dichloromethane (2 x 25 cc). The crude reaction product obtained on taking the organic extract to dryness was separated into its components using preparative alumina tlc eluting with ethyl-acetate-chloroform (1:1). The products isolated were 4β-dihydrocleavamine (5.9 mg), vindoline (2.3 mg), starting dimer (3.4 mg) and desacetylvindoline (10.2 mg). Each of these materials were identified by tlc and ir comparison with samples of authentic material.

Chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (120)

To a solution of 18β-carboamethoxy-4β-dihydrocleavamine (400 mg) in dichloromethane (40 cc) and triethylamine (0.2 cc) cooled in an ice-water bath, was added a solution of tert-butyl hypochlorite in carbontetrachloride (25 cc, 0.05 M) over a period of 45 min. The solution was washed with ice-water (2 x 30 cc), dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give the chloroindolenine as an amorphous solid (440 mg); $\lambda_{max}^{dioxane}$: 292, 275, 227 (log ε 3.44, 3.44, 4.30 respectively); $\nu_{max}^{CHCl_3}$: 2775 cm^{-1} (Bohlmann bonds), 1727 cm^{-1} (ν C=O), 1612 and 1575 cm^{-1} (indolenine ν

C=N); NMR: τ 2.40-2.98 (4H, aromatic), 5.53 (doublet, 1H, C_{18}-H), 6.41 (singlet, 3H, -COOC<u>H</u>$_3$), 9.14 (triplet, 3H, -CH_2C<u>H</u>$_3$). Mass spectrum: main peaks, m/e 376, 374, 138, 124.

<u>Anal</u>. Calcd. for $C_{21}H_{27}N_2O_2Cl$: 374.176. Found: 374.174, (high resolution mass spectrometry).

<u>Dimer (119)</u>

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (400 mg) and vindoline (336 mg) were dissolved in anhydrous methanolic 1.5% hydrochloric acid solution and the resulting solution was refluxed under a nitrogen atmosphere for 3 hours. The solvent was removed under reduced pressure and the residue was partitioned between dichloromethane (100 cc) and aqueous potassium bicarbonate solution (100 cc, 1%). The aqueous phase was extracted with a further amount of dichloromethane (2 x 30 cc) and the combined extracts were dried over anhydrous sodium sulfate. The solvent was removed to give a yellow glass-like residue (823 mg). This material was chromatographed on alumina (100 g); benzene-diethylether (1:1) elution gave the desired dimer as a colourless glass. Crystallization from methanol gave a sample, mp 221-225°C; λ_{max}: 217, 265, 287, 296, 313(sh) (log ε 4.48, 3.93, 3.92, 3.92, 3.78 respectively); $\nu_{max}^{CHCl_3}$: 3430 cm^{-1} (ν N-H), 1730 cm^{-1} (ν C=O), 1630 cm^{-1} (ν C=C); NMR: τ 0.49 (broad singlet, 1H, OH), 1.00 (broad singlet, 1H, N-H), 2.5-3.0 (diffuse, 4H, indole aromatic), 3.05 (singlet, 1H, C_{14}-H), 4.04 (singlet, 1H, C_{17}-H), 4.12 (doublet of doublets, 1H, C_6=C_7HR), 4.67 (singlet, 1H, C_4-H),. 4.72 (broad doublet, 1H, C_7=C_6<u>H</u>), 6.16 (singlet, 3H, -O<u>Me</u>), 6.29 (singlet, 6H, two-COO<u>Me</u>), 6.36 (singlet, 1H, C_2-H), 7.40 (singlet, 3H,

N-Me), 7.96 (singlet, 3H, -OAc), 9.09 (triplet, 3H, C_4'-$CH_2\underline{CH_3}$), 9.34 (triplet, 3H, $C_5CH_2\underline{CH_3}$). Mass spectrum: main peaks, m/e 58, 74, 91, 106, 107, 121, 122, 135, 138.

Anal. Calcd. for M^+, $C_{46}H_{58}O_8N_4$: 794.425. Found: 794.419.

Calcd. for $M^+ + H$, $C_{46}H_{59}O_8N_4$: 795.433. Found: 795.428.

Calcd. for $M^+ + 2H$, $C_{46}H_{60}O_8N_4$: 796.441. Found: 796.437.

Calcd. for $M^+ - 1H$, $C_{46}H_{57}O_8N_4$: 793.417. Found: 793.410.

Calcd. for $M^+ - 2H$, $C_{46}H_{56}O_8N_4$: 792.409. Found: 792.403

(high resolution mass spectrometry).

Cleavage of dimer (119)

The dimer (119) as the hydrochloride (50 mg) was dissolved in anhydrous methanolic 7% hydrochloric acid solution (5 cc), and tin (100 mg) and stannous chloride (100 mg) were added. This mixture was refluxed for 1 hour under a nitrogen atmosphere, and was then basified using ammonium hydroxide solution. To this suspension was added dichloromethane (50 cc) and water (30 cc) and the emulsion formed on shaking was filtered under reduced pressure. The aqueous phase was extracted with a further amount of dichloromethane and combined organic extracts were dried over anhydrous sodium sulfate and taken to dryness. The crude product (53 mg) was separated into its components by preparative alumina tlc using ethylacetate-chloroform (1:1) as eluting solvent. The products obtained were vindoline (12.6 mg), desacetylvindoline (3.2 mg), 18α-carbomethoxydihydrocleavamine (8.1 mg) and 18β-carbomethoxydihydrocleavamine (8.0 mg). The identity of these products was established by a comparison with authentic materials on

tlc; superimposable ir spectra were obtained for all except 18α-carbomethoxy-4β-dihydrocleavamine. The identity of this compound was established by nmr comparison with an authentic sample.

18-Methylene-4β-dihydrocleavamine (79) from 18β-hydroxymethyl-4β-dihydrocleavamine (92)

To a solution of 18β-hydroxymethyl-4β-dihydrocleavamine (200 mg) in dry pyridine (20 cc) was added freshly prepared 3,5-dinitrobenzoyl chloride (110 mg). The resulting red solution was stirred at room temperature for 2 days. Dichloromethane (100 cc) was then added to the reaction mixture and this solution was then washed briefly with aqueous sodium carbonate solution (100 cc, 3%), followed by water (100 cc). The organic phase was dried over anhydrous sodium sulfate and stripped of solvent to give a red gum. Chromatography on alumina (30 g) gave 18-methylene-4β-dihydrocleavamine (43 mg) with benzene as eluting solvent. Elution with benzene-10% diethylether gave starting 18β-hydroxymethyl-4β-dihydrocleavamine (86 mg). The 18-methylene-4β-dihydrocleavamine was identical in all respects with a sample obtained from the fragmentation reaction of dihydrocatharanthinol-O-tosylate.

18-Hydroxymethyl-4β-dihydrocleavamine benzoate (138)

18-Hydromethyl-4β-dihydrocleavamine (200 mg) was dissolved in triethylamine (15 cc) and benzoyl chloride (0.11 cc) was added. This solution was stirred at room temperature for 12 hours. After this time, the triethylamine was removed under reduced pressure and the residue was dissolved in dichloromethane (50 cc). This solution was

washed with aqueous sodium bicarbonate (50 cc, 3%) followed by water (50 cc) and the organic phase was then dried over anhydrous sodium sulfate and stripped of solvent. The residue was chromatographed on alumina (20 g) using petroleum ether-benzene (1:1) as eluant to give 18β-hydromethyl-4β-dihydrocleavamine benzoate (184 mg); λ_{max}: 227, 283, 293, 305(sh), 315(sh), 335(sh); $\nu_{max}^{CHCl_3}$: 1720 cm^{-1} (ν C=O); NMR: τ 1.32 (broad singlet, 1H, N-H), 1.7-3.0 (complex, 9H, indole and benzoate aromatic), 5.35 (broad singlet, 2H, -CH_2OCOR), 5.55 (multiplet, 1H, C_{18}-H).

Dimer (142)

The diol (99) (193 mg) and vindoline (226 mg) were dissolved in an anhydrous methanolic hydrochloric acid solution (18 cc, 1%) and this solution was refluxed under a nitrogen atmosphere for 4 hours. The reaction was then poured into ice-cold aqueous ammonium hydroxide (20 cc, 2N) and the resulting suspension was extracted with dichloromethane (3 x 30 cc). The combined organic extracts were taken to dryness and the residue was chromatographed on alumina (100 g). The dimer (142)(197 mg) was eluted with dichloromethane-1% methanol. Further elution with this solvent gave some desacetyl dimer (12 mg). The dimer (142) crystallized from diethyl-ether; mp 205-215 (dec); λ_{max}: 213, 257, 286, 293, 312(sh) (log ε 4.61, 4.10, 3.95, 3.97, 3.82 respectively); ν_{max}^{nujol}: 3400 cm^{-1} (ν N-H, O-H), 1740 cm^{-1} (ν C=O); NMR: τ 0.47 (broad singlet, 1H, O-H), 2.24 (broad singlet, 1H, N-H), 2.5-3.1 (diffuse, 4H, indole aromatic), 3.30 (singlet, 1H, C_{14}-H), 3.97 (singlet, 1H, C_{17}-H), 4.18 (doublet of doublets, 1H, C_6=C_7-HR), 4.68 (singlet, 1H, C_4-H), 4.7 (broad doublet, 1H, C_7=C_6-HR), 5.56 (broad

doublet, 1H, C'_{18}-H), 6.19 (singlet, 3H, -OMe), 6.27 (singlet, 3H, $-CO_2Me$), 6.36 (singlet, 1H, C_2-H), 7.39 (singlet, 3H, N-Me), 7.99 (singlet, 3H, OAc), 9.24 (triplet, 3H, $C'_4-CH_2\underline{CH_3}$), 9.49 (triplet, 3H, $C_5CH_2\underline{CH_3}$). Mass spectrum: main peaks, m/e 50, 51, 57, 69, 77, 78, 88, 101, 128, 154, 165, 204.

Anal. Calcd. for $C_{44}H_{56}O_7N_4$: 752.415. Found: 752.414 (high resolution mass spectroscopy).

Cleavage of dimer (142)

The dimer (142) (50 mg) was dissolved in an anhydrous methanolic hydrochloric acid solution (5 cc, 6%) and tin (50 mg) and stannous chloride (50 mg) were added. This mixture was refluxed under a nitrogen atmosphere for 3 hours. After this time it was basified by the addition of ammonium hydroxide solution and extracted with dichloromethane (3 x 15 cc). The residue obtained on taking the organic extracts to dryness was chromatographed on alumina (5 g). Elution with dichloromethane gave first vindoline (4.0 mg), followed by isovelbanamine (3.9 mg). Changing to the dichloromethane-1% methanol as eluent, gave desacetylvindoline (3.6 mg), starting dimer (18.0 mg) and desacetyl dimer (8.3 mg). The methanol flush gave a mixture of unidentified polar products (6.3 mg). The above materials were identified by comparison on tlc and ir with authentic samples.

18β-Hydroxy-4β-dihydrocleavamine (132)

4β-Dihydrocleavamine (88 mg) was converted to the corresponding chloroindolenine as previously described. This material was dissolved

in glacial acetic acid (2 cc) and after dissolution, water (1 cc) was added. This solution was stirred at room temperature for 24 hours. After this time it was poured into aqueous ammonium hydroxide solution (10 cc, 5 N) and the resulting suspension was extracted with dichloromethane (3 x 10 cc). The combined extract was taken to dryness and the residue was chromatographed on alumina (20 g). Dichloromethane elution gave first some starting chloroindolenine (20 mg) and further elution gave the desired 18β-hydroxy-4β-dihydrocleavamine (27 mg). This material crystallized from methanol-water, mp 203-205°C. Comparison with an authentic sample, mp 202-205°, showed identical tlc properties and superimposable ir spectra.

Dimer (118), from 18β-hydroxy-4β-dihydrocleavamine (132) plus vindoline

18β-Hydroxy-4β-dihydrocleavamine (132) (106 mg) and vindoline (157 mg) were dissolved in an anhydrous methanolic hydrochloric acid solution (1%, 10 cc) and the solution was refluxed under a nitrogen atmosphere for 4 hours. The reaction mixture was then poured into ice-cold aqueous ammonium hydroxide solution (10 cc, 5 N) and the resulting suspension was extracted with dichloromethane (3 x 30 cc). The combined organic extract was taken to dryness and the residue was chromatographed on alumina (100 g). Dichloromethane elution gave vindoline (10 mg) in the early fraction and further elution gave the dimer (118) (173 mg). Dichloromethane-1% methanol elution gave desacetylvindoline (3 mg) which was followed by desacetyl dimer (15 mg). The major dimeric product, dimer (118), had identical tlc properties and gave the same nmr spectrum as that obtained for the product from

the dimerisation of the chloroindolenine of 4β-dihydrocleavamine (41) with vindoline.

18β-Cyano-4β-dihydrocleavamine (42)

4β-Dihydrocleavamine (1.00 g) was converted to the corresponding chloroindolenine by the procedure already described. This material was dissolved in a solution of fused sodium acetate (10 g) in glacial acetic acid (80 cc) and acetic anhydride (20 cc). This solution was warmed to 60°C for 2 hours. The solvent was removed under reduced pressure; high vacuum was used for the last traces. The residue was flushed through a short column of alumina (20 g as a 2" column) using ethanol. This solvent was removed and the residue was dried under high vacuum. Potassium cyanide (500 mg) was added and into the reaction vessel was distilled dimethylformamide from over barium oxide. This mixture was refluxed for 1 2/3 hour. The solvent was removed under reduced pressure and the dichloromethane soluble material was chromatographed on alumina (200 g). Benzene elution gave the desired 18β-cyano-4β-dihydrocleavamine (42) (224 mg). This material crystallized from methanol, mp 150-152° and showed identical tlc properties and superimposable ir and nmr spectras compared with an authentic sample, mp 150-152°.

Dimer (146), from 18β-cyano-4β-dihydrocleavamine

18β-Cyano-4β-dihydrocleavamine (147 mg) was dissolved in a solution of dichloromethane (17 cc) and triethylamine (0.079 cc) and this solution was cooled to ice-acetone bath temperature. A solution

of tert-butyl hypochlorite in carbon tetrachloride (12 cc, 0.042 M) was added dropwise to the cooled solution, over a 0.5 hour period. The solvent was removed under reduced pressure and an aliquot of benzene (20 cc) was added to the residue and distilled off in order to azeotrope off any water which might be present. To this crude chloroindolenine was added vindoline (217 mg) and this mixture was dissolved in an anhydrous methanolic hydrochloric acid solution (15 cc, 1%). The solution was refluxed under a nitrogen atmosphere for 2 hours. After this time, the solvent was removed, the residue was taken up in water (50 cc) and made slightly basic. This mixture was extracted with dichloromethane (3 x 30 cc) and the combined extracts taken to dryness. The residue was chromatographed on alumina (100 g). Benzene-5% ethyl acetate elution gave unconsumed vindoline (119 mg) and benzene-10% ethylacetate elution gave the dimer (146) (22 mg). This material crystallized from diethyl-ether, mp 204-224°C; λ_{max}: 215, 265, 285, 294, 312(sh) (log ε 4.68, 4.19, 4.02, 4.01, 3.78, respectively); ν_{max}^{nujol}: 3320 cm^{-1} (ν N-H, O-H), 1740 cm^{-1} (ν C=O); NMR: τ 0.7 (very broad, 1H, O-H), 1.90 (broad, 1H, N-H), 2.5-3.0 (diffuse, 4H, indole aromatic), 3.79 (singlet, 1H, C_{14}-H), 3.94 (singlet, 1H, C_{17}-H), 4.20 (doublet of doublets, 1H, C_6=C_7H<u>R</u>), 4.70 (singlet, 1H, C_4-H), 4.80 (broad doublet, 1H, C_7=C_6H<u>R</u>), 6.12 (singlet, 3H, -OCH_3), 6.29 (singlet, 3H, -CO_2<u>CH_3</u>), 6.36 (singlet, 1H, C_2-H), 7.35 (singlet, 3H, N-CH_3), 7.99 (singlet, 3H, -OAc), 9.11 (triplet, 3H, C_4'-CH_2<u>CH_3</u>), 9.45 (triplet, 3H, C_5-CH_2<u>CH_3</u>). Mass spectrum: main peaks, m/e 50, 51, 55, 57, 67, 69, 77, 78, 79, 107, 122, 124, 128, 135, 138.

<u>Anal</u>. Calcd. for $C_{45}H_{55}O_6N_5$: 761.415. Found: 761.413 (high resolution mass spectrometry).

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