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STUDIES RELATED TO THE SYNTHESIS OF BISINDOLE ALKALOIDS OF THE INDOLE-INDOLINE TYPE

BY

ADI MINOO TREASURYWALA

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ABSTRACT

The first part of this thesis describes the synthesis of 3,4-functionalized cleavamine templates bearing a C_{18}-carbomethoxy group. Thus hydroboration of 18β-carbomethoxycleavamine (29) produced two epimeric alcohols; 18α- and 18β-carbomethoxydihydrocleavamin-3-ol (56 and 57). These compounds could be interconverted by using boron trifluoride etherate in benzene. One of these compounds (56) could be oxidized to the corresponding C_3 ketone which is a key intermediate for future work.

The second part describes the research in the area of the so-called dimerization reaction. The generality of a procedure which had been used before was tested. When the chloroindolenine of 4β-dihydrocleavamine and 18-carbomethoxy-4β-dihydrocleavamine were each treated with vindoline in 1.5% methanolic hydrogen chloride, good yields of dimeric products were obtained. These materials have been shown by X-ray to be epimeric at $C_{18'}$ to the natural dimers vincristine (VCR) and vinblastine (VLB). When these conditions were applied to the chloroindolenines of 18β-carbomethoxycleavamine and the 18α- and 18β-carbomethoxycleavaminols (56 and 57), good yields of dimers did not result. A detailed study, which has illuminated the mechanism of this reaction, was thus undertaken. As a result of this study, an improved procedure for the dimerization of such sensitive cleavamine templates was discovered. The insight gained from this study has permitted changes in the reaction conditions which have resulted in the isolation of two dimeric products from a single dimerization reaction. Previously, only one dimer had resulted from such reactions stereoselectively.

Several new and exciting other approaches to the coupling of the indole and dihydroindole portions have been explored. Some of these have uncovered novel and useful avenues for eventually achieving the synthesis of the natural dimers such as VLB, VCR leurosine and leurosidine.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

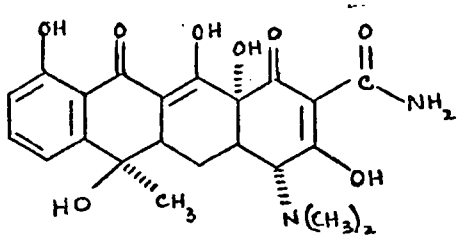
Throughout the history of man, he has been inquisitive about his surroundings. In most cases, this curiousity has led to some form of "progress". Perhaps the oldest preoccupation has dealt with the examination of the chemistry of natural products. The ancient Egyptian discoveries of dyestuffs from plant sources were, surely, one of the earliest fruits of primitive natural products chemistry. Tribal medicine represents another form of these early investigations, and has led to an accumulation of observations about the pharmacological effects of various natural products.

In recent times, one of the main goals of the so-called natural products chemist is the discovery of compounds that are of some interest to man either in the curing of ailments or the control of pests or in some other area. Tribal folk remedies are often starting points of such searches. However, today crude plant preparations have been largely replaced by pure compounds (or a set of pure compounds) to which all, or most, of the activity of the old remedy may be ascribed. Once such a pure active ingredient has been isolated, its structure must be proved before its action may be fully understood. This is often an exacting task requiring detailed spectral studies of the compound and several of its derivatives using, for example, infrared,

ultraviolet, and nuclear magnetic resonance spectroscopy. A tool that has provided great impetus in this area in very recent times is X-ray crystallography.

Once a structure has been unambiguously assigned, a synthesis of the compound may be undertaken. The latter operation may be essential for several reasons. In a case where a clinically useful compound is present in only a minute amount in nature, a synthesis from readily available starting materials may compete favourably as a source of the drug. Usually a synthetic scheme is designed so that a whole family of structurally analogous drugs may be synthesized. In this case it is possible that a derivative, unavailable from the natural source, may have higher activity, with relatively fewer side effects, than the parent compound. Finally, the compound may be of sufficient structural and stereochemical uniqueness to provide an academic challenge to the synthetic chemist.

Amongst the various classes of compounds isolated from nature that are of some pharmacological interest to man, compounds which contain nitrogen atoms as part of the molecule appear to predominate. These basic, nitrogen containing compounds are classified as alkaloids. As a class of compounds, they differ widely in structure as well as pharmacological activity. A few examples are such well-known compounds as tetracycline (1), quinine (2), ephedrine (3), nicotine (4), strychnine (5), and lysergic acid (6).



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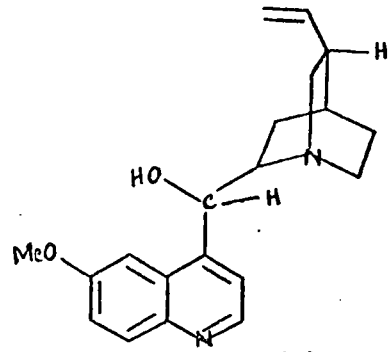

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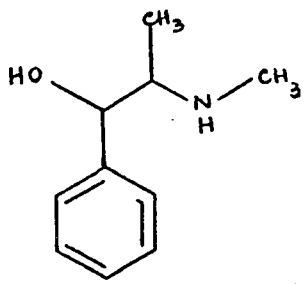

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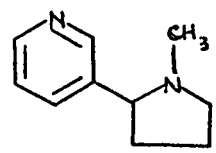

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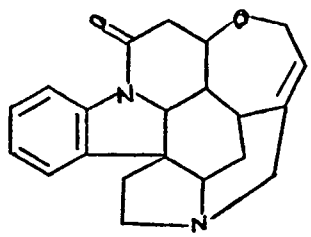

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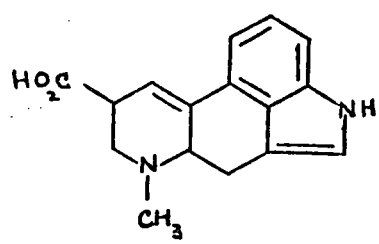

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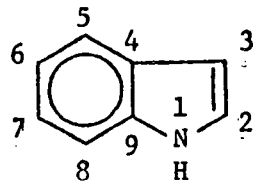
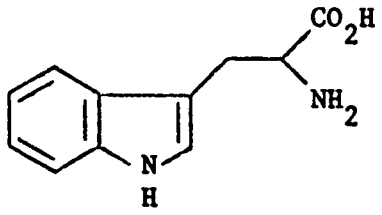
The last two compounds are part of a family of alkaloids which have in common the indole nucleus (7).



(7)

These indole alkaloids are postulated to arise from the amino-acid tryptophan (8) and a "non-tryptophan" unit which in the case of some recent studies, turns out to be a monoterpenoid compound; loganin (9) or its derivatives. The alkaloids which have these two structural units



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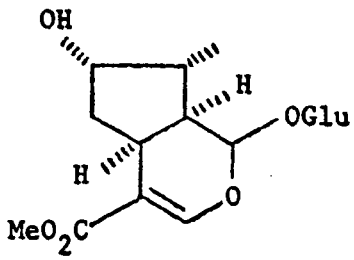
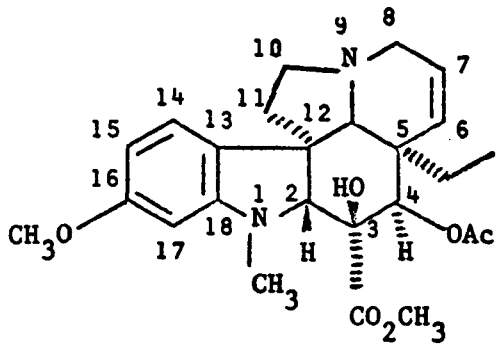


(9)

incorporated into them are C_{20} or C_{21} compounds most of which may be classified as belonging to the Corynanthe, Strychnos, Aspidosperma or Iboga families. Examples of the latter three are strychnine (5), vindoline (10) and catharanthine (11), respectively.



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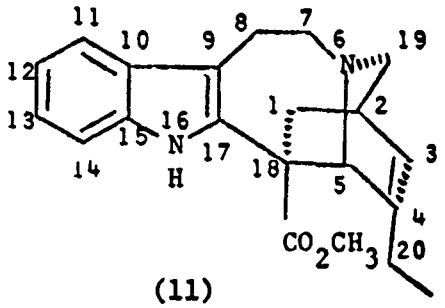

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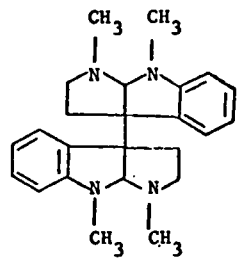
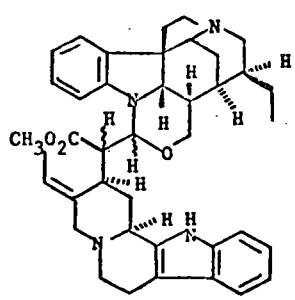
One of the newer groups of indole alkaloids is the bisindole alkaloid family, or the "dimeric" indole alkaloids, as they are sometimes called. More than eighty such compounds are presently known. Structural elucidations of most of these, however, awaited the development of efficient separation techniques and as a result[1] date back only some twelve years. These bisindole alkaloids may easily be

divided into two categories. The first is composed of alkaloids with identical or very closely related components in which the same centres act as linkage points. This group contains the Calycanthaceous and Calabash-curare alkaloids. An example of this family is folicanthine (12).



(12)

The second group of bisindoles consists of those where the alkaloid components are of different structural types, or in which two similar types are linked through different centres. Examples of these are geisospermine (13) and villalstonine (14).



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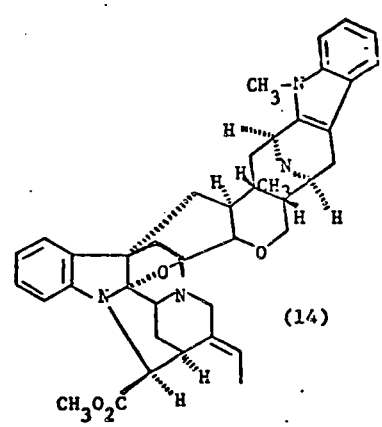
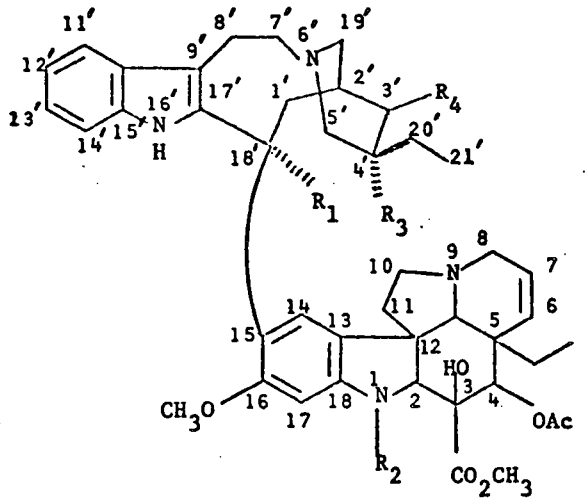


(14)

Recently another such type of bisindole alkaloid has been discovered. This is the so-called "vinblastine" type. The most interesting members of this family are vinblastine (vincaleukoblastine,

VLB) (15), vincristine (leurocristine, VCR) (16), leurosidine (17), and leurosine (18). These four compounds, amongst others, have been



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

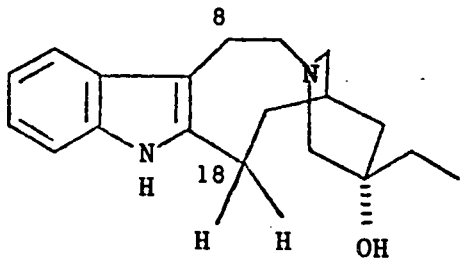
isolated in minute amounts from the common periwinkle plant - Vinca rosea Linn or Catharanthus roseus G. Don - and show marked anti-cancer activity.

The story of the isolation and characterization of these compounds, particularly vinblastine (VLB) and vincristine (VCR) dates back to an observation by W.R.N. Drew et al.[2] in 1929 that the administration of Vinca rosea leaf extracts to rabbits lowered their blood sugar content. Further attempts to study this effect later by two independent groups[3,4] met with varied success. In 1955 Noble and Beer,[5] in an attempt to study this reported hypoglycemic activity discovered

that the plant extracts exhibited marked anti-neoplastic effects. This observation immediately catalyzed a great deal of interest in the constituents of this plant and in 1958 the isolation of the first bisindole alkaloid of the vinblastine type from V. rosea was reported. The compound was leurosidine (17).[6] There followed a rapid succession of reports about the isolation of other members of this category and their clinical activity.

The elucidation of the structures of VLB and VCR was quite stimulating and much was learned about the chemistry of these "dimeric" compounds at the same time. The alkaloids were each found to possess two individual chromophores; an indole and a 6-methoxyindoline in the case of VLB; and an indole and an N-formyl-6-methoxyindoline in the case of VCR. An important landmark in the elucidation of the structure of VLB[7] was the discovery that the infrared spectrum of this alkaloid closely resembled that of an equimolar mixture of vindoline (10) and catharanthine (11); two co-occurring monomeric alkaloids. The structures of these two compounds were, at that time, largely unknown but they were quickly determined by chemical correlations to known compounds and from a study of their mass spectral fragmentation patterns.[8,9] Reduction of VLB and VCR with lithium aluminum hydride gave the same pentahydroxy derivative thus correlating the two alkaloids with each other.[10] It was possible to elucidate the constitution shown (15) for VLB on the basis of chemical and spectroscopic data alone.[9] The relative and absolute configuration had to be determined, however, from an X-ray analysis of VCR monomethiodide.[11,12] Owing to the earlier determination of an incorrect molecular formula (2 hydrogens less) for

VLB and VCR it had been assumed that the indole portion of these "dimers" represents a dihydro-dihydroxy-catharanthine.[10] Establishment of the correct molecular formula, however, made this postulate untenable.[13] Reductive cleavage (in concentrated hydrochloric acid containing stannous chloride and tin) of the "dimers" VLB and VCR led to one and the same indolic base, velbanamine (19), and a different indoline in each case: deacetylvindoline from VLB and des-N_a-methyldeacetylvindoline from VCR. The constitution of 19 available by this reductive acid cleavage of VLB; and in particular, the location of the hydroxyl group was assigned on the basis of the mass spectral fragmentation peak at m/e = 154 which could be shown to arise from the fragment (20) below. The complete structure of velbanamine was



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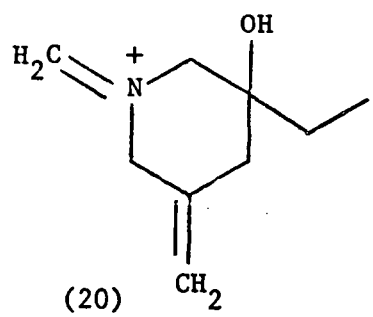
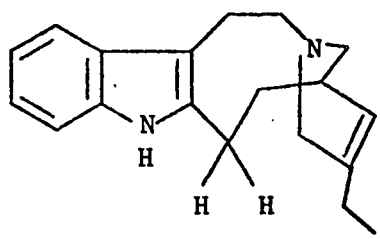


(20)

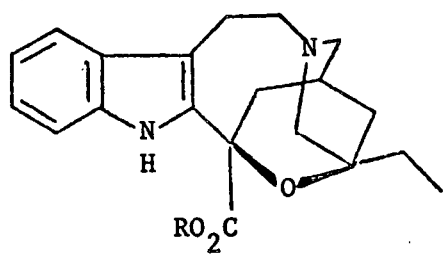
established by correlating it with the compound cleavamine (21) whose structure had been independently proved by X-ray analysis of the methiodide.[14,15]



(21)

Reductive cleavage of VLB in a deuterating medium led to [$^2H_6$]-velbanamine in which four deuterium atoms were attached to the aromatic ring and the remaining two to the C_{18} position or else one each to the C_8 and C_{18} positions.

Acid catalyzed cleavage in the absence of reducing agents led to the amino acid (22) which could be converted to the ester (23) which, in turn, upon treatment with stannous chloride in DCl-D_2O again gave [$^2H_6$]-velbanamine which was identical in every respect to that obtained previously. This excluded C_8 as a possible point of deuteration during cleavage and therefore as a possible site of linkage of the two monomers. This left C_{18} as the only choice for such a linkage.[13]



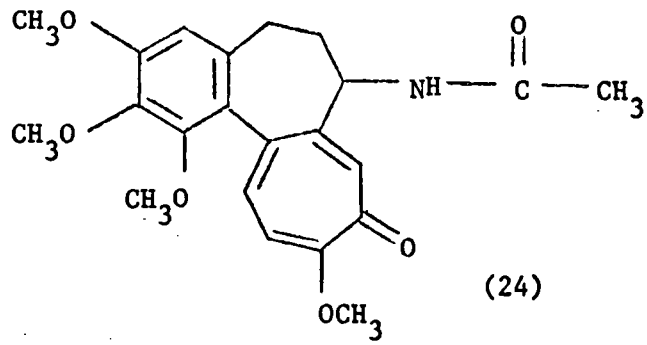
22; R = H
23; R = CH_3

While the structures of these new "dimeric" compounds were being pursued, research was also simultaneously under way to investigate their interesting biological activity.

After Beer and Noble were able to demonstrate that vinblastine was capable of producing severe leukopenia in rats,[5,16-18] a team from the Eli Lilly Laboratories began exploring the possibility that the periwinkles contained other interesting alkaloids of the VLB type. A systematic search resulted in the isolation of a total of seventy-two

alkaloids from this source by a variety of methods. Of these twenty-four were shown to have "dimeric" structures. Only four of this latter group, however, were of any clinical interest in the area of cancer control (structures 15-18 above). A great deal of effort was expended in developing procedures for isolating these compounds [19-34] and detecting them in minute amounts.[35-39]

The mode of action of these drugs is largely unknown even today despite considerable effort by several groups to learn something about it. On the basis of several observations made during these researches, a working hypothesis for their mode of action has arisen and is widely accepted although not yet conclusively established.[40,41] It appears that these compounds may be classified with other structurally diverse drugs such as colchicine (24) as "mitotic poisons".



(24)

The primary effect of these compounds seems to be the inhibition of t-RNA synthesis thus causing in turn a decrease in the protein synthesis. The alkaloids enter the cell only between prophase and metaphase and thus intracellular protein synthesis is blocked at the very time when it is most needed to spread the fibres of the spindle apparatus apart and support them in a fanned out position. Spindle fibres in cells affected by these alkaloids appear tangled and

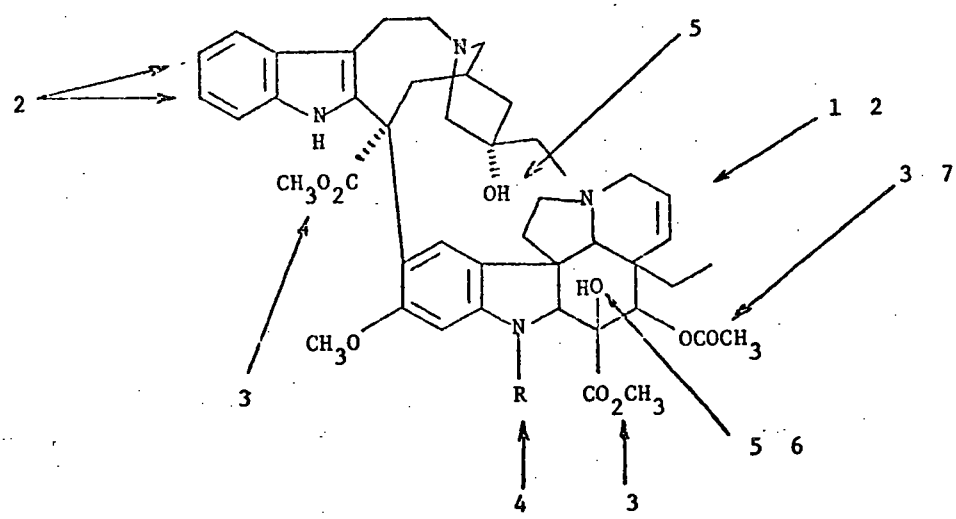
disarrayed. The migration of the chromosomes under such conditions is blocked and inhibition of DNA synthesis as a result of this metaphase arrest is observed.

The exact metabolic path of these drugs in vivo is not known and some attempts to elucidate this path by the administration of radioactively labelled alkaloids to laboratory animals has been undertaken in the past.[42-47] In this area, several important questions remain to be answered. For example, the possibility that it may be a metabolite of the drug which exerts the antitumor effect, has not yet been excluded. Much research is currently in progress to answer such questions.

Some studies have been possible by chemical modifications of VLB itself to yield new drugs. Some of these were shown to have an even better therapeutic index than the parent compound. For example, W.W. Hargrove[48] observed that in initial tests deacetyl VLB was less active and more toxic than VLB. This prompted some speculation that the acetyl group in VLB was important for its activity. This fact, coupled with the observation that deacetyl VLB was relatively easily available, by the hydrolytic action of anhydrous methanol saturated at 0°C with hydrogen chloride, led him to study a series of compounds made by attaching other acyl groups to deacetyl VLB. Chloroacetyl VLB so produced, for example, caused a 127% prolongation of life in mice infected with P-1534 leukemia as opposed to a 25-30% prolongation under similar conditions by the parent compound (VLB) itself. This was indeed a significant finding and several other acyl substituents were examined.[49-53] As is obvious from the number of patents arising out

of this work, the technique was thoroughly exploited for the development of new and potentially more useful drugs than were directly available from nature.

Some other structural modifications were attempted and these are summarized in Figure 1 below.[54] A detailed and systematic study, of the relationship between the structure and activity of these alkaloids must await a general synthetic entry into this skeletal system.



	Reaction	Derivative	Activity Relative to VLB	Toxicity Relative to VLB
1.	Hydrogenation	Dihydro-VLB	1/3	less
2.	Hydrogenation	Hexahydro-VLB	none	none
3.	$LiAlH_4$ Reduction	VLB-carbinol	"	"
4.	Acid Hydrolysis	Desformyl-VCR	"	"
5.	Acetylation (Ketene)	Triacetoxy-VLB	"	"
6.	Acetylation (Ac_2O)	Diacetoxy-VLB	"	"

Figure 1. Structural modifications vs. activity.

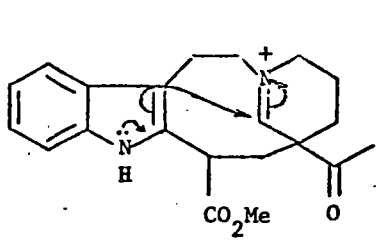
Changes other than in the functional groups on the molecule, however, are less simple to achieve. Two possibilities for the solution of this problem present themselves: (a) Partial degradation of one of the more readily available bisindole alkaloids (probably VLB) followed by structural modification and resynthesis, or (b) synthesis of analogous compounds by appropriate "dimerizations" of preformed Iboga and Aspidosperma type units.

The observed lability of the C_{15} to $C_{18'}$ bond in these alkaloids would suggest that approach (a) is somewhat unfavourable. This lability is to be expected on the basis that the Aspidosperma unit is, after all, a meta-methoxy anilino compound. This renders it nucleophilic at the C_{15} position by virtue of the mesomeric electron donating effect of both the methoxy as well as the nitrogen substituents. Thus it would be expected to be protonated under acidic conditions at this site. Cleavage of the C_{15} to $C_{18'}$ bond then simply neutralizes the positive charge so generated.

This same mesomeric donation which makes the bond cleavage so facile also renders the C_{15} position in the monomeric Aspidosperma unit nucleophilic and a "dimerization" reaction simply involves finding the appropriate conditions by which the above process may be "reversed". Because Kutney and coworkers had developed a general scheme for the synthesis of both the Aspidosperma and Iboga alkaloid types, the approach of affecting a suitable "dimerization" between such templates became attractive.

A key step in the synthesis of these monomeric entities was the transannular cyclization step. This had already been postulated in

1962 by Wenkert[55] in a biosynthetic scheme proposed for these compounds. The relevant part of his postulate is shown below.



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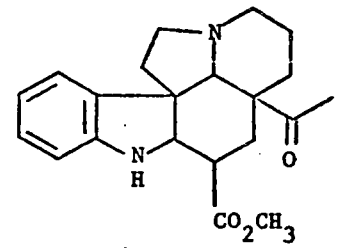

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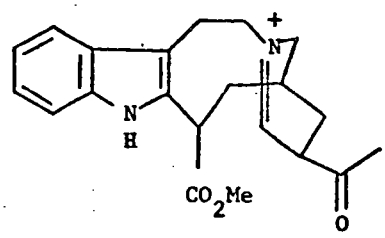
Aspidosperma



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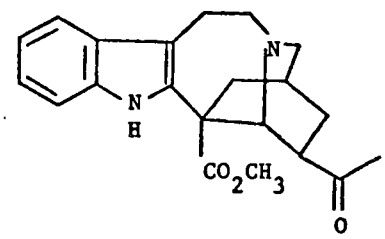

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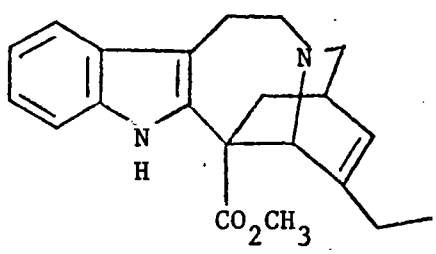
Iboga

Dihydrocleavamine (31) could be obtained from catharanthine (11) via the scheme shown below[56] in moderate yield. Compound 31 provided



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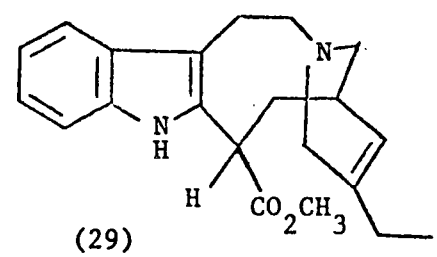

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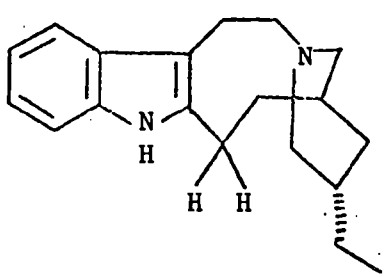

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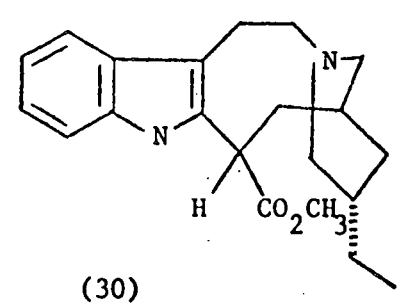
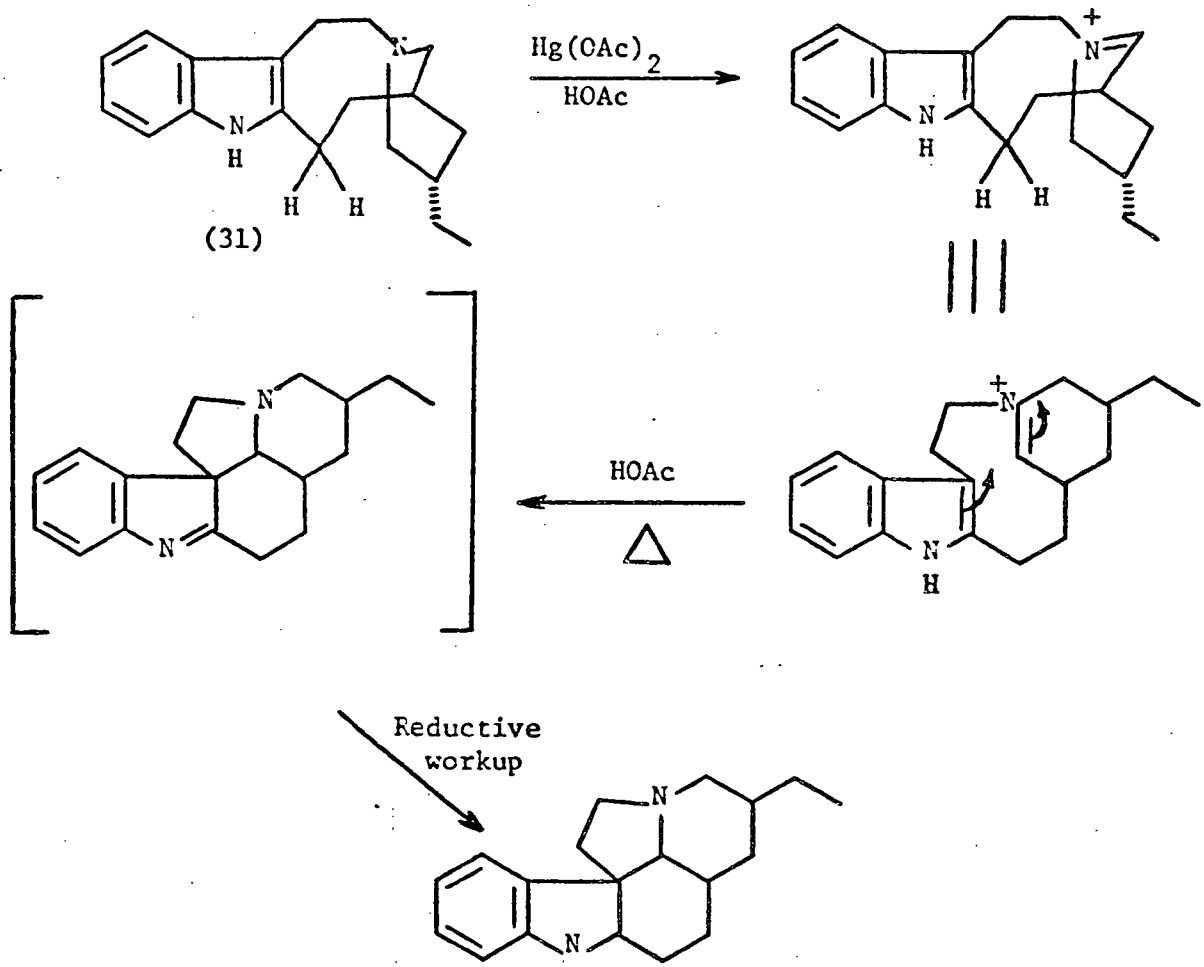


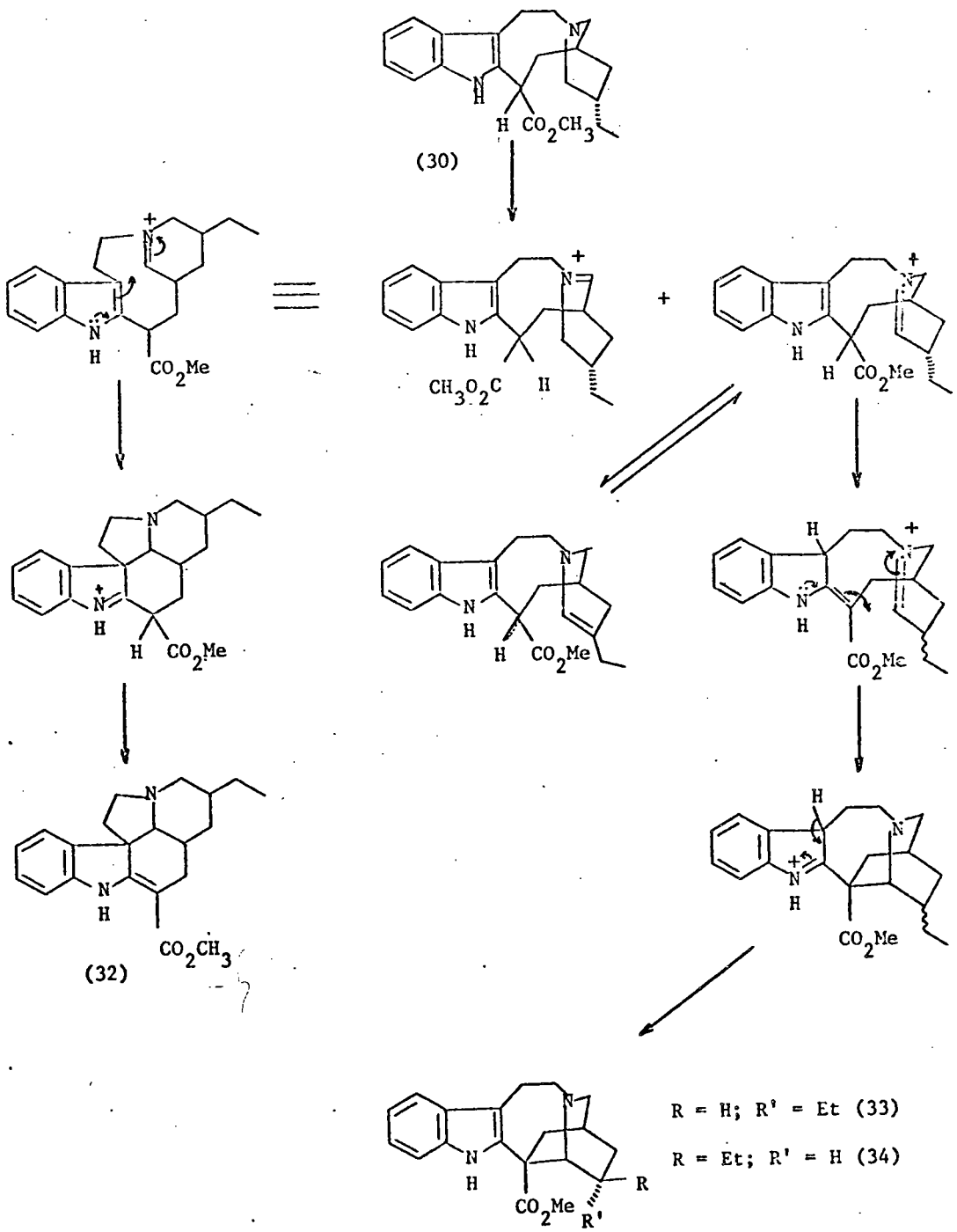
(30)

a good model with which to test Wenkert's hypothesis above. It is well-known that the treatment of many tertiary amines with mercuric acetate converts them to the corresponding iminium species. Treatment of dihydrocleavamine (31) with mercuric acetate in acetic acid yielded the corresponding iminium species which underwent cyclization, when heated, in the manner shown below to yield, upon reductive workup, the Aspidosperma skeleton.[57] When the analogous reaction was carried out

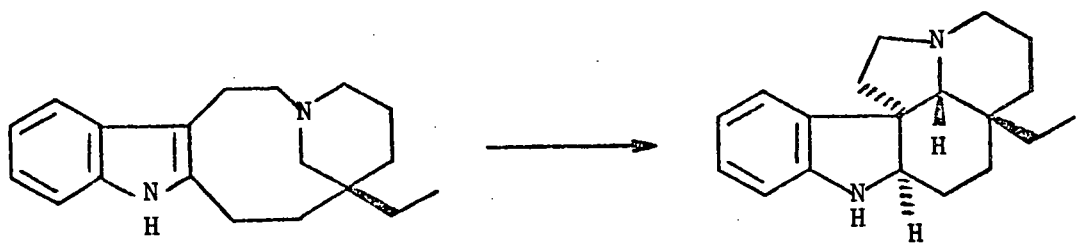


(31)

on 18-carbomethoxydihydrocleavamine (30), pseudovincadifformine (32), dihydrocatharanthine (33) and its 4-ethyl epimer coronaridine (34) were all isolated.[58]



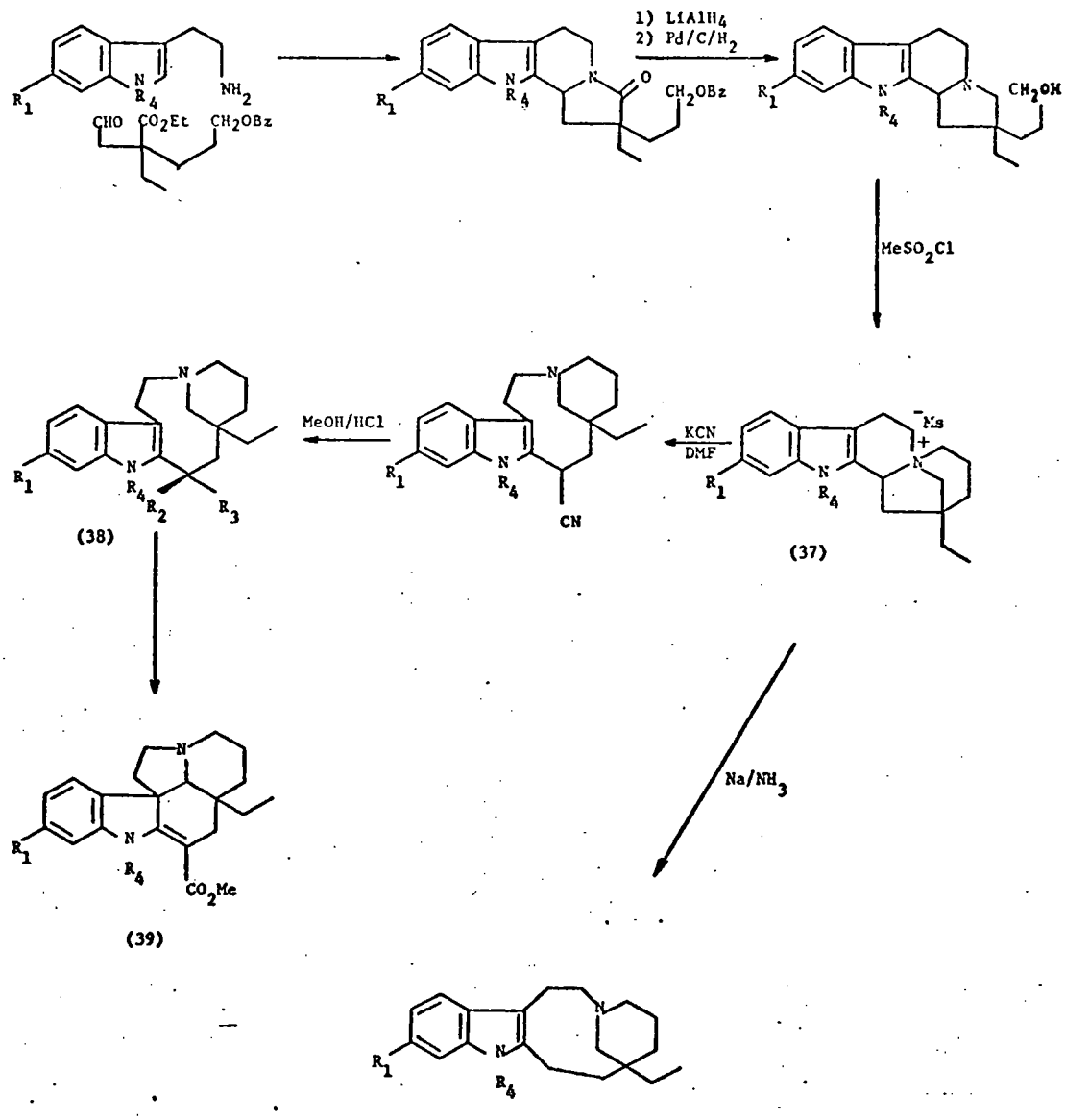
Clearly the Aspidosperma skeleton so synthesized is aberrant in that it is an ethyl isomer of the natural compounds. The natural skeleton embodied in the structure of aspidospermidine (36) was synthesized however by the same transannular cyclization of quebrachamine (35). This cyclization is of great interest because it has been shown



(35) (36)

to be completely stereospecific by both chemical and X-ray studies.[59] Thus the proper choice of stereochemistry of the lone asymmetric centre in the starting material makes available either of the two known stereochemical series of the Aspidosperma skeleton.

The utility of this approach to the synthesis of Iboga and Aspidosperma systems rested on the availability of the appropriate nine-membered ring compounds. The synthesis of intermediate-sized rings has been known, for some time, to be fraught with difficulties. However in this particular case, these difficulties were overcome by the skillful use of the nitrogen atom present in the ring system. The key intermediate for this synthetic approach was the quaternary salt (37) (Figure 2). Ring opening of this intermediate was shown to occur readily under reducing conditions (sodium in liquid ammonia) to give dl-quebrachamine (35). The appropriate ethyl isomer of (37) under similar conditions could be made to yield dl-dihydrocleavamine



(38) R_1	R_2	R_3	R_4	
H	CO_2CH_3	H	H	vincadine
H	H	CO_2CH_3	H	epivincadine
H	CO_2CH_3	H	CH_3	vincaminoreine
H	H	CO_2CH_3	CH_3	vincaminorine
OCH_3	CO_2CH_3	H	CH_3	vincaminoridine
	H	CO_2CH_3		

(39) R_1	R_4	
H	H	vicadifformine
H	CH_3	minovine

Figure 2. Kutney's total synthesis of the Aspidosperma skeleton.

(31).[60-62] The introduction of a carbomethoxy group was achieved by finding conditions to achieve the ring opening of compound 37 by the nucleophilic attack of cyanide ion followed by hydrolysis and esterification. The resulting intermediate when transannularly cyclized yielded the Aspidosperma skeleton bearing a carbomethoxy group at the appropriate position. A similar approach was possible with an ethyl isomer, as shown in Figure 3, to yield an entry into the Iboga skeleton. Here the decarbomethoxy compounds were synthesized first followed by the introduction of cyanide on the appropriate chloroindolenines. This approach was first discovered by Buchi and coworkers and was reported in his synthesis of voacangine.[63] It is an important reaction that has been greatly utilized in the course of subsequent work and will be described in detail in a subsequent section of this thesis.

Hydrolysis and esterification of the resulting nitrile afforded carbomethoxydihydrocleavamine (30) which could be cyclized, as mentioned before, to coronaridine (34) and dihydrocatharanthine (33).

Since catharanthine is a readily available alkaloid, and since it bears the required functionality for further elaboration towards the cleavamine-type part of the VLB-type "dimers", its use as a relay compound became attractive. In order to achieve this goal two criteria needed to be satisfied: (1) A total synthesis of catharanthine needed to be achieved, and (2) Some means of converting the rigid pentacyclic Iboga skeleton of catharanthine to the tetracyclic cleavamine system was necessary.

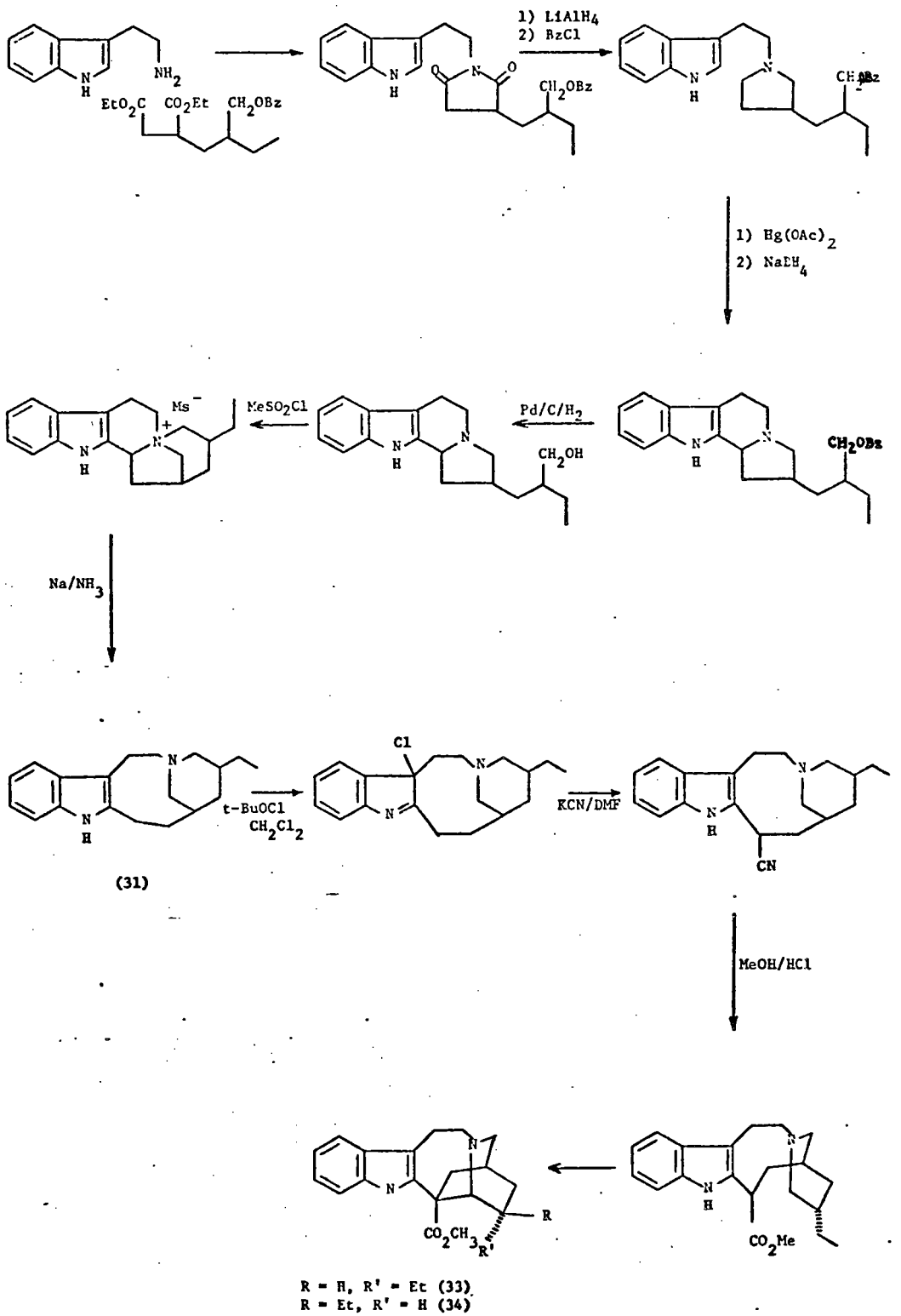


Figure 3. Kutney's total synthesis of the Iboga skeleton.

The former of these two objectives was achived by an interesting sequence.[64] This sequence also made available several previously inaccessible functionalized cleavamine systems such as isovelbanamine (47) and velbanamine (19). These were obviously very closely similar to the materials required to make the naturally occurring dimeric alkaloids.

The starting material was dihydrocatharanthine (33) available in racemic form by the total synthesis described above or in optically active form from the hydrogenation of catharanthine (11).[65] The latter was reduced with lithium aluminum hydride to the alcohol (40). Treatment of the tosylate of this compound (41) with triethylamine resulted in an interesting fragmentation (shown in Figure 4) to give the secodiene (42) in 62% overall yield. This compound could be converted to the triol (44) by standard methods, and the latter could in turn be converted to the ketol (45) by cleavage of the vicinal diol with sodium periodate. The ketol could be partially reduced to the diol (46) or else the "benzylic" hydroxyl group could be removed to yield isovelbanamine (47) isomeric at the C_4 position with the cleavage product of VLB; velbanamine (19) to which it could be easily converted by treatment with aqueous sulfuric acid. Treatment of compound 47 on the other hand with concentrated sulfuric acid led to the expected dehydration of the tertiary alcohol to cleavamine (21). Introduction of the carbomethoxy group by the previously described method resulted in 18β-carbomethoxycleavamine (29) which could be transanularly cyclized to catharanthine thus completing the total synthesis of this compound.[66] It should be mentioned that the synthesis of velbanamine

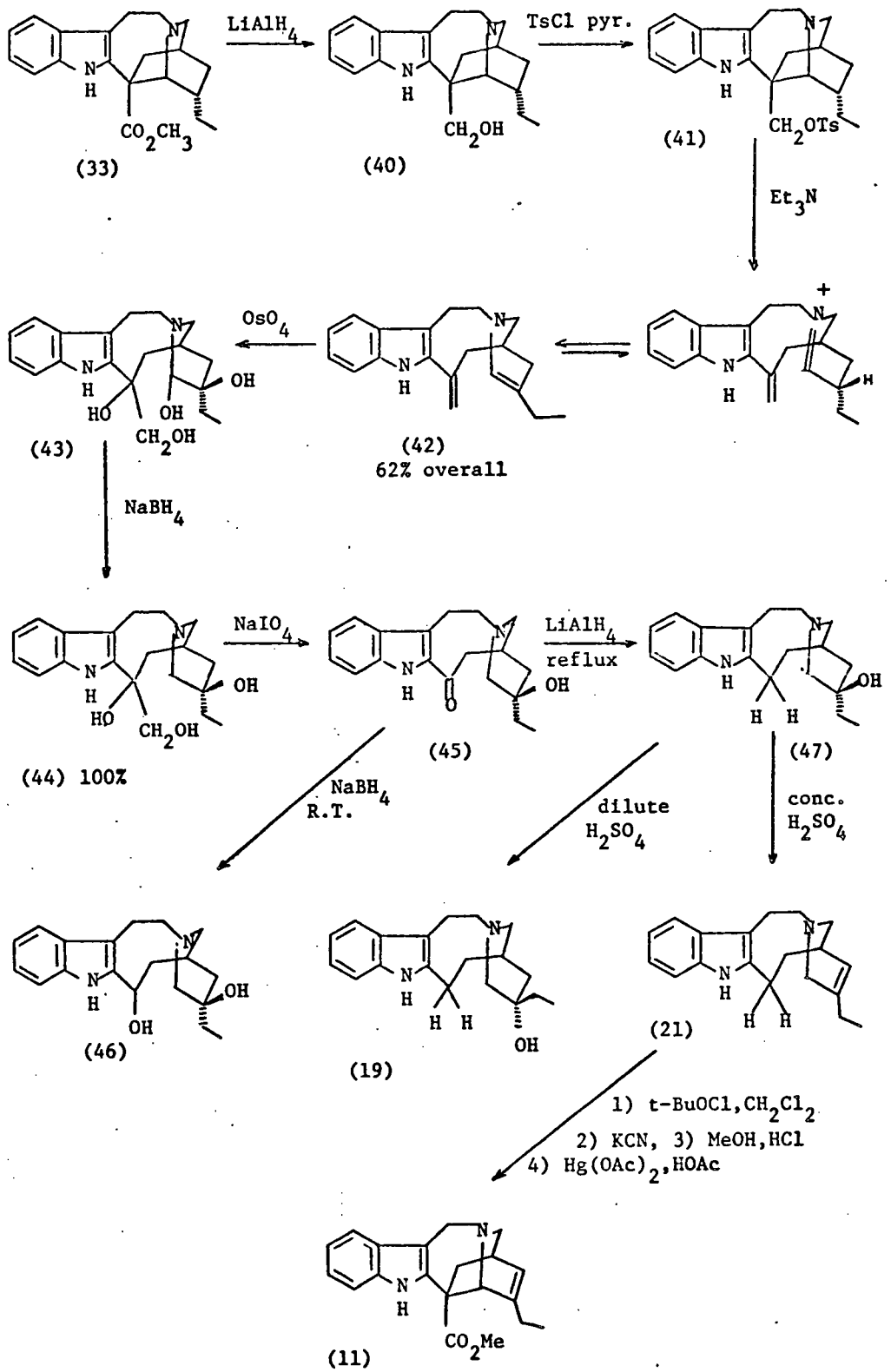


Figure 4. Kutney's total synthesis of Catharanthine.

and catharanthine using a totally different approach was reported almost simultaneously by Buchi and coworkers.[67,68]

There only remained, the development of a high yielding ring opening reaction that would convert catharanthine into carbomethoxycleavamine. This reaction had been reported using zinc in acetic acid but the yield and stereochemical purity of the resulting carbomethoxycleavamine was not good enough to render it synthetically useful. The discovery that the treatment of catharanthine hydrochloride in refluxing glacial acetic acid with an excess of sodium borohydride under carefully controlled conditions resulted in a 65% overall conversion to 18β-carbomethoxycleavamine was thus a discovery of singular importance.[69]

In summary then, methods for the development and elaboration of both "halves" of the natural VLB type dimers had been developed in our laboratories. The remaining requirements could be summarized as follows:

(1) To find a mild and general method of coupling both these monomeric units together at the appropriate centres so as to yield a junction which was stereochemically equivalent at the $C_{18'}$ position to the stereochemistry found in all naturally occurring oncolytic dimers (namely in the $C_{18'}$ carbomethoxy dimers - the $C_{18'}$-R stereochemistry).

(2) To elaborate the previously described scheme so as to obtain the exact functionality at the C_3, and/or C_4, positions of the carbomethoxycleavamine skeleton so that dimerization would produce compounds which were either the natural products themselves or else

C_{18}, epimers of them.

(3) To obtain a total synthesis of vindoline.

The description of the progress towards the goals (1) and (2) is the subject of this thesis. Goal (3) is an exacting one and is currently under study by several workers in our laboratories.

DISCUSSION

The research embodied in this section may be conveniently divided into two major categories: (1) Some work was required to modify the monomeric species available from previous work in these and other laboratories in order to obtain monomeric indole moieties which closely resembled the appropriate parts of the natural oncolytic alkaloids. The work towards this goal is treated together for purposes of convenience and is contained in Part I of this discussion. (2) There remained, in the area of the so-called "dimerization reaction", three major questions which were largely unanswered. These were: (a) What is the stereochemistry at $C_{18'}$ of the bisindole alkaloid resulting from the "dimerization reaction" conditions so far employed?[70,71] (b) What can be said of the mechanism of this dimerization reaction? And finally, (c) once enough is known about the mechanism of this dimerization reaction, what modifications and/or new approaches may be attempted in order to ensure that a stereochemistry about $C_{18'}$ similar to that of the natural compounds is obtained? The efforts to shed some light on these questions represent a major portion of this thesis and are embodied in Part II.

Part I

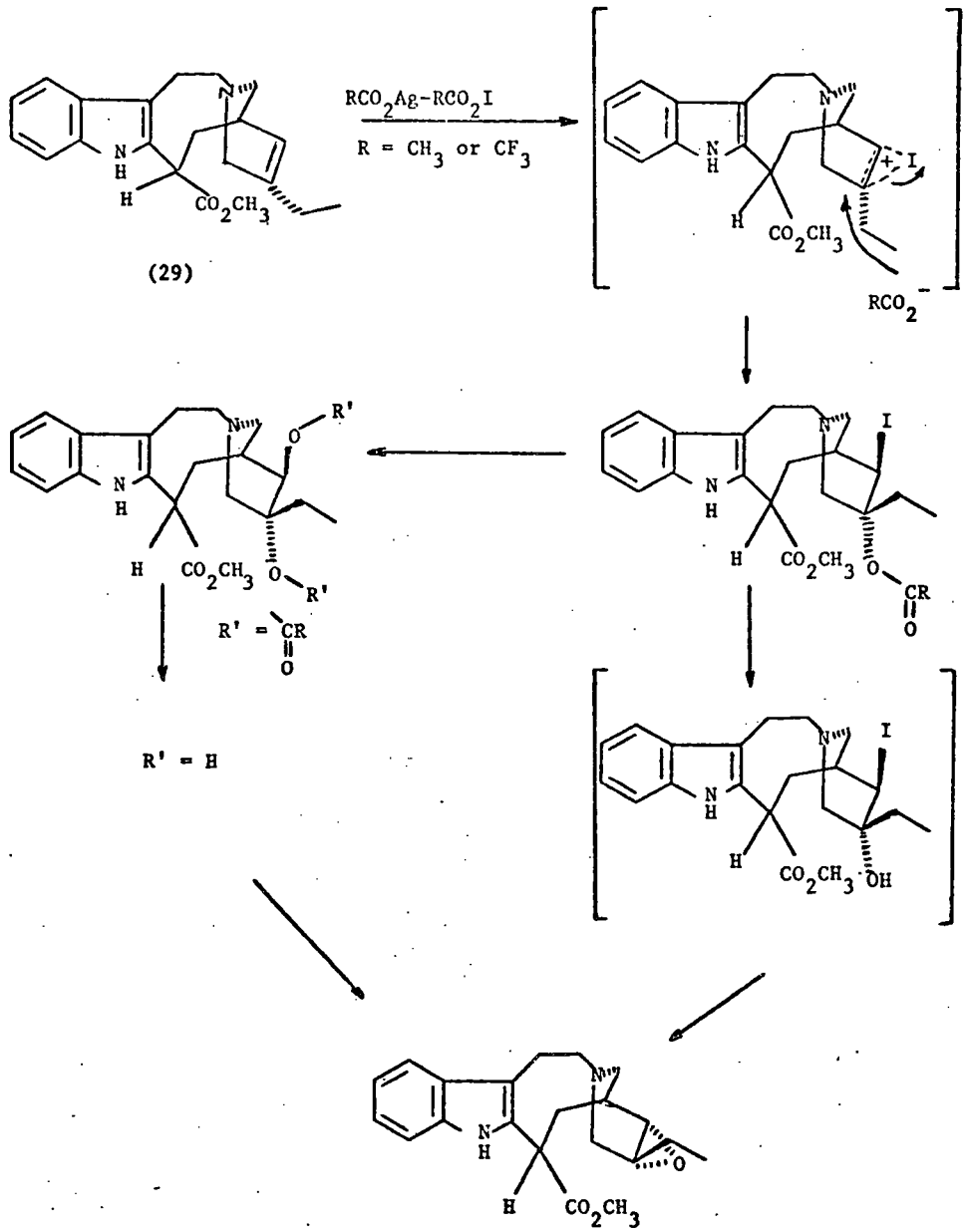
The four known oncolytic dimers (15-18) all contain some oxygen functionality at $C_{3'}$ and/or $C_{4'}$. The synthesis of any of these indolic templates therefore required a compound with some suitable "handle" at one or both of these positions which could easily be transformed into the appropriate oxygen functionality. The presence of a double bond at precisely this position in 18-carbomethoxycleavamine and its ready availability from the reduction of catharanthine with sodium borohydride in acetic acid, as mentioned earlier, made it a prime candidate. This observation together with the numerous methods available in the literature for converting olefins to the appropriate oxygen functionality suggested that this approach may be a rewarding one.

An attempt was thus made to explore the conditions of direct "hydration" of the double bond of 18-carbomethoxycleavamine. If this could be achieved in the Markovnikoff sense then 18-carbomethoxy-isovelbanamine or -velbanamine would become available and the possible epimerization of the former, if it formed, could be investigated. Velbanamine itself had already been synthesized; in our laboratory by the epimerization of synthetic isovelbanamine,[64] and in the laboratory of G. Buchi[68] by the reaction of ethyl magnesium bromide on the corresponding C_4 ketone. These reactions indicated that the stereochemistry of the C_4 alcohol in velbanamine was the more stable one. Indeed an examination of the molecular model of velbanamine and the X-ray structure of VCR monomethiodide showed that when the piperidine ring was in the most stable chair conformation, the C_4 ethyl group was equatorial in velbanamine (and VCR) and axial in isovelbanamine.

These results thus supported the view that 18-carbomethoxyisovelbanamine should in principle be epimerizable to 18-carbomethoxyvelbanamine.

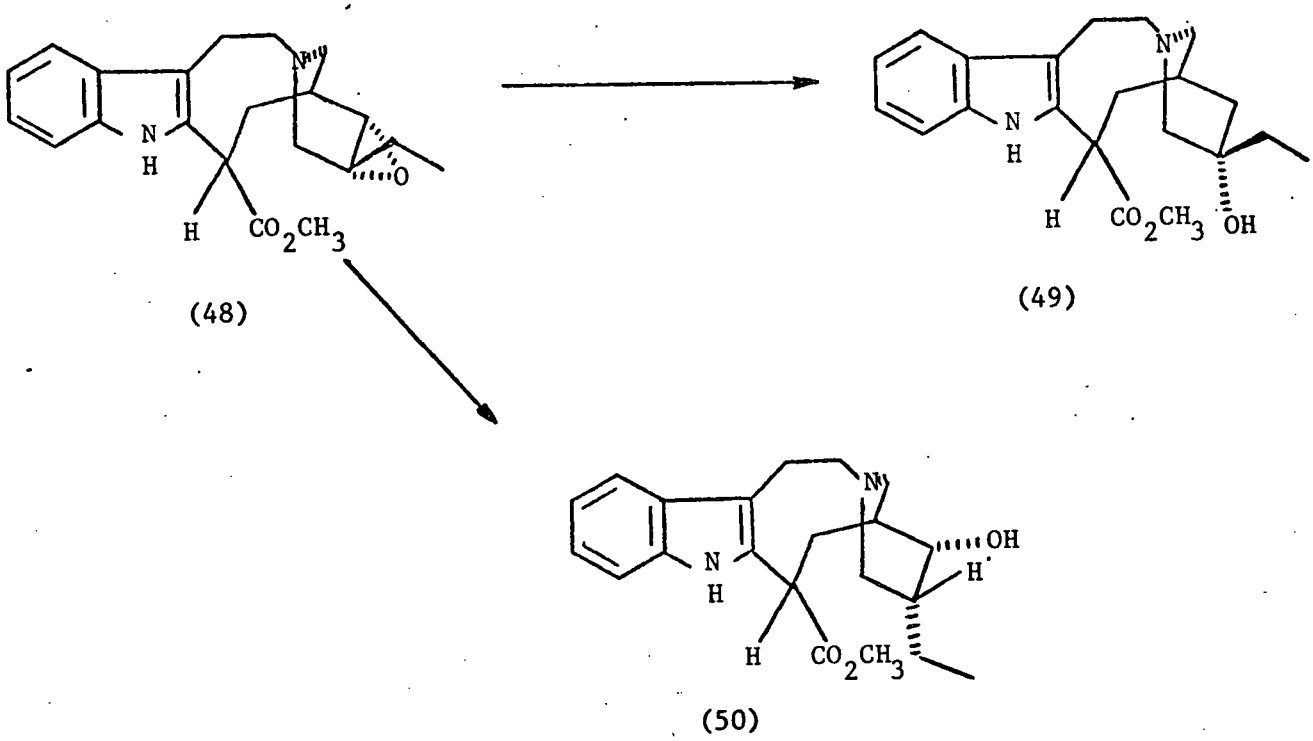
It was obvious that conditions which would yield directly the velbanamine type of stereochemistry at C_4 needed to be investigated first. If such reaction conditions were successful they would circumvent an epimerization at this centre altogether. The addition of acylhypoiodites to olefins is indeed a well documented reaction,[72] and in some cases the trans-diaxial halohydrin ester may be obtained. Since the reaction conditions required for such a reaction are usually mild in terms of time and temperature, it was resolved to attempt to use them here. The accepted mechanism for this reaction requires attack of a "positive iodine" species upon the π-system of a double bond from the less hindered side to yield a cyclic iodonium ion. In our case, it was thus expected that attack would occur from the side closest to N_b (vide infra). Finally this cyclic iodonium ion could be attacked in a concerted manner by the acyl species to provide the trans-diaxial halohydrin ester. Some cases had been reported where this intermediate could be isolated and transformed to the halohydrin which could then be closed to the appropriate epoxide by treatment with a base. Following through this anticipated reaction pathway, would result in the β-epoxide (48), as shown below. In a majority of cases, however, the trans-diaxial diester resulted. This could easily be converted to the corresponding diol. Such a product in the case in point would result in a trans diol in which one alcohol was secondary and the other tertiary. Conversion of the secondary alcohol to a suitable leaving group followed by displacement by the

adjacent alcoholic oxygen would again result in compound 48.



Compound 48 would be an extremely valuable one because it is exactly the indole template required for the synthesis of one of the natural dimers - leurosine (18). Furthermore, it could be converted by appropriate manipulation to 18-carbomethoxyvelbanamine (49) as well as the corresponding secondary alcohol (50), each of

which is the required template for the other dimers VLB (15) and leurosidine (17), respectively.



(48)

(49)

(50)

Several attempts to induce this reaction to proceed as desired were made. Temperatures between 0° and 40°C (refluxing methylene chloride) were investigated together with changes in reaction times from 0.5 hours to overnight. The efforts were all uniformly useless. In each case unchanged starting material was recovered to the extent of 94% of the initial weight. This was proved to be the case by the comparison of the ir and nmr spectra of the reaction product with starting material. One possible explanation of the inertness of this molecule to these reaction conditions is that the incoming iodonium ion is too bulky to attach itself to the π-system of the 3,4 double bond. An alternative and somewhat preferable explanation is that the

displacement of the intermediate cyclic iodonium ion at the C_4 position would be an energetically highly unfavourable step requiring attack by the carboxylate anion from the side which would be anticipated to be quite sterically hindered. If the reason for the failure of this reaction involves steric crowding on the β-face of the double bond then the hope of directly obtaining a $C_4\beta$ alcohol could not be realized.

Thus attention was turned to an investigation of the possible synthesis of a $C_4\alpha$ alcohol which could then be epimerized to the desired one at some later stage. Previously, Kutney and coworkers[73,57,58] had investigated in detail, the effect of mercuric acetate on indole moieties (vide supra). They had established that the primary effect of mercuric acetate on cleavamine-type molecules lacking a 3,4 double bond, in acetic acid, was the oxidation of N_b in the molecule to the corresponding iminium species. It remained to investigate this reaction under the conditions of oxymercuration usually employed for the hydration of double bonds.[74-76] The reaction of N_b would be reversed upon treatment with sodium borohydride in aqueous alcohol. The oxymercuration reaction required just such a workup in order to break the carbon-mercury bond formed during the reaction. It was thus felt that this reactivity of N_b towards mercuric acetate constituted no problem in this reaction and may indeed have the added advantage of protecting this centre from any other side reactions.

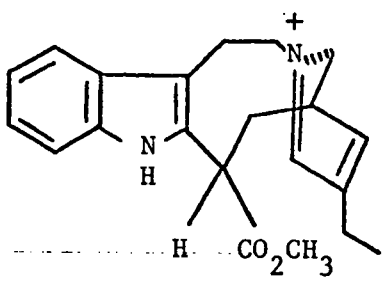
As a model, catharanthine was treated in aqueous tetrahydrofuran (1:1) with two equivalents of mercuric acetate under reflux for 2.5 hours. Workup by reduction with sodium borohydride resulted in a

precipitation of mercury which was filtered off. Only one half of the material could be recovered. However, the examination of this reaction mixture showed that roughly half of it had been converted to another more polar compound. This may have been the desired alcohol on the basis of its infrared spectrum which showed a shoulder at 3300 cm^{-1} on the usual indole N-H absorption at 3440 cm^{-1}.

On the basis of this result, several attempts to duplicate this employing 18-carbomethoxycleavamine were made. This compound was treated with two equivalents of mercuric acetate in aqueous tetrahydrofuran under reflux. The crude reaction mixture, after borohydride reduction, contained only one major spot. An nmr investigation of this reaction mixture showed that this compound was starting material. In particular, no exchangeable protons were encountered in the nmr upon shaking with deuterium oxide. No change in this reaction mixture could be detected when it was attempted to acetylate it using acetic anhydride and pyridine. These facts ruled out the possibility that even any minor constituents of the mixture were alcohols. Finally, purification of the major compound was possible using thin layer chromatography on alumina. An infrared comparison of this product with authentic 18β-carbomethoxycleavamine rapidly established that it was unchanged starting material. Several other attempts to induce this reaction to proceed as desired also failed.

Formation of N_b-C_5 iminium species in the flexible tetracyclic cleavamine system results in the formation of a dihydropyridinium ion. This delocalized system deactivates the 3,4 double bond towards electrophilic attack. Thus the formation of the intermediate acetoxy-

mercurium ion may not be facile. In other words, the positive charge in the iminium system is delocalized over the entire diene system of the dihydropyridinium ring thus depleting the electron density of the 3,4 double bond and of course correspondingly reducing its reactivity towards electrophiles. The relative apparent "success" in the rigid pentacyclic system of catharanthine, on the other hand may result from the failure of it to form an iminium ion because of the strain associated with the formation of such an intermediate under these particular reaction conditions. Kutney and coworkers[73] have studied the rearrangement of catharanthine in concentrated acid to cleavamine and decarbomethoxycatharanthine. In this study they implicated a similar iminium species to the one required here in the tetracyclic case (51). An equivalent one in the pentacyclic case has, however, never been reported. The formation of the analogous iminium (52) in the rigid system would clearly violate Bredt's Rule and would thus be much more difficult to form.



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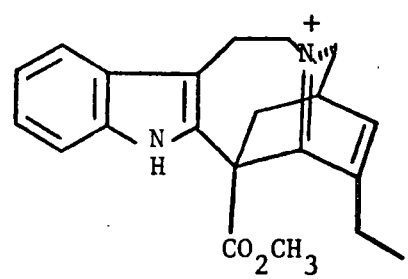


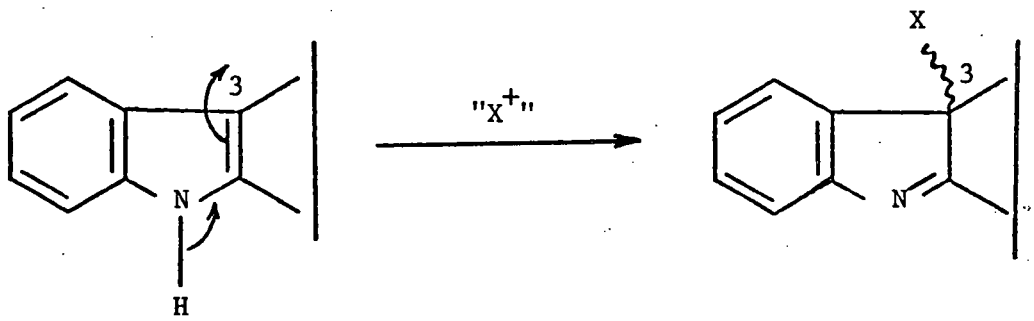
(52)

Under the conditions of the reaction the primary reaction of carbomethoxycleavamine then, is the formation of the dihydropyridinium species. This reverts to starting material when treated with sodium borohydride. On the other hand this reaction is suppressed or eliminated in the more rigid system and so the more normal oxymercuration results.

It was thus clear that the Markovnikoff hydration of the 3,4 double bond could not be readily achieved, and attention was turned to the possible factors affecting any form of electrophilic hydration of the 3,4 double bond. Several factors needed to be considered. First of all, the double bond to be functionalized is in a sterically crowded environment thus mitigating against the approach by large electrophilic groups of relatively low reactivity. Molecular models revealed, furthermore, that if an electrophile is to approach the olefin in a plane perpendicular to the plane of the double bond so as to maximize overlap with the π-system; it may only enter from the side closest to the N_b atom. Indeed this had been previously corroborated in our laboratory.[69] The catalytic hydrogenation of 18-carbomethoxycleavamine with Adam's catalyst at atmospheric pressure and room temperature in ethyl acetate yielded only 18-carbomethoxy-4β-dihydrocleavamine. Thus it was established that hydrogenation had occurred from the side closest to N_b. Functionalization of the 3,4 double bond should therefore be expected to yield compounds where the oxygen functionality is epimeric to that obtained in the natural dimers.

Secondly, the presence of a basic nitrogen atom may be expected to complicate matters somewhat by its own intrinsic reactivity towards some electrophilic reagents. The reactivity of such basic nitrogen atoms to peracids to yield N-oxides is well documented.[77] This reactivity is utilized later in this thesis and will be discussed at that point. Suffice it to say, here, that one would expect to obtain a reaction between any sufficiently reactive electrophile and the lone pair of electrons present on this nitrogen.

Finally, the reactivity of the indoles to electrophiles is well known. For example, these compounds react readily with sources of "positive halogen" such as N-bromosuccinimide, N-chloroacetamide, and t-butyl hypochlorite[78-83] to incorporate halogen atoms into the C_3 position of the indole nucleus with the formation of indolenines. This reaction has been mentioned previously in this thesis and forms the basis of most of the work in Part II of the discussion.



Thus, in summary, the direct functionalization of 18-carbomethoxy-cleavamine (29) to the necessary indole templates for the synthesis of the naturally occurring dimers, without the intermediacy of an epimerization of the oxygen functionality introduced, was unlikely. Furthermore the introduction of oxygen functionality must be performed in such a way that the other functionalities in the molecule are either unaffected or else are converted in situ to a derivative from which the parent functionality may be easily regenerated.

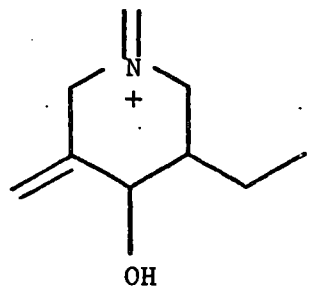
It is known that olefins react with diborane in solution to form stable boron adducts where boron has attached itself to the less-substituted terminus of the double bond. Oxygen may then be readily substituted for the boron by the use of alkaline hydrogen peroxide to give the so-called least substituted alcohol. Since the attack by boron is rapid and since the steric requirements of this reaction are

minimal, it was decided to attempt to hydroborate 18-carbomethoxy-cleavamine. Treatment of 18β-carbomethoxycleavamine with an excess of diborane in tetrahydrofuran under rigourously anhydrous conditions at 0°C followed by slow warming to room temperature resulted in a good yield of the boron adducts. This material could be treated with an aqueous alkaline solution of hydrogen peroxide to yield, after workup, a greater than 75% yield of two alcohol amine-boranes. These amine-boranes had resulted from the attachment of the electrophilic boron atom to the basic N_b atom. Absorptions in the infrared spectra of such amine-boranes at 2375 cm^{-1} (ν B-H) and 1170 cm^{-1} with 2260 cm^{-1} overtone (δ B-H) facilitates their detection in a reaction mixture. This was not an unexpected result and had been encountered previously during the hydroboration of similar alkaloids.

Indeed other workers in our laboratory had perfected a method for the removal of such amine-boranes by refluxing a mixture of the substrate and triethylamine in tetrahydrofuran for 2.5 hours.[84] Such treatment of the crude reaction mixture resulted in an overall total yield of two alcohols of roughly 70%. These were available in a ratio of approximately 1:1 and were readily separable by column chromatography on deactivated alumina.

An examination of their infrared spectra showed that they both possessed an absorption at approximately 3600 cm^{-1} distinct from the indole N-H absorption at about 3440 cm^{-1}. They were thus both alcohols. Furthermore a comparison of these spectra showed that they were indeed closely related. The ease with which both were acetylated established their identity as secondary alcohols; and their ultraviolet spectra

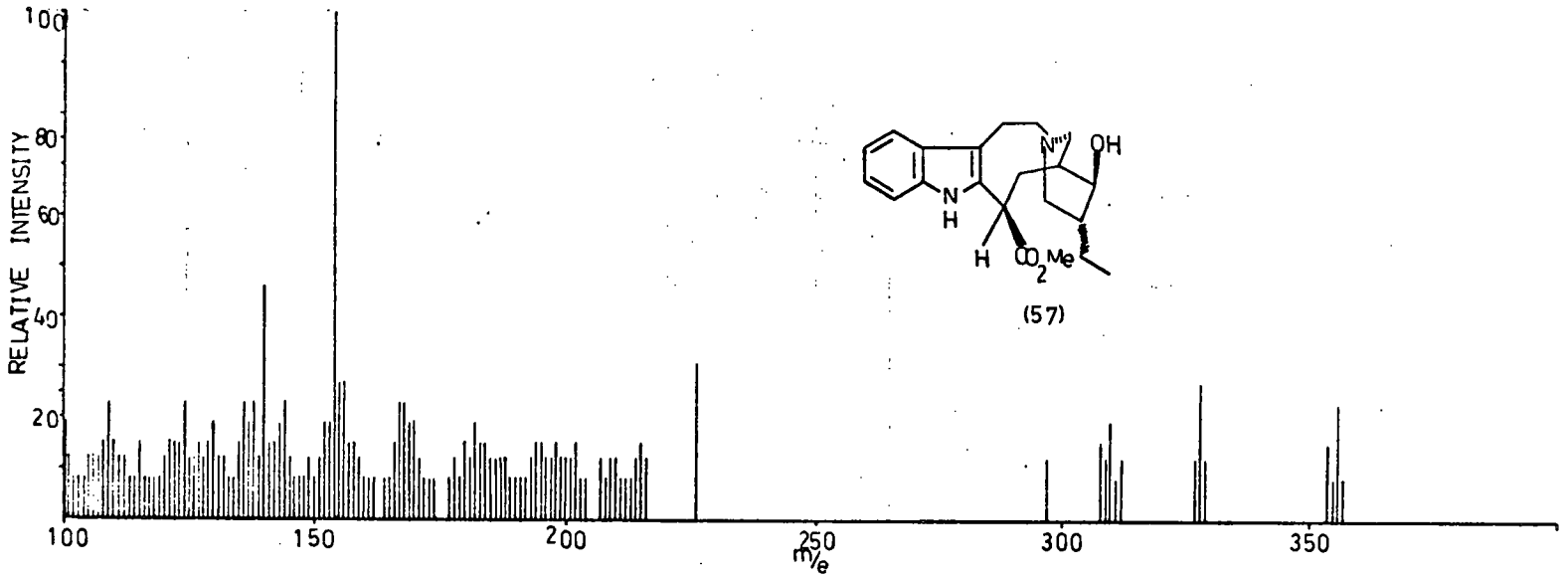
were typically indolic in both cases, eliminating any possibility that the indole chromophore had been somehow modified during the reaction. The best evidence in favour of their assigned structures was however available from their mass spectral fragmentation patterns. These were entirely in accord with that expected on the basis of previous work in this area[65] and confirmed the presence of an alcohol moiety in the piperidine ring system by the presence of an intense peak at m/e = 154 attributable to the fragment 53 shown below.



m/e = 154

(53)

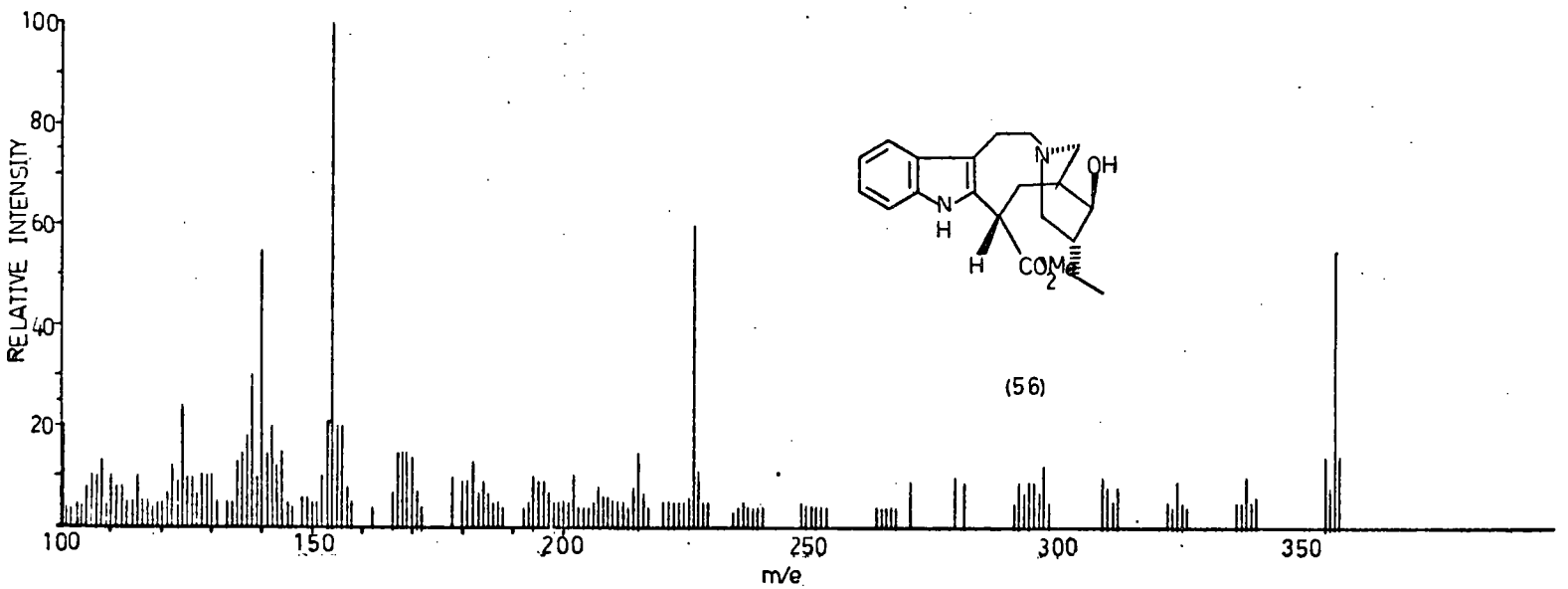
The reasons for the isolation of two alcohols instead of one was not immediately obvious. The starting material was optically pure, being obtained in one stereospecific step from a known natural alkaloid. Since two new optical centres were created during the hydroboration sequence at C_3 and C_4 the possibility of diastereomers needed consideration. However, it is well established[85] that the addition of boron and hydrogen to adjacent carbon atoms of an olefin occurs from the same side. Thus the stereochemistry at the two new centres was fixed in a relative sense. Furthermore, replacement of boron by oxygen is also known to be entirely stereospecific resulting in the overall retention of configuration at that centre. In other words, mechanistic arguments required the alcohol at C_3 and the ethyl group at C_4 to be trans. Two possible compounds could be drawn with this



(57)

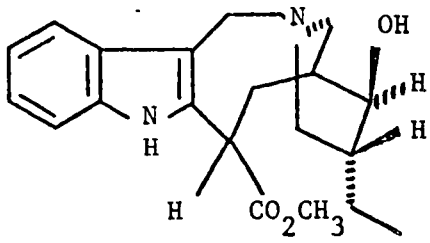
Figure 6. Mass spectrum of 18α alcohol (57).

Figure 5. Mass spectrum of 18β alcohol (56).



(56)

requirement and these are shown as 54 and 55. Past experience tended



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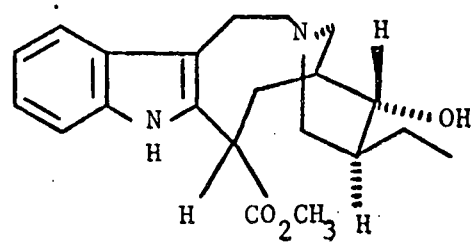


(55)

to rule out compound 55 as a possible one on the basis that it would require approach of the reagent from the more hindered side (see above). Thus it was necessary to conclude that one of the asymmetric centres already present in the molecule had been epimerized during the course of the reaction. Since two products had appeared after treatment with diborane, and before any further treatment, it was necessary to assume that diborane itself was responsible for the isomerization. Indeed treatment of either alcohol or either amine-borane separately with aqueous alkaline hydrogen peroxide or with triethylamine under reflux failed to yield a mixture of the two corresponding products.

As part of an attempted total synthesis of indole and dihydroindole alkaloids, Kutney and coworkers had cleaved catharanthine to four isomeric 18-carbomethoxydihydrocleavamines. It was possible at this time to assign the stereochemistry at the C_{18} and C_4 positions to each of these compounds chiefly on the basis of nmr, in particular, the chemical shifts of the signal attributable to the C_{18} proton and the C_4 ethyl group. It was shown that the series of 18β-carbomethoxydihydrocleavamines exhibited a one proton multiplet in the region

τ 4.5-5.0 attributable to the C_{18} proton. In contrast, in the 18α-carbomethoxydihydrocleavamines this C_{18} proton appeared as a multiplet in the region τ 6.0-6.2. The seemingly anomalous values in the 18β series, were explained by the argument that in this case the C_{18} proton is in close proximity to the N_b atom and may be deshielded by its lone pair. This argument had been used previously by Mokry and Kompis to assign the stereochemistry of some other related Vinca alkaloids.[86] It could be noticed furthermore that within each series of C_{18} isomers the $C_4\alpha$ compound exhibited a three proton triplet, attributable to the methyl group of the C_4 ethyl, at slightly lower frequency than the corresponding $C_4\beta$ compound. (See Table I). Finally, the absorptions of the ethyl-methyl of both C_4 epimers were at higher frequency in the $C_{18}\alpha$ series than the corresponding ones in the $C_{18}\beta$ series. Thus by a simple examination of the nmr spectra of

Table I. Chemical shifts of cleavamine-type compounds

C-18 carbomethoxy	C-4 ethyl α ethyl	C-4 ethyl α $C_{18}H$	C-4 ethyl β ethyl	C-4 ethyl β $C_{18}H$	Name
β	τ = 9.09;	4.53	τ = 9.12;	4.98	18-carbomethoxy-dihydrocleavamine
			τ 9.08;	5.00	18-carbomethoxy-dihydrocleavaminol
α	τ = 9.33;	6.13	τ = 9.45;	6.12	18-carbomethoxy-dihydrocleavamine
			τ = 9.44;	6.00	18-carbomethoxy-dihydrocleavaminol

cleavamine-type compounds one could assign the stereochemistry at both C_{18} and C_4 positions. The generality of this approach had been proved by its use in the corresponding 18-cyano- and 18-methoxy-dihydrocleavamines[87] and indeed, was utilized in the structure elucidation of three dimeric compounds, deoxy VLB-'A', deoxy VLB-'B' and deoxy VLB-'C'.[71]

A similar examination of the nmr spectra of the two alcohols available from the hydroboration above (see Figures 7 and 8), readily revealed them to be epimeric at C_{18} and not at C_3 and/or C_4. In fact by analogy it appeared that both compounds bore a $C_4\beta$ ethyl group, indicating that attack had been from the side closest to the N_b atom in support of the previous steric arguments. Acetylation of both alcohols independently, showed that the previous assignments of signals to the C_{18} proton were indeed correct by virtue of the fact that they were unchanged by such treatment. Both compounds were independently decarboxylated using aqueous concentrated hydrochloric acid and were shown to be identical to one another by superimposible infrared, nmr, and elemental analysis together with melting point of the corresponding acetates. Furthermore, each was independently also shown to be completely identical to an authentic sample of 4β-dihydrocleavaminol acetate prepared by the hydroboration of cleavamine itself.[84] Finally, the interconversion of these two alcohols to a mixture of both compounds by the use of boron trifluoride-etherate in benzene under reflux put this assignment on firm ground. This method of epimerization of the C_{18} carbomethoxy group had been used previously in the interconversion of 18α- and 18β-carbomethoxydihydrocleavamines.[69]

It is tempting to speculate that the mechanism of this known

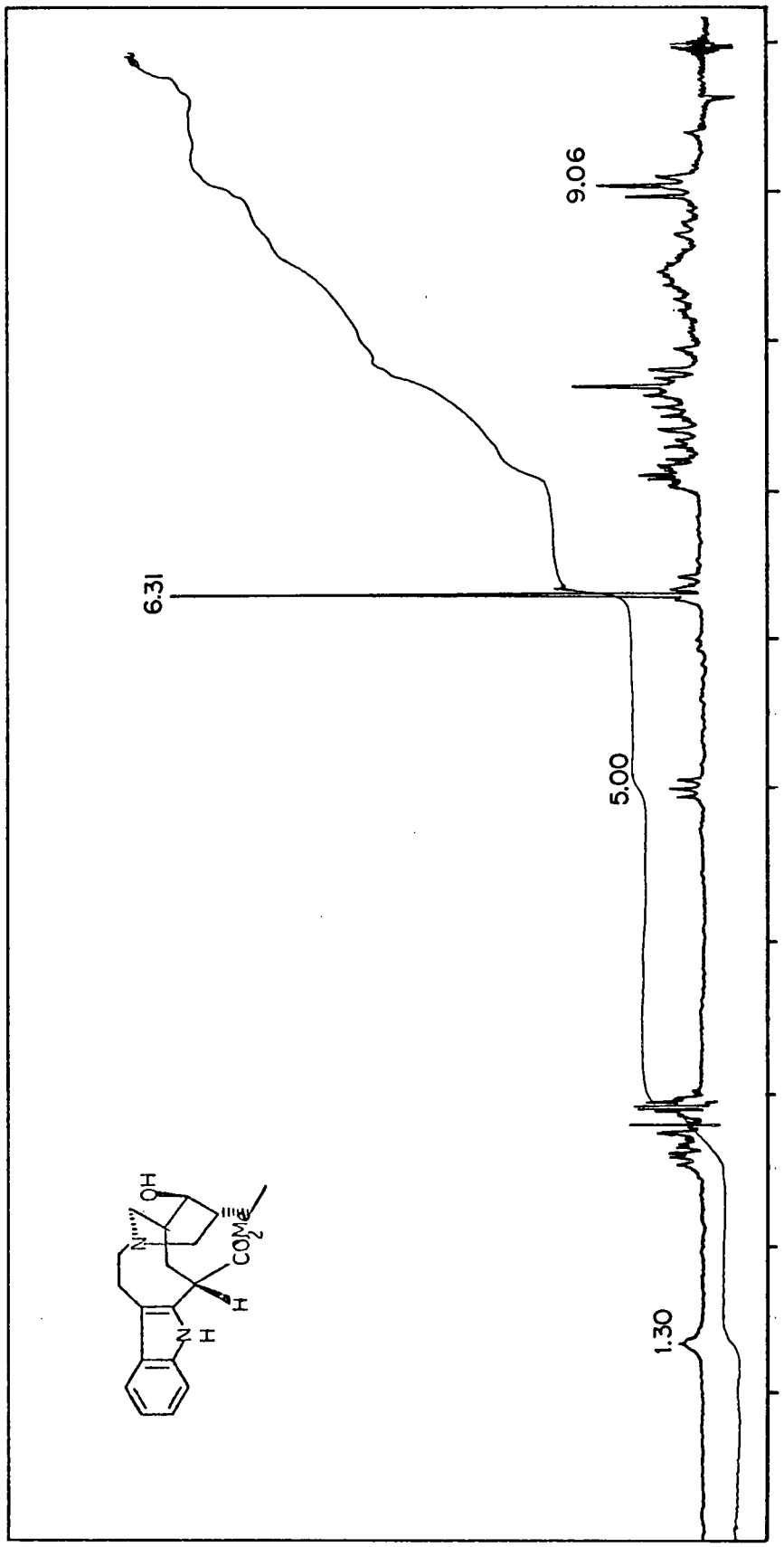


Figure 7. Nmr spectrum of 18β alcohol (56).

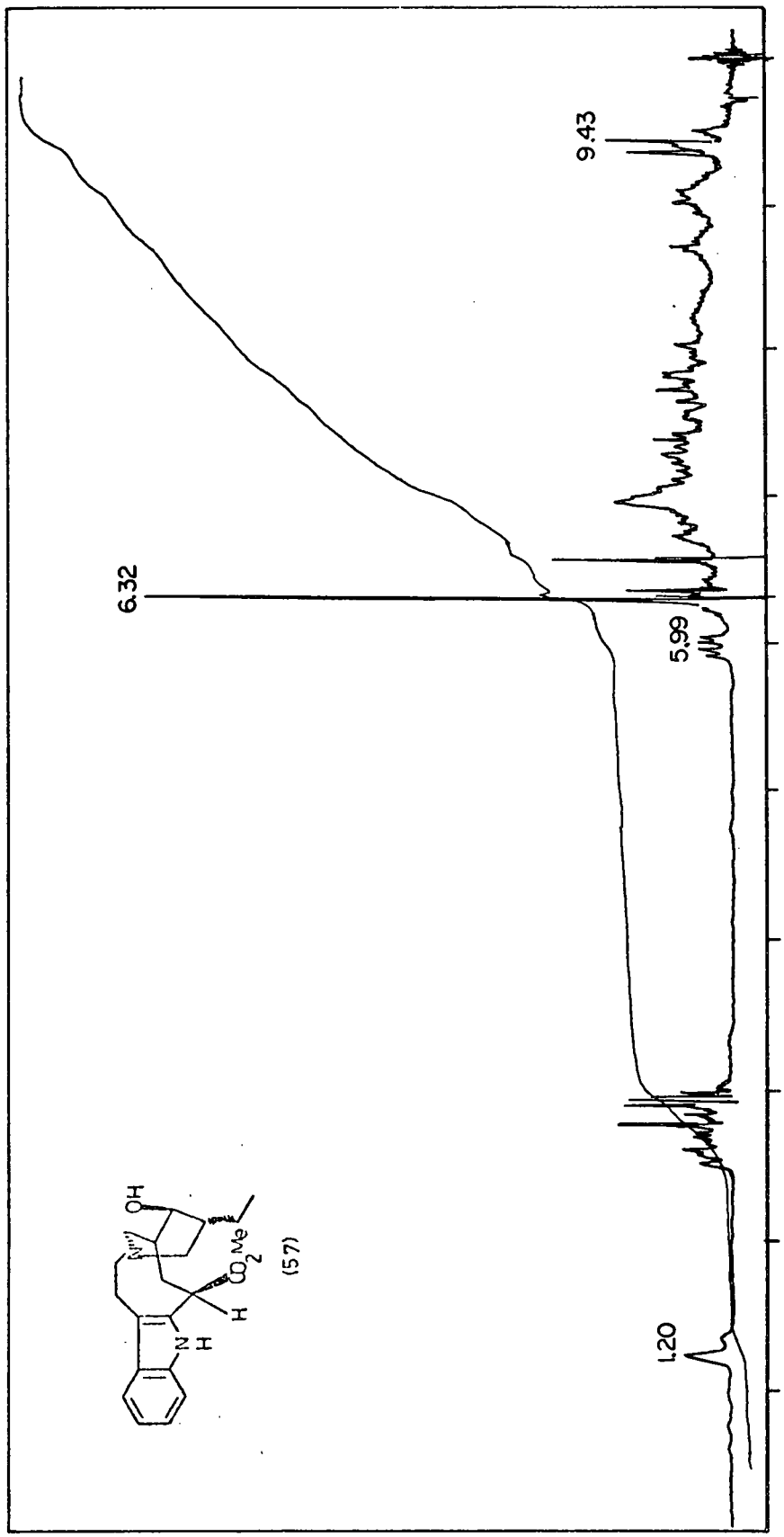
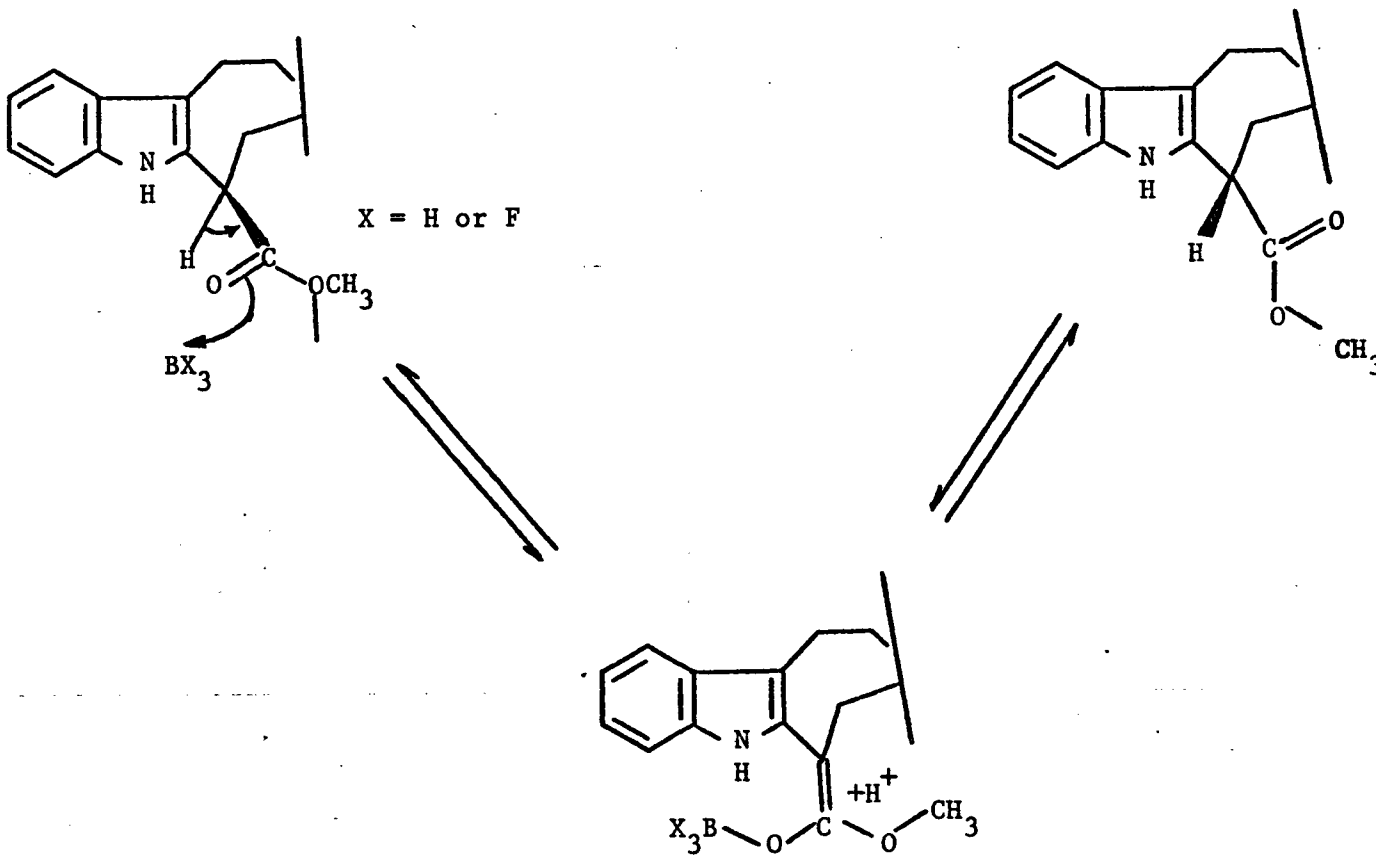
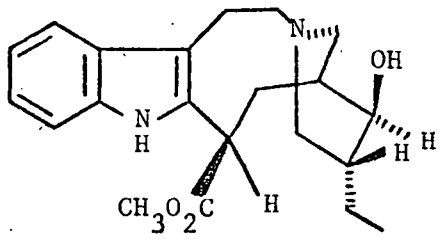


Figure 8. Nmr spectrum of 18α alcohol (57).

epimerization of the carbomethoxy group is analogous to that occurring during hydroboration. In each case an electron-deficient boron-containing molecule attaches itself to the electron-rich carbonyl oxygen of the carbomethoxy group, thus forcing the loss of a proton from the C_{18} position reversibly. This deprotonation-reprotonation mechanism finally results in a thermodynamically controlled mixture of epimers.

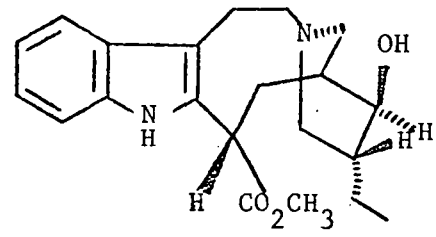


Thus compounds 56 and 57 could be obtained in one step from 18β-carbomethoxycleavamine. This constituted, then, a direct functionalization of the 3,4 double bond in the 18-carbomethoxycleavamine series and is the first and only one reported thus far.



(57)

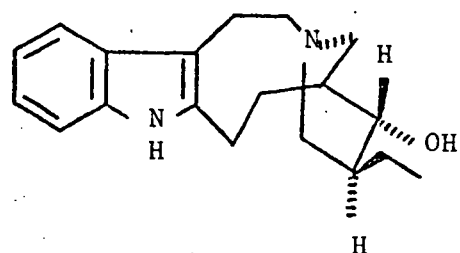
18α-carbomethoxy-4β-ethyl-dihydrocleavamin-3α-ol



(56)

18β-carbomethoxy-4β-ethyl-dihydrocleavamin-3α-ol

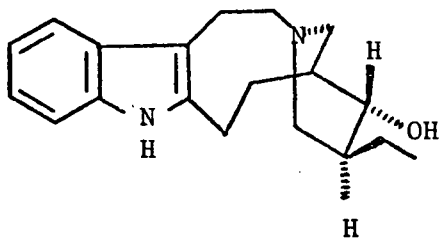
These compounds were of central importance for two reasons. First of all, the natural oncolytic dimer, leurosidine (17) had been postulated to have an indole portion,18-carbomethoxyvinrosamine with exactly such a secondary alcohol functionality.[88] During the structure elucidation of leurosidine, however, the stereochemistry at C_3, and C_4, could not be definitively established. However, on the basis of analogy with the structures of VLB (15) and VCR (16) the structure of vinrosamine (58), available from the cleavage of leurosidine (17), was assigned as shown below.



(58)

There existed now for the first time a real possibility to correlate the products of the hydroboration above with the compound available from the cleavage of leurosidine and thus prove the stereo-

chemistry at C_3 and C_4 of the naturally derived compound. Towards this end, it would be necessary to obtain all four possible compounds with differing stereochemistry at C_3 and C_4. These are shown below.



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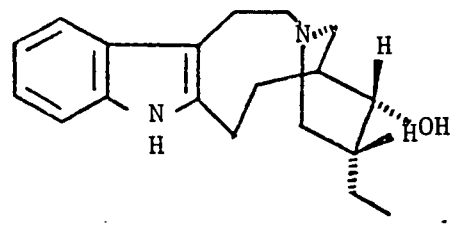

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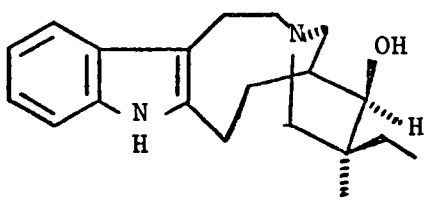

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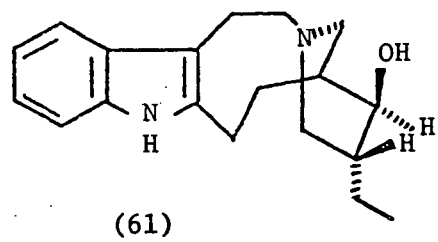


(61)

Compound 61 is available from the direct hydroboration of cleavamine[84] or, alternatively, by decarboxylation of either alcohol available from the above hydroboration. Oxidation of this secondary alcohol to the corresponding ketone provided the key intermediate for the synthesis of compounds 58, 59 and 60 stereoselectively. Thus epimerization of the adjacent C_4 position using strong base or Lewis acids such as boron trifluoride-etherate should yield an entry into the $C_4\alpha$-ethyl series. The epimerization of the other adjacent position at C_2 is impossible since the necessary enol or enolate double bond violates Bredt's rule.

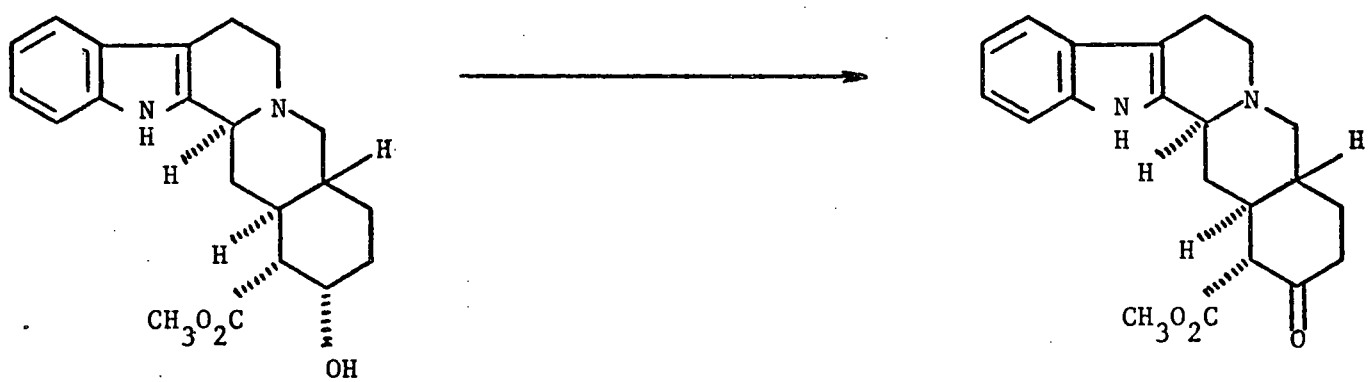
Subsequent reduction with a suitable hydride source should result in the delivery of hydride from the less hindered side, namely that closest to N_b, and should thus yield the $C_3\beta$ alcohols. By combining the epimerization at C_4 with this reduction at C_3, compounds 58 and 60 should be secured. Perhaps the isomerization of the C_4 ethyl group may sufficiently alter the conformation of the piperidine ring so as to allow reduction, at least partially, from the face furthest away from N_b and so permit the isolation of compound 59 as well. If not, other means of arriving at that compound could be devised.

Although significant, this was not the only use that compounds 56 and 57 furnished in the overall synthetic aim. The oxidation of the secondary alcohol in these compounds would provide a compound of the most central and vital importance to the entire project. This 3-keto-carbomethoxycleavamine compound should be utilizable; as outlined above in the decarbomethoxy case; in the production of four isomeric templates bearing a carbomethoxy group at C_{18}. These, when coupled with vindoline, should provide the first synthetic dimers bearing oxygen functionality at $C_{3'}$. In fact one of these compounds would necessarily be identical to leurosidine at least at the $C_{3'}$ and $C_{4'}$ positions and should represent the closest analogue of the natural compounds so far synthesized.

Secondly, this 3-keto compound should activate the adjacent C_4 position and allow placement of a suitable precursor of an epoxide and eventually the tertiary alcohol; thus allowing an entry into the C_4 functionalized templates required for the synthesis of the other natural dimers. In short, this 3-keto-carbomethoxycleavamine would in principle

make available all the templates necessary to synthesize not only all the known natural dimers but also other closely related analogues previously unavailable from nature. Finally, decarboxylation of this ketone should yield another direct entry into the compounds mentioned above. All these steps are summarized in Figure 9. The oxidation of the alcohols 56 and 57 to the compound 62 proved to be remarkably difficult.

Albright and Goldman had reported[89] that the use of mixtures of acid anhydrides and dimethyl sulfoxide (DMSO) proved to be a mild and effective method for the conversion of alcohols to ketones in various indole containing compounds. For example yohimbine (67) could be transformed to yohimbinone (68) in 80% yield in twelve hours at room temperature using a twenty-fold excess of acetic anhydride in DMSO. The use of benzoic anhydride afforded an even greater yield under the same conditions.



(67) (68)

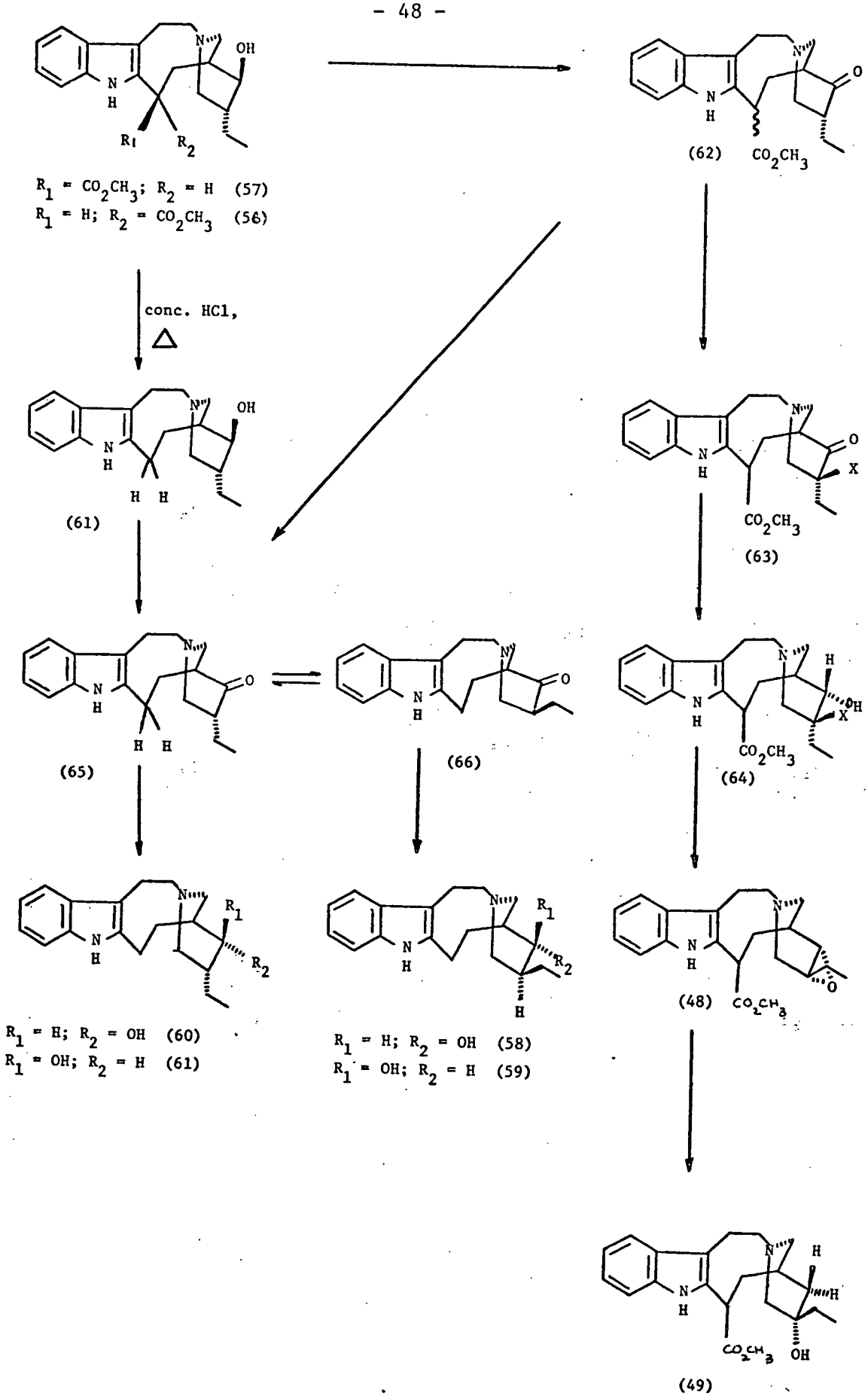


Figure 9. Proposed uses of hydroboration products 56 and 57.

These authors claimed this to be the method of choice for the oxidation of alcohols in sensitive indole alkaloids. However, they were quick to caution against the use of these reagents in cases where "alcohols are rapidly esterified under the conditions of the reaction..."[89]

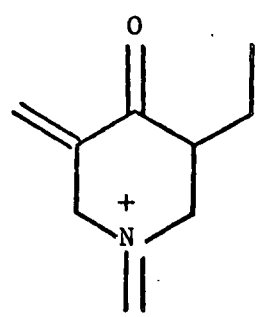
The compound 56 was treated with a five-fold molar excess of acetic anhydride in DMSO for a period of eighteen hours at room temperature. Workup afforded two major compounds which proved to be the unreacted starting material and the corresponding C_3 acetate, both of which could be readily identified by spectral comparisons with the authentic materials. On this basis it was felt that the competing esterification could be blocked by the use of benzoic anhydride and so these conditions were attempted. Treatment of compound 56 with an excess of benzoic anhydride in dimethyl sulfoxide for twenty-three hours at room temperature followed by workup afforded an 80% recovery of the corresponding benzoate ester. The identity of this compound could easily be deduced by inspection of the aromatic portion of its nmr spectrum. Several attempts were made to find conditions which would yield the desired ketone *via* this reaction but it appeared that under conditions mild enough to suppress esterification, no reaction occurred at all and the starting material was recovered unchanged.

An oxidizing agent of roughly the same mildness and without the concomitant problem of esterification is the complex of sulfur trioxide and pyridine in DMSO as solvent. This reagent was first described by von Doering.[90] Reaction of the sulfur trioxide-pyridine complex in DMSO with compound 56 at room temperature for periods ranging from twenty-four hours to six days resulted in a near quantitative recovery

of starting material together with traces of decomposition products which were polar. The latter lacked the normal indole absorptions in their uv spectra.

Several other attempts at accomplishing this apparently simple conversion resulted uniformly in failure. Another worker in our laboratory,[91] for example, investigated the various chromium based oxidation techniques such as Collins and Jones oxidations and met with the same result. Finally, after several conditions had been attempted, and rejected, the Moffat conditions[92] using dicyclohexylcarbodiimide, phosphoric acid and dimethyl sulfoxide were attempted. It had been reported in passing that leurosidine itself could be oxidized under these conditions to the corresponding ketone.[88] Submission of both compounds 56 and 57 to this oxidation under very carefully controlled conditions resulted in an interesting discovery. Under identical conditions compound 56 could be oxidized in an optimal yield of 50% to the desired ketone (62) whereas compound 57 was recovered unchanged. A satisfying explanation of this observation is as yet unavailable. This does not, however, constitute any serious limitation on this method because as mentioned earlier compound 57 can be epimerized to 56. Thus, the overall conversion of the products of the hydroboration to 62 in reasonable yield had now been achieved. As mentioned previously this compound provides an opportunity for the study of several aspects of its chemistry which are of pivotal importance to the overall synthetic scheme. Work in our laboratories is currently under way to extend this finding as outlined in Figure 9.

Preliminary studies have indicated that 18β-carbomethoxy-4β-cleavamin-3-one (62) cannot easily be converted to a compound of the type 63. Upon treatment with one equivalent of base, it yields the corresponding anion resulting from removal of the indolic N-H proton as the major, and in many cases the only product. This, of course, enhances the reactivity of the indole-3-position towards electrophiles. In fact attempts to add two equivalents of base followed by electrophiles such as bromine or sources of "positive" halogen have resulted primarily in the destruction of the indole chromophore as evidenced by ultraviolet spectroscopy. Attempts to trap any enolate that may form by the formation of enol acetates under similar basic conditions have also met with little success. Reactions under acidic conditions such as the use of isopropenyl acetate with a catalytic amount of p-toluenesulfonic acid and bromine in acetic acid have resulted in destruction of the indole chromophore as well. It was found, however,[93] that treatment of the ketone (62) with sodium hydride in 1,2-dimethoxy-ethane followed by quenching with deuterium oxide resulted in the formation of mono- and di-deuterio compounds. This conclusion was arrived at by a systematic mass spectral study. It was found that, in accordance with the expected mass spectral fragmentation[65,94,13] for cleavamine-like compounds, the most intense peak in the mass spectrum of compound 62 was at m/e=152 which could be assigned to the fragment shown below.



m/e = 152

In the monodeuterated compound this peak was expected to appear at 153 provided that this material had been deuterated in the piperidine portion. A measurement of the relative intensities of the peaks at m/e = 152 and 153 of the crude reaction mixture from the above deuteration experiment compared to the relative intensities of these peaks in the undeuterated starting material showed that it was 63% undeuterated in the piperidine portion and 37% monodeuterated in the piperidine ring. Thus, it was established that the desired enolate had indeed formed under these conditions. Efforts to utilize these reaction conditions to introduce some useful functionality at C_4 have so far not succeeded.

From the above discussion then, the apparently straightforward "direct" functionalization of the carbomethoxycleavamine system at C_4 has not borne fruit yet. The more demanding and laborious task of systematically protecting all of the sensitive and reactive functionality in the molecule prior to C_4 functionalization, and then deprotection again after this step, appears necessary. Others workers in our laboratory are currently investigating this area.

Part II

In this part, the developments in the area of the so-called dimerization reaction will be discussed. Several different approaches have been investigated and an attempt will be made to present them in a concise and logical sequence. As is the nature of research of any kind at this level, several other new and exciting approaches remain to be explored and, at the end of this section, these will be summarized and their relative merits and disadvantages discussed. Some of the earlier chemistry in the area of indole alkaloids bears strongly on the complexion of this work. Thus it may be useful to discuss it here.

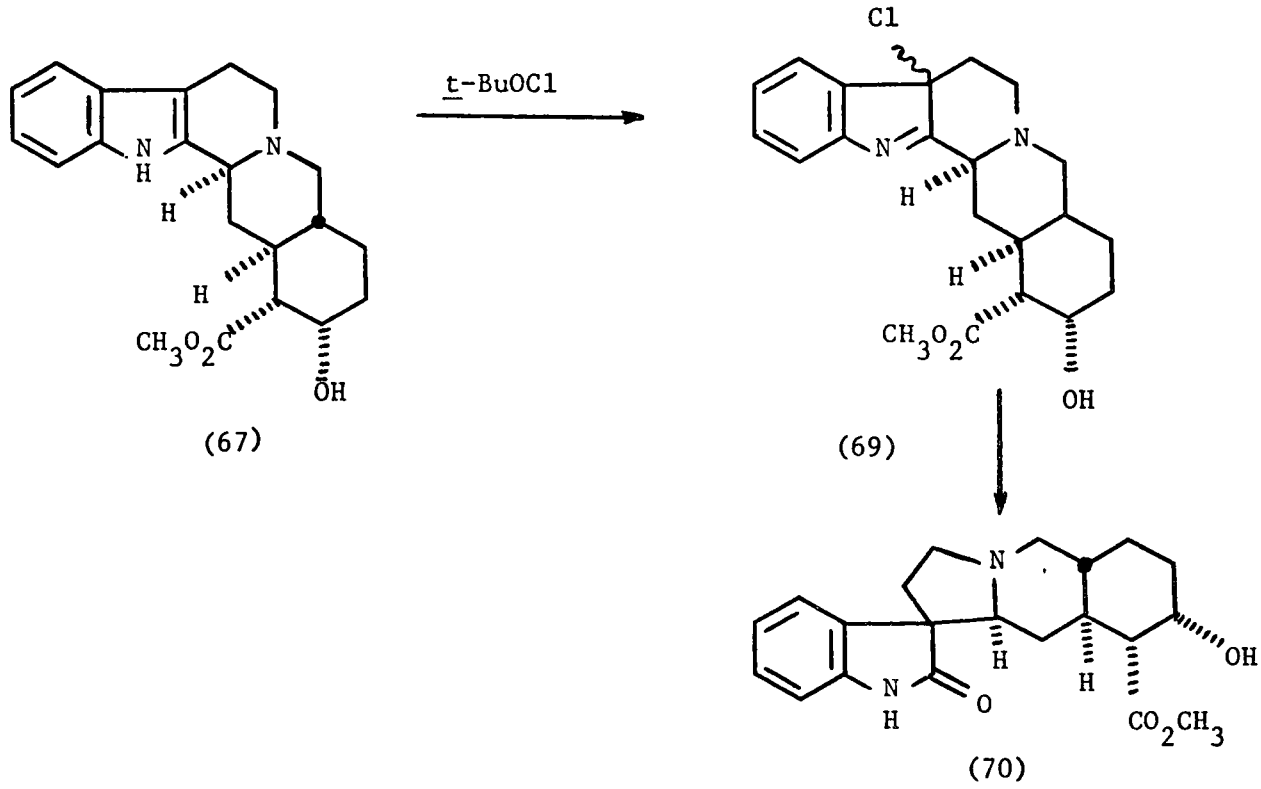
In 1956 W.O. Godtfredsen and S. Vangedal[81] reported that treatment of yohimbine (67) and related alkaloids with tertiary butyl hypochlorite led cleanly to a compound having the following properties:

(1) It had no indole-N-H absorption in its infrared spectrum (around 3440 cm^{-1}).

(2) It had none of the usual oxidizing properties of an N-chloro-compound.

(3) Hydrogenation over platinum in ethylene glycol monomethyl ether regenerated the parent compound.

It was some time before Finch and Taylor[95] assigned the structure of the chloroindolenine (69) to this compound and were able to show that it rearranged upon treatment with aqueous acid or base, followed by hydrolysis of the intermediate, to the oxindole (70). This was shown to be a general reaction for most indole containing compounds. With this observation the chloroindolenine chemistry of indoles was



born and it has grown to be one of the more important and synthetically useful reactions in this field. It was also shown that other indolenines such as the hydroxyindolenines[96] could be formed; and that they reacted in an exactly analogous way to chloroindolenines when treated with acids or bases.

The chloroindolenine formation reaction has found application in the synthesis of 2-acylindoles.[97] Dolby and coworkers converted the tetracyclic amine (71) to the corresponding chloroindolenine (72) by standard methods. A mixture of separable epimers about the carbon-chlorine bond was obtained. These compounds were converted to the quaternary methiodide salts (73) and each in turn was treated with sodium acetate in aqueous ethanol. Hydrolysis of the product resulted, in each case, in the isolation of two compounds (74) and (75), which were shown not to be interconvertible. A "reasonable mechanistic scheme" to rationalize the formation of these compounds was presented and it is summarized in Figure 10.

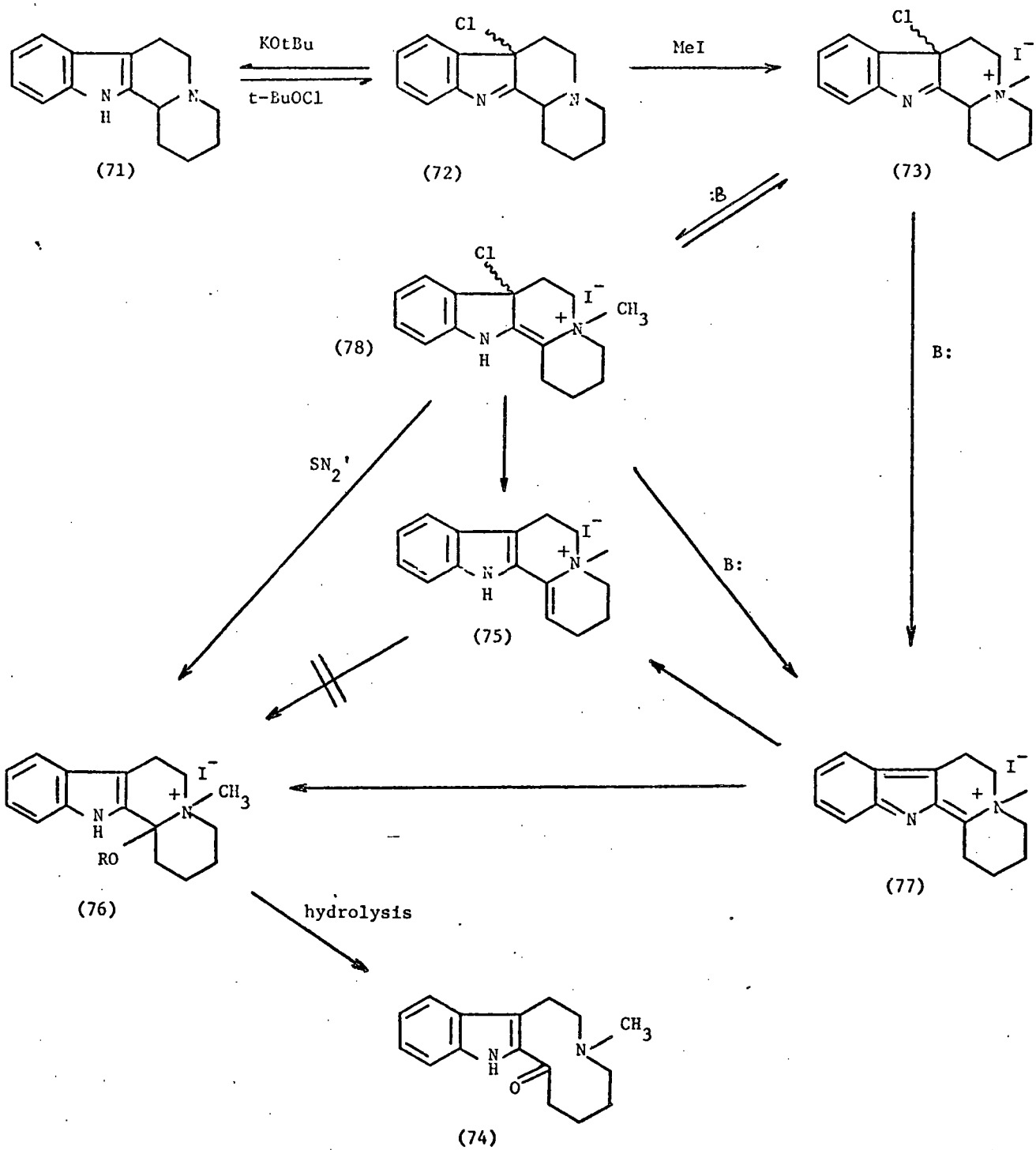
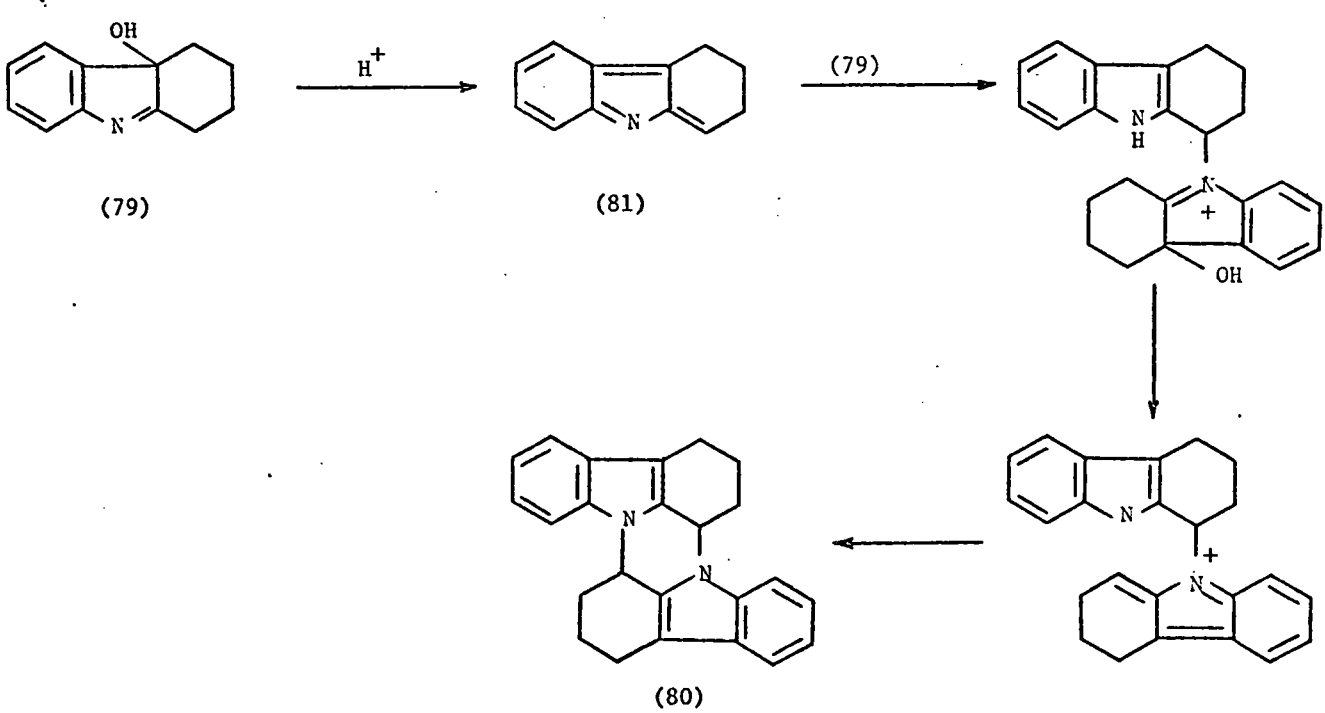


Figure 10. Dolby's mechanism for the formation of the 2-acylindole (74).

It was pointed out that this proposal was in agreement with the general mechanistic scheme of Taylor.[79] Further, it was stated that intermediate 77 was an attractive one because the alternative pathway of concerted SN_2' displacement on compound 78 must involve nucleophilic attack on a tertiary centre. Allylic rearrangement of this compound *via* the carbonium ion intermediate was unlikely in this case because the carbonium ion would be a doubly charged species with adjacent positive charges in one of the possible resonance contributors. The ion 77 is an interesting one since similar ions have been postulated by several workers in this area.

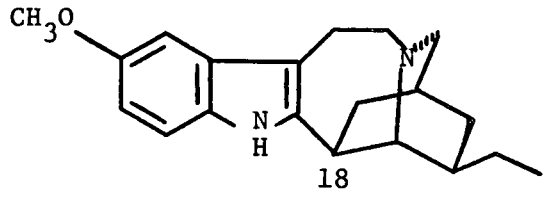
Another application of this reaction was made by Buchi and Manning.[98] Following the report of Patrick and Witkop[96] that 11-hydroxytetrahydrocarbazolenine (79) was converted to the dimer (80) by treatment with an acid they reasoned that this conversion had probably occurred *via* the intermediate imine (81) as shown in Figure 11.



(80)

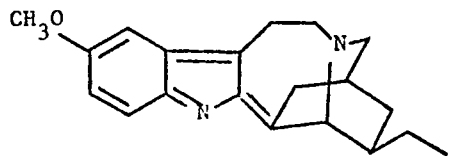
Figure 11. Proposed path for the formation of 80 from 79.

Application of this type of reaction to ibogaine (82) allowed them to introduce such functionalities as hydroxyl, methoxyl and nitrile into the C_{18} position. Thus treatment of the parent base (82) with t-butyl hypochlorite in solution resulted in the formation of the chloroindolenine in excellent yield. Exposure of the chloroindolenine to a solution of potassium cyanide in methanol resulted in a good overall conversion to the corresponding 18-cyano-ibogaine. Hydrolysis and esterification of this last compound led to the synthesis of voacangine (compound 82 bearing a carbomethoxy group at C_{18}): a natural product. Similarly, treatment of the chloroindolenine with methanolic



(82)

hydrogen chloride resulted in a conversion to 18-methoxy-ibogaine whereas treatment with dilute aqueous hydrochloric acid resulted in the corresponding 18-hydroxy-compound. The latter could be converted to the methoxy compound by treatment with acidic methanol and the conversion of the methoxy- to the hydroxy-compound was achieved when the former was exposed to aqueous mineral acid. These interconversions were all considered to proceed via the ubiquitous imine (83), analogous to compounds 77 and 81 above, or its conjugate acid. In the pentacyclic iboga skeleton this ion has much built-in strain, and so the alternative delocalized ion (84) was also proposed here as an intermediate.



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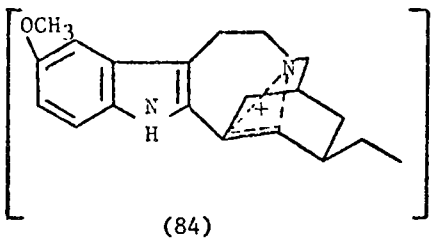


(84)

This approach was later utilized by Buchi et al.[68] to introduce an ester function at the C_{18} position during a total synthesis of catharanthine (11). In our laboratories, this approach has been improved and extended to provide an entry into the cleavamine skeleton bearing a carbomethoxy group at C_{18}.[61,87] Thus formation of the chloroindolenine of 4β-dihydrocleavamine (31) in the usual way, followed by introduction of a nitrile resulted in 18-cyano-4β-dihydrocleavamine which could be hydrolyzed and esterified to 18-carbomethoxy-4β-dihydrocleavamines (33) and (34); (see Figure 3). An analogous sequence in the cleavamine series[64] led to a total synthesis of 18β-carbomethoxycleavamine, and in turn, completed the total synthesis of catharanthine (11) (see Figure 4).

In summary, the indoles can be smoothly converted to indolenines which can react further in one of two ways when exposed to hydroxylic solvents under acidic conditions. These are shown in Figure 12. The products are those of substitution on one hand and rearrangement on the other. It has been proposed[98] that pathway A is preferred when it is sterically possible to accommodate the leaving chloride and the incoming nucleophile cis to one another, since this is the necessary

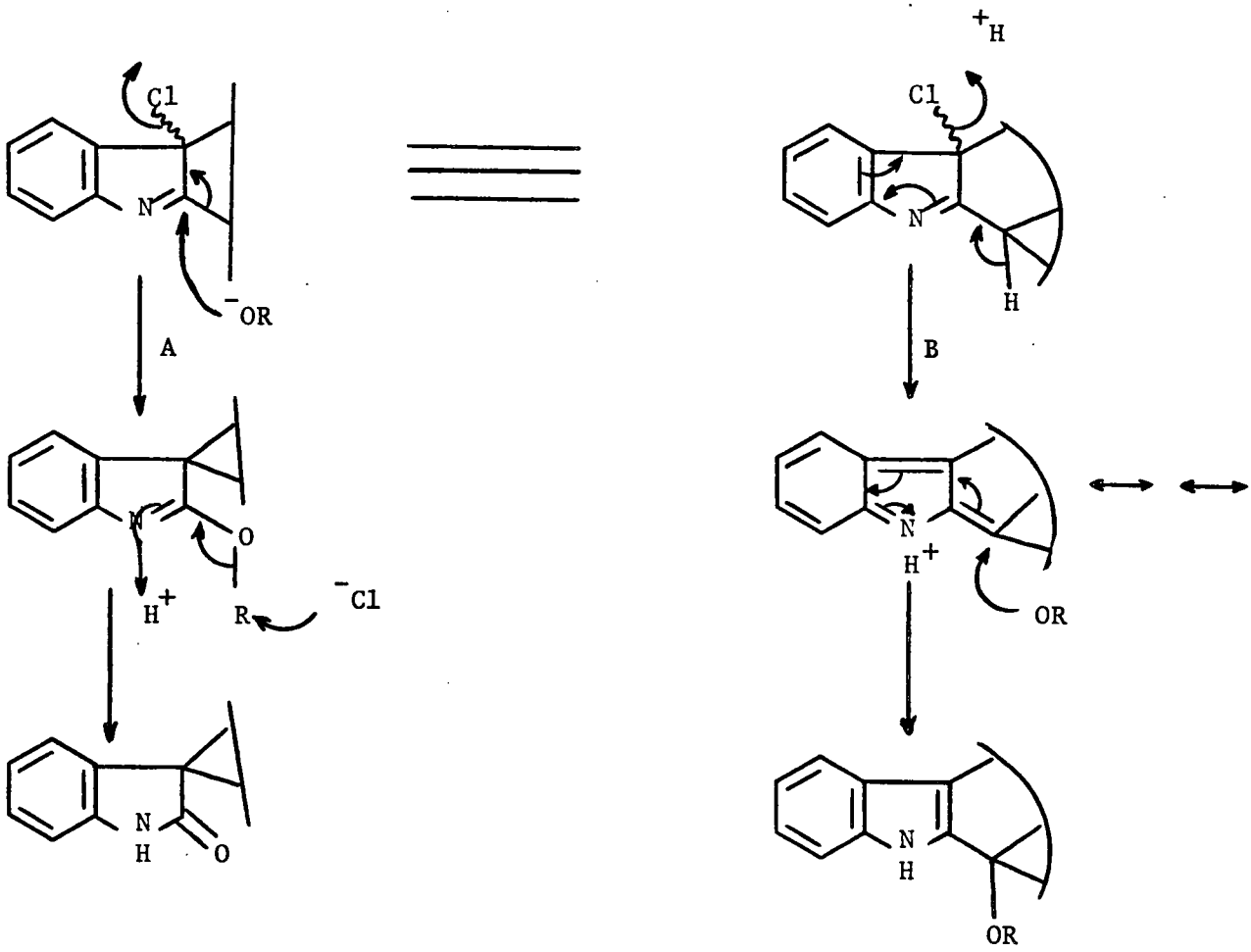
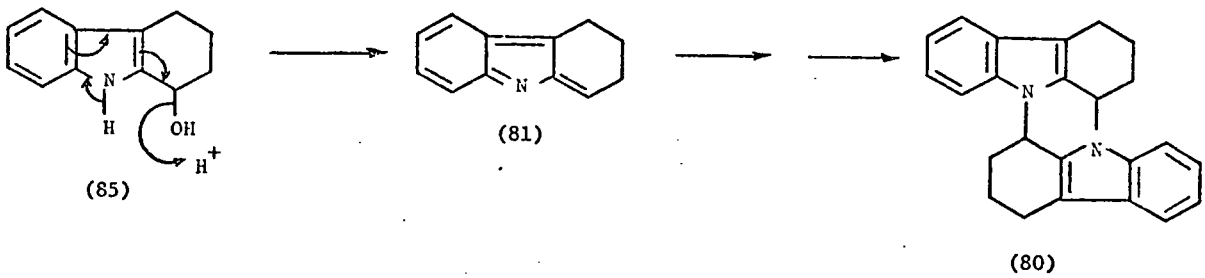


Figure 12. Pathways of reaction of indolenines.

condition for the concerted, stereospecific rearrangement postulated. Indeed in several cases where this rearrangement occurs, it has been shown to be completely concerted and stereospecific. Whenever the above condition cannot be met, a stepwise elimination of HCl followed by a nucleophilic substitution occurs as shown in path B.

Turning our attention now to another aspect of the "dimerization reaction", it had been reported[99] that the natural dimers could be cleaved into their corresponding monomeric templates by treatment with acids in the presence of reducing agents such as stannous chloride and tin. Indeed this cleavage reaction has played an important role in the structural elucidations of natural[10,13,88] as well as synthetic[70] dimers. The mechanism for this cleavage reaction was not fully understood. Part of the work in this section of the thesis provides some clarification of the cleavage reaction and has important mechanistic overtones for the so-called dimerization reaction. These are discussed at a later stage. Buchi and coworkers[100] reasoned that a dimerization between two halves simply involved a formal reversal of the above cleavage reaction. Previously, Plant and coworkers[101] had reported that the dimerization of compound 85 to the dimer (80) under acidic conditions occurred with ease. The mechanism of this reaction was believed to be closely parallel to that shown in Figure 11 and indeed the same intermediate (81) was viewed as being responsible for this conversion in both cases. Along these lines, Buchi and coworkers treated dregamine (86) with sodium borohydride and obtained dregaminol (87) stereoselectively in 80% yield. This compound is analogous to compound 85 above and it was felt that it could be dimerized with voacangine (88) under acidic conditions by creating a



(85) (81) (80)

similar ion. Indeed treatment of dregaminol (87) in methanol containing 1% hydrogen chloride with one equivalent of voacangine (88) resulted in a 50% yield of dihydrovoacamine (89),[102] identical with authentic material obtained from the hydrogenation of naturally available voacamine (90). The only other reported dimeric product from this reaction was a positional isomer of dihydrovoacamine, namely dihydrovoacamidine (91). This sequence is summarized below in Figure 13. The reaction was extended to the synthesis of other members of this family of dimeric indole alkaloids by the use of suitably functionalized templates with the same skeletal features. In each case, in agreement with the above scheme, only one epimer of the C_{13} junction and one epimer of the C_{11} junction was isolable. The stereoselectivity of these reactions, and that of the borohydride reduction step, may have been due to the severe steric strain associated with the approach of a nucleophile from the same side as the carbomethoxy group-bearing bridge.

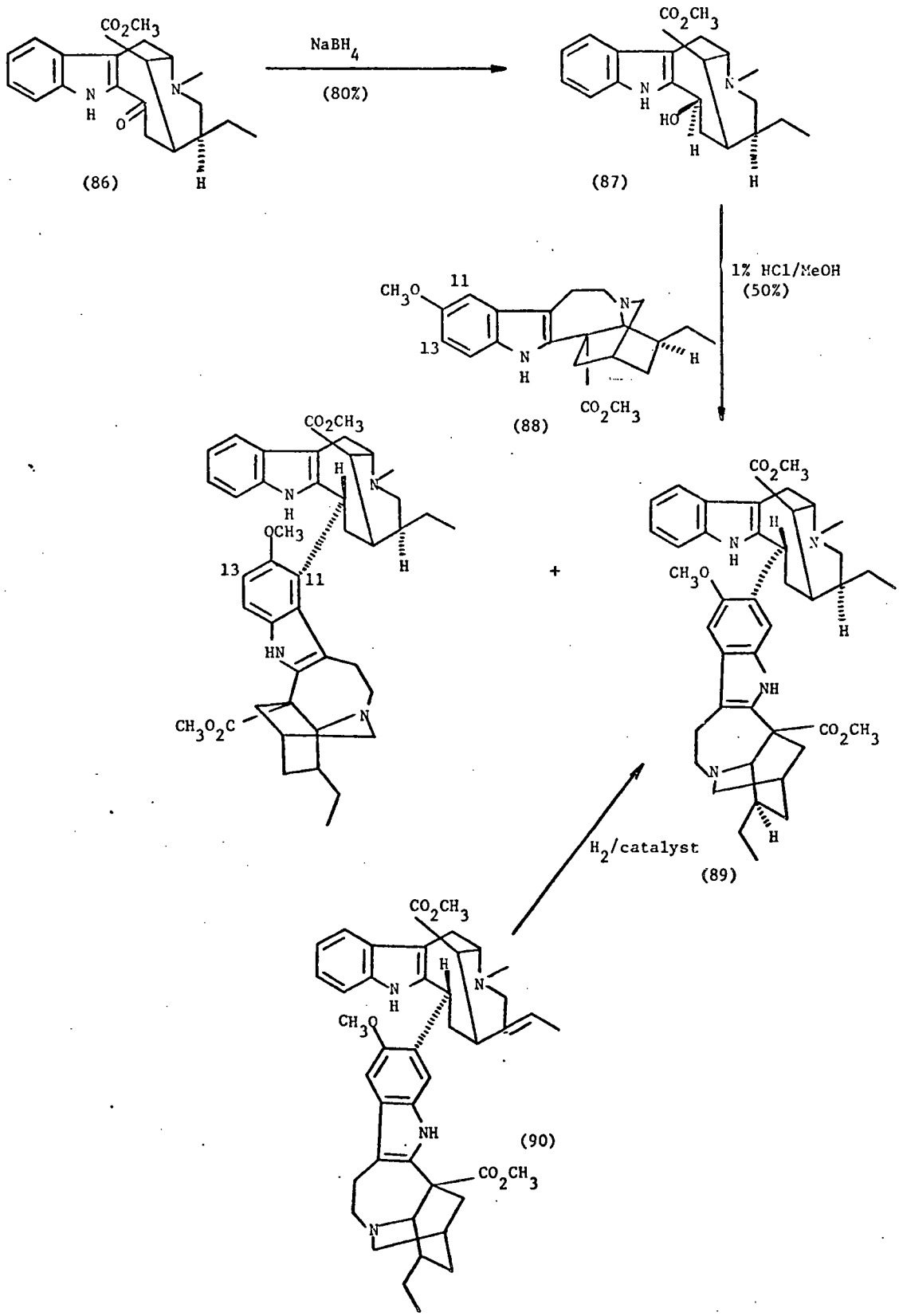
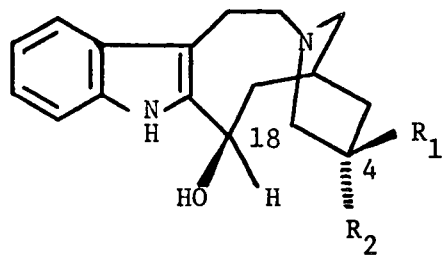


Figure 13. Buchi's partial synthesis of dimeric Voacanga alkaloids.

In an analogous study Harley-Mason and Rahman[103] reported the synthesis of two epimeric 18-hydroxy-dihydrocleavamines (92 and 93), epimeric about the C_4 position only.



R_1= $-CH_2CH_3$ R_2=H (92)
R_1=H R_2=CH_2CH_3 (93)

When the mixture of these two epimers was treated with one equivalent of vindoline (10) in methanol containing 1% hydrogen chloride; two separable dimers could be isolated. Had the dimerization not been stereoselective, each C_4, isomer would have yielded two C_{18}, epimers and thus four diastereomeric compounds would be expected. The complete stereoselectivity is more difficult to explain in this case than in the previous case of voacamine (90). Indeed a planar, long-lived carbonium ion appears quite unlikely in both cases.

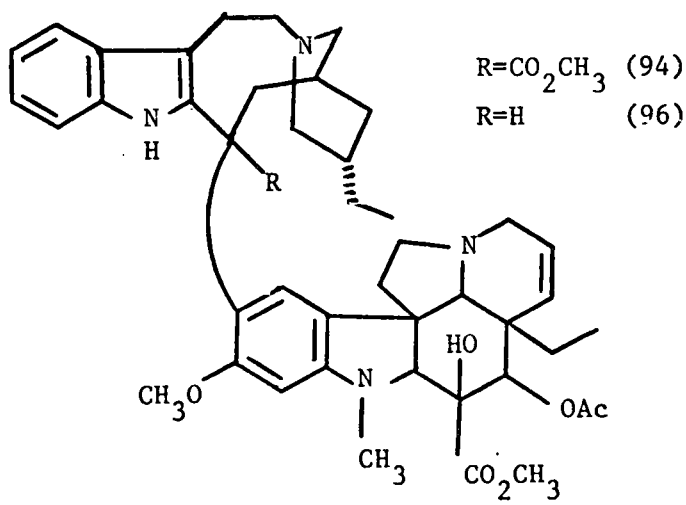
In 1968, in an attempt to elucidate the structures of two naturally derived compounds designated deoxy VLB'A' and deoxy VLB'B', the Lilly group carried out an interesting series of transformations.[71] First, deoxy VLB'A', upon cleavage with concentrated hydrochloric acid using stannous chloride and tin, yielded deacetyl vindoline and 4α-dihydrocleavamine. Leurosine (18) ($C_{3',4'}$ epoxy dimer) could be treated with

Raney nickel in ethanol under reflux to yield an isomer - deoxy VLB'B' - which could be shown by an analogous cleavage reaction to contain the 4β-dihydrocleavamine moiety. Deoxy VLB'A' and 'B' were thus designated as C_4, ethyl epimers of each other. In an attempted partial synthesis of deoxy VLB'B', these workers transformed 4β-dihydrocleavamine to its chloroindolenine by standard methods. Treatment of this compound with vindoline in 1.5% methanolic hydrogen chloride resulted in the isolation of a single dimeric compound. This product was a third compound - deoxy VLB'C'. The conversion of all three compounds to their decarbomethoxy - deacetyl hydrazides showed that decarbomethoxy-deacetyl-deoxy VLB'A' and 'B' hydrazides were not isomeric about $C_{18'}$ because their nmr spectra clearly showed the same chemical shift for the new $C_{18'}$ proton at about τ 6.5. On the other hand, decarbomethoxy-deacetyl-deoxy VLB'C' hydrazide was isomeric at $C_{18'}$ to both these compounds as evidenced by a displacement of its $C_{18'}$ proton by about 1 τ unit downfield to τ 5.5. Further, an attempted isomerization of any of these compounds in 1% methanolic hydrogen chloride in the deoxy VLB hydrazide series failed. The treatment of decarbomethoxy-deacetyl-VLB hydrazide itself, however, with 1.5% methanolic hydrogen chloride achieved epimerization to the corresponding $C_{18'}$ epimer. These conclusions were all based solely on the chemical shift of the $C_{18'}$ proton introduced during the hydrazide formation. It was thus impossible to decide which one of a pair of epimeric dimers possessed a stereochemistry at the critical $C_{18'}$ centre which was similar to that obtained in the natural dimers (this will henceforth be called the natural stereochemistry at $C_{18'}$ and the corresponding epimer will be designated as the unnatural stereochemistry).

A recent X-ray study, kindly performed by Professor J.C. Clardy[104] in collaboration with our laboratory, has been immensely useful in this and other areas. It will be discussed at a later stage.

In our laboratories it was discovered that the treatment of chloroindolenines of cleavamine-type molecules with vindoline in 1.5% methanolic hydrogen chloride resulted in a good overall conversion to dimeric material.[70] This reaction has come to be called "the dimerization reaction". At the time this research was initiated, what has been described above is all that was known regarding this dimerization reaction. Several important questions about the details of this reaction remained unanswered.

As has been mentioned previously, in two series (voacanga and VLB-type) the dimerization reaction involving, possibly, an imine of the type depicted in compounds 77, 81 or 83, had yielded, stereoselectively only one product in the hands of several workers. The coupling of 18-carbomethoxy-4β-dihydrocleavamine and 4β-dihydrocleavamine with vindoline in our own laboratory <u>via</u> their respective chloroindolenines had yielded in each case a single dimeric compound 94 and 96 respectively.



$R=CO_2CH_3$ (94)

R=H (96)

Before any other investigations could be performed, it was imperative to determine if an epimeric dimer was present at all in these reaction mixtures. An exhaustive search of the reaction mixtures from the above dimerizations was carried out. One hundred percent by weight of the reaction mixture was accounted for. All products, other than the dimers actually isolated previously and mentioned above, were found to be monomeric and could be classified as indole or dihydroindole on the basis of their ultraviolet spectra. Thus, the stereoselectivity of this reaction was established to be 100% in both cases, and the possibility that the desired dimers were being formed in either case, even in minute amounts, was ruled out.

Attention was now turned to an investigation of the generality of the dimerization reaction. In order to achieve a synthesis of the natural compounds it was clear that at some stage, the dimerization of 3,4-functionalized cleavamine templates would be necessary. Since such templates were already available from the work described in Part I of this Discussion, it seemed logical to use them in an attempt to test the generality of the dimerization conditions. The products would provide, for the first time, synthetic dimers bearing functionality at the same position as the natural compounds. As such they should be useful in the overall study of structure-activity relations in this series of drugs. In view of the work reported in Part I, the logical choices of indole templates for this investigation were 18β-carbomethoxycleavamine (29) and the 18-carbomethoxy-4β-dihydrocleavamin-3α-ols (56 and 57). These compounds were now readily available <u>via</u> the route already described and would serve well for a test of the generality of the so-called

dimerization reaction.

In our laboratories, in the past, several different chloroindolenines had been prepared from cleavamine-type compounds. In each case, these materials could be identified by the following properties:

(a) In the infrared spectra of these compounds the sharp absorption at about 3440 cm^{-1} attributable to the indole N-H stretch of the parent compound had disappeared.

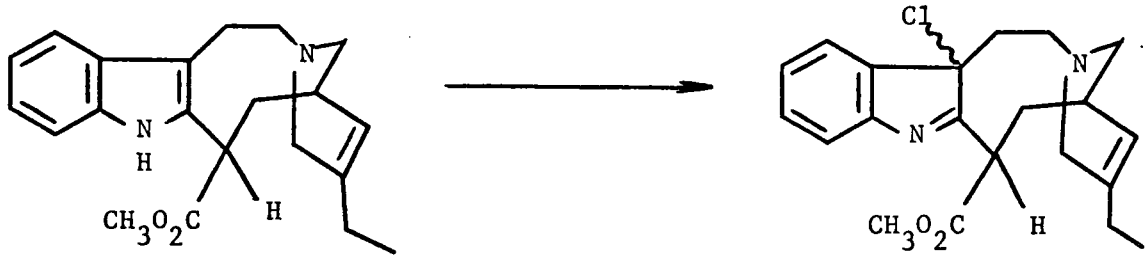
(b) The ultraviolet spectra of these compounds showed a change from the normal indole absorptions at 293, 285, 278 and 226 nm (log ε 3.8, 3.9, 3.8 and 4.5 respectively) to 227, 260 and 303 nm (log ε 4.3, 3.5 and 3.4 respectively) when these spectra were measured in isooctane solution. These changes were rather subtle, and could only be observed when the spectra were run in isooctane.

(c) When developed on an alumina tlc plate using the solvent system amenable to the detection of the parent compound, they appeared always as slightly more polar spots. These compounds gave a remarkably bright pink color when sprayed with ceric sulfate whereas the parent compounds were a light, almost invisible blue. Upon spraying another similarly developed plate with antimony pentachloride in carbon tetrachloride the parent base appeared as an intense brown or green spot whereas the chloroindolenine was undeveloped.

These three methods, were sensitive and provided a convenient method for monitoring the progress of a chloroindolenine-forming reaction.

Proceeding, in a logical fashion then, the chloroindolenine of 18ß-carbomethoxycleavamine (98) was prepared. The reaction was performed in the usual way by reacting a dilute solution of t-butyl hypochlorite

in carbon tetrachloride at low temperature, slowly, with a solution of 18β-carbomethoxycleavamine (29) in methylene chloride. The reaction proceeded almost as a titration and upon checking the reaction mixture by the tlc technique described above, it was estimated to have proceeded in greater than 95% yield. The material could be purified to yield an analytical sample by column chromatography on alumina. This material could be shown to have all the spectral and physical properties in accord with its postulated structure as the chloroindolenine of 18β-carbomethoxycleavamine (98). This purification of the chloroindolenine



(29) (98)

however, proved to be a costly one. Only a 30% recovery of the pure chloroindolenine could be realized together with a large amount of other new decomposition products in subsequent fractions. Thus, after the initial characterization it was resolved to react the "crude" (but usually greater than 90% pure) chloroindolenine directly in the next step. The above result was not entirely an expected one. t-Butyl hypochlorite is a powerful source of "positive" halogen and as such, is a strong electrophile. Thus, it may have reacted with the 3,4 double bond instead of, or as well as, the indole moiety. The complete and instantaneous reaction at the indole, to form the chloroindolenine, is a testimonial of the extreme reactivity of this chromophore towards such

reagents.

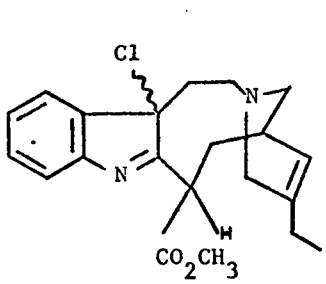
The achievement of chlorination of 18-carbomethoxycleavamine, was encouraging and an exploration of whether dimerization would also occur under the usual conditions was undertaken. The chloroindolenine (98) was prepared as above. This time however, when the reaction was deemed to be complete, by the various criteria described above, it was worked up by simply evaporating the solvents at 0°C under high vacuum to yield a foam. To this foam, one equivalent of vindoline was added followed by a solution of 1.5% hydrogen chloride in methanol. The reaction mixture was then refluxed for three hours. However, workup, followed by a check of the crude reaction mixture by tlc, afforded the rather disappointing evidence that there was no detectable dimeric material. Starting materials of both halves were easily identified by their R_f values and characteristic color-reactions. Deacetyl vindoline and vindoline accounted for virtually all of the dihydroindole type material. The only new compound in the reaction mixture had a characteristic blue color-reaction on tlc to antimony pentachloride in carbon tetrachloride. This material was purified and examined spectroscopically. Though its structure is as yet unknown, no single piece of evidence supported the view that it was dimeric. Thus the ultraviolet spectrum of this compound was that of a dihydroindole. Since vindoline and deacetyl vindoline already accounted for all of the Aspidosperma portion it was clear that this substance was derived from the chloroindolenine. The nmr spectrum of this compound was also reminiscent of a C_{15} substituted Aspidosperma-type skeleton. However the mass spectrum lacked all of the characteristic fragmentation of the Aspidosperma alkaloids and showed a molecular ion peak at m/e = 370. Indeed, treatment of the

chloroindolenine (98) in absolute methanol with only a trace of acid as catalyst resulted in a conversion to this compound together with other decomposition products. Treatment of vindoline, on the other hand, in methanolic hydrogen chloride, resulted only in a conversion to a mixture of vindoline and deacetyl vindoline. The structure of the latter could be proved by comparing it with an authentic sample as well as by acetylating it under the usual conditions (acetic anhydride and pyridine at room temperature) to yield again, vindoline. Thus the origin of this blue spot was established. Though several schemes may be written for the derivation of the requisite skeleton from the chloroindolenine none of these was compatible with all the available evidence. Because of its non-dimeric nature, this particular compound was not studied in detail.

In spite of this rather discouraging initial result, it was felt that the dimerization should proceed, at least in part, and thus another reaction was performed. This time, instead of the usual purification by column chromatography on alumina, a separation on the basis of molecular size was attempted using gel permeation chromatography on Sephadex LH-20. Several advantages were anticipated by this deviation from the normal workup procedures. First, the packing material was completely inert, in the sense that no chemical reaction between the substrate and the packing material could be visualized. Secondly, a steady elution with only one solvent was required so that this solvent could be purified thoroughly before beginning the chromatography so as to rid it of any detrimental impurities. Third, the column was essentially indefinitely reusable. If an adequately efficient column and solvent were found, it could be repeatedly used for the routine

separation of all dimerization reaction mixtures. Finally, since the larger molecules were expected to be displaced through the gel first, the dimers would be quickly removed from contact with the column; thus correspondingly diminishing the chance of decomposition on the column.

By this technique, from a reaction mixture of 800 mg a sample of 60 mg of the dimer (99) of 18-carbomethoxycleavamine and vindoline could be isolated pure (10% yield). This proved to be the optimal yield that could be realized and various other attempted improvements failed to increase the amount of this compound formed in any dimerization reaction



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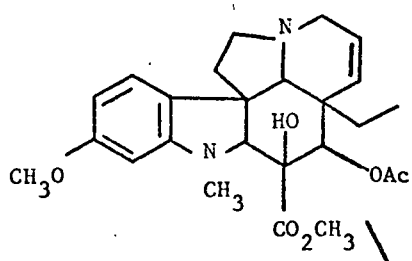

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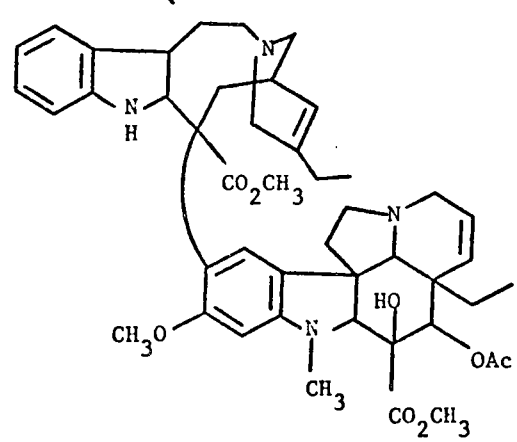


(99)

using these conditions. The ultraviolet spectrum of this compound was an exact summation of indole and dihydroindole absorptions and was almost completely superimposable on that of compounds 94 and 96 as well as the known natural dimers, VLB and VCR. Thus, the ultraviolet spectrum alone provided a strong support for the structure of 99. The nmr spectrum (Figure 14) of this compound similarly was exactly as expected on the basis of previous experience (see Figure 15 for the nmr spectrum of VLB and Figure 16 for that of compound 94). The position of dimerization was established as being between C_{15} on vindoline and C_{18} on 18-carbomethoxycleavamine by, (a) the disappearance of the signal attributable to the C_{18} proton in the starting material at τ 5.62 and (b) the disappearance of the doublet of doublets corresponding to the C_{15} proton of vindoline at τ 3.70 (J = 2.5 and 8 cps) (see Figure 17) and the accompanying collapse of the signals attributable to the protons at C_{14} and C_{17} to singlets due to a lack of ortho- and meta-coupling respectively.

The complete details of the structure of this compound were proved primarily in two ways. First, a sample of compound 99 was cleaved using 7% methanolic hydrogen chloride containing stannous chloride and tin. The products were compared on the basis of thin layer chromatography and infrared spectroscopy after isolation of each component. Cleavamine (21), vindoline (10), deacetyl vindoline, and starting dimer (99) were the only compounds isolated. Thus, there had been no unexpected rearrangements during dimerization, and compound 99 indeed did have the structure shown. It was now necessary to establish the stereochemical relationship at $C_{18'}$ in this compound to that in compound 94. To this

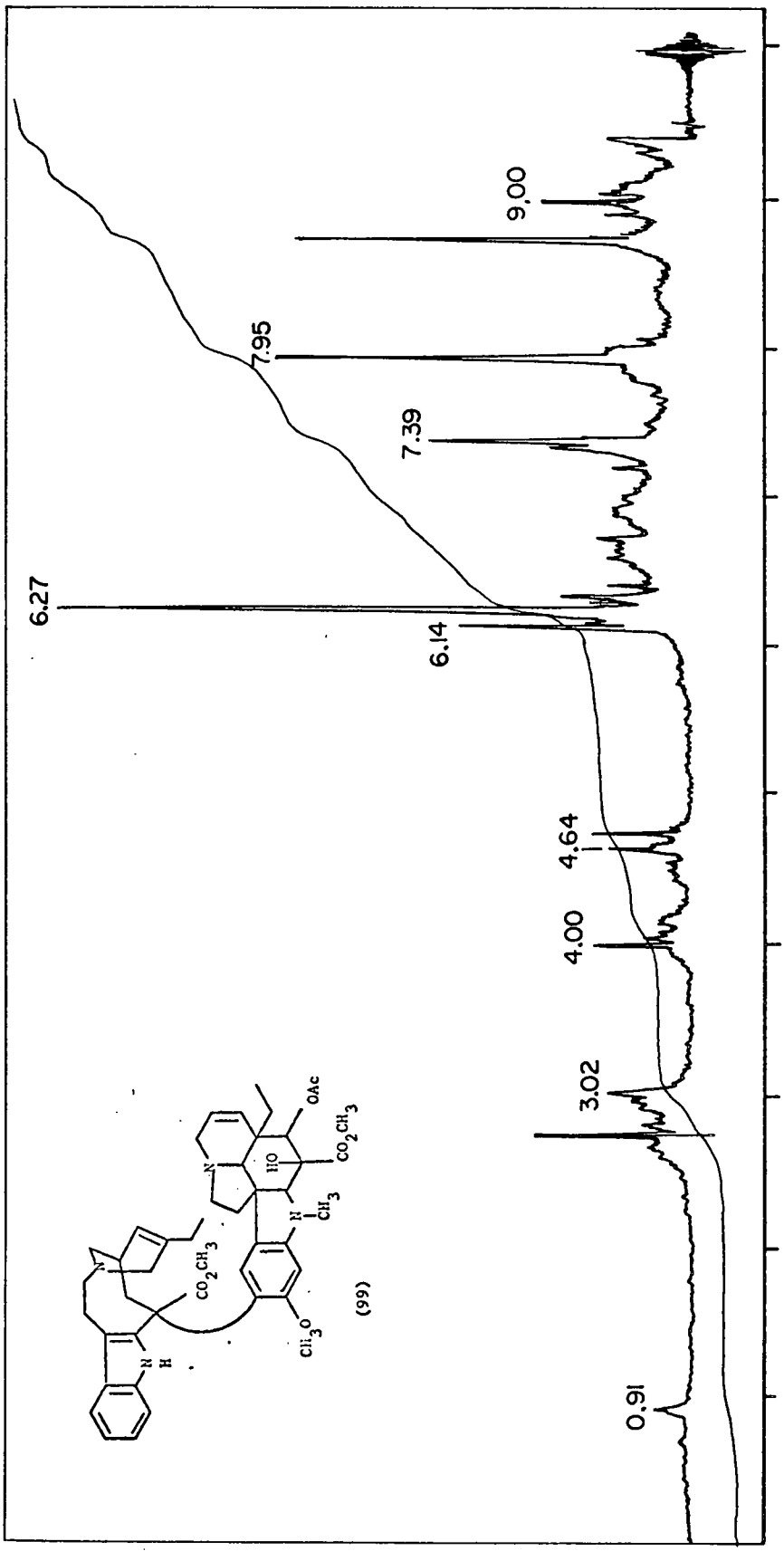


Figure 14. Nmr spectrum of dimer (99).

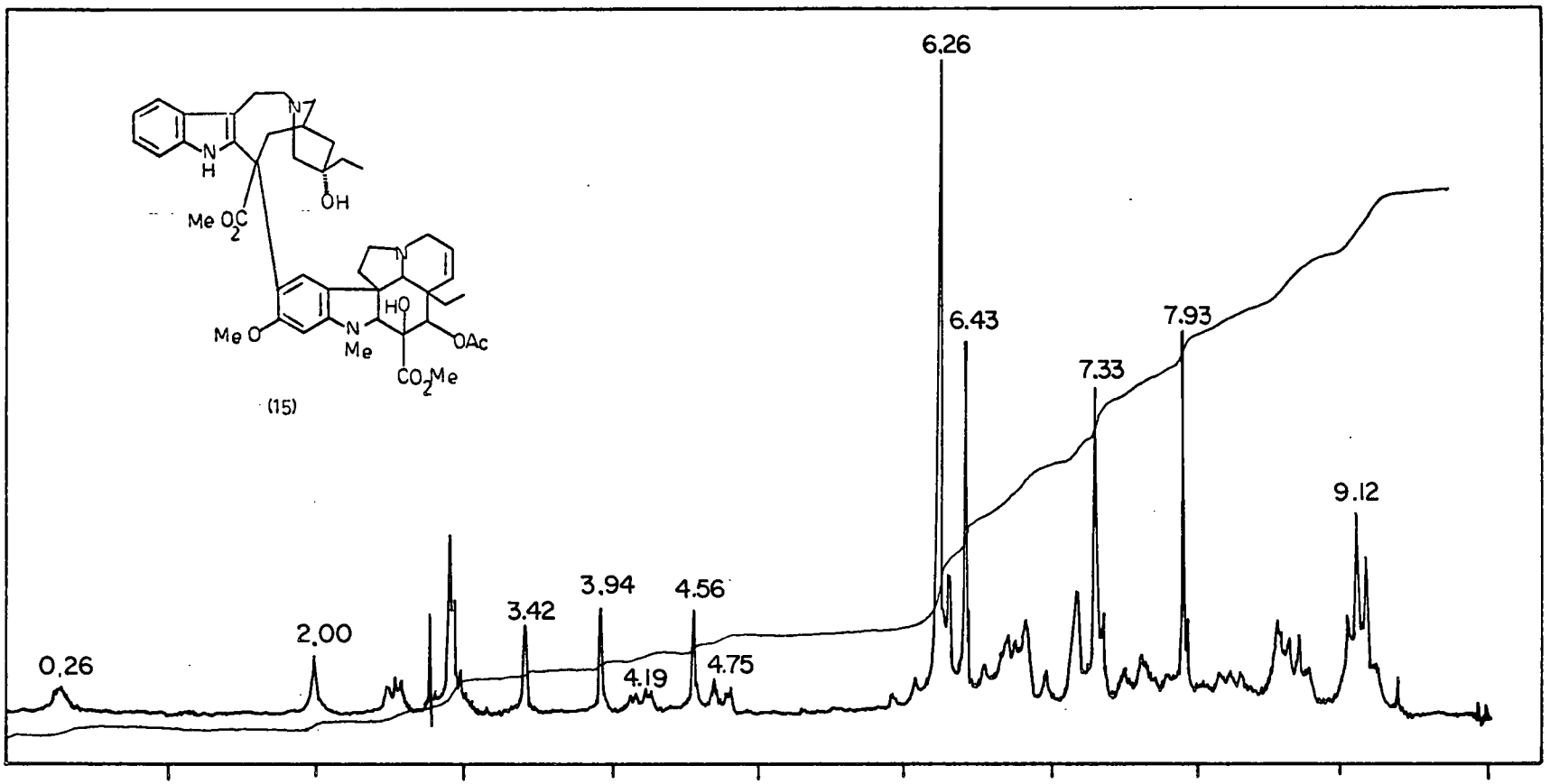


Figure 15. Nmr spectrum of vinblastine (VLB) (15).

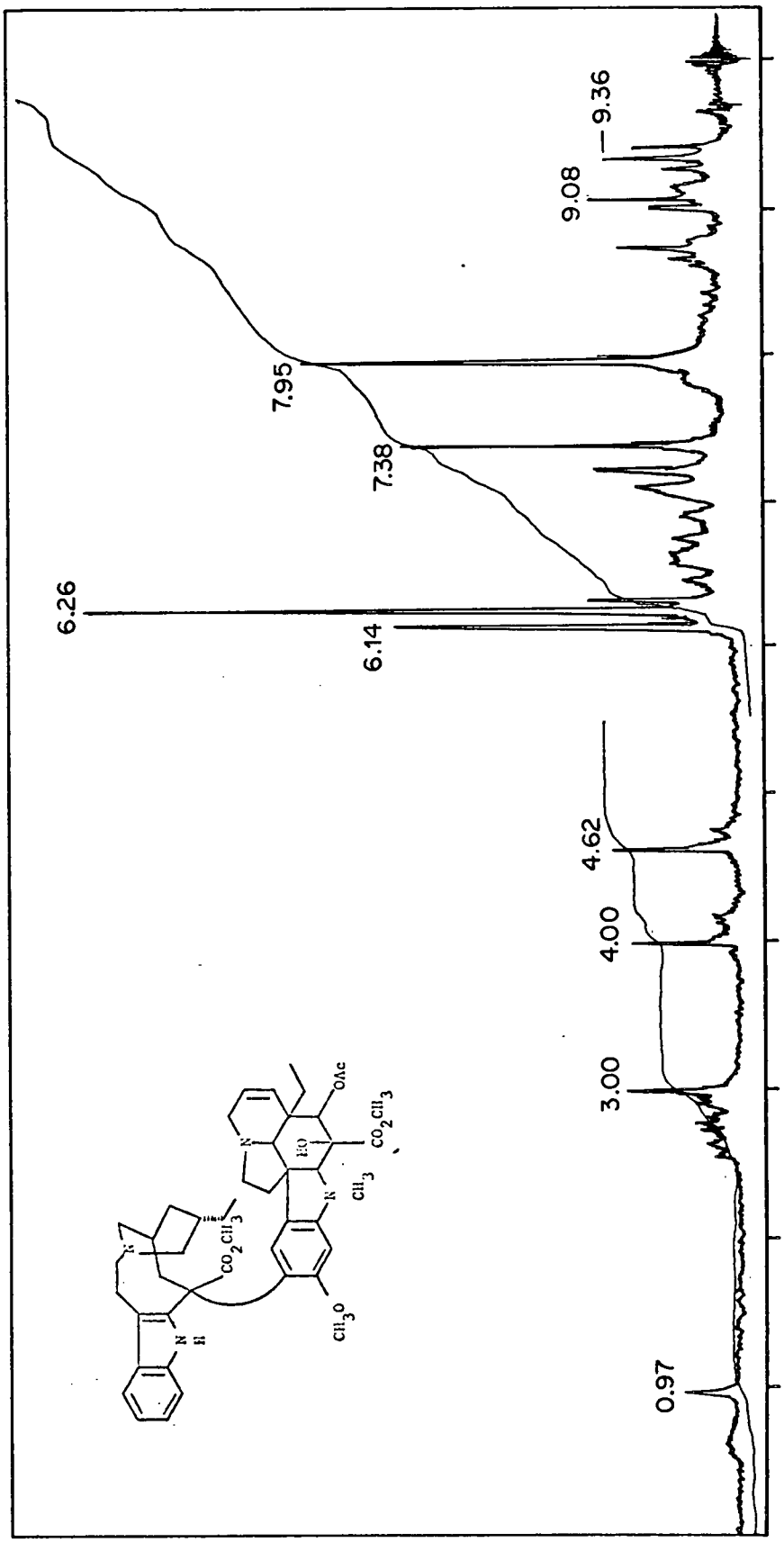


Figure 16. Nmr spectrum of dimer (94).

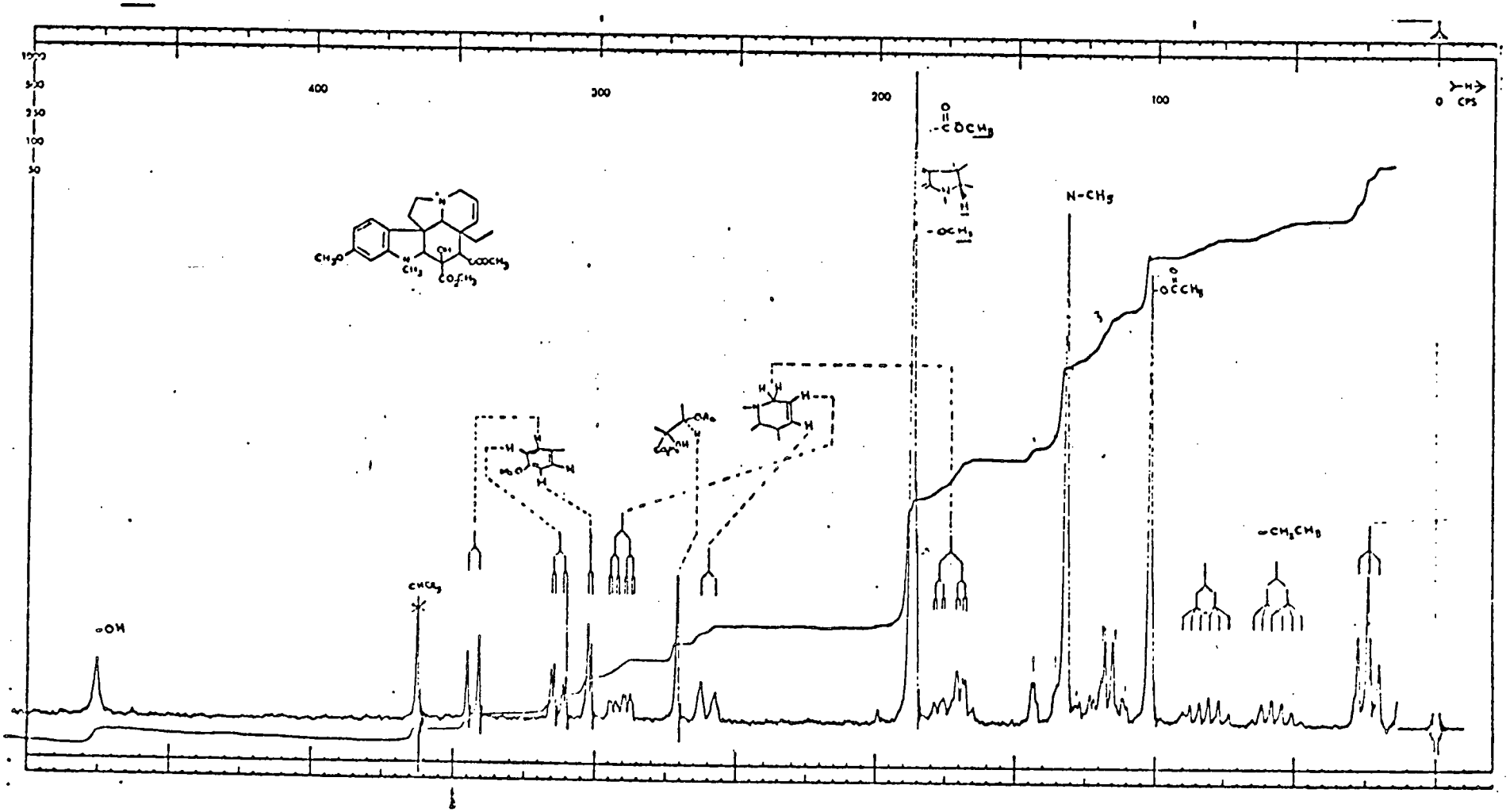


Figure 17. Nmr spectrum of vindoline (10).

end, a series of reactions culminating in a direct correlation of compounds 94 and 99 was executed.

Vindoline (10) was hydrogenated by standard methods to yield dihydrovindoline (100). Compound 100 was treated with the chloroindolenine (101) prepared from 18-carbomethoxy-4β-dihydrocleavamine (30), under the usual dimerization conditions to yield (in approximately 70% yield) the tetrahydro analogue (102) of compound 99. This compound was fully characterized by physical and spectroscopic methods, all of which were entirely in accordance with the postulated structure. Of particular value, in this case, was the nmr spectrum which is shown in Figure 18. Now compound 94 was hydrogenated in ethanol over Adam's catalyst on a quantitative basis. The uptake of one mole of hydrogen was noted and, upon workup, a dimer was isolated which was identical in every respect; including superimposable nmr, ir, and mass spectral fragmentation as well as undepressed mixed melting point; with compound 102 obtained above. The stereochemistry about the $C_{18'}$ position for compound 102 was thus unambiguously established as being the same as that in compound 94. Similar treatment of compound 99 yielded, again, a compound which was identical in every respect to compound 102 obtained by both of the above routes. This sequence is summarized in Figure 19 below and conclusively establishes the structure of compound 99 and 102 in every detail including the relative stereochemistry about $C_{18'}$. The latter was again shown to be the same as in compound 94 in both cases.

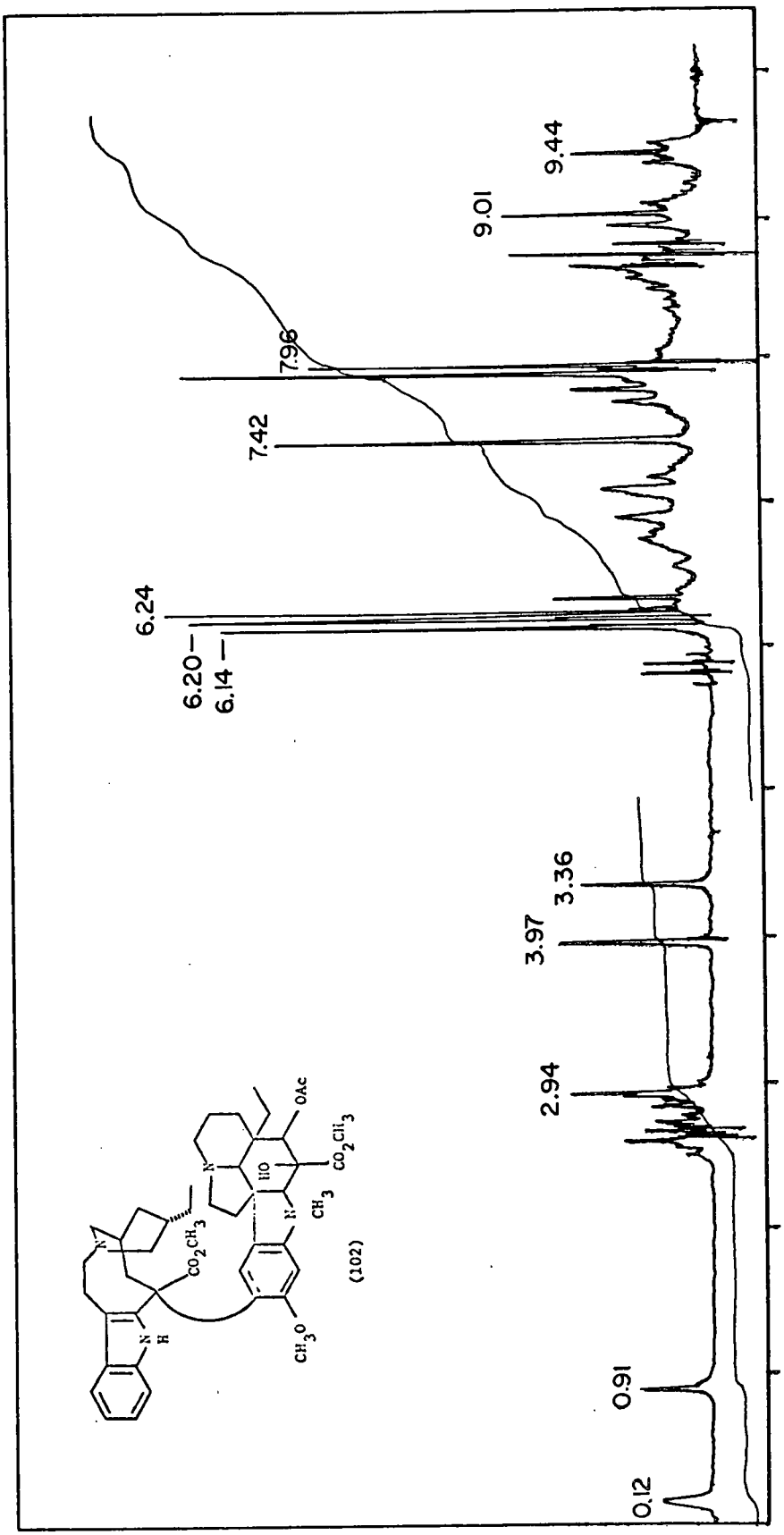


Figure 18. Nmr spectrum of dimer (102).

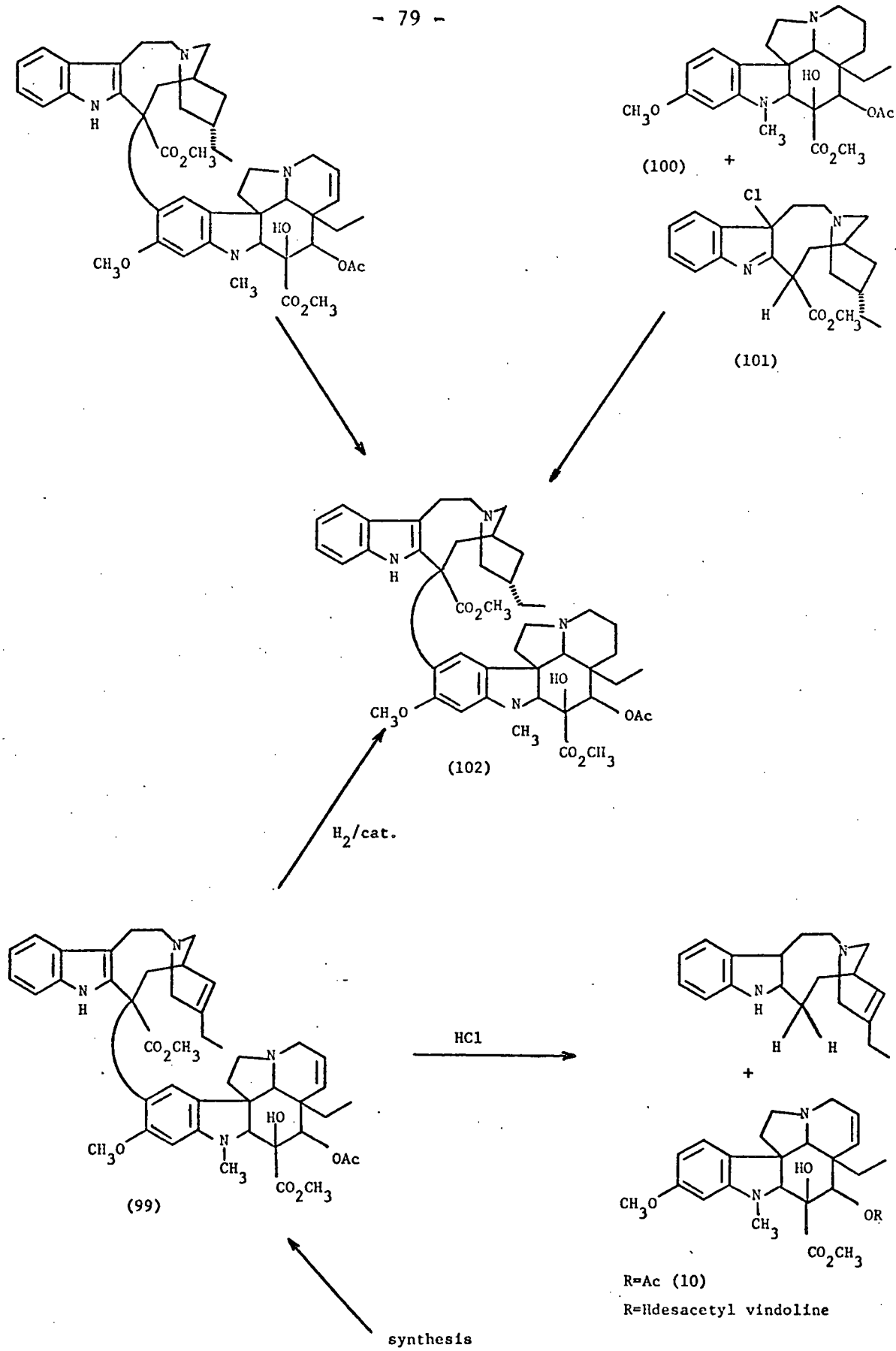
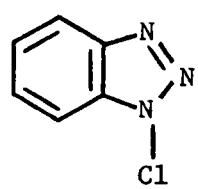


Figure 19. Proof of the structure of compound 99.

It should be pointed out that the conditions of hydrogenation used, namely using the free bases and absolute ethanol, caused severe losses of material resulting in a poor recovery of the product of any given dimer hydrogenation. This was undoubtedly due to the attachment of the amine portion of these dimers to the platinum catalyst. The poisoning of catalysts by amines is in fact a well-known phenomenon. Other groups of workers[105,106] have circumvented this problem by performing the hydrogenation of VLB in ethanol containing some hydrogen chloride. The formation of the hydrochloride salts blocks any possible interaction between the amine and the catalyst and makes the reaction synthetically useful.

The low yield of compound 99 obtained by the usual dimerization reaction sequence prompted a study of some other modifications of this procedure with the aim of increasing this yield. The first area to be investigated was the chloroindolenine formation. The use of 1-chlorobenzotriazole (103) as a mild and selective chlorinating agent for various indoles had been reported[107] and it was resolved to see if



(103)

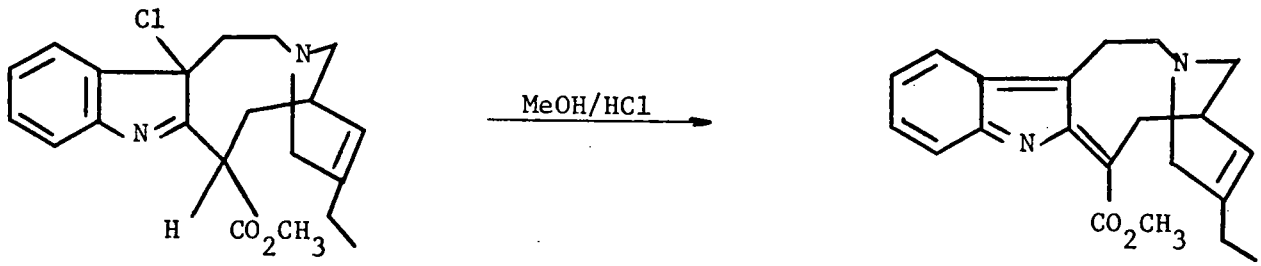
this agent could affect the yield of the dimer formed in the subsequent step. The chloroindolenine was prepared in the prescribed manner using this reagent, and after workup, was subjected to the usual dimerization in 1.5% methanolic hydrogen chloride. The yield of

isolated dimer was 8.0%. Thus, on the basis of the purity of the resulting chloroindolenine as well as the yield of the dimer formed, there was nothing to be gained in this case by using the newer reagent.

Since the problem of low yield seemed to centre on the dimerization reaction itself, it was decided to attempt, empirically, to find the optimum conditions for the formation of the desired dimer in this case. Variations of reaction temperature and time of reaction resulted in some improvements in the yield of the desired material. The optimum conditions were realized when a 1.5% methanolic hydrogen chloride solution of the reactants was stirred at room temperature for ten days. The resulting crude reaction mixture could then be purified to afford a 20% yield of dimer 99. The so-called blue spot was correspondingly diminished in amount in this case. A parallel standard dimerization reaction performed on the same sample of chloroindolenine afforded an 8% yield of the dimer and proved that this modification had indeed resulted in a genuine improvement of the overall yield of desired material. It should be pointed out that Rahman[108] has recently independently reported the synthesis of compound 99 by standard methods. No yields are quoted and no attempts to optimize reaction conditions were reported.

The presence of acid catalysts may interfere with the course of the dimerization reaction when the indole template bears some functionality at the 3,4 positions, by promoting elimination of oxygen functionalities for example, or rearrangements in the carbon skeleton itself. Indeed, such rearrangements must be involved in the formation of the so-called blue spot material mentioned above. If the acid catalyst could be replaced by some other agent that would function similarly, then a

substantial increase in the overall yield of dimers in such cases must result. By analogy to the reasoning of other workers mentioned previously, it was felt that the hydrogen chloride functions to protonate the chlorine atom of the chloroindolenine, thus initiating the formation of ion 104 which bears a strong resemblance to ions 77, 81 and 83. If some other, non-acidic material could be used to achieve



(98) (104)

this goal then the dimerization could essentially be performed under neutral conditions. The silver (I) ion is just such a material since it is expected to react irreversibly with chloride to precipitate silver chloride. Silver nitrate is a good source of silver(I) ions and the anionic species left in solution (NO_3^-) is not expected to be strongly nucleophilic in comparison to vindoline itself. Silver nitrate, however, proved to be insoluble in most organic solvents. Ethyl ether was found to dissolve significant amounts of the reactants as well as silver nitrate and the initial reaction was performed in this solvent. Unfortunately, even after several hours of reflux, no dimeric material could be detected. Several other attempts to induce this reaction to proceed by using various co-solvents such as dimethyl sulfoxide and triethylamine also failed. It appears that the ability of the silver ion to combine with the chlorine atom bound to carbon, and force cleavage

of the carbon-chlorine bond is extremely limited. This may be due to the steric bulk of the silver(I) ion. Indeed, the general concept of this approach may still prove to be a valid one. However, for the time being it was abandoned.

The attainment of a low overall yield of the desired dimer in the $\Delta^{3,4}$ compound could, at this stage, be interpreted in one of two ways. Either it was the result of some interactions peculiar to this compound alone and as such completely irrelevant to the general scheme; or it was the first indication that 3,4 functionalized indole templates may undergo different side reactions under the dimerization conditions. In order to cast some light on which one of these two alternatives was correct, a dimerization of the alcohols (56 and 57) was imperative.

18β-Carbomethoxy-4β-dihydrocleavamin-3α-ol (56) was treated in the usual way with t-butyl hypochlorite and the formation of the chloroindolenine was again monitored by the tlc technique mentioned above. Compound 56 showed the expected reactivity to this reagent and the chloroindolenine (105) was formed almost as soon as the reagent had been added. This crude chloroindolenine, which was greater than 80% pure, was not purified further but instead, was treated with one equivalent of vindoline which had been dissolved in methanol containing 1.5% hydrogen chloride. The resulting mixture was refluxed for three hours and then worked up and chromatographed on deactivated alumina to yield the desired dimer (106) in an overall yield of 8%. A similar reaction run simultaneously was purified by gel filtration using the same Sephadex LH-20 column mentioned above with methanol as eluent. In this case, an 18% overall yield of the same dimer (106) could be realized. This demonstrated that, in cases where the overall yield of dimeric

material was not high, sephadex chromatography did indeed possess the anticipated advantages over the more commonly used adsorbent.

A second, and perhaps more important, deduction could be made from the above experiments. In all the 3,4 functionalized cleavamine templates so far investigated, the yield of dimers, resulting from the conventional reaction conditions, was dramatically lower than in the 3,4 dihydro cases where the yield was usually approximately 50-70%. It could be shown, by an exactly analogous reaction sequence that the 18α-carbomethoxy alcohol (57) could also be dimerized in the same way to afford again, an overall yield of 15-20% of dimer 106. The identity of this dimer was proved by its uv and nmr spectra (Figure 20) in an analogous way to the dimer 99 in the 18-carbomethoxycleavamine case. Mass spectral data obtained were also entirely in accord with this structure and confirmed its dimeric nature (Figure 21). Cleavage of compound 106 under the usual conditions resulted in the isolation of dihydrocleavaminol, vindoline, and deacetyl vindoline which were identified by tlc and ir comparisons with the authentic materials, together with traces of the starting 18-carbomethoxydihydrocleavaminols (56 and 57) which were identified by tlc comparisons alone because of the small amounts of these materials present. An attempted dimerization of both the 18-carbomethoxy alcohols as their acetates in two separate reactions failed to yield any detectable dimeric material at all. There appears to be no truly satisfactory explanation of this observation although some explanations involving steric or conformational arguments may be offered.

In summary, it had been established that (a) in every case so far examined, the stereochemical outcome of the conventional dimerization

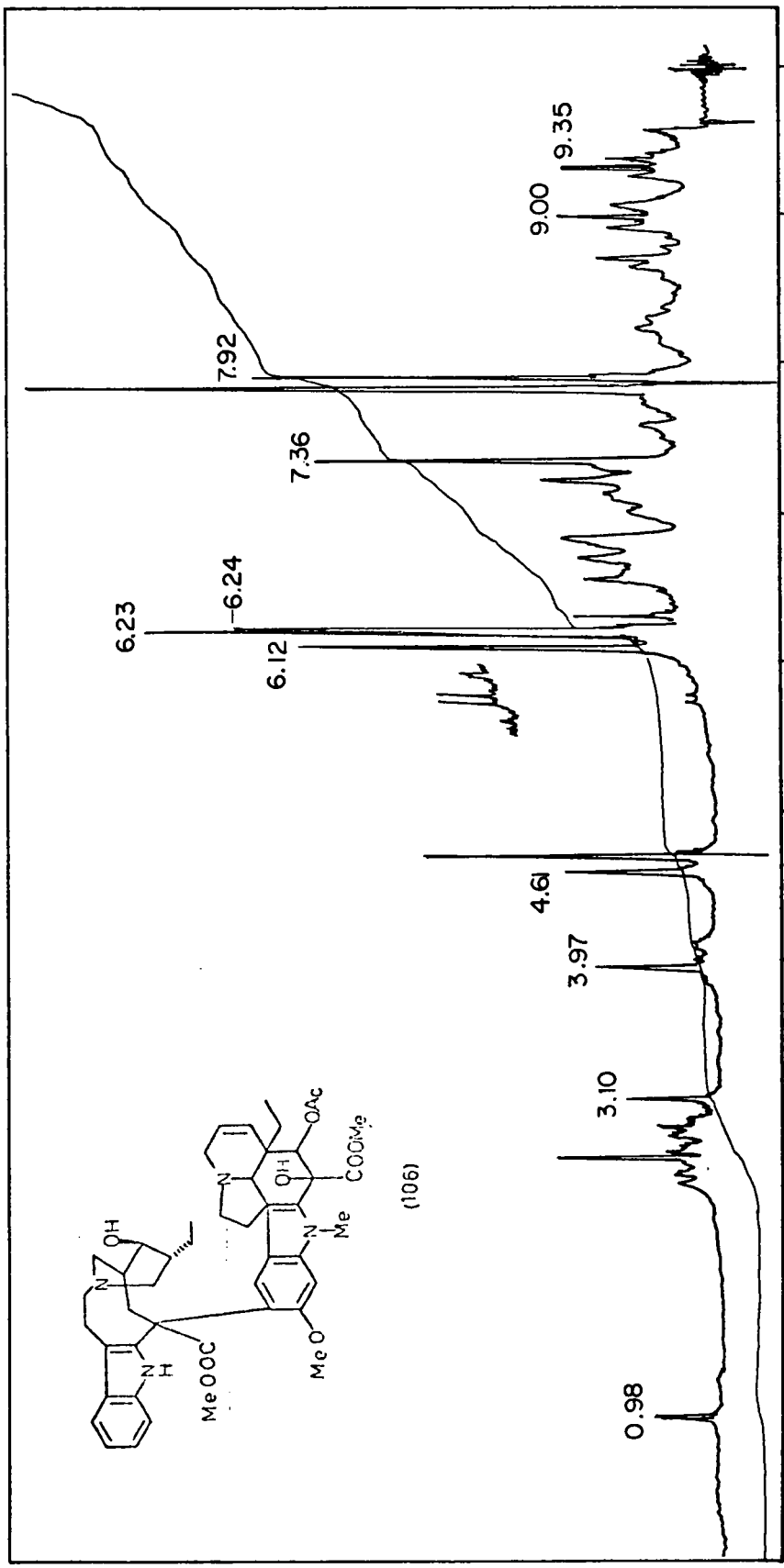
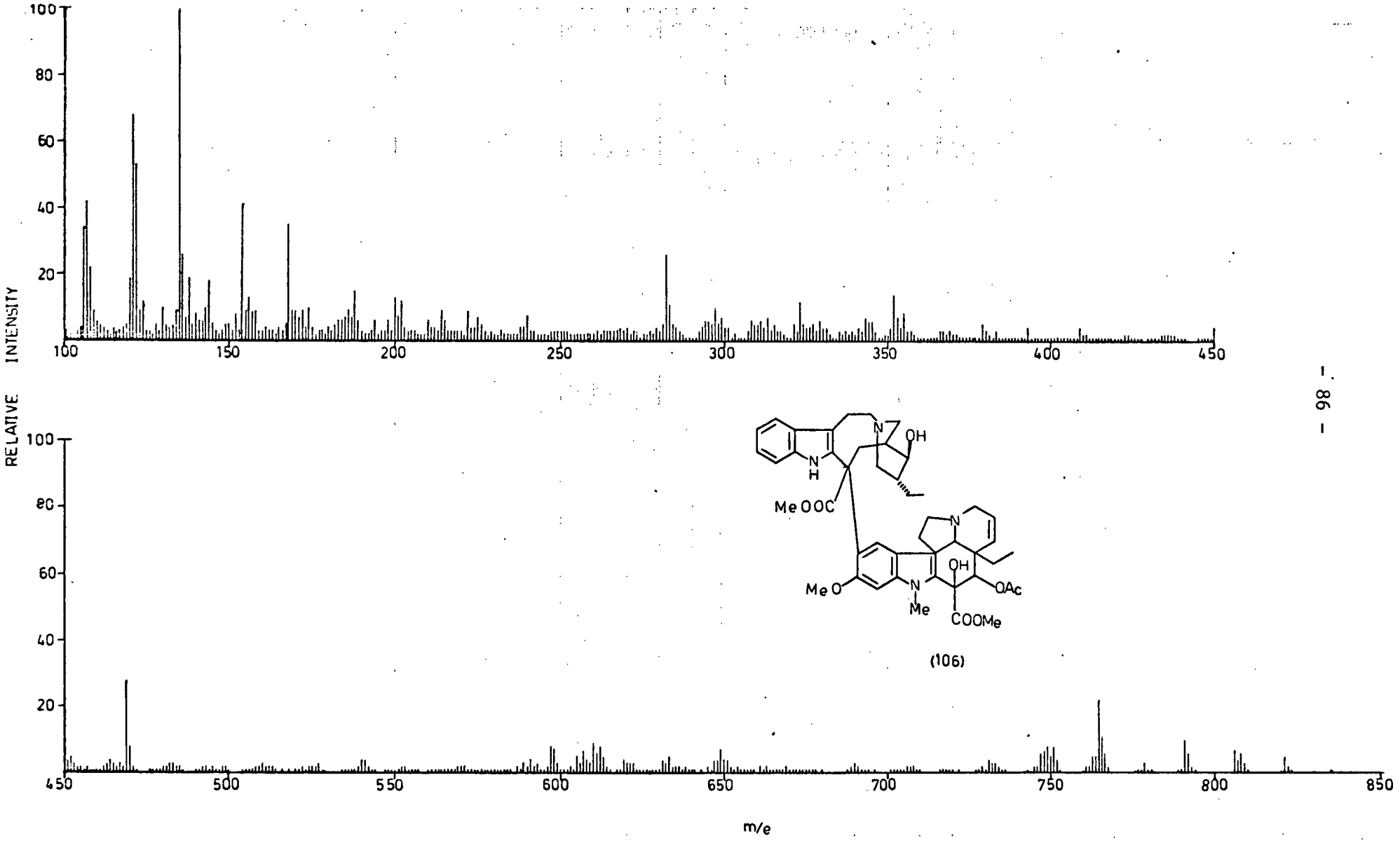


Figure 20. Nmr spectrum of dimer (106).



(106)

Figure 21. Mass spectrum of dimer (106).

reaction was such that only one epimer at $C_{18'}$ was produced entirely stereoselectively and (b) the 3,4 functionality of the type examined so far resulted in some complication in the normal reaction sequence so that the yield of dimer was dramatically reduced. It was resolved to investigate closely the so-called "conventional" dimerization technique in order to learn something about the details of the reaction itself as well as to investigate the conditions of optimum reaction in much greater detail than previously. It was felt that perhaps a study of the mechanism of this dimerization reaction combined with a thorough understanding of the effects of various factors such as temperature, solvents, etc. may be critical in the development of any new and better approaches to dimerization. The study of the synthetic dimers of several types had, furthermore, pointed to an unnatural stereochemistry at $C_{18'}$ in these compounds. The detailed and thorough study mentioned above may lead to an understanding of why this was the case and what could be done to change this situation. Dimers with an unnatural stereochemistry about $C_{18'}$ were not entirely undesirable since such compounds would prove useful for the overall study of structure-activity relationships. Indeed dimer 96, for example, has been recently found to possess a therapeutic index at least as good as, if not better than VCR (16).[109] This compound may soon compete successfully with the rare and expensive naturally derived drug as a clinical anti-cancer agent.

This study was visualized as serving a dual purpose: (a) If a method of dimerizing sensitive cleavamine templates under mild conditions in high yield could be found, these compounds would become available for the first time for clinical evaluation as synthetic analogs of the natural drugs. As such they would be expected to contribute to the

understanding of the structure-activity relationships in this series. (b) If the mechanism of this reaction could be sufficiently understood to enable changes in the overall stereoselectivity of the dimerization step so as to yield dimers with the alternative stereochemistry at $C_{18'}$, then a major breakthrough would surely have been achieved.

18β-Carbomethoxy-4β-dihydrocleavamine (107) was chosen as the substrate for this study for several reasons. First, it was a compound bearing the C_{18} carbomethoxy group required for the eventual synthesis of natural dimers. It could thus be used as an effective model for any study of the yield or stereochemical effects of any change in reaction conditions because it would possess all the functionality necessary at the critical centre ($C_{18'}$). Second, it lacked any complication in the sense that it possessed no 3,4 functionality. By using compound 107, for the time being one could concentrate on improving the reaction conditions in a case where the isolation of the dimeric product would not be unduly laborious. Finally, and perhaps most important of all, a direct X-ray structure was available for the product of the conventional dimerization in this series (vide infra) so that correlations between it and any new product or products, that may result, would be on a sound basis.

Since one of the goals of this study was to attempt to reduce, as far as possible, the stereoselectivity of the dimerization step, it was necessary to anticipate in advance the changes which would be induced in the various spectra of a dimer by a change in its stereochemistry at $C_{18'}$, in the $C_{18'}$, carbomethoxy dimer series. A comparison of the ir, nmr, and uv spectra of compound 94 with VLB (15)[84] (see

Figure 16 and 15 for these nmr spectra and Figure 22 for a uv comparison) showed that the degree of potential usefulness of any of these techniques for distinguishing between possible epimers at $C_{18'}$ was, in ascending order: uv < ir < nmr. In other words, the ultraviolet spectra of these two compounds were virtually superimposable with only a slight shift of the absorptions of one compound relative to those of the other. This shift could conceivably be diagnostic of a change in stereochemistry at $C_{18'}$ but it could just as easily be due to some other difference between these molecules. The infrared spectra similarly, were not informative with regard to this point because, as expected, the change in stereochemistry could not substantially alter the absorptions of so large and complex a molecule except in the fingerprint region (1430-910 cm^{-1}).

In contrast, the nmr spectral comparison showed small but definite differences particularly in the position of the aromatic C_{14} and C_{17} protons of vindoline which could only be rationalized in terms of the stereochemical difference about $C_{18'}$ between these two compounds. This was coupled with slight shifts in the position of the C_{16} methoxyl group of vindoline which would also be expected to be affected by changes at the $C_{18'}$ position. Furthermore, the ethyl-methyl of the vindoline C_5 position provided yet another difference between the two spectra. The protons mentioned above are all on the vindoline portion and since this portion is completely unchanged in both the compounds being compared these differences must represent a certain sensitivity of these protons to the stereochemistry at $C_{18'}$. The protons of the indole template were singularly uninformative. For example, the $C_{4'}$ ethyl-methyl remained remarkably constant throughout this series appearing at τ 9.13

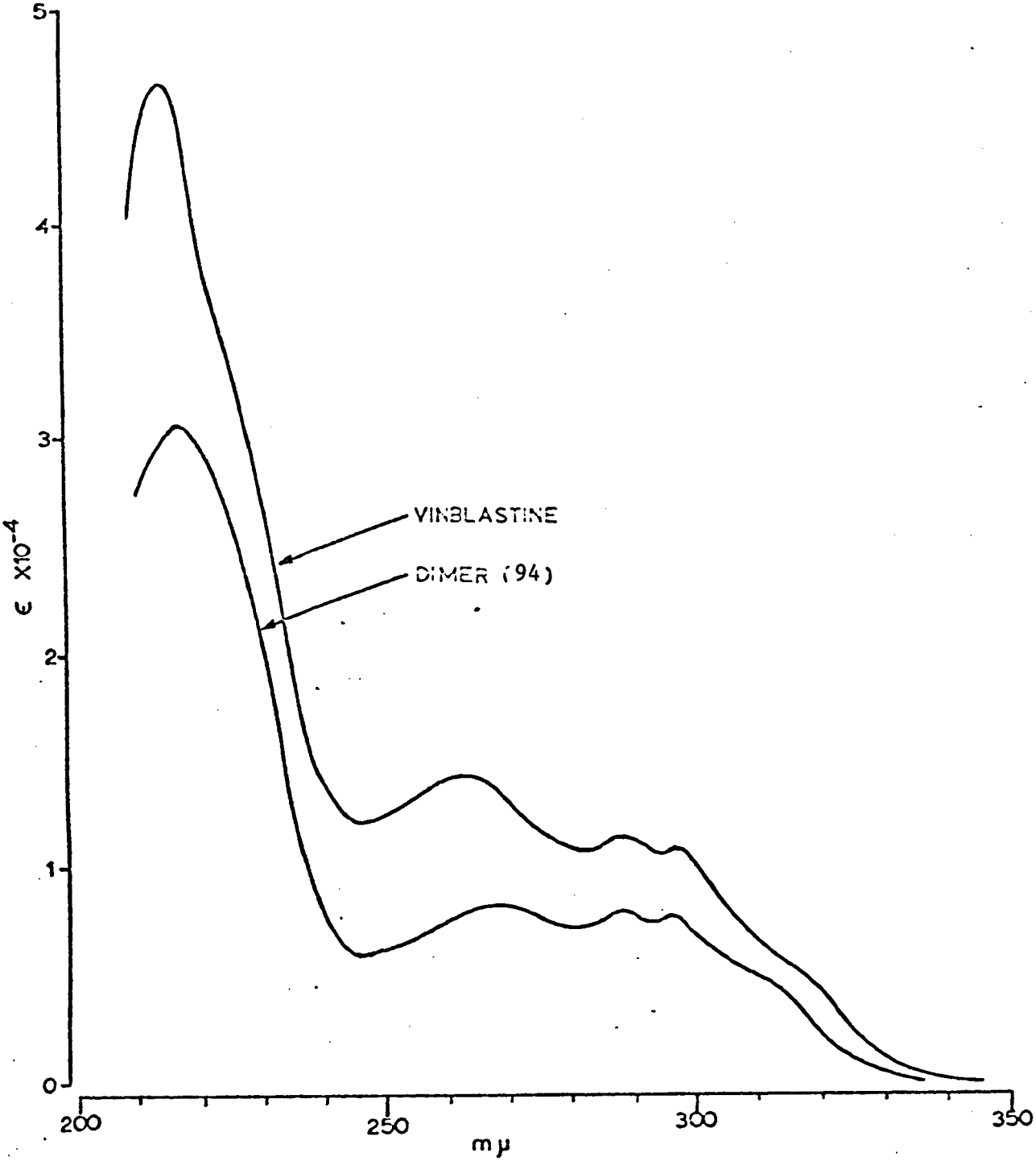
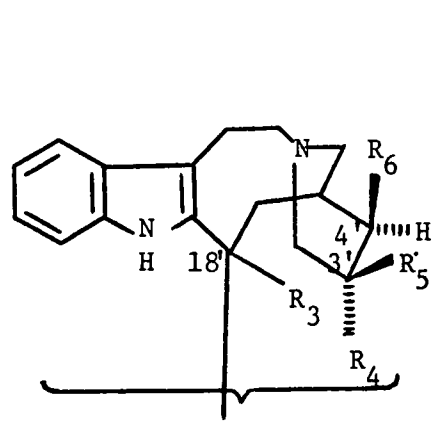


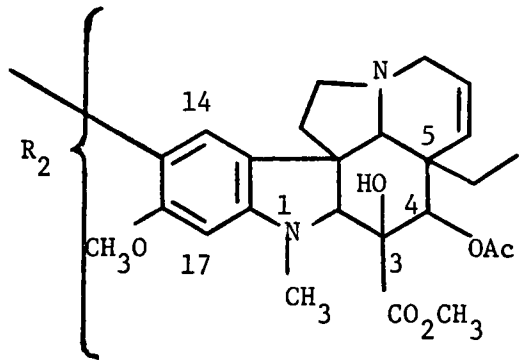
Figure 22. Uv comparison of VLB (15) with dimer (94).

in dihydrocleavamine, at τ 9.17 in the corresponding dimer (96), at τ 9.09 in compound 94 and still unshifted at τ 9.12 when the $C_{4'}$ position had a tertiary alcohol attached to it as in VLB. These similarities and differences in the nmr data of the pertinent centres of a series of compounds are summarized in Figure 23.

Some remarkable facts emerge from the data presented. First, it would appear, that the synthetic dimers are closer than VLB (15) to the corresponding monomers with regard to the chemical shifts of all the protons listed. Thus the whole chart may be viewed as a set of self-consistent numbers to which VLB is an exception. Since VLB may differ from the above dimers in its stereochemistry at $C_{18'}$ it is tempting to consider the differences as being due to this change. A detailed study of these chemical shifts is of some value. The most dramatic shift is that of the C_5 ethyl-methyl of the vindoline part. In the synthetic dimers it appears in the region of τ 9.4-9.5. However, in VLB it is shifted to τ 9.12. This is not readily explicable but may be viewed in terms of some interaction through space between this ethyl group and the indole template in the natural dimer which is lost when the $C_{18'}$ epimeric dimers are synthesized. The proton on C_{14} is the closest one on the vindoline unit to the critical junction and would be expected on this basis to be the one most sensitive to changes in the stereochemistry at $C_{18'}$. Indeed, it demonstrates a dramatic shift from τ 3.12 in vindoline to τ 3.42 in VLB (15). Surprisingly, the change between its chemical shift in VLB and that in the synthetic dimers is not so great, although it is consistently lower (τ 3.32 to τ 3.02) and in some cases is even shifted in the opposite sense from

Figure 23. Nmr comparison of various monomers and dimers.





Compounds	No.	C_5 $-CH_2\underline{CH}_3$	C_{17} H	C_{14} H	C_4 H	$C_{18'}$ and C_3 $-CO_2CH_3$	C_{16} -OMe	N_1 -Me	$C_{4'}$ $-CH_2\underline{CH}_3$
R_1H: R_3=R_5=R_6=H,R_4=Et	31								9.13
R_1H: R_3=CO_2CH_3(β), R_5=R_6=H,R_4=Et	107					6.25			9.12
R_1H: R_4=Et,R_3=CO_2CH_3(β),R_5;R_6= ═	29					6.42			8.96
R_1H: R_3=CO_2CH_3(β),R_4=Et,R_5=H,R_6=OH	56					6.31			9.06
R_2H:	10	9.55	3.98	3.12	4.58	6.26	6.26	7.38	
R_1R_2: R_3=R_5=R_6=H,R_4=Et	96	9.39	3.92	3.32	4.66	6.14	6.28	6.36	7.35	9.17
R_1R_2: R_3=CO_2CH_3,R_5=R_6=H,R_4=Et	94	9.34	4.05	3.05	4.67	6.29	6.29	6.16	7.40	9.09
R_1R_2: R_3=CO_2CH_3,R_5,R_6=═, R_4=Et	99	9.40	4.00	3.02	4.64	6.27	6.27	6.14	7.39	9.00
R_1R_2: R_3=CO_2CH_3,R_5=H,R_6=OH,R_4=Et	106	9.40	4.00	3.11	4.63	6.25	6.25	6.14	7.39	9.02

Figure 23 (continued)

Compounds	No.	C_5 $-CH_2\underline{C}H_3$	C_{17} H	C_{14} H	C_4 H	C_{18}, and C_3 $-CO_2CH_3$		C_{16} -OMe	N_1 -Me	$C_{4'}$ $-CH_2\underline{C}H_3$
R_1R_2: R_3=H,R_4=Et,R_5=OH,R_6=H		9.49	3.97	3.30	4.68	6.19	6.27	6.27	7.39	9.24
R_1R_2: R_3=CO_2CH_3,R_4=OH,R_5=Et,R_6=H	15	9.12	3.94	3.42	4.56	6.26	6.26	6.43	7.33	9.12

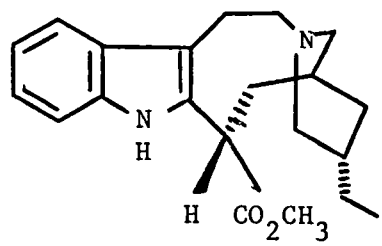
vindoline to the corresponding shift from vindoline to VLB. The C_{17} proton also shows a slight but distinct shift from τ 3.94 in VLB to higher field in the synthetic dimers. This shift is thus in the opposite sense to that observed for the C_{14} proton. The adjacent C_{16} methoxyl also shows a distinct and consistent shift from τ 6.43 in VLB to lower field and more towards its position in vindoline at τ 6.26. Similarly the N_a methyl is shifted to higher field in the synthetic dimers from τ 7.33 in VLB towards its position in vindoline at τ 7.38. Finally, a slight shift is also observed in the C_4 proton between the synthetic and natural dimers. In summary, the following groups were potential diagnostic tools for the determination of stereochemistry about $C_{18'}$: (a) the C_5 ethyl-methyl, (b) the C_{17} and C_{14} aromatic protons, (c) the C_{16} methoxyl and the N_a methyl and perhaps, (d) the C_4 proton. All of these signals were closer to the monomers in the synthetic dimers analyzed than in VLB.

To gain any insight into the many facets of the conventional dimerization reaction, it was necessary to set up a rigid sequence of steps which would represent the standard or norm. This sequence would have to be followed exactly to determine the yield and type of dimeric material obtained. It would have to be sufficiently refined and reproducible so as to yield reliable results, and finally, it would have to be quick and convenient in order to facilitate the rapid analysis of any given reaction mixture. The series of experiments described below used, as a basis, the standard dimerization reaction conditions. Each experiment was repeated twice to check its reproducibility. In every case, the overall yield of dimer obtained in these duplicate runs agreed within ± 5%. In those cases where no dimeric material

was isolated, reproducibly, the standard conditions were repeated using the same reagents and the yield of this reaction was used as a means to determine the validity of such results. The reactions were all performed on the same scale (50 mg of each monomer). Since the standard conditions play such a pivotal role in this study it is perhaps appropriate to describe them in some detail here.

The conventional reaction may be divided into two parts (a) chloroindolenine formation and (b) coupling reaction.

(a) Chloroindolenines were formed by dissolving 18β-carbomethoxy-4β-dihydrocleavamine (107) in a fixed volume of dry methylene chloride



(107)

containing one equivalent of triethylamine, cooling it to 0°C and adding one equivalent of an ice-cold solution of 0.05 M t-butyl hypochlorite in carbon tetrachloride over a period of twenty minutes. After this time the tlc check, described previously, was applied and the reaction was routinely found to have proceeded to completion. Evaporation of the solvent at 0°C under high vacuum usually resulted in a foam.

(b) To this foam was added an equal weight of vindoline followed by a solution of 1.5% hydrogen chloride in methanol under a stream of nitrogen, and the whole was plunged into a preheated oil bath at 70°C,

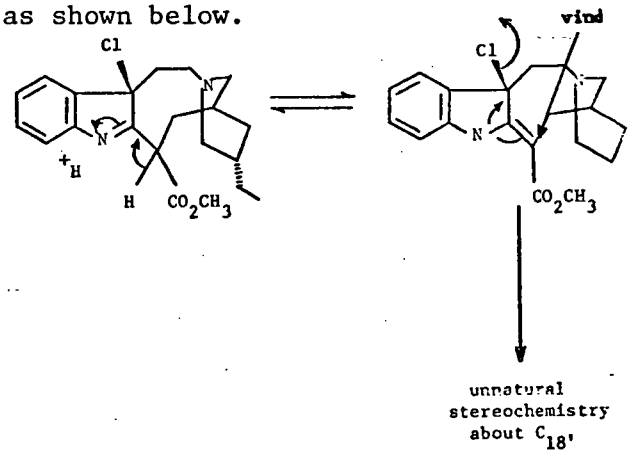
and allowed to reflux for three hours. Workup, followed by column chromatography of the reaction mixture according to a rigidly standardized procedure resulted in the isolation of dimer 94 in 65% yield. The nmr spectrum of this compound was taken to be the standard spectrum to which all subsequent experimental results were compared.

The first question that needed to be clarified in this study concerned itself with whether the dimerization reaction was stereospecific or stereoselective with regard to the stereochemistry at C_{18} in the cleavamine template. There had already been some indications that the stereochemistry at the C_{18} position did not play any role in the stereochemistry of the resulting dimers. Work in the decarbomethoxy series where this stereochemistry is non-existent, for example, had yielded only one dimer (96), and furthermore, the attempted dimerization of the C_{18} epimeric alcohols (56 and 57) had resulted in one and the same dimer from either starting alcohol. In order to prove this in the 3,4 dihydro series, 18α-carbomethoxy-4β-dihydrocleavamine was dimerized in the standard way to afford a 45% yield of isolated dimer which was proved to be identical, by nmr, to compound 94. Thus, it could be concluded that, in all the cases analyzed so far, the reaction proceeded stereoselectively with regard to the C_{18} position to yield attack by vindoline from only one side of the cleavamine template. The yield of dimeric material isolated was slightly but distinctly lower when the starting material possessed an 18α-carbomethoxy group than when it possessed an 18β-one.

The previous reports of chloroindolenine-forming reactions in the literature[97] required the addition of one equivalent of triethylamine. The function of this reagent in the reaction had never been explored.

Thus, an entirely parallel dimerization experiment to the above was performed on compound 107 without the addition of triethylamine at the chlorination step. The resulting dimer was unchanged in stereochemistry and overall yield (65%). There was, in fact, an added advantage that the solvents were much easier to remove at the end of the chlorination step in this case.

Although it had been shown that the dimerization step proceeded stereoselectively with respect to the C_{18} stereochemistry, it was possible that the reaction was concerted and thus stereospecific with regard to the stereochemistry of the carbon-chlorine bond of the chloroindolenine. One mechanism that had been proposed[84] to account for this was as shown below.



It had been pointed out that, based on this mechanism, a mixture of two dimers could be visualized in two ways: (a) The chlorination could occur <u>a priori</u> from either face of the molecule to yield two isomeric chloroindolenines which, upon concerted displacement would yield two dimers or (b) the tautomerization of the indolenine double bond could lead to cis and trans isomers about the C_{18} centre. Concerted displacement would then produce two dimers again. The isolation of only one dimer in all the cases studied implied one of several alternatives.

It was possible under the conditions of chlorination, that the steric bulk of the chlorinating agent caused prohibitive interactions on the α-face of the molecule so that the chloroindolenine was itself formed stereoselectively. This situation then governed the stereochemistry of the dimer formed according to the concerted mechanism shown above. There was some support for this hypothesis from the fact that these chloroindolenines appeared to behave as single compounds on several tlc systems. Spectroscopic techniques as well, had never suggested the presence of two isomeric materials. Furthermore, by examining the molecular model of the starting material it was possible to see that the β-face was slightly more accessible than the corresponding α-face.

The formation of only a single dimer, however, necessitated more than mere stereoselectivity of the chlorination step. It was necessary to assume, further, that none of the cis-trans isomerization mentioned above occurred prior to the dimerization under the reaction conditions. This assumption could also be justified by an examination of the steric factors associated with placing a trans double bond in a nine-membered ring. It could be shown that the most favourable carbon-chlorine stereochemistry when coupled with the double bond stereochemistry which would yield the preferred cis double bond in the nine-membered ring, was such that concerted attack by vindoline occurred from the β-face to give exclusively the unnatural stereochemistry at $C_{18'}$ in accordance with the experimental results.

If this mechanism was operating, and dimer formation was indeed a concerted and stereospecific reaction, then the problem of obtaining

two epimeric dimers from any given reaction could be rephrased simply as a problem of reducing the stereoselectivity of the chlorination step. Two possible strategies for the achievement of this goal could be presented. The use of smaller, less stereoselective, and perhaps more reactive chlorinating agents appeared to be a logical direction in which to proceed. Together with this, a change in reaction temperatures upwards from 0°C was appropriate. This latter approach was anticipated to cause a greater mobility in the nine-membered ring of the tetracyclic substrate so that, perhaps, the α-face could become as accessible as the β-face and chlorination would occur less selectively.

The standard dimerization conditions were used on several chloroindolenine reaction mixtures prepared by the use of different chlorinating agents. The most important results are summarized in Table II. It is clear from this table that the use of even the smallest and least

Table II. Effect of different chlorinators on the yield and stereochemistry of dimers produced.

Reagent	Solvent	Time to completion (min)	Temperature °C	Yield of dimer(%)	Type of dimer
1-chlorobenzotriazole	benzene	20	20	60	"unnatural"
"	methylene chloride	20	0	60	"unnatural"
0.05 M t-BuOCl in CCl_4	"	15	49	50	"unnatural"
N-Chlorosuccinimide	"	20	25	50	"unnatural"
N-Chloroacetamide	"	20	25	50	"unnatural"
NaOCl(H_2O) (household bleach)	" (two phase)	60	25	30	"unnatural"

discriminatory chlorinating agents failed to produce even the faintest trace of any new dimer when the products were coupled in the standard way with vindoline. This pointed to the fact that perhaps the stereochemistry of the carbon-chlorine bond did not govern the outcome of the dimerization reaction.

Further support for this hypothesis was obtained when the use of t-butyl hypochlorite as a chlorinating agent was examined in greater detail. Initially, this reagent had been used as a dilute solution, where the rate and stereoselectivity would be expected to be diffusion controlled. Thus, in order to produce the opposite effect, the use of neat t-butyl hypochlorite in this reaction was examined. One equivalent of this compound, neat, was injected directly into a solution of compound 107 in methylene chloride at 0°C using a microsyringe. The yield of chloroindolenine formed was not seriously altered by this procedure but it was found that a slow addition of the dilute solution of the reagent was slightly preferable. The yield of dimer obtained upon subsequent standard dimerization was 55%, and the stereochemistry about C_{18}, was again unaltered.

The temperature of this reaction was the final variable left to be examined. Reaction temperatures between 0°C and 77°C were used by refluxing the substrate in the appropriate mixture of methylene chloride and carbon tetrachloride, followed by the addition of neat t-butyl hypochlorite. This data is summarized in Table III. It is obvious from this table that the yield of dimer resulting from coupling chloroindolenines so derived with vindoline is sensitive to the temperature of the chlorination step but that the stereochemical outcome is completely unaltered by changes in the temperature at which the chlorination is performed.

Table III. Temperature of chlorination vs. yield and type of dimer.

Solvent Methylene chloride:carbontet.		Tempera-ture °C	Yield of dimer (%)	Type of dimer
1	1	0	65	"unnatural"
1	0	50	45	"unnatural"
3	2	50	10	"unnatural"
1	1	55	5	"unnatural"
0	1	77	No dimer	
1	4	72	No dimer	

Two possible explanations for this phenomenon may be offered. It was possible, although quite improbable, that the steric factors governing the stereoselectivity of this reaction were such that all the above efforts to obtain two epimeric chloroindolenines had failed. This possibility could only be conclusively ruled out by the isolation and separation of two such epimeric compounds, both of which could then be dimerized independently. This objective has not been achieved yet.

If the formation of two epimeric chloroindolenines had occurred at least to some extent in a minimum of one of the above conditions, then it was necessary to conclude from the above data that the stereochemistry of the carbon-chlorine bond was irrelevant to the outcome of the reaction. This was later proved by the isolation and dimerization of an intermediate lacking a carbon-chlorine bond (vide infra). The previously proposed concerted mechanism was thus untenable. This deduction had an important bearing on the direction of further efforts.

It meant that these efforts to change either the stereochemistry or the yield of the dimerization reaction must concentrate on the area of the coupling reaction itself and not on the chloroindolenine-forming step. Attention was thus turned to this part of the dimerization reaction.

Several factors were involved at this stage. In order to reach a clear understanding about the role that they played, a systematic study of each of these factors was necessary. The temperature of the coupling reaction was the first variable to be examined. It was conceivable that, regardless of the exact nature of the transition state, the stereoselectivity of this coupling step was a reflection of the steric interactions experienced by vindoline when it approached the α-face of the molecule in order to lead to the natural stereochemistry at $C_{18'}$. If these were considerably greater than those on the β-face, then at lower temperatures when conformational mobility was diminished in the intermediate, attack would occur exclusively on the less hindered β-face to produce only the unnatural stereochemistry at $C_{18'}$. If this line of reasoning was correct, the attack upon the α-face of the molecule may be favoured by increasing the temperature of this step so as to supply greater conformational mobility to the system.

One disadvantage of this approach was that the chloroindolenine could be expected, on the basis of the work mentioned above, to be heat-sensitive. Thus, the yield of dimeric material resulting from dimerizations at higher temperatures may be expected to be substantially diminished. Coupling reactions were performed at several temperatures ranging from room temperature to 140°C. These reactions were monitored

by tlc until a maximum amount of dimer had been formed and then worked up in the usual way. This time of optimum reaction provided a measure of the relative rate of the reaction. In the case of the higher temperatures, this was slightly more inconvenient and necessitated several runs. When the optimum time had been deduced another reaction was run, its product isolated, and the yield determined. The pertinent data derived from this sequence of experiments is summarized in Table IV. It can be seen that the "rate" of this reaction increased as the

Table IV. Effect of the temperature of the coupling step on the yield and stereochemistry of dimer.

Temperature (°C)	Time (hr)	Yield (%)	Stereochemistry
140	0.5	0	
100	0.25	30	"unnatural"
68	2.5	65	"unnatural"
25	18	75	"unnatural"

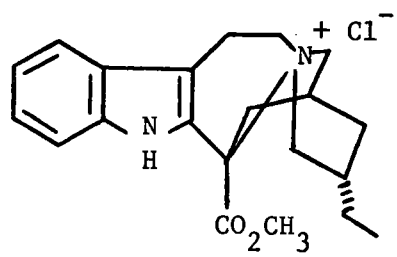
temperature was increased. The yield, as had been expected, was inversely correlated with the temperature, being optimal at the relatively lower temperatures. Unfortunately, the stereochemistry at C_{18}, of the resulting product was completely independent of the temperature of the coupling step. Thus, again the hope of altering the stereochemical outcome of this reaction, based on the above arguments, had been frustrated.

The reaction mixture resulting from the reaction at room temperature, however, was remarkably simple in nature consisting of vindoline, deacetyl vindoline, and baseline material as the minor components of the reaction and the dimer (94) as the only major component. As mentioned previously, these reaction conditions had been discovered to be preferable to the conventional ones in the $\Delta^{3,4}$ case as well. They thus represent a genuine improvement upon the standard reaction. Some progress in one of the two goals of the study had thus been achieved.

Vindoline itself had previously demonstrated a remarkable inertness to 1.5% methanolic hydrogen chloride (vide supra). The fate of the chloroindolenine during the coupling step may have been critical in determining the overall yield of dimeric material isolated. It was possible that when this compound was mixed with vindoline and then treated with the acidic methanol, the chloroindolenine decomposed partially before it had an opportunity to dimerize. Vindoline was dissolved in a small amount of the solvent in a separate flask and added slowly to the chloroindolenine. When this addition was complete at room temperature, the remaining amount of the solvent was added and the normal dimerization was performed. A slight improvement in the overall yield of dimer 94 was realized by this approach (70%) but it was not sufficiently significant to be classified as a real advance particularly in view of the added inconvenience associated with this modification.

An interesting discovery was made, however, upon inverting the direction of this addition. When the chloroindolenine was mixed with 1.5% methanolic hydrogen chloride and then added to solid vindoline, the yield

of dimer isolated after the standard dimerization appeared to be remarkably dependent upon the length of time for which the chloroindolenine had remained in this solution prior to the addition, and the temperature at which this solution had been maintained. In fact, if the chloroindolenine solution was allowed to reflux for five minutes, or stand at room temperature for an hour, or at 0°C overnight, the yield of dimeric material isolated after the standard coupling step was reduced to zero. Such experiments with the chloroindolenine under the above conditions revealed by tlc that it had completely disappeared and had been replaced by a polar, salt-like material which had the characteristic uv absorptions of an indole. On the basis of similar work in our laboratories,[111] the structure 108 below was tentatively assigned to this compound on the basis of its nmr and uv spectra. All attempts to dimerize this so-called quaternary salt in a separate step failed completely under the standard



(108)

conditions. It should be pointed out that the chloroindolenine was quite unreactive in the absence of acidic or basic catalysts, and could be recovered unchanged when refluxed in dry, distilled acetone or rigorously anhydrous methanol (distilled first from magnesium and then from a small amount of sodium) for periods in excess of six hours.

Clearly then, the acid catalysts reacted with the chloroindolenine to produce an intermediate which was quite reactive and which then reacted with vindoline or any other nucleophile that may be present or else forced the intramolecular condensation with N_b to yield compound 108 above. The work described at the beginning of this part of the discussion suggested the nature of this intermediate to be 109 and it was thus postulated to arise as shown in Figure 24. Intermediate 109 is strongly reminiscent of compounds 77, 81 and 83 proposed by others in closely analogous cases (vide supra). This mechanism was supported by the fact that the C_{18} alcohols, when solvolyzed in the presence of vindoline, yielded the same product[84,103] as could be obtained from the chloroindolenine approach implicating the same intermediate in both cases.

Only one further experiment needed to be performed in order to prove this mechanism. It was necessary to trap this reactive compound in some way. This trapped form could be isolated and then used in an independent step to regenerate compound 109 in the presence of vindoline and allow normal dimerization to proceed. If the yield and stereochemical outcome of such an experiment were the same as the standard one, then this postulate would be strongly supported. Fortunately, such an experiment was indeed possible. Treatment of the chloroindolenine of compound 107 with 1% methanolic hydrogen chloride under milder conditions (0°C for ten minutes) yielded a rapid and visible reaction with the initially clear light yellow solution becoming a deep wine-red colour. Evaporation of the solvent under high vacuum at 0°C afforded a reddish-brown solid which was proved to be compound 110 shown below. Its identity

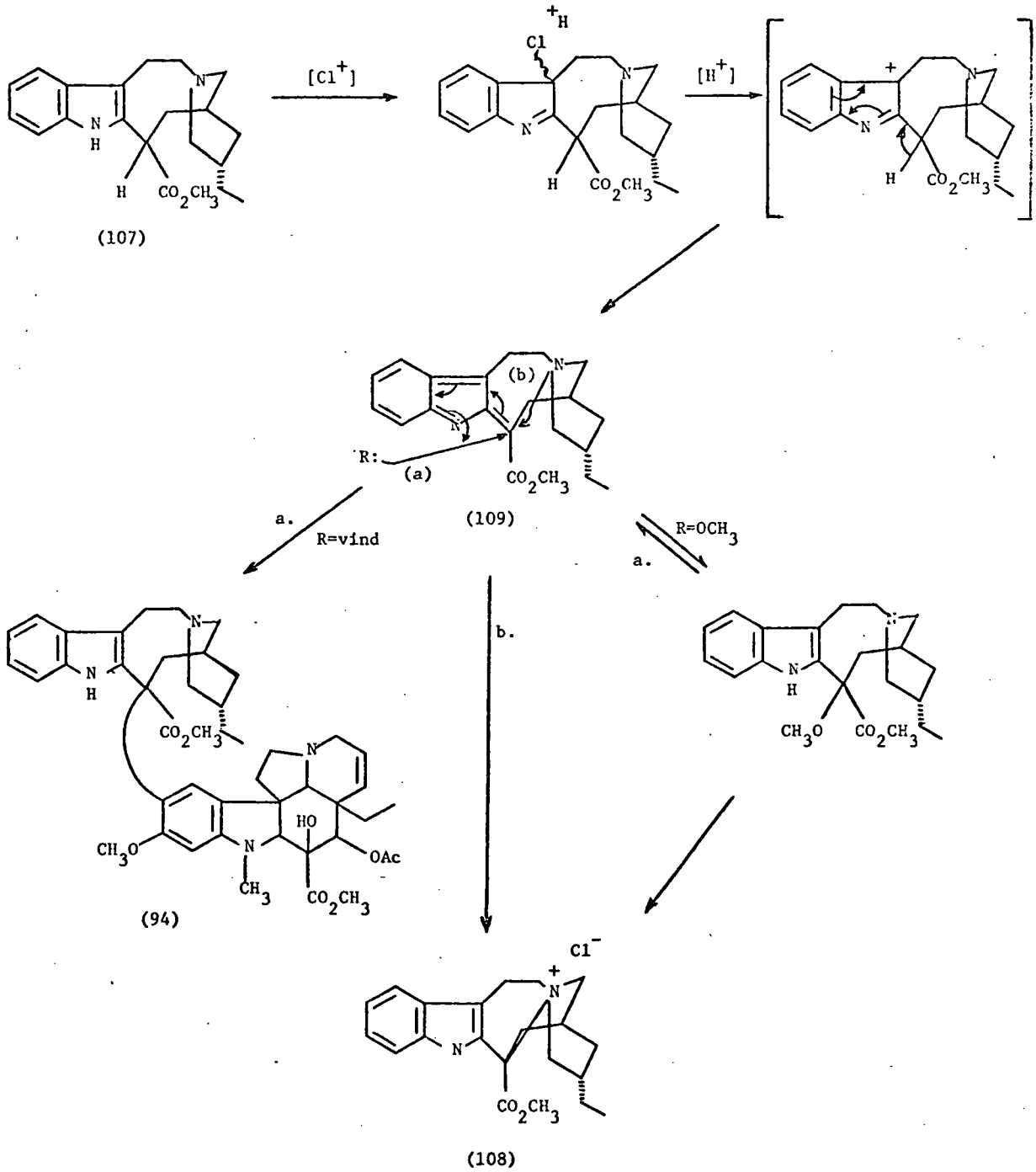
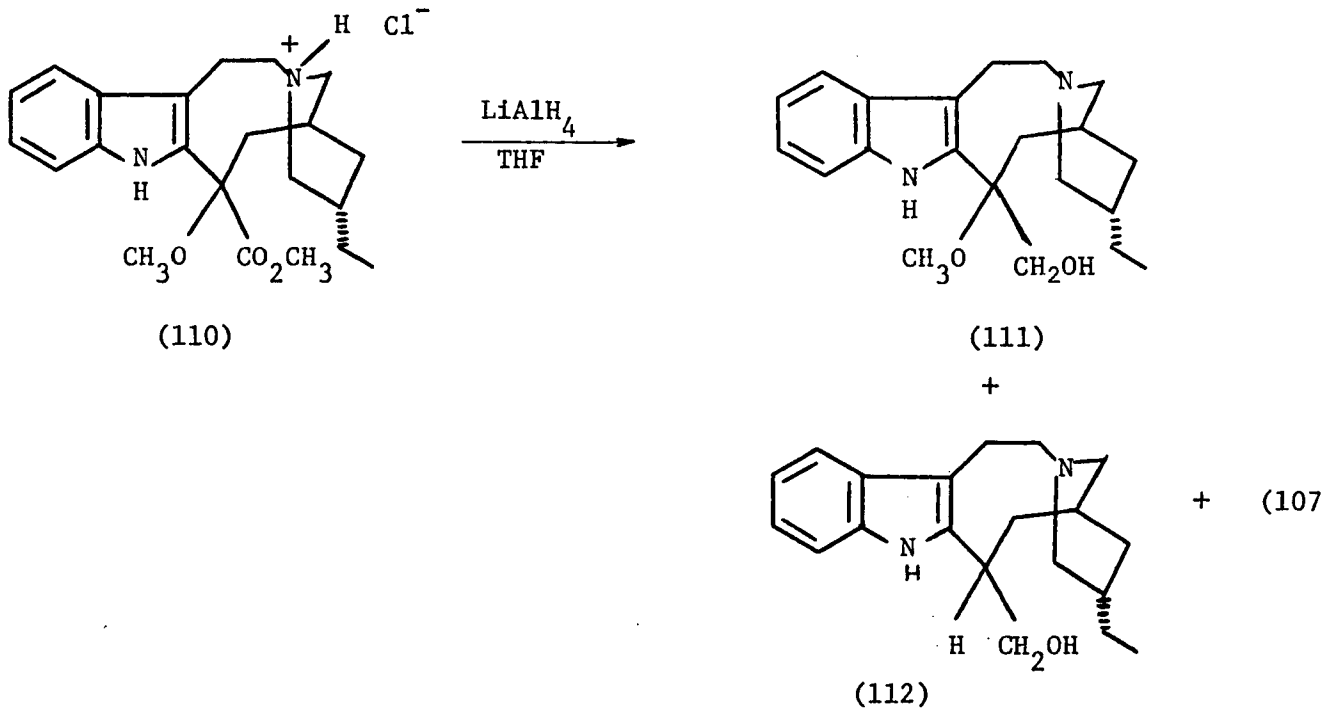


Figure 24. Proposed mechanism of the standard dimerization.



(110) (111) + (112) + (107)

could be inferred by an examination of the nmr spectrum of the compound as the free base which showed the expected disappearance of the C_{18} proton as well as the appearance of the new three proton singlet due to the C_{18} methoxyl group. When converted to the free base, compound 110 was quite unstable and decomposed, upon standing for several hours, to the quaternary salt (108). Its structure could however be conclusively established by reducing it with lithium aluminum hydride to the stable vicinal alcohol-ether (111) which was completely characterized by nmr (see Figure 25), ir and uv as well as elemental analysis after crystallization from ethyl acetate and high and low resolution mass spectrometry (see Figure 26). The reduction was however somewhat difficult because compound 110 was characteristically insoluble in any etherial solvent as the hydrochloride salt. The addition of lithium aluminum hydride

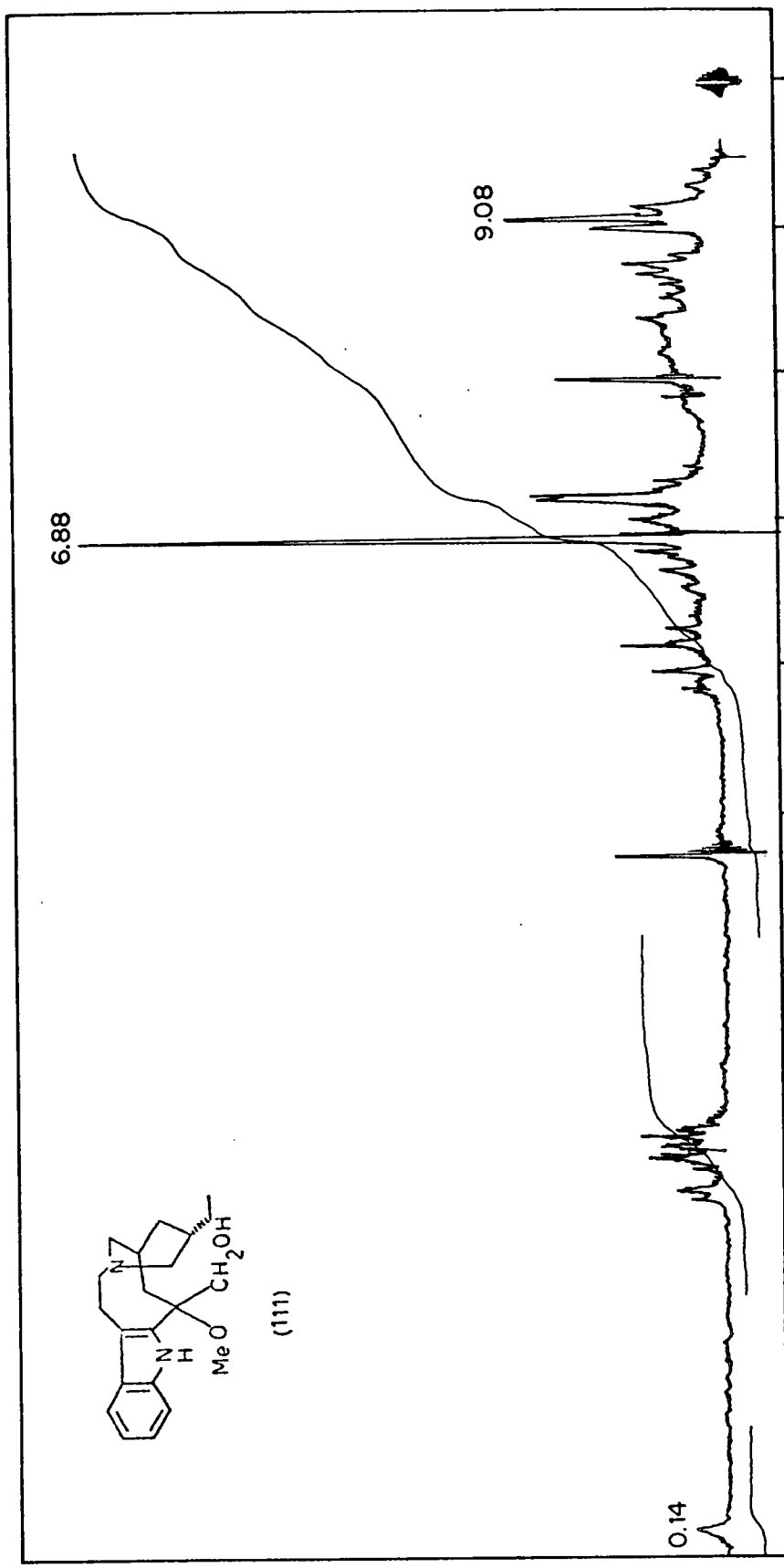
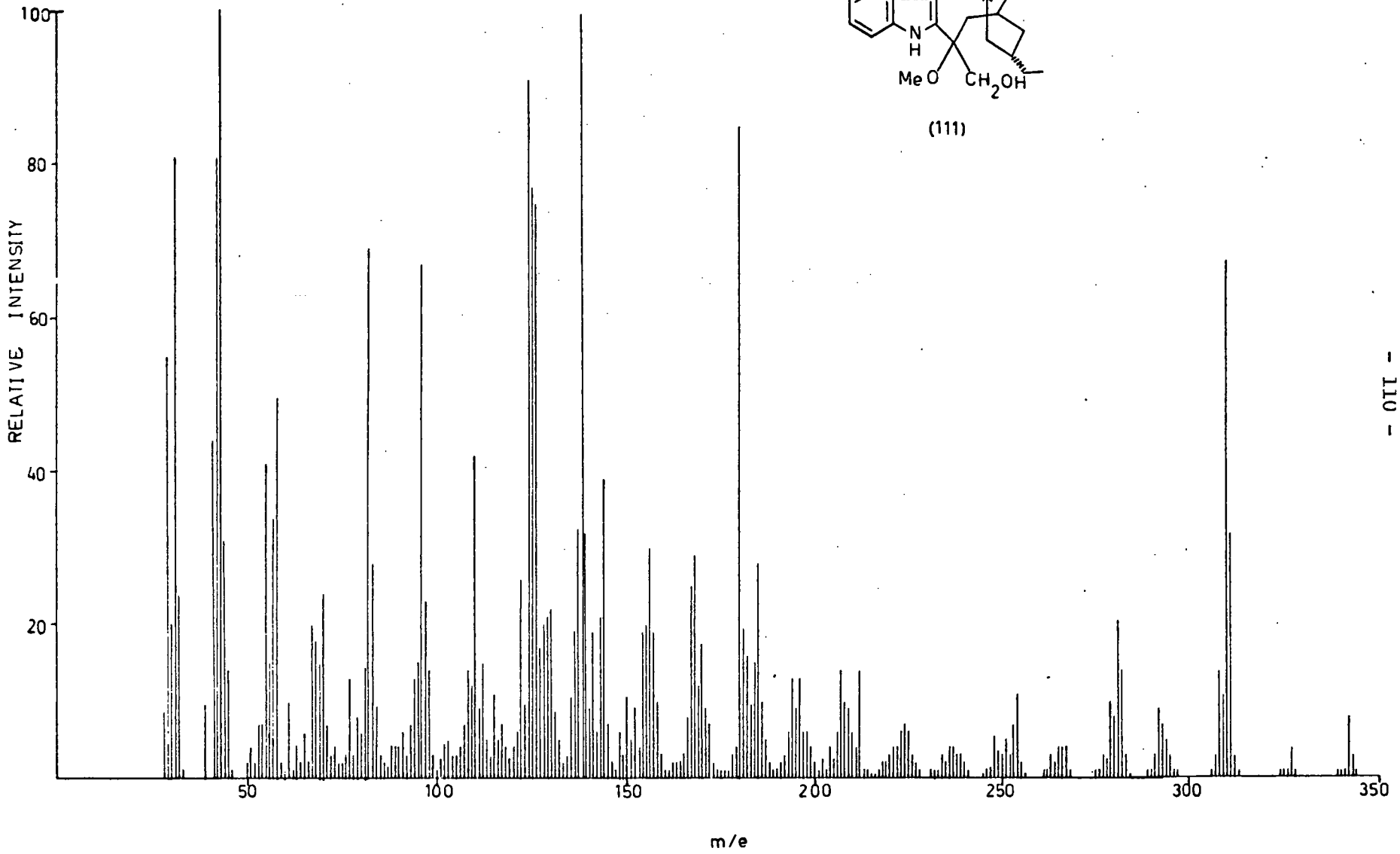


Figure 25. Nmr spectrum of the vicinal alcohol-ether (111).



(111)

Figure 26. Mass spectrum of the vicinal alcohol-ether (111).

generated the free base which then dissolved. However, generation of the free base allowed quaternization to yield compound 108 to compete effectively in the basic medium with reduction to afford compound 111. This was further complicated by the fact that attack by the active "hydride" agent could occur at the C_{18} position directly as well to yield dihydrocleavaminol (112) from hydrogenolysis of the methoxyl group with concomitant reduction of the ester functionality. The isolation of the desired compound (111) in overall 37% yield from this reduction, however, proved the identity of the previous product (110).

In a parallel experiment, compound 110 was produced exactly as above and dissolved in 1.5% methanolic hydrogen chloride containing vindoline. Dimerization in the standard way afforded a 55% yield of dimeric material whose stereochemistry was the same as that obtained in the standard reaction. The compound 109 was thus most probably a real reaction intermediate during the standard dimerization reaction. The use of this concept allowed the extension of the reaction to permit the introduction of several C_{18} substituents other than methoxyl. These compounds will be discussed at a later stage.

This postulated mechanism is consistent with all the facts so far obtained about the dependence, for example, of the reaction on such factors as the temperature of the coupling reaction etc. It also explains the observed lack of correlation between the stereochemistry at either the C_{18} or the indole-3-position of the chloroindolenine, and the stereochemistry of the isolated dimer. Furthermore, this postulated mechanism is entirely consistent with the previous postulates by other workers (vide supra) in closely similar cases. Using the mechanistic

scheme of Figure 24 the stereoselectivity of the standard reaction could be viewed in one of two extreme ways. If it were possible to decide which one of these was operating then suitable means for reducing this stereoselectivity could be devised.

The attack of vindoline on the compound under the conditions of the reaction may be viewed as the normal Friedel-Crafts alkylation of the meta-methoxy-anilino system of vindoline by the reactive group. The details of such a reaction may be depicted as shown in Figure 27. If this reaction sequence is visualized as proceeding essentially irreversibly, then the stereochemistry of the product must reflect the initial side of approach preferred by vindoline. It is reasonable to assume that vindoline enters the reaction site orthogonal to the existing π system so as to maximize overlap. An examination of a molecular model of the intermediate revealed that the molecule was considerably more crowded on its concave, α-face, than on the corresponding convex, β-face. Thus the exclusive attack by vindoline on the β-face of this molecule to yield dimers of the unnatural stereochemistry was understandable. This explanation implied that the reaction was proceeding essentially under kinetic control and therefore that the usual stereochemistry about $C_{18'}$ was the kinetic product.

At the other extreme, it may be argued that the reaction may <u>a priori</u> proceed entirely reversibly under the conditions of the reaction to yield the thermodynamically most stable product. Certainly, the protonation of the C_{15} position of a dimer under highly acidic conditions may be visualized to yield the same intermediate (113) shown in Figure 27. The cleavage of the C_{15}-$C_{18'}$ bond, instead of the loss of this proton,

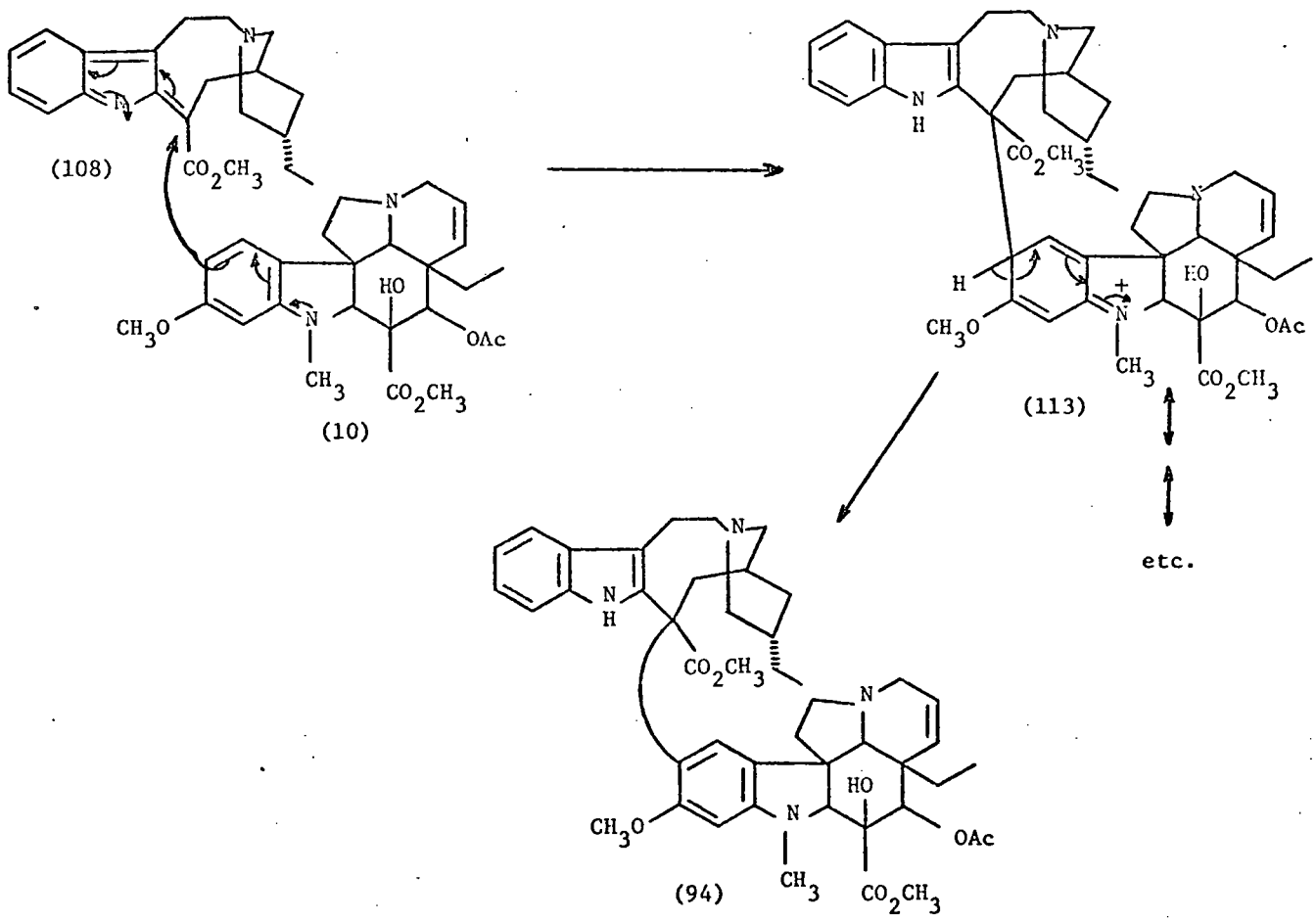


Figure 27. Proposed details of the coupling step.

would effectively reverse the dimerization reaction to yield intermediate 109 and vindoline. The cleavage reaction can now be explained in the light of the proposed mechanism in exactly this way. Under these conditions the intermediate 109 is immediately reduced by the stannous chloride and tin in situ, and so it is trapped, and the equilibrium reaction is thus "drained" over to the side of monomeric cleavage products.

It should be mentioned in passing that this cleavage reaction was possible without the use of the reducing agent only in the case of VLB (15). In this case, the C_4, alcohol participated to trap the monomeric species analogous to 109 as the C_4-C_{18} ether (22). In the light of the proposed mechanism now, the apparent side reaction of the 3,4 functionalized templates could be explained exactly analogously. It was conceivable that the 3,4 functionality may act in a similar way as in the case of VLB above, under the dimerization conditions, towards an intermediate analogous to 109 to trap it in some way and thus block its participation in the dimerization reaction.

Although the concentration of acid used in the cleavage conditions was obviously high enough to allow the reaction to become reversible, it was not clear whether the reaction was proceeding according to kinetic or thermodynamic control under the standard dimerization conditions. Molecular models of the two epimeric dimers were studied closely, in an attempt to discover if the secondary interactions embodied in any one epimer about the C_{18}, centre were distinctly greater than those in the other. Although it must be admitted at the outset that such studies in a molecule of this size and complexity may be misleading, it should be mentioned that these models failed to show any great differences in the stability of one epimer over the other. Thus, if the reaction were proceeding under thermodynamic control, the isolation of only one dimer was not readily explicable. In summary, it appeared that under the reaction conditions employed in the standard dimerization, the coupling of vindoline to compound 109 proceeded under kinetic control to yield only the kinetic product of the reaction.

Two strategies for modifying the result of this reaction, on the assumption that it was proceeding according to kinetic control, could be conceived.

(a) If the reaction mechanism was indeed as described above, the nature of the transition state would affect the nature of the product. It was thus conceivable that the use of different solvents and Lewis acids may sufficiently alter the nature of this transition state so as to permit a change in the overall stereochemistry of the dimer obtained. Several Lewis acids and solvents were investigated. The reactions were run at three temperatures - room temperature, 70°C, and the reflux temperature of the solvent employed. In each case, the progress of the reaction was monitored by tlc and worked up when optimal dimerization had occurred. These results are summarized in Table V. For purposes of brevity, only the optimal temperature in each case is reported. It soon became clear that the change in this transition state was not a simple matter and attention was thus turned to a more systematic approach.

(b) If the reaction mechanism was as shown in Figure 27, the degree of reversibility of the reaction, and therefore the extent of thermodynamic control, was dependent on the concentration of acid present in the reaction medium. In other words, the formation of the intermediate 109 under the usual conditions may be viewed as the rate limiting step. Once this ion was formed the next steps would be rapid and essentially irreversible. An increase in the concentration of the acid present should function to slow down the step which converts intermediate 113 to compound 94 because, in simple terms, the loss of a proton into the medium (or the overall loss of H-Cl) would become less

Table V. Effect of solvents and Lewis acids on the dimerization reaction.

Solvent	Lewis acid	Temp. (°C)	Time (hr)	Yield (%)	Stereochemistry
Trifluoroacetic acid	-	25	48	5	"unnatural"
N,N-Dimethyl-formamide	HCl	25	19	60	"unnatural"
Tetrahydrofuran	HCl	66	2.5	25	"unnatural"
Dioxane	$HClO_4$	25	48	5	"unnatural"
Methanol	HCl	70	3	65	"unnatural"
Methanol	HBr	25	48	50	"unnatural"
Methanol	$ZnCl_2$	25	48	0	
Methanol	SiO_2	25	48	0	
Benzene	$AlCl_3$	25	48	0	
Benzene	BF_3	80	1.75	5	"unnatural"

facile as the concentration of HCl in the medium was increased. Furthermore, this increase of the activation energy for the loss of a proton may eventually become sufficiently great that carbon-carbon bond cleavage was competitive. In such a case, the kinetics may be viewed as a rapid conversion of the chloroindolenine to the intermediate 109 irreversibly, which would then slowly be converted to the intermediate 113 reversibly. Compound 113 would then lose a proton reversibly in the rate determining step to form dimer 94, or its epimer at $C_{18'}$, or else a mixture of these compounds.

A study of the yield and stereochemical outcome of the conventional dimerization reaction as a function of acid concentration was thus undertaken. Concentrations of acid below 1.5% were used to see whether or not the same dimer was formed under these conditions which better favoured kinetic control. At very low acid concentrations however, the rate determining formation of intermediate 109 was sufficiently slow to permit the decomposition of the chloroindolenine irreversibly to other products and thus the yield of dimeric material obtained was dramatically reduced. In the limiting case, when the dimerization was attempted in absolute methanol it was found that the chloroindolenine and vindoline could be recovered unchanged as virtually the only products after several hours of refluxing.

At the other end of the scale however, the gradual increase in the acid concentration up to 5% revealed no detectable change in the stereochemistry of the dimeric product. A remarkable discovery was made upon performing the reaction according to the cleavage conditions previously reported during the characterization of compound 94.[84] This dimer had been completely cleaved by refluxing it for only one hour in anhydrous 7% methanolic hydrogen chloride containing stannous chloride and tin. When the chloroindolenine of compound 107 and vindoline were refluxed together for 3.5 hours in 7% methanolic hydrogen chloride the major products, in contrast to the above result, were the dimer 94 and its deacetyl analogue. Minor products included vindoline, deacetyl vindoline and baseline material.

Upon purification of the dimeric fractions, it rapidly became obvious that they contained another material which was almost inseparable

from the dimer 94. This material had a characteristic colour-reaction on tlc to antimony pentachloride in carbon tetrachloride. All of the synthetic dimers so far analyzed showed a brown colour-reaction to this reagent which was invariant with time. This compound, on the other hand, was distinguishable from compound 94 in the following way: when initially sprayed with this reagent both compounds showed up as indistinguishable brown spots. The new compound gradually turned pink while the spot corresponding to compound 94 did not change. After much effort and extensive decomposition, this compound could be purified from dimer 94 by the use of high pressure liquid chromatography on a specially prepared column of deactivated alumina. Mass spectral studies on this purified fraction revealed a molecular weight of 794 which was exactly the same as the molecular weight of dimer 94. The Fourier-Transform nmr of this compound was not directly comparable to the normal spectrum of compound 94. This was due to the fact that in the extremely dilute solution of the compound in deuteriochloroform it reacted with the residual acid in this solvent to partially form a salt. This salt thus modified the spectrum observed. The phenomenon was not entirely new in our laboratories and had been observed by other workers as well. The nmr of a mixture of the new compound with compound 94 prior to separation was concentrated enough that this problem was not serious here. This spectrum was extremely useful. It showed that the new compound did not lack any of the functionality present in dimer 94. The chemical shifts of the protons which were expected to be diagnostic of changes in stereochemistry about $C_{18'}$ were all exactly as expected for the $C_{18'}$ epimer of compound 94. On this basis, this

compound was tentatively postulated to be the desired natural stereochemistry dimer. It should however be emphasized that this assignment was by no means definite. The complete structural assignment of this compound must await its isolation in larger quantities to permit further analyses such as normal nmr, cleavage to the monomers, and, perhaps, finally even X-ray crystallography. Nevertheless, the isolation of a new dimeric compound under these conditions even in trace amounts is an extremely useful and gratifying result since it supports the predictions of the theoretical arguments mentioned above.

If these predictions were indeed being borne out in the above experiment, it may be argued that the epimerization of compound 94 to a mixture of these compounds must be possible under the reaction conditions. Indeed, submission of dimer 94 to identical conditions yielded a reaction mixture which was exactly the same as that obtained above and from which a similarly low (< 10%) yield of the new dimer was isolable. Unfortunately, all attempts to improve the yield of this compound by increasing the acid concentration beyond 7% failed. Furthermore the overall yield of dimeric material slowly decreased as the acid concentration was increased beyond 15%. These results are summarized in Table VI. Conditions for improving this reaction are currently being sought in our laboratories.

Table VI. Effect of acid concentration on the coupling reaction.

% HCl in methanol	Time (hr)	Yield (%)	Type of dimer
1.5	3.5	30	"unnatural"
1.6	3.5	36	"unnatural"
2.0	3.0	45	"unnatural"
5.0	2.0	56	"unnatural"
7.0	3.5	50	mixture
15	3.5	40	mixture
30	3.0	30	mixture
60	3.0	25	mixture

The second goal of this study, namely to improve the yield of the dimerization under possibly milder conditions, was also fortuitously realized. During this study, the methanolic hydrogen chloride had been prepared by adding the calculated volume of purified acetyl chloride to anhydrous methanol which had been cooled to 0°C. These two compounds reacted instantly to yield methyl acetate and hydrogen chloride. Thus, during the study of the effects of acid concentration mentioned above, successively larger aliquots of acetyl chloride had been added. For the sake of completeness, a conventional dimerization in acetyl chloride as solvent at its reflux temperature (51-52°C) was attempted. The chloroindolenine and vindoline readily dissolved in this solvent. However, as the reaction proceeded, dimer 94 precipitated. Workup consisted merely of removing the solvent under vacuum to leave a

whitish powder. Dissolution in an organic solvent, (methylene chloride) basification, and extraction resulted in a near quantitative yield of the dimer 94. If this reaction were run at room temperature for a longer period of time it should constitute a mild and much improved synthesis of dimers bearing sensitive indole templates. Indeed, initial results in the 18-carbomethoxycleavamine case to yield dimer 99 have been encouraging. Thus, the goal of a milder and higher yielding reaction for the dimerization of sensitive indole templates had also been realized.

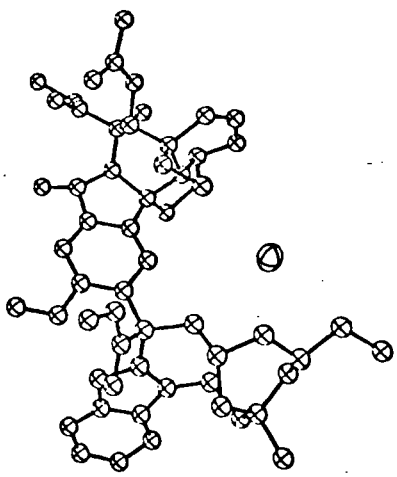
In summary, this laborious and painstaking study has borne fruit in several areas. It has permitted, for the first time a proper and detailed understanding of the overall dimerization reaction. This insight has allowed the prediction of ways and means of arriving at the natural stereochemistry. Finally, a mild and high yielding dimerization reaction is in hand. All of these findings are expected to contribute immensely to the synthetic goals of this endeavour.

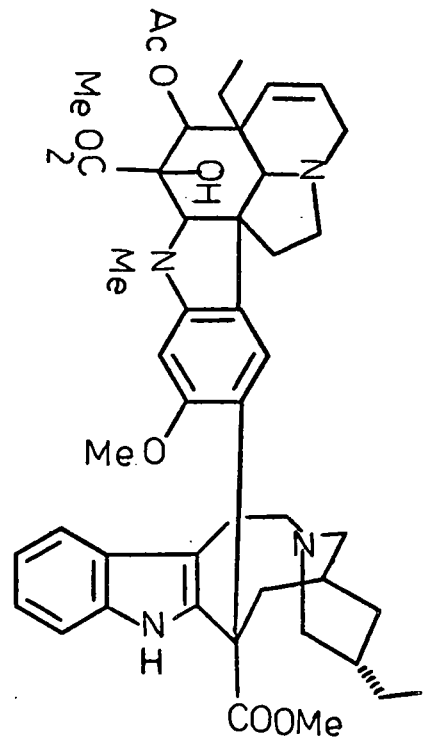
There remained, however, one question of pivotal importance. This dealt with the conclusive establishment of the absolute stereochemistry of the crucial $C_{18'}$ centre. It had been apparent for some time that although speculations based on various spectra were possible and indeed were probably correct, concrete proof in this area could only be furnished by an X-ray study of one of these dimers. Recently, Professor J.C. Clardy of Iowa State University expressed an interest in performing such a study. Dimer 94, which was the major compound from all the experiments just described, was converted to its crystalline monomethiodide. An X-ray study has revealed that this compound is indeed isomeric to the natural dimers at $C_{18'}$.[104] Its structure may thus be

more properly represented as shown in Figure 28 (95). Thus the previous deductions, on the basis of nmr data, about the stereochemistry at $C_{18'}$ were proved to be completely correct. The structure of the dimers 102 and 99 were also secured by this study. From the evidence presented in Figure 19, it was clear that the stereochemistry about the $C_{18'}$ centre in these cases was also the unnatural one. On this basis, as well as on the basis of the nmr data presented in Figure 23, it may be deduced that indeed, in all the synthetic dimers made in the standard way, the stereochemistry about $C_{18'}$ is unnatural.

This generalized statement was supported by a second X-ray study performed on dimer 96 - the compound lacking the $C_{18'}$ carbomethoxy group but otherwise identical in every respect to compound 94 above. Dimer 96 has recently stimulated a great deal of interest. Upon testing it for antineoplastic activity,[109] this compound demonstrated activity against P_{388} lymphocytic leukemia in mice at dose levels comparable to vincritine (VCR). In fact, most recent evidence appears to suggest that it may possess a sufficiently good therapeutic index to warrant its consideration as a clinically useful drug for man. If dimer 96 is as useful an anticancer agent as initial tests have indicated, it should compete successfully with the extremely expensive natural drug, VCR, to become the first clinically important synthetic dimer ever to be reported in this series.

This activity could be explained in three ways <u>a priori</u> (a) It could be an indication that the $C_{18'}$ carbomethoxy group is of no consequence as far as structure-activity relations are concerned. (b) A somewhat more intriguing possibility was that, in the case of the





(95)

Figure 28. X-ray structure of dimer (95).

$C_{18'}$ hydrogen dimers, the stereochemistry at this centre was inverted relative to compound 94 in the synthetic material. (c) Finally, it is possible that the biological system of the host mouse is able to perform this inversion to a natural stereochemistry dimer even if the stereochemistry is initially unnatural. A mechanism for this inversion may be as shown in Figure 29. The $C_{18'}$ proton is benzylic with respect

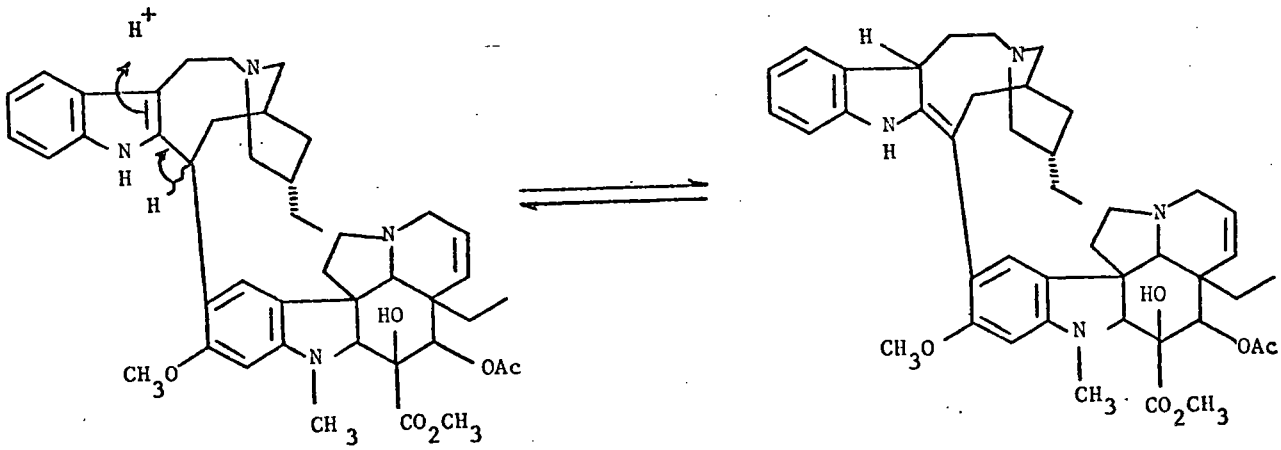


Figure 29. A mechanism for the epimerization of dimer (96).

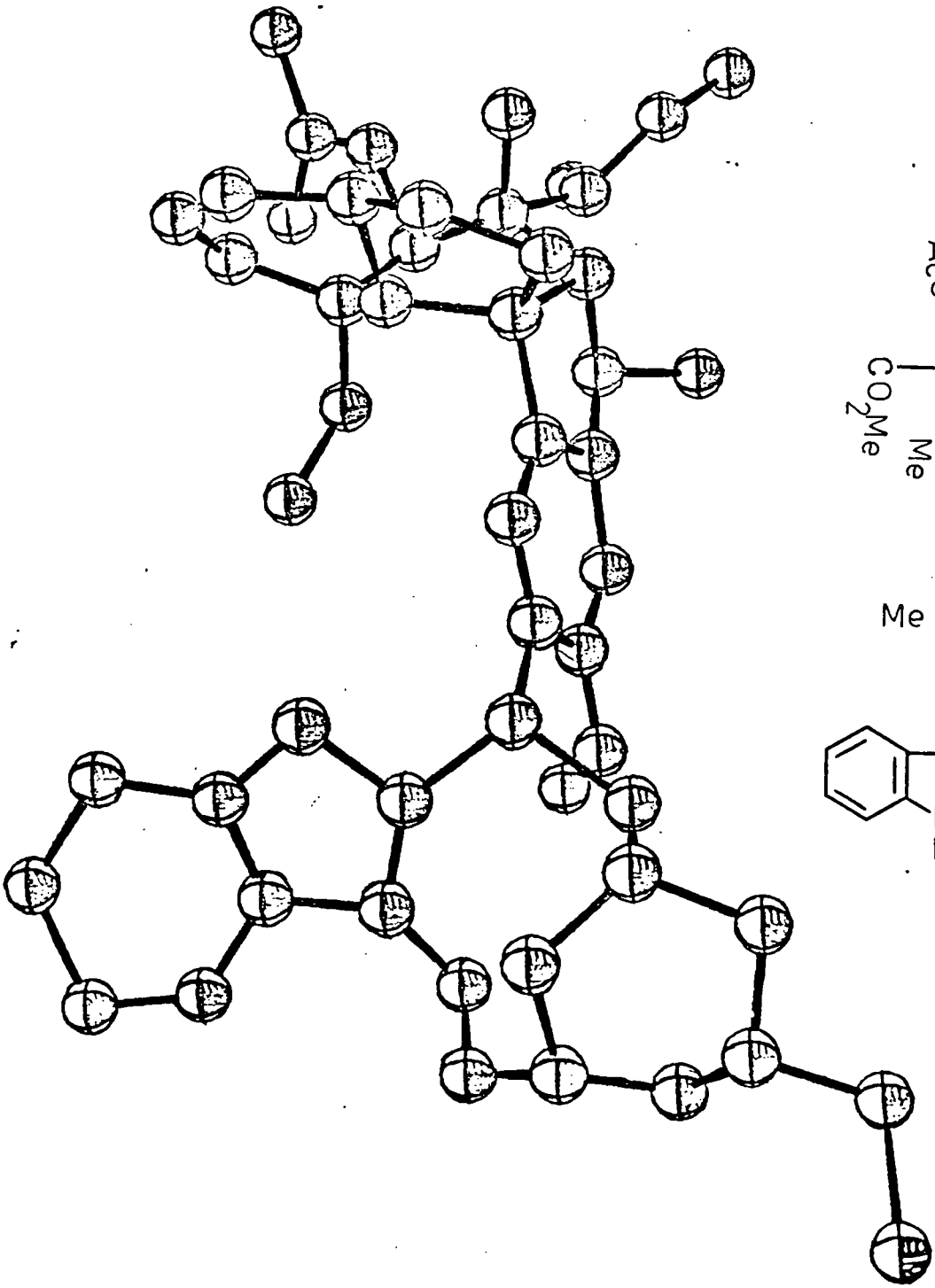
to the vindoline portion and "allylic" with regard to the indole portion. The loss of this proton should thus generate a rather stable centre at $C_{18'}$ and reprotonation from the less hindered β-face would lead to the epimeric dimer. Since this mechanism could, in principle, occur in vivo, or in vitro under the reaction conditions it was necessary to prove the structure of compound 96 by X-ray crystallography. To this end, compound 96 was transformed into its crystalline monohydrobromide and then subjected to X-ray analysis. The results confirmed that the stereochemistry was indeed the unnatural one as shown in

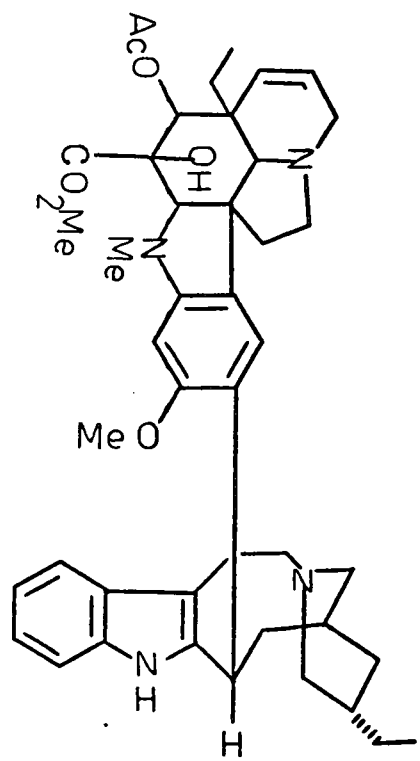
Figure 30. This compound is now more properly represented as structure 97. The possibility that this epimerization may have occurred under the reaction conditions was thus ruled out.

This X-ray analysis also provided some concrete evidence for earlier work in other laboratories. As mentioned previously, the Lilly group had elucidated the structures of three dimeric compounds: deoxy VLB-'A', -'B' and -'C'. They had shown that 'A' and 'B' were not $C_{18'}$ epimers of each other but that both were epimeric to deoxy VLB-'C' at this centre. This claim was based on an nmr comparison of their decarbomethoxydeacetoxy hydrazides. However, at that time they were unable to assign the stereochemistry at this centre as either natural or unnatural with any degree of certainty. Since deoxy VLB-'C' and compound 97 are in fact the same compound, this X-ray study enables one to assign the unnatural stereochemistry to this compound about $C_{18'}$ and, therefore, the natural stereochemistry to the compounds in the deoxy VLB-'A' and -'B' series.

Both these X-ray studies have thus provided vital information in this area of chemistry and have replaced speculation by a concrete framework upon which much more rational discussions may be based.

It is possible that the carbomethoxy group at $C_{18'}$ is unimportant to the overall activity of any dimer or even that of a hydrogen at this centre is necessary for biological activity. It would seem logical to synthesize the decarbomethoxy (at $C_{18'}$) analogs of dimers 99, 102, and 106 at some future date and to examine their anticancer activity. This would enable a more thorough evaluation of the importance of the above finding and may also simultaneously provide other drugs with





(97)

Figure 30. X-ray structure of dimer (97).

perhaps a different spectrum of activity.

While the study just described was under way, other avenues of approach that might yield the natural stereochemistry dimers by a mild and possibly high-yielding step, were simultaneously being considered and explored. Some of these studies have shown great promise and are currently under active scrutiny by other workers in our laboratories. It is useful at this stage to describe these concepts and the general pattern of thought behind them as well as to mention the initial experiments that have been performed.

The linking of two carbon atoms, inter- or intra-molecularly, may, in principle be visualized as occurring in one of three possible ways:

(a) Carbon atom A may function as the nucleophile and may attack atom B which has been suitably activated as an electrophilic centre.

(b) Carbon atom B may function as the nucleophile and attack atom A or,

(c) The two centres may come together by a free radical coupling reaction.

All of the work thus far described in this thesis and indeed, all of the work reported in this series of alkaloids in the literature, has concentrated on variations of the first approach in which the C_{15} position of vindoline functions as the nucleophile. This was, in many respects, the logical choice from the point of view of a synthetic plan because this position is electron rich. Furthermore, the activation of the C_{18} position of the indole template as an electrophile is facile.

Some other avenues of approach which also utilize vindoline as the nucleophile were attempted in our laboratory. The work mentioned above

concentrated almost exclusively on conditions, for the coupling reaction, that were essentially acidic. Such conditions would of course favour the non-concerted mechanism via intermediate 109 as outlined in Figures 24 and 27. The stereochemical outcome of such a mechanism has already been amply demonstrated. What would be the stereochemical outcome, however, under conditions that were neutral or basic and which did not favour the formation of such an intermediate? The normal Friedel-Crafts alkylation of aromatic systems under such conditions is difficult if not impossible. Some modifications were necessary in order to furnish an answer to the above question.

It was found that vindoline reacted spontaneously with sources of "positive" halogen in solution. The carefully controlled addition of 1.00 mole equivalents of N-bromosuccinimide to a solution of vindoline in methylene chloride at 0°C resulted in the isolation of 15-bromo-vindoline (114) as the only product. It could be completely characterized by its ir, nmr, and uv spectra together with elemental analysis and mass spectroscopy. The most informative spectra in this case were the nmr (Figure 31) and the mass spectra (Figure 32). The former showed that all the functionality of the vindoline moiety was intact and that the doublet of doublets at τ 4.2 attributable to the C_{15} proton of vindoline had disappeared. Furthermore, the C_{14} and C_{17} proton signals had collapsed to singlets, and had shifted slightly from their positions in vindoline at τ 3.12 and 3.98 respectively to τ 2.86 and 3.87 respectively as would be expected by the introduction of bromine at the C_{15} position. The mass spectrum showed the expected isotope ratio of 1:1 for the molecular ion peak attributable to the isotopic ratio of the two isotopes of

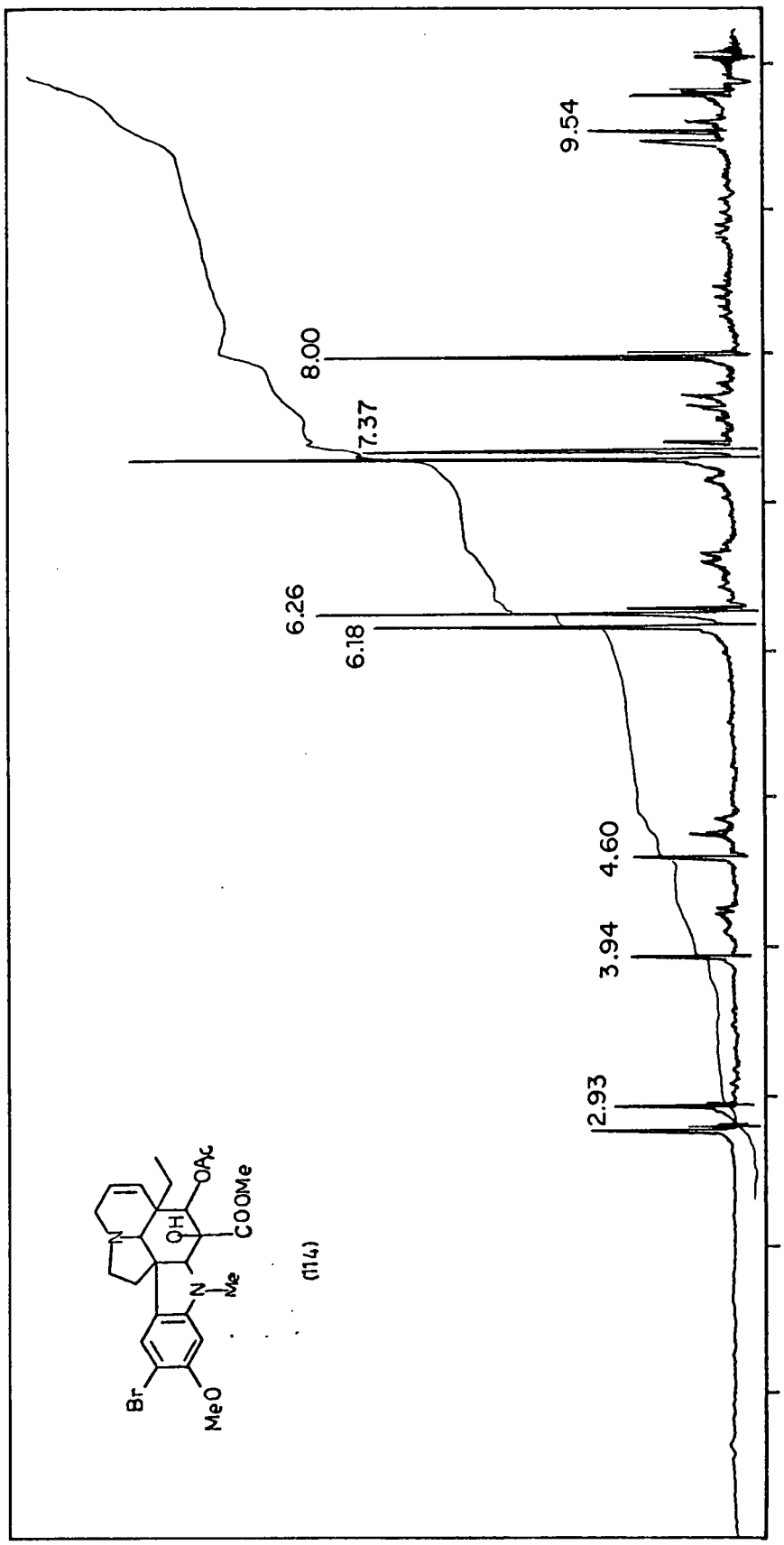


Figure 31. Nmr spectrum of 15-bromovindoline (114).

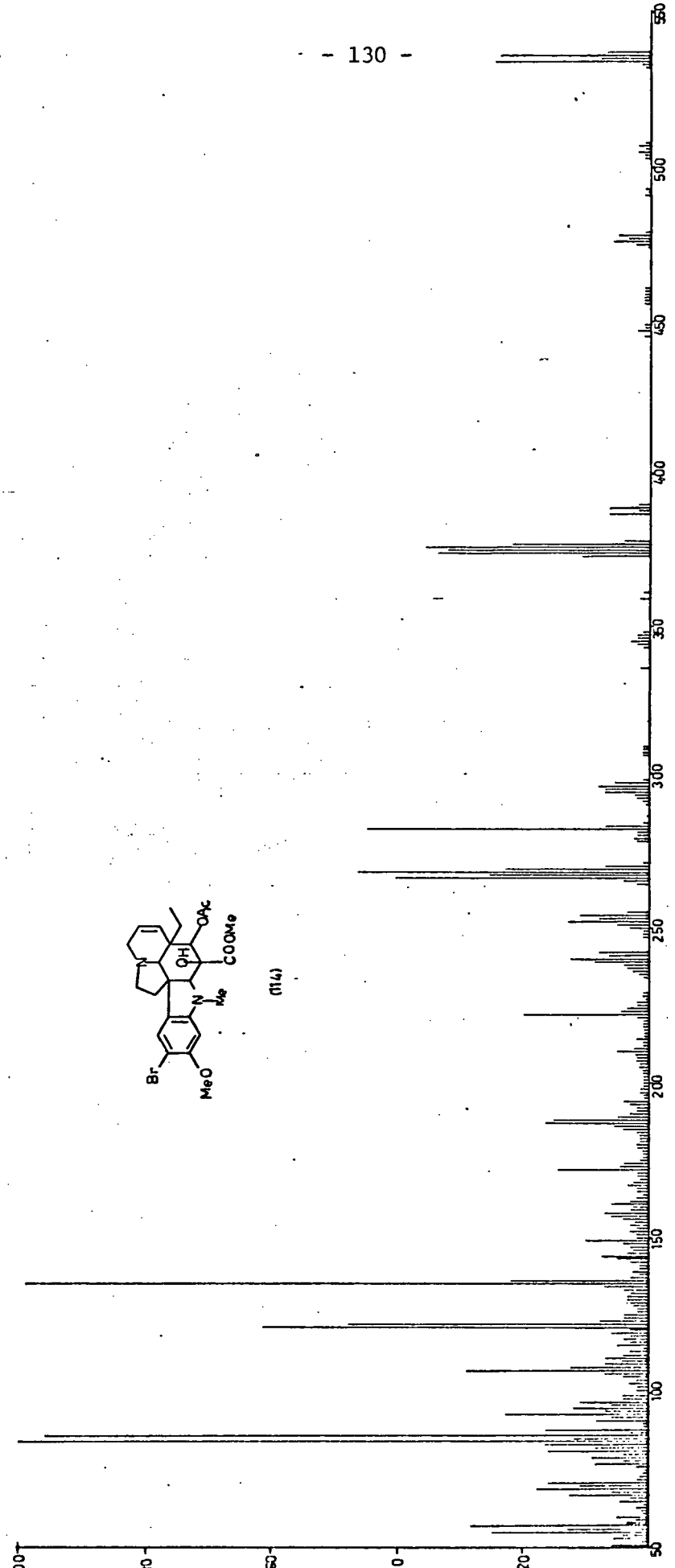


Figure 32. Mass spectrum of 15-bromovindoline (114).

bromine, thus confirming the identity of this compound. In addition, the fragmentation of vindoline has been carefully studied and the established fragmentation scheme is as shown in Figure 33. This fragmentation pattern was virtually completely supported by the mass spectrum of the 15-bromovindoline. All the fragments containing the aromatic portion appeared as two peaks in a 1:1 ratio at 79 and 81 units higher than the corresponding peaks in vindoline. The peaks corresponding to the fragments not containing the aromatic ring were unshifted relative to vindoline. Thus, the complete structure of this compound could, in theory, be deduced from either the nmr or mass spectrum alone.

H. Gilman and coworkers[113] had previously treated related aromatic halides, particularly bromides, with n-butyl lithium under controlled conditions and had obtained high yields of the corresponding aromatic organolithium compounds. For example, treatment of dibromocarbazole (115) according to the scheme shown in Figure 34 afforded a high yield of the corresponding diacid by this approach. If it were possible to make the corresponding anion from 15-bromovindoline, this position would be further activated towards dimerization as well as making it possible for the reaction to be performed under basic conditions where the mechanism may be different from the one portrayed before. Vindoline is a large and complex molecule bearing several functionalities such as carbomethoxy and acetoxy groups which are themselves susceptible to attack by organolithium compounds. If the anion could be made with a stoichiometric amount of n-butyllithium under relatively mild conditions, however, the most reactive centre should react first. According to

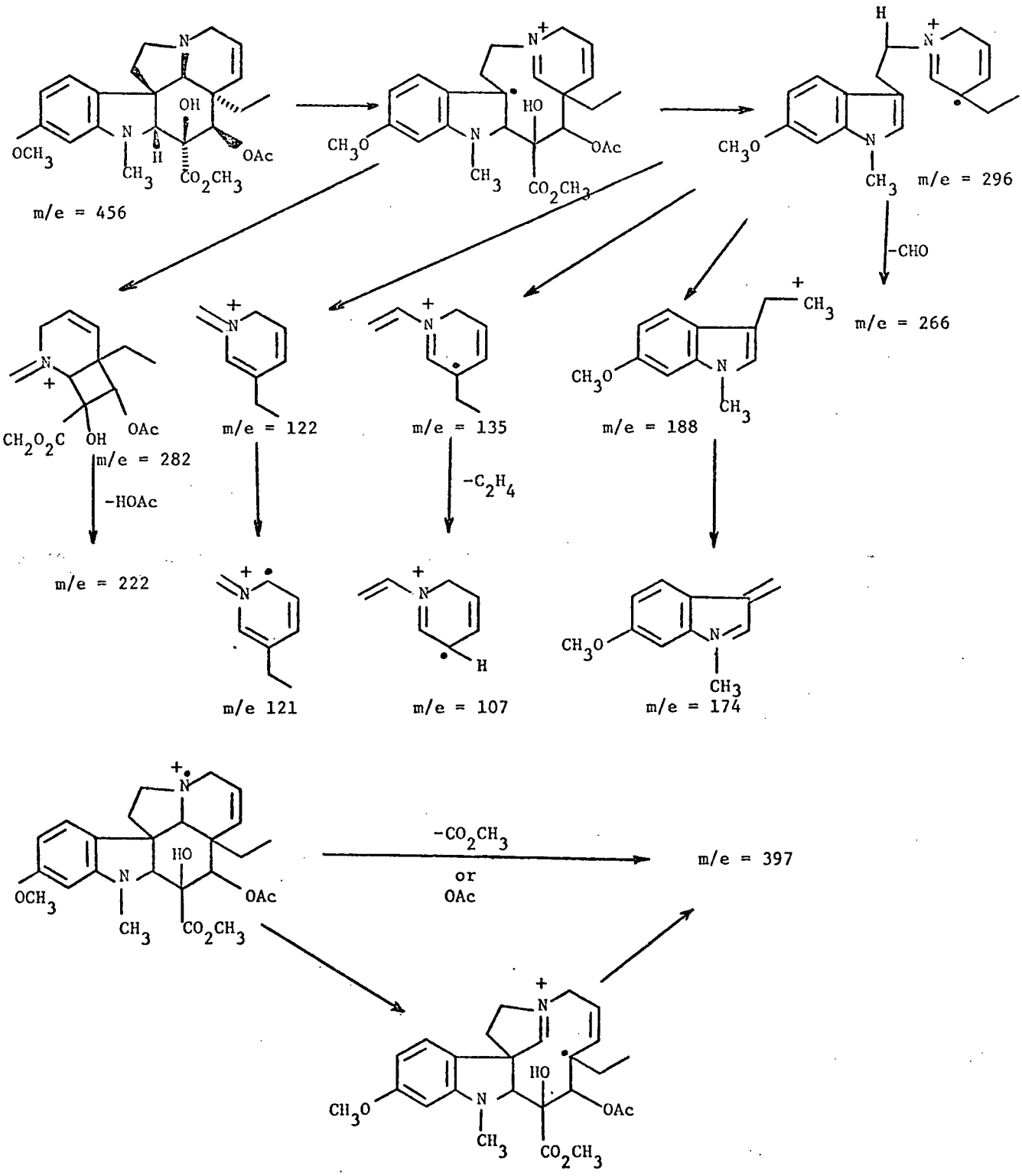
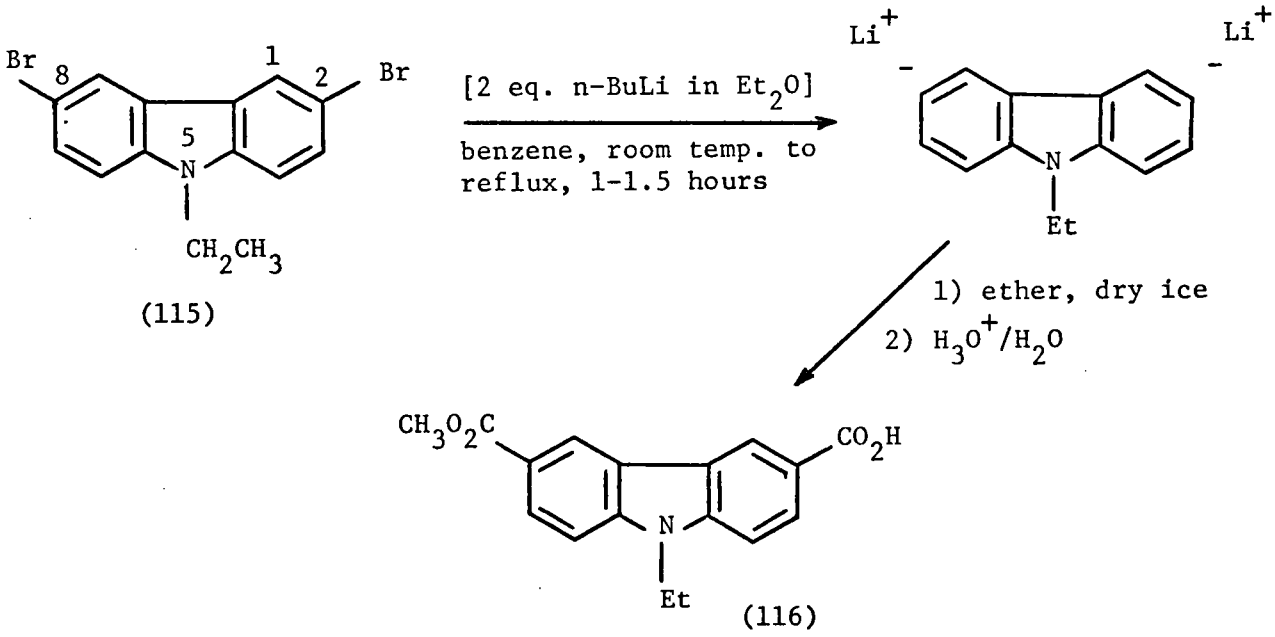


Figure 33. Mass spectral fragmentation scheme for vindoline.[110]



Starting material	Time	Temp.	Product	Yield %
2-bromocarbazole	60 min	reflux	2-lithiocarbazole	57.8
5-ethyl-2-bromocarbazole	70 min	reflux	5-ethyl-2-lithio-carbazole	71.1
5-ethyl-2-iodocarbazole	20 hr	reflux	5-ethyl-2-lithio-carbazole	67.0
5-ethyl-2,8-diiodocarbazole	75 min	reflux	5-ethyl-2,8-di-lithiocarbazole	79.0

Figure 34. Gilman's synthesis of compound 116.[112]

previous work in the literature, this was expected to be the C_{15} bromine atom. Once halogen-metal interconversion had occurred, the anion of vindoline would be too bulky to participate in intermolecular condensations at the C_3 carbomethoxy or acetoxy groups of another vindoline molecule. Molecular models revealed that both these groups were in a rather sterically crowded environment. One further factor favoured such an approach. Under appropriate conditions, n-butyllithium has been used to act as a strong base rather than a nucleophile to produce, for example, the enolate anions of carbonyl groups. Thus, it was felt that under the reaction conditions chosen for the halogen-metal interchange perhaps a nucleophilic condensation by the n-butyl anion onto the carbomethoxy or acetoxy groups could also be ruled out. Since the vindoline moiety has an active hydrogen in the C_3 alcohol functionality, however, an extra mole equivalent of the base would be required in order to form the lithium alkoxide at this position and consume this acidic hydrogen.

The initial reaction performed exceeded all expectations. The anion was produced in tetrahydrofuran, by the gradual addition of two mole equivalents of a 2.0 M solution of n-butyl lithium in hexane at -78°C. Warming to 20°C followed by quenching the reaction with water provided a crude product which by nmr and tlc consisted mainly of vindoline and deacetyl vindoline as judged by the changes in the signals attributable to the C_{14} and C_{17} positions as well as the appearance of a new C_{15} quartet. In particular, the C_{15} bromo compound had completely disappeared. The deacetylation reaction may have occurred either during the reaction or during the workup when lithium hydroxide was generated. When the

anion was formed as above and quenched with acetyl chloride, the acyl group was incorporated and lithium chloride precipitated. To provide concrete proof that both these reactions did not, for example, proceed via a benzyne intermediate, it was necessary to obtain a condensation of this anion onto a ketone. A non-enolizable ketone was necessary because the anion may function simply as a strong base to form the enolate and thus no apparent reaction would occur. Benzophenone was chosen since it had been employed before as a test for such anions. The condensation of this compound onto vindoline, however, proved to be an extremely capricious reaction and appeared to work only when the entire reaction was carried out in one flask in a benzene solution at room temperature. Under these conditions, a reaction mixture could be isolated from which the major compounds could be identified as deacetyl vindoline and a product that might have been the diphenyl-deacetyl vindoline-carbinol on the basis of its nmr spectrum. Firm evidence for the formation of this compound, however, is as yet lacking.

In any event, it was decided to attempt to react this compound with the chloroindolenine of compound 107. It was possible that under the conditions employed, a concerted reaction may occur and this may be reflected in the final stereochemistry of the dimeric product. The chloroindolenine was formed in the usual way and then reacted with a two-fold excess of the anion of vindoline which had been made, as described above, in tetrahydrofuran. Workup followed by purification afforded a 10% overall yield, based on the chloroindolenine, of the dimer 94 as the only isolable dimeric product. Clearly, the small amount of free lithium ions present in solution had catalyzed a non-concerted loss of the chloride as lithium chloride to generate the same intermediate (109)

as in the case of the acid catalyzed reactions. The subsequent dimerization of this with the vindoline generated by the abstraction of the C_{18} proton of the chloroindolenine by the vindoline anion would account for the dimeric product. Recently, F.G. Bordwell and T.G. Mecca[114] have presented a strong case against the existence *in vitro* of SN_2' reactions *per se*. Certainly, the results described above cannot be used to discount the claims of these workers, and may indeed confirm and support them.

Several other modifications were attempted. For example, the possibility of a non-concerted loss of chloride may be circumvented by using the reaction described earlier to introduce a C_{18} substituent such as methoxyl or acetoxyl in a separate step. This could then be displaced in a concerted SN_2 reaction by the vindoline anion. The centre at which displacement was to occur however, was a tertiary one. Although in some cases, concerted displacements adjacent to an ester group were known to occur even at such centres, some difficulties were anticipated here primarily because of the bulk of the incoming nucleophile. Nonetheless, this experiment was performed with 18-methoxy- and 18-acetoxy-carbomethoxy-4β-dihydrocleavamines produced by reacting the chloroindolenine of compound 107 with a 1.5% solution of hydrogen chloride in methanol and acetic acid respectively. In both cases, the formation of the C_{18} substituted material was rapid and occurred in high yield. These compounds failed to react in the desired way, however, with the anion of vindoline under a wide variety of conditions. Finally, in an attempt to see whether these compounds would react at all with organo-lithium reagents, the C_{18} acetoxy compound was reacted with an excess of

methyl lithium. The reaction mixture was found to be extremely complex. The laborious separation and identification of the major products afforded the rather disappointing result that none of the desired C_{18} methyl compound had been formed. On the basis of this discouraging evidence, this particular approach was abandoned.

It was felt, however, that the presence of a carbonyl at C_{18} may facilitate attack by the anion onto this position and may yield two epimeric C_{18}, hydroxy dimers. These compounds would not only be interesting in themselves in a clinical sense but could easily be converted, by hydrogenolysis of the C_{18}, alcohol, to a C_{18}, hydrogen dimer epimeric at C_{18}, to dimer 97 for which X-ray evidence was now available. Furthermore it might be possible to replace the hydroxyl functionality with a carbon-carbon linkage which may be converted to the C_{18}, carbomethoxy group. This could, for example, be a nitrile group. If this reaction were performed in a non-concerted SN_1 manner, the intermediate would be similar to 109 above. If it is assumed that the same stereoselectivity operates in this intermediate as operated during the conventional dimerization reaction, then the incoming nitrile must attack the β-face of the molecule to produce the natural stereochemistry about C_{18}, in the resulting nitrile dimer.

The approaches envisioned for the synthesis of the required acylindole are outlined in Figure 35. Each one of these approaches has certain difficulties associated with it and preliminary experiments have revealed some of these difficulties in each case. Approach A appeared to involve the most direct extension of known chemistry. Indeed this whole sequence had been carried out (R = OAc) from compound 107 to

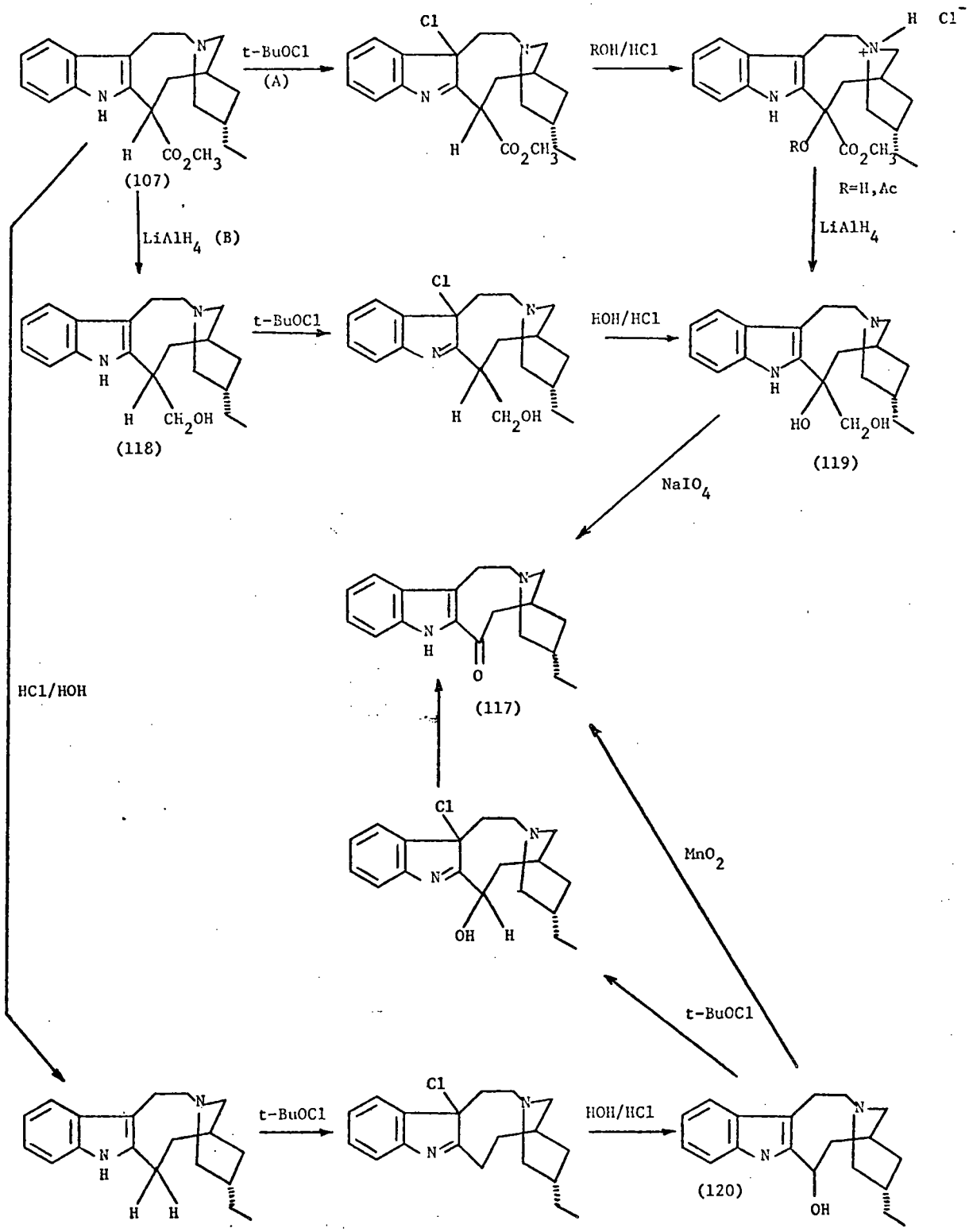


Figure 35. Approaches to the synthesis of the acylindole (117).

117 as part of the proof of the structure of the C_{18} acetoxy compound. As mentioned previously in the description of a similar reaction in the C_{18} methoxy series (vide supra) the hydride reduction of the C_{18} substituted compound proceeded in low overall yield to give a rather complex reaction mixture. In agreement with this observation, the overall yield of compound 117 isolated by route A from compound 107 was roughly 10%. Both pathways B and C represent attempts to circumvent this low yielding reduction step. In pathway B this was accomplished by reducing the ester prior to any C_{18} functionalization. Though the reduction proceeded in nearly quantitative yield in this case to afford compound 118, it was found that any treatment of the corresponding chloroindolenine of this compound under acidic conditions resulted in the formation of the C_{18} aldehyde (121) as the major product (70%) together with the desired diol as a minor product (30%). The mechanism for this reaction is thought to be as shown in Figure 36. This phenomenon had been noted in a similar case in our laboratories. The aldehyde was easily identified on the basis of its nmr spectrum (Figure 37) as well as the carbonyl absorption in its ir spectrum. It could be reduced with sodium borohydride in ethanol to the starting primary alcohol (118), thus proving its structure to be as assigned. Attempts to block such a reaction by suitably masking the primary alcohol functionality are currently in progress in our group.

Pathway C effectively removes the problematic centre in pathway A and B entirely. Thus, in theory, it should be devoid of any of the technical problems of the other two schemes. One problem that may be anticipated here involves the final oxidation step. Though on the surface

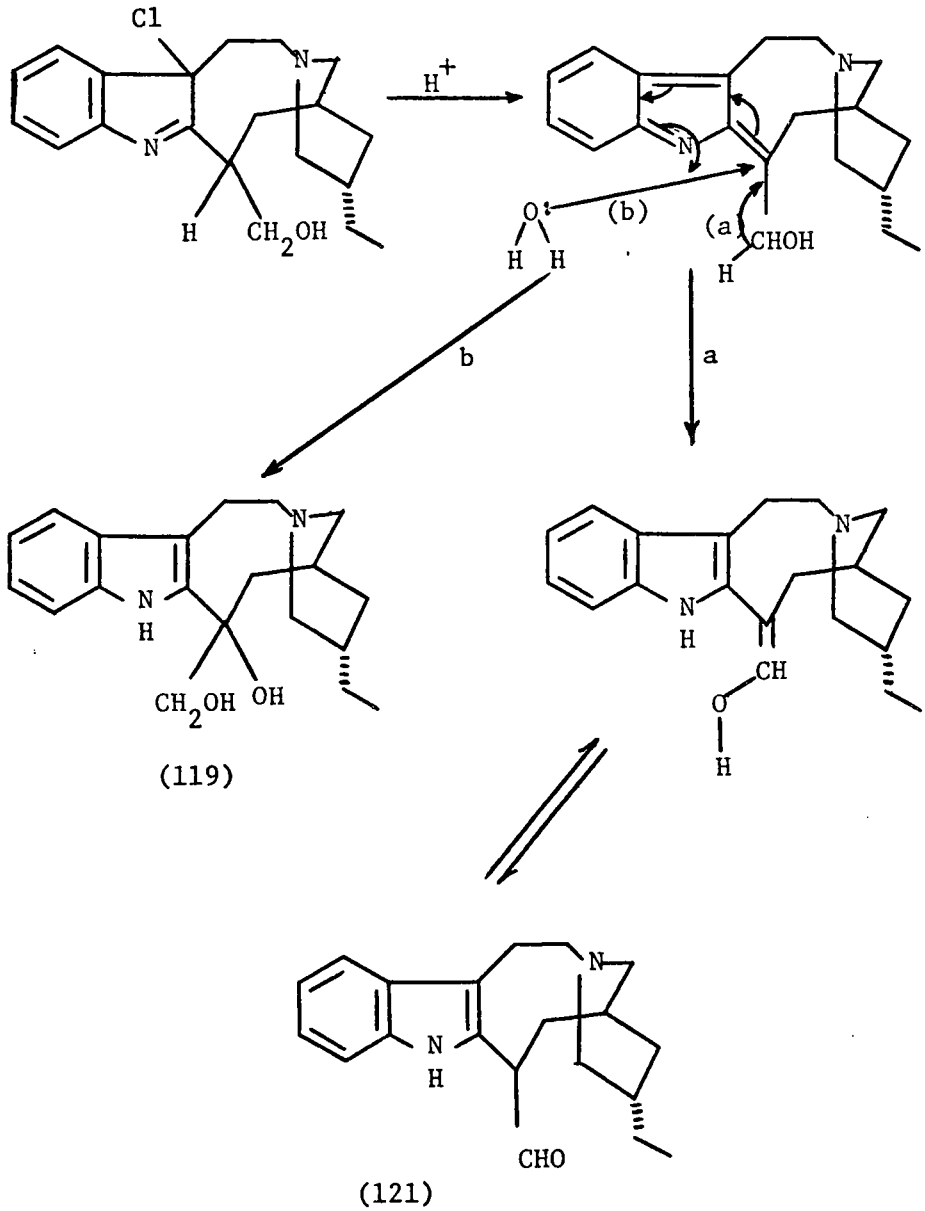


Figure 36. Reactions of the chloroindolenine of compound 118 under acidic conditions.

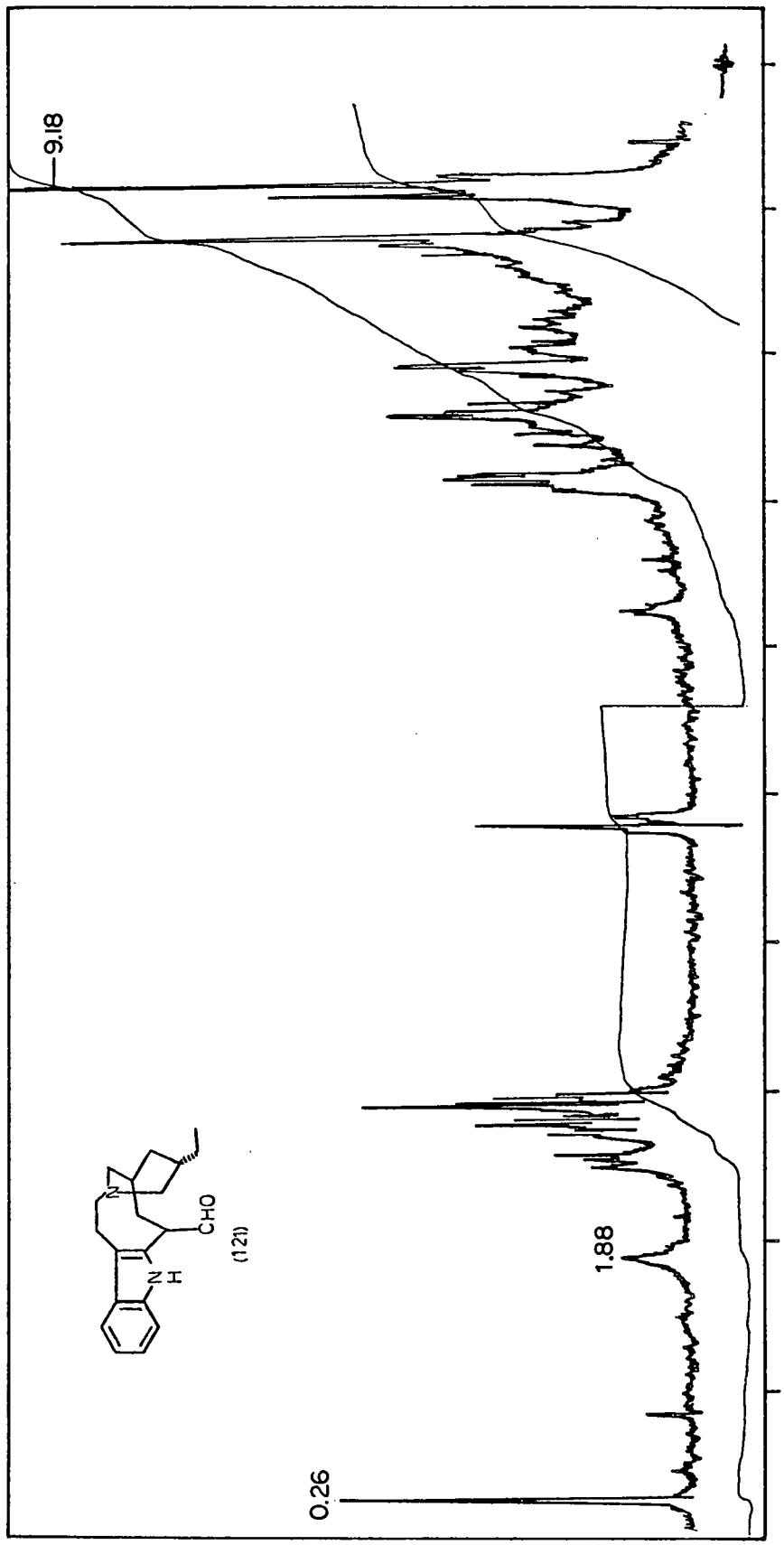


Figure 37. Nmr spectrum of aldehyde (121).

it may appear to be the trivially simple oxidation of an "allylic" secondary alcohol (120); past experiences with indole alkaloids in our laboratories (some of which are mentioned in Part I of this discussion) would suggest that the indole chromophore is remarkably susceptible to oxidation itself. One method of achieving this oxidation would be to utilize this reactivity of the indole by forming the chloroindolenine of compound 120 again. This may be solvolyzed in a non-hydroxylic solvent such as chloroform or dichloromethane. Such conditions would force intramolecular participation by the lone pair of electrons on the C_{18} alcohol oxygen to regenerate aromaticity and produce the desired 2-acylindole in one step. Even the use of hydroxylic solvents such as methanolic hydrogen chloride would result in either the formation of the acylindole directly via the intramolecular reaction mentioned above or else would result in the formation of a hemiketal structure via solvent participation which would be converted in situ, or upon workup, to compound 117.

Another promising and useful sequence, that has been developed by other workers in our group involves essentially a cyanylation of the preformed dimer 97. This approach is particularly appealing because it turns the previously undesirable stereoselectivity of the coupling reaction to advantage by allowing the same stereoselectivity to guide the approach of cyanide onto the molecule. Thus, if the chloroindolenine of the dimer 97 is treated under mildly acidic or neutral conditions with a source of cyanide, this nucleophile must attack the resulting intermediate from the β-face, exclusively, to form a $C_{18'}$ cyano dimer which bears the natural stereochemistry about $C_{18'}$. Thus, in effect,

the order of introduction of the carbomethoxy group and the vindoline moiety onto the cleavamine template has been reversed by this approach relative to the dimerization of compound 107 with vindoline.

Until recently, this approach was plagued by two major drawbacks: (a) a reaction to form the $C_{18'}$ cyano dimer in reasonably high yield was unavailable and (b) when this dimer was produced in small amounts and isolated, it was found to be remarkably sensitive to acidic conditions. Thus the conditions required for the conversion of the nitrile to the corresponding carbomethoxy group were such that cleavage and decomposition of this dimer might be anticipated.

Recently, the first problem has been eliminated by the skillful use of tetra-n-butylammonium cyanide as a source of cyanide which is readily soluble in organic solvents. When the chloroindolenine of dimer 97 and the above reagent were refluxed in 1,2-dichloroethane, they reacted smoothly to produce the desired cyano dimer apparently in reasonable yield. The second problem may be overcome by using the above approach to introduce, not a nitrile but, for example, an aldehyde masked as the tetra-n-butylammonium salt of 1,3-dithiane. This could be easily converted to the aldehyde and thence to the $C_{18'}$ carbomethoxy group. This strategy holds great promise for the future.

A rather different approach to dimerization has evolved during the course of these studies which is truly attractive from an aesthetic and academic point of view as well as from the purely practical and pragmatic one. V. rosea is probably one of the most studied plant systems. As mentioned in the introduction of this thesis, extensive efforts have been made to isolate, characterize and test all of its

alkaloids. Despite this fact, and after the isolation and characterization of roughly 120 alkaloids, there has never been any report of the isolation of a monomeric tetracyclic indole template resembling cleavamine. If the plant synthesized and coupled such monomers in two separate steps, their isolation from Nature, at least in small amounts, should be possible. On the other hand, the rigid pentacyclic Iboga skeleton epitomized by catharanthine (11) is quite abundant in the plant system. The in vivo dimerization may thus be viewed as a formal ring opening of catharanthine by a nucleophile-vindoline to generate a dimer bearing the cleavamine skeleton in a single step. Functionalization of the 3,4 double bond would then complete the synthesis of the natural dimers. In fact such functionalization may activate the catharanthine skeleton and initiate the dimerization. In the laboratory, the conversion of catharanthine to 18-carbomethoxycleavamine is accomplished with sodium borohydride in glacial acetic acid. The cleavamine template is then activated, through the indole portion, towards nucleophilic attack by vindoline at the C_{18} position. The two steps - ring opening and coupling - are separated in vitro. What would be the stereochemical outcome, however, if a reaction could be devised where these steps were reunited as in Nature?

An inspection of molecular models revealed that if vindoline could be used to displace the C_{18}-C_5 bond in a trans-coplanar fashion, the resulting dimer would possess the natural stereochemistry about $C_{18'}$. This was indeed an exciting postulate for two reasons. From a purely academic standpoint it meant that the achievement of an in vitro fragmentation of the kind envisaged would be tantamount to a biomimetic dimerization.

Even more important, however, was the fact that dimers could be obtained by such a reaction directly from the two most abundant alkaloids of the plant - catharanthine (11) and vindoline (10). Attention was thus turned towards achieving this goal. It was obvious, from the outset, that a suitable leaving group was necessary at C_4, and that a particular stereochemistry of this leaving group was absolutely mandatory in order to make a concerted fragmentation possible. The general scheme is outlined in Figure 38, together with some of the anticipated side reactions.

The proper choice of a method of placing such a leaving group on the C_4 position may also permit the simultaneous functionalization of the C_3 position thus permitting the synthesis of a 3,4 functionalized dimer of the correct stereochemistry at $C_{18'}$. The stereochemistry of the C_4 functionality would almost certainly be α on the basis of all the previous work described in Part I. This was fortunately exactly the required one to permit the desired dimerization reaction. The first choice of an activating group at the 3,4 position was the diol resulting from the osmylation of catharanthine. Clearly if this diol could be produced, the tertiary C_4 alcohol could be solvolyzed under acidic conditions to yield exactly the carbonium ion of the type shown in compound 123, and thus permit the desired fragmentation to occur. The advantage of this approach was that it would yield a dimer bearing an oxygen functionality at the $C_{3'}$ position in the same stereochemistry as was already available in dimer 106 derived from the hydroboration products (56 and 57) of 18-carbomethoxycleavamine. Thus any differences between the dimer generated by this approach and dimer 106, may be

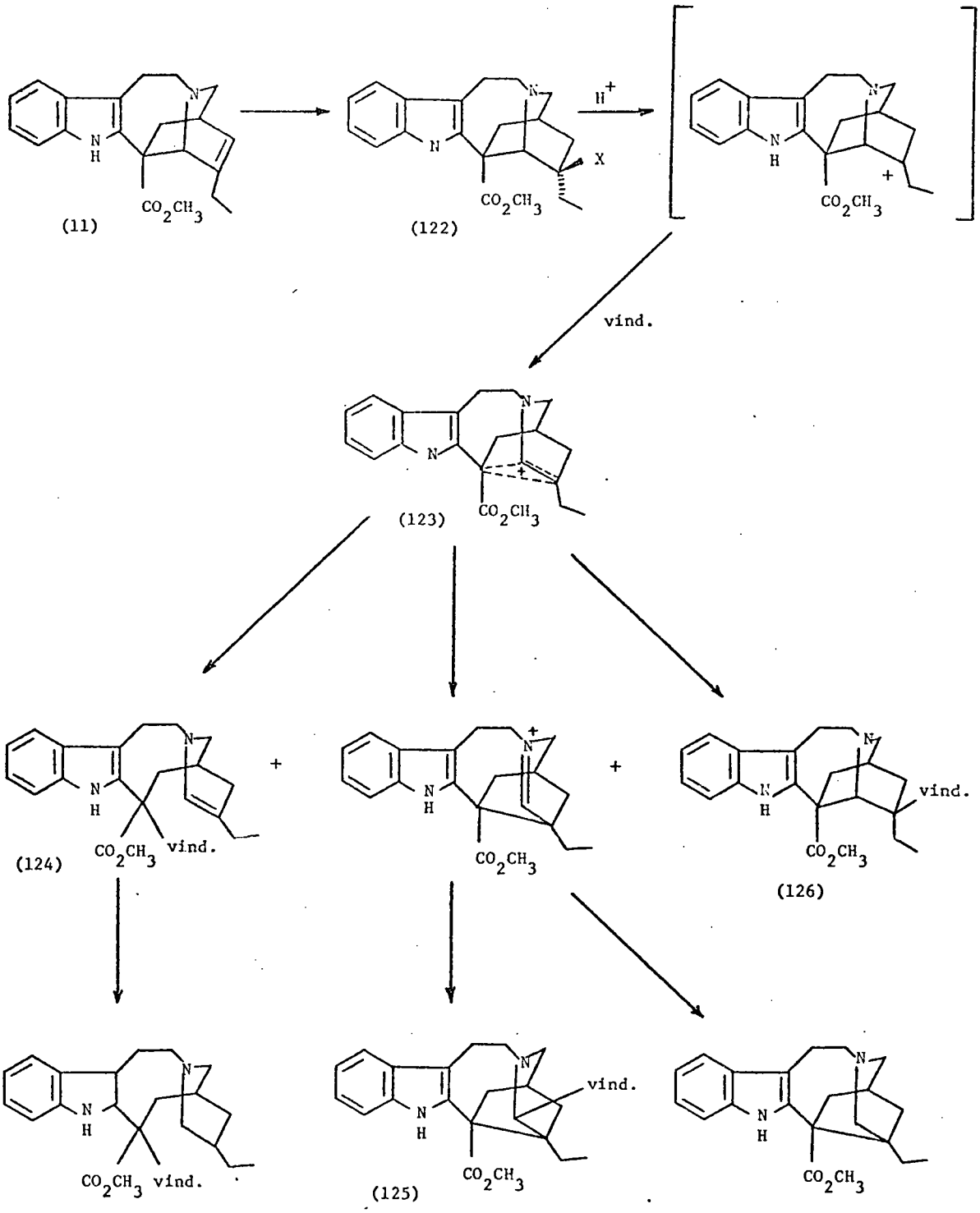


Figure 38. General approach to the biomimetic dimerization.

interpreted as being indicative of some change in stereochemistry about the $C_{18'}$ position.

Several attempts to osmylate this sterically hindered double bond under a variety of conditions, resulted in no reaction under milder conditions, and destruction of the indole chromophore under more stringent conditions. Attention was turned to other means of achieving the same goal. Epoxidation was the next choice. It rapidly became clear that the treatment of catharanthine, as the free base, with 1.0 equivalent of m-chloroperbenzoic acid yielded, almost exclusively, the N-oxide of catharanthine. The structure of this compound was not at all a simple matter to prove. Upon attempted purification by column chromatography or even by recrystallization from neutral organic solvents, it rearranged cleanly to another compound. The structure of the rearrangement product is not known with certainty beyond the fact that it possesses an exchangeable proton in the nmr spectrum, other than the indolic N-H, indicating that it may be an alcohol. This is corroborated by the ir spectrum. However, upon producing catharanthine N-oxide and hydrogenating it immediately, without purification, the N-oxide grouping could be hydrogenolyzed to generate dihydrocatharanthine which was identical in every way to the authentic material prepared by the direct hydrogenation of catharanthine itself.

Therefore, it was necessary to protect the basic N_b atom prior to any attempt to epoxidize the 3,4 double bond of catharanthine. When catharanthine hydrochloride in dry methylene chloride was treated with an excess of m-chloroperbenzoic acid, the major product in the reaction mixture was not the N-oxide nor the starting material. This product

was purified and treated with vindoline in a biomimetic reaction using 1.0 N hydrochloric acid at room temperature for ten days. It yielded a reaction mixture from which a dimeric compound could be isolated in 30% yield. Nmr (Figure 39) and mass spectral (Figure 40) studies were rather confusing however. They indicated that the 3,4 double bond was intact and that another benzylic or olefinic proton had been generated. In fact, it appeared that dimerization may not have occurred at C_{18}, but rather at C_5 as shown below (Figure 41). In a separate experiment, it could be shown that the reagent had attacked the indole portion of the compound to produce fragmentation to a tetracyclic intermediate which had subsequently undergone dimerization. Reaction of the chloroindolenine of catharanthine[107] under the same conditions also produced the same dimer in similar yield; thus demonstrating the above postulate. Furthermore, the treatment of dihydrocatharanthine hydrochloride with m-chloroperbenzoic acid followed by dimerization with vindoline in 1.0 N hydrochloric acid also produced a similar dimer lacking the 3',4' double bond but otherwise quite analogous to the previous dimer. The same sequence repeated on the chloroindolenine of dihydrocatharanthine confirmed and supported the above claim.

In summary, it was clear from these results that the order of reactivity within the catharanthine moiety towards electrophilic reagents is $N_b > N_a > \Delta^{3,4}$ and at present the fragmentation approach, though fascinating and potentially useful, must await another study currently under way in our laboratories to block the other two reactive centres, epoxidize the 3,4 double bond, and then systematically deprotect both centres. This study is visualized as serving a dual purpose: (a) In the

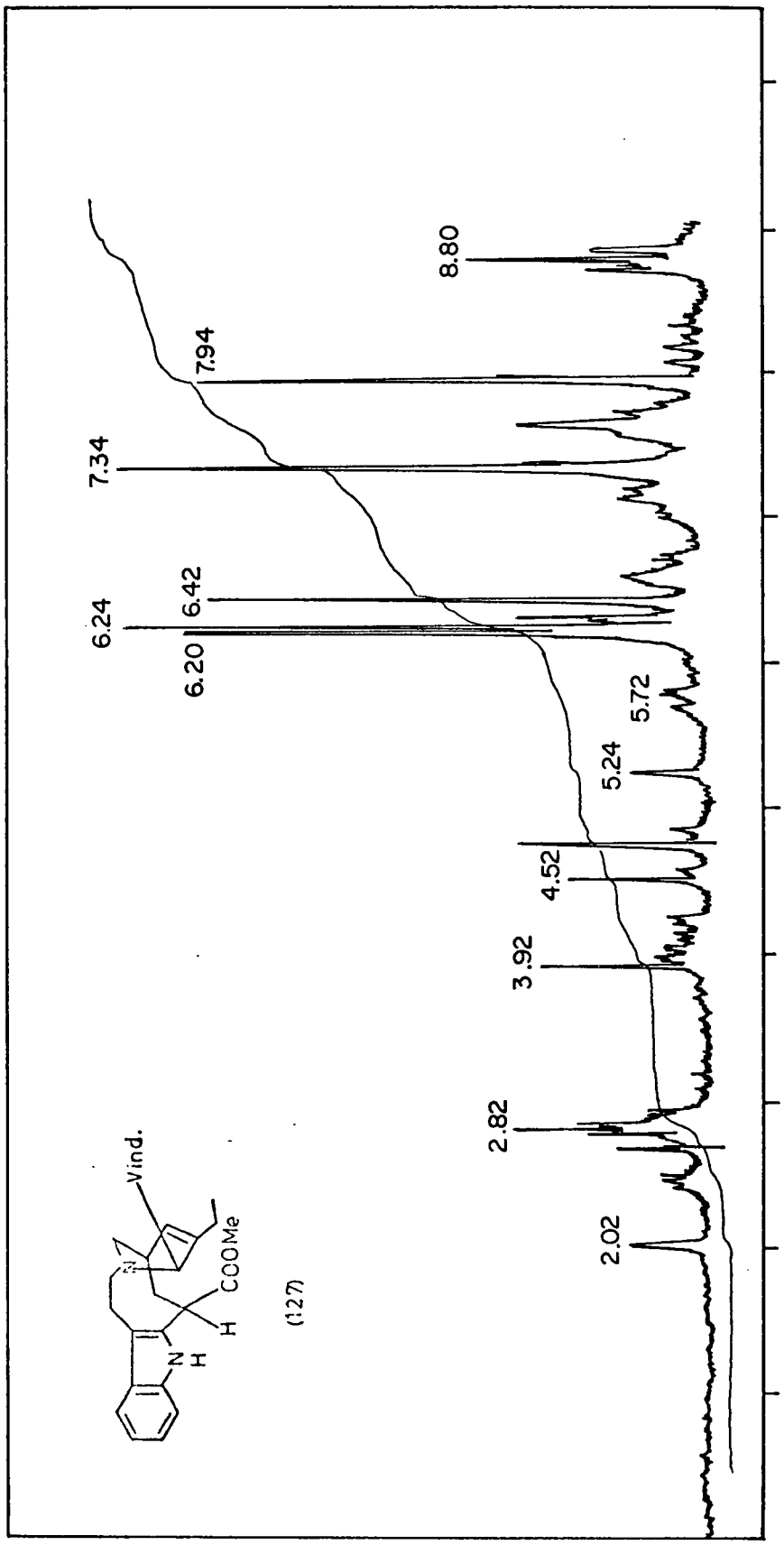


Figure 39. Nmr spectrum of dimer (127).

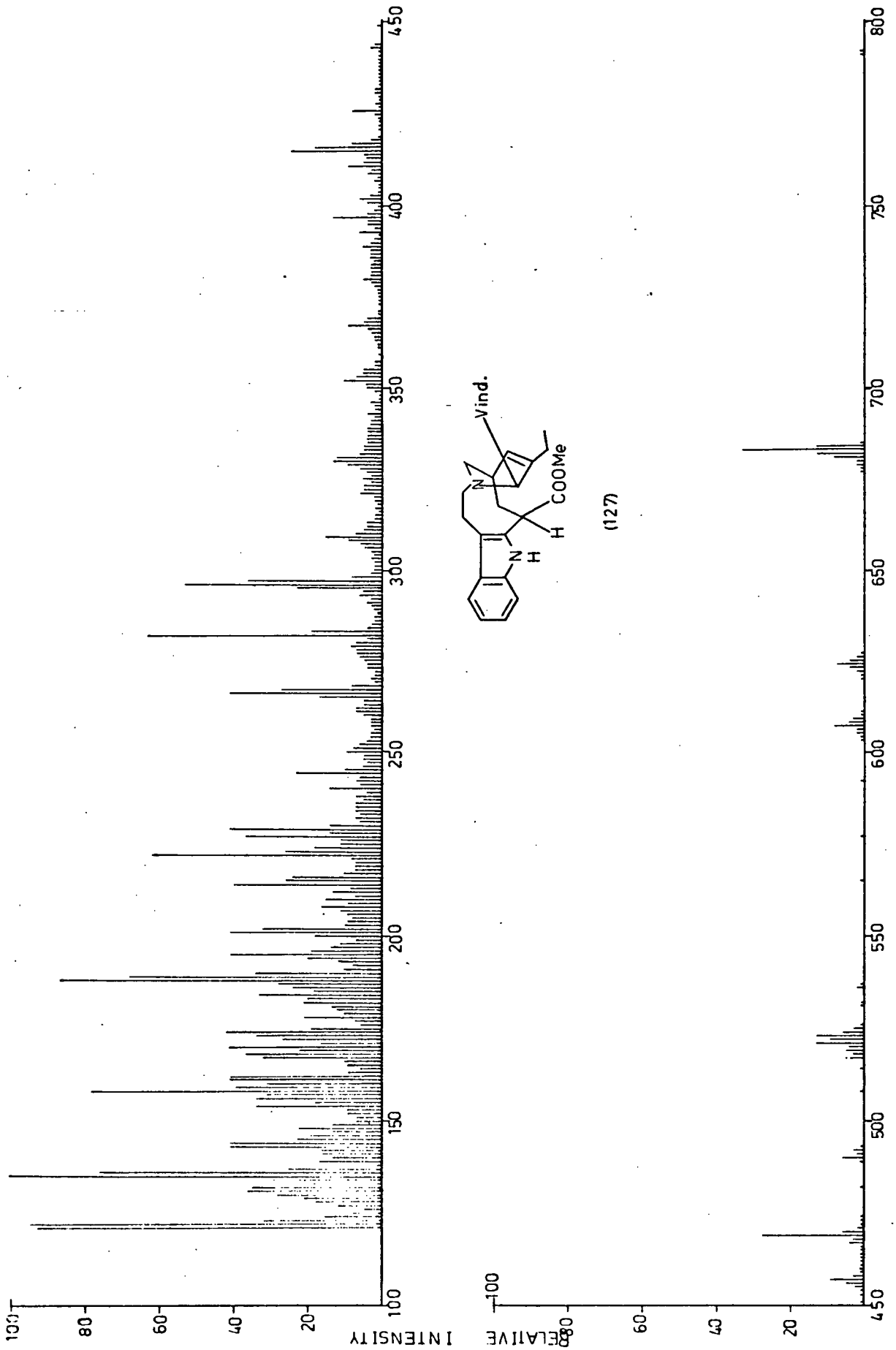
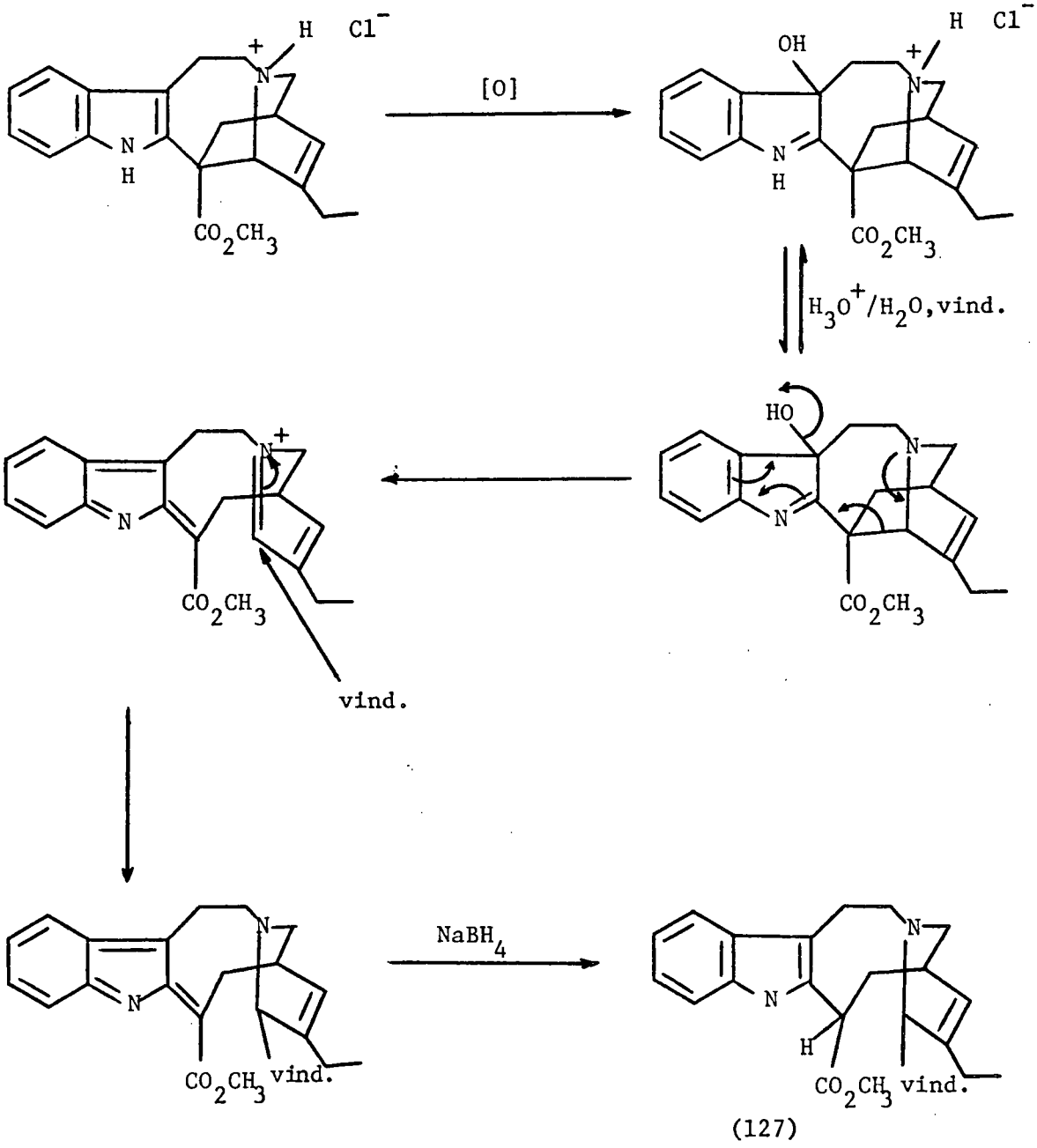


Figure 40. Mass spectrum of dimer (127).



(127)

Figure 41. Proposed mechanism for the formation of dimer (127).

tetracyclic cleavamine template, if a method of epoxidizing the 3,4 double bond, after blocking all possible side reactions, is found then a critical intermediate for the synthesis of the indole portion of the various natural dimers would have been synthesized. (b) If an epoxide can be introduced into the 3,4 position of the rigid catharanthine skeleton, then the first opportunity for testing this interesting fragmentation scheme would become available.

In conclusion, when this work was initiated, three vital questions remained unanswered. As a result of the efforts of this research all of these questions have been answered. The questions dealt with the stereochemical outcome of the conventional dimerization, the mechanism of this reaction and the development of new methods for performing this step. The question of stereochemistry in the conventional reaction has been conclusively established in several cases as a result of the X-ray studies mentioned. The details of the mechanism of this reaction have been elucidated by the study of the dimerization of 18β-carbomethoxy-4β-dihydrocleavamine (107). Finally, new methods and concepts have been explored which may lead to an improvement in the yield or an inversion of the stereochemistry of the synthetic dimers. These results have been obtained by slow and painstaking study rather than by a series of fortuitous discoveries. However, if a single one of these avenues bears fruit at some future date in terms of providing a synthesis of the target molecule, this will be a sufficient reward for any efforts that may have been, or may need to be, spent.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet spectra (uv) were recorded on a Cary 11 spectrophotometer using ethanol as solvent unless otherwise specified. Infrared spectra (ir) were recorded on a Perkin-Elmer Model 21 and Model 137 spectrophotometers. Nuclear magnetic resonance (nmr) spectra were recorded in deuteriochloroform at 100 MHz on Varian HA-100 and XL-100 instruments and the chemical shifts are given in Tiers τ scale with reference to tetramethylsilane as the internal standard. Mass spectra were recorded on an Atlas CH-4B or an AE-MS-902 mass spectrometer. Analyses were carried out by Mr. P. Borda of the microanalytical laboratory, The University of British Columbia. Woelm neutral alumina (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 carbon tetrachloride antimony pentachloride solution and aqueous ceric sulfate solution as stated. Woelm neutral alumina (activity III) was used for column chromatography (unless otherwise indicated).

18-Carbomethoxy-4β-dihydroclevamin-3α-ol (56) and (57)

To a solution of 18β-carbomethoxycleavamine (2.2792 g, 6.44 mmoles) in anhydrous tetrahydrofuran (THF) (100 ml) contained in a 250 ml 3-necked round-bottomed flask cooled to 0°C and under a brisk stream of dry nitrogen, was added a solution of diborane in THF (9.6 ml of a 1.5 M solution) over a period of 30 minutes with stirring. After the addition was complete the pale yellow solution was allowed to come to room temperature during a further 1 hour. Removal of the solvent in vacuo at not higher than 20°C afforded a whitish-yellow gum. This material was taken up in a further portion of freshly distilled THF (100 ml) and cooled again to 0°C. Basic hydrogen peroxide (4 ml of a solution containing 9 ml of 30% hydrogen peroxide and 1 ml of 10% by weight aqueous sodium hydroxide) was added with stirring over 10 minutes. Removal of the solvent resulted again, in a yellowish gum which was partitioned between water and dichloromethane. The aqueous phase was washed with several portions of dichloromethane (4 x 40 ml). The combined organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure to yield a light foam (2.2 g).

This material was dissolved again in dry THF (100 ml), distilled triethylamine (4.5 ml) was added and the whole refluxed for 1.5 hours under a positive pressure of dry nitrogen. Evaporation of the solvent in vacuo resulted in a yellow gum (3.0 g). This was immediately chromatographed on alumina (200 g). Elution was begun with benzene, dichloromethane (1:1). Elution with pure dichloromethane afforded 18β-carbomethoxy-4β-dihydrocleavamin-3α-ol (56) pure and crystalline (574.5 mg) in fractions 12 to 22. This compound was recrystallized from methanol, mp 158-163° dec. λ_{max}^{EtOH} nm:

293, 285, 278 (sh), 226 (log ε 3.8, 3.9, 3.8, 4.5 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3442, 2920, 1718, 1460, 1331, 1305, 1260, 1155, 1040, 905; Nmr: τ 1.13 (broad singlet, 1H, N-H), 2.40-3.00 (diffuse multiplet, 4H, aromatic), 5.00 (triplet, J ∿ 7 cps, 1H, C_{18} H), 6.31 (singlet, 3H, C_{18} $-CO_2CH_3$), 6.32 (quartet, J = 10 cps, 1H, C_3H), 9.06 (triplet, J = 7 cps, 3H, $-CH_2CH_3$); mass spectrum: main peaks at m/e = 356, 226, 154, 140, 138, 124.

Anal. Found: C, 70.74%; H, 7.99%; N, 7.66%. Calcd. for $C_{21}H_{28}N_2O_3$: C, 70.76%; H, 7.92%; N, 7.86%. High resolution mass spectrometry: Found, 356.2017; Required for $C_{21}H_{28}N_2O_3$, 356.2098.

Further elution with dichloromethane, ethyl acetate (1:1) yielded 18α-carbomethoxy-4β-dihydrocleavamin-3α-ol (57) (929.2 mg) in fraction 23 and 74.5 mg of the compound slightly contaminated with other materials in fraction 24, mp 110-112°C. λ_{max}^{EtOH} nm: 293, 285, 276 (sh), 226 (log ε 3.8, 3.9, 3.8, 4.5 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 2920, 1720, 1460, 1330, 1165, 1010; Nmr: τ 1.20 (broad singlet, 1H, N-H), 2.46-3.06 (diffuse multiplet, 4H, aromatic), 5.99 (multiplet, 1H, C_{18}H), 6.32 (singlet, 3H, C_{18}-CO_2CH_3), 9.43 (triplet, J = 7 cps, 3H, $-CH_2CH_3$); mass spectrum: main peaks at m/e = 356, 327, 226, 154, 140, 138, 124.

Anal. High resolution mass spectrometry: Found, 356.2129; Required for $C_{21}H_{28}N_2O_3$ (M^+), 356.2098. The total yield of alcohols was 69.5%.

Epimerization of 18α- and 18β-Carbomethoxy-4β-dihydrocleavamin-3α-ol to an Equilibrium Mixture

To a solution of 18β-carbomethoxy-4β-dihydrocleavamin-3α-ol (56) (150 mg) in benzene (10 ml) was added borontrifluoride etherate (1 ml)

and the whole system maintained at reflux under a positive pressure of nitrogen for 6 hours. At the end of this time the reaction mixture was cooled to room temperature and then poured into an aqueous solution of 10% sodium bicarbonate at 0°C. The two phases were separated and the aqueous phase was again washed with dichloromethane (2 x 50 ml). The combined organic phase was dried over sodium sulfate, filtered, and evaporated under reduced pressure to yield a whitish foam (151 mg). This material was chromatographed on alumina (neutral Woelm III). Elution with methylene chloride afforded 70 mg of the starting compound (56). Gradual increases in the solvent polarity to a mixture of methylene chloride and ethyl acetate (1:1) afforded the C_{18} epimeric alcohol (57) (77 mg) together with some minor fractions containing mixtures of these two compounds. These compounds could be identified by comparison with authentic materials.

18β-Carbomethoxy-4β-dihydrocleavamin-3α-acetate

18β-Carbomethoxy-4β-dihydrocleavamin-3α-ol (56) (150 mg) was dissolved in a 1:1 mixture (15 ml) of acetic anhydride and pyridine. The reaction mixture was allowed to stir at room temperature overnight. It was then poured onto ice cold 10% sodium bicarbonate and extracted with methylene chloride. The aqueous layer was further extracted once with methylene chloride. The combined organic phases were dried over anhydrous sodium sulfate and filtered. Removal of the solvent in vacuo followed by evacuation under high vacuum for several hours yielded 18β-carbomethoxy-4β-dihydrocleavamin-3α-acetate (147 mg) which could be purified by recrystallization from ethyl acetate. $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 2940, 1720, 1460, 1370, 1330, 1300, 1020 (broad); Nmr: τ 1.35 (broad

singlet, 1H, N-H), 2.46-3.04 (diffuse multiplet, 4H, aromatic), 4.90 (multiplet, 2H, C_{18}-H and C_3-H), 6.33 (singlet, 3H, C_{18}-CO_2CH_3), 7.97 (singlet, 3H, C_3-OAc), 9.13 (triplet, J = 7 cps, 3H, $-CH_2CH_3$).

18α-Carbomethoxy-4β-dihydrocleavamin-3α-acetate

18α-Carbomethoxy-4β-dihydrocleavamin-3α-ol (57) (150 mg) was dissolved in a mixture (1:1) of acetic anhydride and pyridine. The reaction mixture was allowed to stir overnight at room temperature. It was then poured onto ice cold 10% sodium bicarbonate and extracted with methylene chloride. The aqueous layer was further extracted once with methylene chloride. The combined organic phase was dried over anhydrous sodium sulfate and filtered. Removal of the solvent in vacuo followed by evacuation under high vacuum for several hours yielded 152 mg of a reaction mixture consisting mainly of 18α-carbomethoxy-4β-dihydrocleavamin-3α-acetate. This compound was purified by column chromatography over deactivated alumina (grade III). $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 2920, 1720, 1460, 1365, 1250 (broad), 1160, 1005; Nmr: τ 1.26 (broad singlet, 1H, N-H), 2.42-3.02 (diffuse multiplet, 4H, aromatic), 5.51 (broad multiplet, 1H, C_3-H), 6.04 (triplet, J ∿ 5 cps, 1H, C_{18}-H), 6.32 (singlet, 3H, C_{18}-CO_2CH_3), 8.01 (singlet, 3H, C_3-OAc), 9.42 (triplet, J ∿ 7 cps, 3H, $-CH_2CH_3$).

4β-Dihydrocleavaminol Acetate from 18α- and 18β-Carbomethoxy-4β-dihydrocleavamin-3α-ols (56 and 57)

18α-Carbomethoxy-4β-dihydrocleavamin-3α-ol (24.8 mg) and 18β-carbomethoxy-4β-dihydrocleavamin-3α-ol (23.6 mg) were taken separately in two identical flasks and were treated with 3 ml each of 5 N HCl. These

flasks were then placed under a positive pressure of dry nitrogen and immersed into an oil bath at 90°C with stirring. After 7 hours the darkly coloured reaction mixtures were cooled to room temperature and poured into ice cold 10% sodium carbonate. Extraction with several aliquots of methylene chloride followed by drying of the combined organic phase (in each case separately) over anhydrous sodium sulfate and filtration and evaporation of the solvent in vacuo yielded 26.8 mg of crude 4β-dihydrocleavamin-3α-ol from the 18α series and 23.1 mg of 4β-dihydrocleavamin-3α-ol from the 18β series.

These were immediately acetylated in the usual way to yield after purification pure crystalline 4β-dihydrocleavamin-3α-acetate (6.5 mg) from the 18α series and 4β-dihydrocleavamin-3α-acetate (6.4 mg) from the 18β series. Both these compounds were identical to one another as judged by mixed melting points which were undepressed as well as completely superimposable infrared spectra both as chloroform solutions as well as nujol mulls. In the latter case, both spectra were shown to be superimposable independently with a nujol spectrum of authentic 4β-dihydrocleavamin-3α-acetate obtained from the hydroboration of cleavamine and the acetylation of the product of this reaction. Samples from the decarboxylation reactions: λ_{max}^{MeOH} nm: 292, 286, 278, 226 (log ε roughly 3.8, 3.9, 3.8, 4.5 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470, 2920, 1725, 1460, 1370, 1250 (broad), 1020 (broad); ν_{max}^{nujol} cm^{-1}: 3400, 2920, 2850, 1725, 1460, 1378; Nmr: τ 2.16 (broad singlet, 1H, N-H), 2.4-3 (broad multiplet, 4H, aromatic), 4.90 (multiplet, 1H, C_3-H), 6.40 (multiplet, 1H, C_{18}-H), 7.97 (singlet, 3H, C_3-OAc), 9.17 (triplet, 3H, C_4-CH_2CH_3); mp 136-139°C. Authentic material: ν_{max}^{nujol} cm^{-1}: 3400, 2920, 2850, 1725, 1460, 1378 (actually completely superimposable in every detail when run at the same

time as the above samples.) Nmr: τ(reported) 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-H), 6.4 (complex multiplet, 1H, C_{18}-H), 9.17 (triplet, 3H, $-CH_2CH_3$).

18β-Carbomethoxy-4β-dihydrocleavamine-3-one (62)

Dicyclohexylcarbodiimide (4.414 g, 21.6 mmoles) was dissolved in dry dimethyl sulfoxide (35 ml, reflxued over barium oxide and then distilled and stored over type 4A molecular sieves) and 10 ml of anhydrous phosphoric acid (standing over phosphorous pentoxide) was added. Compound 56 (2.040 g, 5.72 mmoles) was dissolved in dry dimethyl sulfoxide (25 ml) and added to the above mixture slowly with stirring under a stream of dry nitrogen. After allowing the reaction to proceed for 26 hours at room temperature it was worked up as follows. The crude reaction mixture was poured into water (160 ml), ether (80 ml) and 1 M phosphoric acid (4 ml) with cooling in an ice bath and stirring. The resulting suspension of dicyclohexylurea was filtered and washed with water and ether. This acidic solution was extracted with ether (5 x 125 ml). Evaporation of the solvent in vacuo yielded a yellow solid (1.4 g) which could be identified as a mixture of the desired ketone (roughly 55%) and dicyclohexylurea. Basification and re-extraction of the aqueous phase with methylene chloride yielded the unreacted starting material heavily contaminated with dicyclohexylurea.

Preparative thin layer chromatography of the impure ketone on alumina (methylene chloride elution) yielded an analytical sample of 18β-carbomethoxy-4β-dihydrocleavamin-3-one (62). λ_{max}^{EtOH} nm: 292, 284, 277 (sh),

226, and a small peak at 332 (log ε 3.8, 3.9, 3.8, 4.5, 1 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3000, 1725, 1520, 1460, 1425, 1340, 1320, 920; Nmr: τ 1.30 (broad singlet, 1H, N-H), 2.50-3.00 (multiplet, 4H, aromatic), 5.38 (doublet, J ∿ 10 cps, 1H, C_{18}-H), 6.32 (singlet, 3H, C_{18}-CO_2CH_3), 9.10 (triplet, J ∿ 8 cps, 3H, C_4-CH_2CH_3); mass spectrum: main peaks at m/e = 354, 295, 257, 224, 215, 182, 169, 152, 151, 140.

Chloroindolenine of 18β-carbomethoxycleavamine (98)

To a solution of 18β-carbomethoxycleavamine (102 mg) in absolute benzene (2 ml) was added, at room temperature, with stirring 1-chlorobenzotriazole (94.6 mg) in benzene (2 ml) over a period of 20 minutes. After this addition was complete the remaining 1-chlorobenzotriazole was rinsed in with a further portion of benzene (0.5 ml) over a period of 5 minutes. The reaction mixture was then partitioned between benzene and ice cold water (10 ml of each). The benzene layer was separated, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to yield a yellowish-brown foam (115 mg). This material was quickly columned through a plug of alumina (neutral Woelm III) with benzene elution followed by dichloromethane and then finally methanol. The pure chloroindolenine was eluted first from the column (29 mg, ∿ 30%). λ_{max}^{EtOH} nm: 335 (sh), 291 (sh), 285, 260 (sh), 222; $\nu_{max}^{CCl_4}$ cm^{-1}: 2990, 1790(sh), 1749, 1675, 1620, 1575, 1455, 1425; Nmr: τ 2.35-3.40 (multiplet, 4H, aromatic), 4.6 (broad doublet, J ∿ 8 cps, 1H, C_3-olefinic), 5.65 (broad doublet, J ∿ 10 cps, 1H, C_{18}-H), 6.38 (singlet, 3H, CO_2CH_3). The remainder of the material was unidentifiable and corresponded to decomposition on the column. Prior to chromatography a tlc check of the reaction mixture revealed that it contained >90% of

the desired material.

Dimer 99

Vindoline (400 mg) was dissolved in anhydrous 1.5% methanolic hydrogen chloride (75 ml) prepared by the addition of purified acetyl chloride (9.25 ml) to anhydrous methanol (400 ml). This solution was added quickly, under a stream of nitrogen, to the chloroindolenine of 18β-carbomethoxycleavamine (98) (400 mg). The reaction mixture rapidly turned to a deep wine-red colour when refluxed for 2.5 hours under a positive pressure of nitrogen. After this time it was cooled to room temperature, diluted with water and cautiously basified with potassium bicarbonate. When it was weakly basic, it was extracted (4 x 40 ml) with dichloromethane and the combined organic layer was dried over anhydrous sodium sulfate and filtered. Evaporation of the solvent under reduced pressure afforded a brown foam (800 mg). This material was introduced onto a column of Sephadex LH-20 prepared in the following way. The gel-beads were allowed to swell by stirring them in a beaker containing methanol for 4 hours at room temperature. The resulting slurry was packed into a column 36" x 1" diameter. This column was allowed to settle for 1 hour and then stablized under run conditions overnight. The flow rate at this stage was 2 ml/minute. Several 10 ml fractions were collected after the sample was introduced onto the column. Fractions 7 to 10 inclusive contained the desired dimer (99) (60 mg). λ_{max}^{EtOH} nm: 310(sh), 292, 286(sh), 259, 221(sh), 212 (log ε 3.8, 3.9, 3.9, 3.9, 4.4, 4.5 respectively); Nmr: τ 0.70 (broad singlet, 1H, N-H), 0.91 (broad singlet, 1H, OH), 2.55-3.00 (multiplet, 4H, aromatic), 3.02 (singlet, 1H, C_{14}-H),

4.00 (singlet, 1H, C_{17}-H), 4.10 (doublet of doublets, J ∿ 10, 4 cps, 1H, C_7-H), 4.50 (multiplet, 1H, C_3,-H), 4.64 (singlet, 1H, C_4-H), 4.70 (doublet, J = 10 cps, 1H, C_6-H), 6.14 (singlet, 3H, C_{16}-OMe), 6.27 (singlet, 6H, C_{18},-CO_2CH_3 and C_3-CO_2CH_3), 7.39 (singlet, 3H, N_1-CH_3), 7.95 (singlet, 3H, C_4-OAc), 9.00 (triplet, J ∿ 8 cps, 3H, C_4,-CH_2CH_3), 9.40 (triplet, J ∿ 8 cps, 3H, C_5-CH_2CH_3); mass spectrum: main peaks at m/e = 106, 107, 108, 121, 122, 135, 136, 149, 188, 282, 339, 335, 669, 791, 792.

Anal. High resolution mass spectrometry: Found, 792.4103; Required for $C_{45}H_{56}O_8N_4$, 792.4098.

Further fractions contained this same dimer contaminated with small amounts of blue spot (25 mg). Total yield of dimer could be estimated at 10% from this reaction. The major new product of the reaction was the so-called blue spot (obtained in fraction 12-20). Blue spot: λ_{max}^{EtOH} nm: 307, 295(sh), 257, 212 (log ε 3.8, 3.8, 4.0, 4.3 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 2960, 2840, 1740, 1620, 1500, 1468, 1440, 1380, 1260, 1150(broad), 900, 880; Nmr: τ 2.00-3.10 (multiplet, 4H), 3.27 (singlet, 1H), 3.99 (singlet, 1H), 4.20 (diffuse multiplet, 2H), 4.59 (singlet, 1H), 4.80 (diffuse multiplet, 2H), 6.28 (singlet, 7H), 7.42 (singlet, 3H), 8.02 (singlet, 3H), 9.72 (triplet, J = 8 cps, 3H); mass spectrum: main peaks at m/e = 370, 356, 355, 290, 289, 69, 57, 55, 50, 44, 43, 41.

Anal. C, 66.16%; H, 7.05%; N, 6.31%.

Dimer 99 by Modified Conditions

The chloroindolenine (98) of 18β-carbomethoxycleavamine was made by injecting neat t-butyl hypochlorite (0.07 ml) into a solution of 18β-

carbomethoxycleavamine (200 mg) in refluxing dichloromethane (6.5 ml) containing triethylamine (0.1 ml). The resulting yellowish-orange solution was stirred for 30 minutes at reflux, then cooled to room temperature and evaporated to dryness under reduced pressure to yield a light brown foam.

This compound was dissolved in dichloromethane and divided into two equal portions which were again taken to dryness. To each of these portions was added a solution of vindoline (100 mg) in 1.5% methanolic hydrogen chloride (20 ml). One portion was heated to reflux for 3.5 hours. After this period it was cooled to room temperature and cautiously added to an ice cold aqueous solution of sodium bicarbonate (10%, 50 ml), and extracted with dichloromethane (3 x 25 ml). The combined organic phase was dried over anhydrous sodium sulfate filtered and evaporated to dryness to yield a brown foam (200 mg) from which dimer 99 (16.4 mg) was purifyable by column chromatography on alumina (neutral Woelm III) by elution with benzene containing 15% ethyl acetate (8% yield).

The other portion was allowed to stir at room temperature for 10 days. After this period, the solvent was removed under reduced pressure to yield a green gum which was taken up in 0.1 M citric acid (25 ml, resulting pH = 1.8) and extracted with benzene (25 ml). The benzene layer was isolated and evaporated to dryness to yield 27 mg of monomeric materials identified by tlc and uv. Then, the pH was adjusted stepwise to 10.7 and a benzene extraction performed at each step. The dimer 99 enriched fractions were found to be between pH values of 4.5 and 5.4 (74 mg). Total weight recovery was 192.3 mg from 200 mg or 96.5%. The dimer enriched fractions were combined and purified by preparative tlc

on alumina (ethyl acetate) to yield pure dimer 99 (40 mg) identical in every respect to that obtained previously (20% yield).

Dimer 102 from 18-Carbomethoxydihydrocleavamine and Dihydrovindoline

18β-Carbomethoxy-4β-dihydrocleavamine (353.7 mg) was dissolved in dry benzene (20 ml) and a solution of 1-chlorobenzotriazole (200.4 mg) in benzene (10 ml) was added over 10 minutes with stirring at room temperature, and then rinsed in with a further portion of benzene (10 ml) added over 5 minutes. The solvent was removed in vacuo after this period to yield the chloroindolenine as a yellowish foam.

This material was dissolved in benzene (4 ml) and added slowly to a solution of dihydrovindoline (372 mg) in 1.5% methanolic hydrogen chloride (6 ml) maintained at 50°C under a stream of nitrogen. The chloroindolenine was rinsed in with a further protion of benzene (2 ml). The reaction mixture immediately changed from a light blue colour to a brownish-red. After 2.5 hours the reaction was cooled to room temperature poured cautiously onto an ice cold solution of 10% aqueous sodium bicarbonate (50 ml) and extracted with dichloromethane (3 x 30 ml). The combined organic phase was dried over sodium sulfate, filtered and taken to dryness under reduced pressure to yield a brownish foam (726 mg).

This material was chromatographed on 75 g of alumina (neutral Woelm III). Benzene containing 20% ethyl acetate eluted the desired tetrahydro-dimer (102) in fractions 6 through 14 (443.6 mg, 61% yield) pure, together with several subsequent fractions containing this material in impure form. λ_{max}^{EtOH} nm: 310, 295, 287, 260, 216 (log ε 3.8, 3.9, 3.9, 4.0, 4.5 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460(broad), 2960, 1740, 1620, 1500, 1465, 1435, 1375, 1160, 1040, 1110; Nmr: τ 0.91 (singlet, 1H, N-H), 2.50-2.92

(multiplet, 4H, aromatic), 2.95 (singlet, 1H, C_4-H), 3.97 (singlet, 1H, C_{17}-H), 4.36 (singlet, 1H, C_4-H), 6.14 (singlet, 3H, C_{16}-OMe), 6.20 (singlet, 3H, CO_2CH_3), 6.24 (singlet, 3H, CO_2CH_3), 7.42 (singlet, 3H, N_1-CH_3), 7.90 (singlet, 3H, C_4-OAc), 9.01 (triplet, J ∿7 cps, 3H, $-CH_2CH_3$), 9.44 (triplet, J ∿ 6 cps, 3H, $-CH_2CH_3$); mass spectrum: main peaks at m/e = 824, 810, 796, 794, 768, 753, 739, 594, 578, 471, 429, 398, 384, 382, 370, 368, 367, 355, 354, 352, 342, 340, 339, 338, 336, 326, 312, 310, 298, 297, 295, 294, 291, 284, 281, 268, 210, 209, 194, 182, 180, 170, 169, 168, 167, 156, 154, 144, 143, 138, 136, 133, 125, 124, 110, 105. Mp 221-224°C dec.

Anal. High resolution mass spectrometry: Found, 796.441961; Required for $C_{46}H_{60}O_8N_4$, 796.441088.

Cleavage of Dimer 99

Dimer 99 (9.0 mg) was dissolved in anhydrous 7% methanolic hydrogen chloride (4 ml) and tin (39 mg) and stannous chloride (36 mg) were added. The reaction mixture was refluxed for 2 hours, and then cooled, diluted with water, and basified with ammonium hydroxide. This basic solution was extracted with dichloromethane (4 x 20 ml). Drying of the combined organic phase over anhydrous sodium sulfate, filtration, and evaporation of the solvent under reduced pressure afforded a brown foam. This material could be separated by preparative tlc on alumina using chloroform as the developer to yield cleavamine (3 mg), vindoline (3 mg), deacetyl vindoline (1 mg) and starting dimer (1.1 mg) as the only isolable materials. These could all be identified by infrared and melting point comparisons with the authentic materials.

Dimer 106

To a solution of 18β-carbomethoxy-4β-dihydrocleavamin-3α-ol (56) (250 mg) in dichloromethane (25 ml) containing triethylamine (1 ml) was added a solution of t-butyl hypochlorite (40 ml of a 0.02 M solution) in carbon tetrachloride at 0°C with stirring over 1.5 hours. Evaporation of the solvent at 0°C under high vacuum afforded a reddish-brown foam.

Vindoline (150 mg) was dissolved in 1.5% methanolic hydrogen chloride (50 ml) and added, under a stream of nitrogen, to the chloroindolenine above, and the whole refluxed for 2.5 hours. After this time it was cooled to room temperature, diluted with water, basified with solid potassium bicarbonate and extracted with dichloromethane (4 x 25 ml). The combined organic phase was dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a foam (402 mg).

This material was chromatographed on alumina (neutral Woelm III) to yield, upon elution with benzene containing 25% ethyl acetate, the desired dimer (106) as a whitish glass (40 mg, 8% yield). λ_{max}^{EtOH} nm: 315, 295, 286, 262, 225(sh), 216 (log ε 3.8, 3.9, 3.9, 3.9, 4.4, 4.5 respectively); Nmr: τ 0.58 (broad singlet, 1H, N_a'-H), 1.02 (singlet, 1H, C_3-OH), 2.50-3.00 (multiplet, 4H, aromatic), 3.11 (singlet, 1H, C_{14}-H), 4.00 (singlet, 1H, C_{17}-H), 4.10 (multiplet, 1H, C_7-H), 4.63 (singlet, 1H, C_4-H), 4.60 (multiplet, 1H, C_6-H), 6.14 (singlet, 3H, C_{16}-OCH_3), 6.25 (singlet, 6H, 2 x CO_2CH_3), 7.39 (singlet, 3H, N_1-CH_3), 7.75 (singlet, 3H, C_4-OAc), 9.02 (triplet, J ∿ 7 cps, 3H, CH_2CH_3), 9.40 (triplet, J ∿ 7 cps, 3H, $-CH_2CH_3$); mass spectrum: main peaks at m/e = 810, 765, 469, 352, 323, 282, 202, 200, 188, 156, 154, 144, 138, 136, 135, 124, 122, 121, 107.

Dimer 106 by Sephadex Chromatography

18β-Carbomethoxy-4β-dihydrocleavamin-3α-ol (250 mg) was converted to its chloroindolenine according to the procedure above and dimerized with vindoline (200 mg) in 1.5% methanolic hydrogen chloride (100 ml) by refluxing the reaction mixture for 2.75 hours. After this time it was worked up as above to afford the crude reaction mixture as a light yellowish-brown foam (500 mg). This material was introduced onto the same Sephadex LH-20 column mentioned previously. A 2 ml/minute flow rate was maintained and 10 ml fractions were collected. Fractions 12,13 and 14 afforded the desired dimer (106) slightly contaminated with blue spot material. This crude dimer could be purified by preparative tlc to afford the pure dimer (106) (92.3 mg, 18.4% yield).

Cleavage of Dimer 106

Dimer 106 (13 mg) was dissolved in anhydrous 7% methanolic hydrogen chloride and tin (35 mg) and stannous chloride (50 mg) were added. The reaction mixture was refluxed for 1.5 hours and then cooled, diluted with water and basified with ammonium hydroxide. The resulting basic solution was extracted with dichloromethane (4 x 20 ml) and the combined organic phase dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a foam. From this material, vindoline, deacetyl vindoline, 4β-dihydrocleavaminol, and dimer 106 could be isolated by preparative tlc, and alcohols 56 and 57 could be identified in trace amounts.

18-Methoxy-18-carbomethoxy-4β-dihydrocleavamine hydrochloride (110)

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (250 mg) in dichloromethane (25 ml) in a 100 ml round-bottomed flask was added, at 0°C with stirring, t-butyl hypochlorite (200 micro/t.). Tlc check (alumina, benzene, ceric sulfate spray, then alumina, benzene, antimony pentachloride in carbon tetrachloride spray) revealed that the starting material had been completely replaced by the chloroindolenine. The solvent was removed under reduced pressure at room temperature to yield a whitish foam.

To this material was added 1.5% methanolic hydrogen chloride (8 ml) at 0°C with stirring. The reaction mixture was a deep wine-red colour. After stirring for 0.5 hours at 0°C a tlc check (alumina, benzene, uv, then ceric sulfate spray) revealed that the chloroindolenine had completely disappeared, and had been replaced by a slightly more polar major spot. Evaporation of the solvent under high vacuum at 0°C afforded a reddish-brown powder identified as the desired compound (110) chiefly on the basis of its nmr: τ 2.35-3.00 (multiplet, 4H, aromatic), 4.85 (broad singlet, 1H, N_b^+-H), 5.70 (triplet, J ∿ 7 cps, 1H), 6.18 (singlet, 3H, C_{18}-CO_2CH_3), 7.00 (singlet, 3H, C_{18}-OMe), 9.20 (broad unresolved triplet, 3H, C_4-CH_2CH_3). This material was quite stable and could be recrystallized from non-hydroxylic solvents such as purified ethyl acetate. It was however quite unstable as the free base.

18-Methoxy-18-hydroxymethylene-4β-dihydrocleavamine (111)

Compound (110) (255 mg) was dissolved in freshly distilled dry tetrahydrofuran (30 ml) and cooled to room temperature. Lithium aluminum

hydride (250 mg, 27 mole equivalents) was added in four equal portions with stirring. After 1.75 hours, the excess lithium aluminum hydride was destroyed by the dropwise addition of a saturated aqueous solution of sodium sulfate at 0°C (an improvement in the recovery of material was realized by using solid $Na_2SO_4 \cdot 10H_2O$ instead). When no further effervescence was detectable upon the addition of additional drops of the aqueous solution, the reaction mixture was filtered under vacuum through a bed of celite. A white, almost crystalline, residue was collected and returned to the flask. Some more tetrahydrofuran was added and the whole refluxed gently on a steam bath and then filtered again. The combined filtrate was evaporated to dryness under reduced pressure to afford a white foam (232.7 mg).

This material was chromatographed on alumina (neutral Woelm III). Elution with gradually increasingly polar solvent combinations yielded the desired compound (111) (115 mg) in semipure form by eluting with ethyl acetate. This material was re-chromatographed in a similar manner to yield compound 111 pure (recrystallized twice from ethyl acetate, 100 mg, 40% yield), mp 139-141°C. λ_{max}^{EtOH} nm: 292, 285, 279 (sh), 225 (log ε 3.8, 3.9, 3.8, 4.5 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3410, 2920, 1490, 1465, 1440, 1375, 1340, 1305, 1155, 1140, 1080, 1040; Nmr: τ 0.14 (broad singlet, 1H, N-H), 2.40-3.00 (multiplet, 4H, aromatic), 6.88 (singlet, 3H, C_{18}-OMe), 9.08 (triplet, J ∿ 7 cps, 3H, C_4-CH_2CH_3); mass spectrum: main peaks at m/e = 342, 311, 310, 271, 185, 181, 180, 168, 156, 144, 139, 138, 137, 126, 125, 124, 122, 110.

Anal. Found: C, 71.33%; H, 8.81%; N, 7.60%. Calcd. for $C_{21}H_{30}N_2O_2 \cdot 1/2EtOAc$: C, 71.47%; H, 8.87%; N, 7.25%. High resolution

mass spectrometry: Found, 342.2282; Required for $C_{21}H_{30}N_2O_2$, 342.2306; Found, 341.2203; Required for M^+-1, 341.2228; Found, 340.2122; Required for M^+-2, 340.2150; Found, 138.1276; Required for $C_9H_{16}N_1$, 138.1282.

Study of the Mechanism of the Conventional Dimerization Reaction

Each of the experiments below have been checked for reproducibility and where no dimers could be observed, the quality of the reagents has been tested by repetition of the standard reaction with yield of isolated dimer obtained as 60% ± 5%.

Experiment 1

Standard Dimerization

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (40.4 mg) in methylene chloride (4 ml) and triethylamine (0.02 ml; one drop) cooled in an ice-water bath, was added a solution of t-butyl hypochlorite in carbon tetrachloride (3 ml of a 0.05 M solution) over a period of 45 minutes. The solution was washed with ice-water (2 x 3.0 ml) dried over anhydrous Na_2SO_4 and the solvent removed in vacuo to give the chloroindolenine as a foam (44.0 mg).

The chloroindolenine above and vindoline (31.1 mg) were dissolved in anhydrous methanolic 1.5% hydrogen chloride (2 ml) and the resulting solution was stirred at room temperature under a nitrogen atmosphere and then refluxed for a further 2 hours.

The solvent was removed in vacuo and the residue partitioned between methylene chloride and aqueous 10% sodium bicarbonate solution.

The aqueous phase was extracted with further portions of methylene chloride (2 x 10 ml) and the combined organic extracts were dried over sodium sulfate. The solvent was removed to give a light yellow foam (∿ 70 mg). This material was chromatographed on Woelm III alumina (∿ 10 g). Benzene, ethyl acetate (4:1) elution gave the desired dimer (94) in 65% yield (45.0 mg). This material was analyzed by T-60 nmr.

Experiment 2

Standard Dimerization of Pure 18α-Carbomethoxy-4β-dihydrocleavamine

To a solution of 18α-carbomethoxy-4β-dihydrocleavamine (38.6 mg) in methylene chloride (4 ml) and triethylamine (one drop) cooled in an ice-water bath was added a solution of t-butyl hypochlorite in carbon tetrachloride (2.5 ml of a 0.05 M solution) over a period of 1 hour. The solution was washed with ice-water (2 x 3 ml) dried over anhydrous sodium sulfate and the solvent removed in vacuo to give the chloroindolenine as a whitish foam (40.0 mg).

The above chloroindolenine and vindoline (31.2 mg) were dissolved in anhydrous methanolic 1.5% hydrogen chloride (2 ml) and the resulting solution was stirred at room temperature for 30 minutes under a nitrogen atmosphere and then refluxed for a further 2.5 hours.

The solvent was removed in vacuo and the residue partitioned between methylene chloride and aqueous 10% sodium bicarbonate solution. The aqueous phase was extracted with further portions of methylene chloride (3 x 10 ml) and the combined extracts were dried over sodium sulfate. The solvent was removed in vacuo to give a light brownish foam (∿ 70 mg). This material was chromatographed on Woelm III alumina

(∿ 10 g). Benzene, ethyl acetate (4:1) elution afforded dimeric material in 45% yield. This material was found to be identical to dimer 94 by nmr.

Experiment 3

Dimerization with the Chloroindolenine Formation in Refluxing Methylene Chloride

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (43.5 mg) in dichloromethane (5 ml) maintained at reflux temperature by immersion in an oil bath kept at 50°C, was added neat t-butyl hypochlorite (16.8 mg) with a microsyringe all at once under a nitrogen atmosphere. The yellow coloured solution was refluxed for a further 5 minutes, then cooled to room temperature washed with ice-water (2 x 15 ml) dried over anhydrous sodium sulfate and the solvent removed in vacuo to yield a foam.

The above chloroindolenine and vindoline (33.0 mg) were dissolved in anhydrous methanolic 1.5% hydrogen chloride (2.3 ml) and the resulting solution was stirred at room temperature for 30 minutes under a nitrogen atmosphere and then refluxed for a further 2 hours. The solvent was removed in vacuo and the residue partitioned between methylene chloride and aqueous 10% sodium bicarbonate. The aqueous phase was then further extracted with portions of methylene chloride (2 x 10 ml). The combined organic phase was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to yield a foam (64 mg). This material was chromatographed on 8 g of Woelm III alumina. Benzene, ethyl acetate (4:1) elution gave dimeric material in

roughly 35% yield. This was found to be identical to dimer 94 by nmr.

Experiment 4

Experiment 3 with Triethylamine

A solution of 18β-carbomethoxy-4β-dihydrocleavamine (40.8 mg) in methylene chloride (4.5 ml) containing triethylamine (one drop) was maintained at reflux temperature by immersion in an oil bath kept at 50°C. To this solution was added t-butyl hypochlorite (14.0 mg) all at once under a nitrogen atmosphere and the reflux continued for a further 5 minutes. The yellow solution was then cooled, washed with ice-water (2 x 15 ml) dried over anhydrous sodium sulfate and the solvent removed in vacuo to yield a light brown foam (71.6 mg).

This material was columned on 9.5 g of Woelm III alumina in the usual way to yield ∿ 40% dimeric material. This was identified by nmr as dimer 94.

Experiment 5

Experiment 4 using a Solution of Chlorinating Agent in Carbon Tetrachloride

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (40 mg) in methylene chloride (4 ml) containing triethylamine (one drop) heated to reflux in an oil bath, was added a solution of t-butyl hypochlorite in carbon tetrachloride (3 ml of a 0.05 M solution; bath temperature 49°C) under nitrogen over a period of 15 minutes. The reaction mixture was cooled, washed with ice-water (3 x 2 ml) dried over anhydrous sodium sulfate and the solvent removed in vacuo to yield a light foam.

The standard dimerization with 32 mg of vindoline and 2 ml of anhydrous methanolic 1.5% hydrogen chloride followed by the usual column resulted in an isolation of a 50% yield of dimer which was identified by nmr to be dimer 94.

Experiment 6

Experiment 3 using a Mixture of Methylene Chloride, Carbon Tetrachloride (1:4) as Solvent

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (20.7 mg) in 3.5 ml of carbon tetrachloride and 1 ml of methylene chloride heated to reflux in an oil bath at 72°C, was added t-butyl hypochlorite (20 mg) all at once under a nitrogen atmosphere. After 5 minutes the reaction mixture was cooled to room temperature and washed (2 x 10 ml) with ice water. The organic phase was dried over sodium sulfate, filtered and the solvent evaporated to yield a greenish-brown foam.

The above chloroindolenine and vindoline (15 mg) were dissolved in 1 ml of anhydrous methanolic 1.5% hydrogen chloride and dimerized in the usual way. No dimer could be isolated by subsequent column chromatography. This experiment was checked in the usual way for reproducibility and then the standard dimerization was repeated to yield dimer 94 in 60% yield.

Experiment 7

Experiment 6 with 1:1 Mixture of Methylene Chloride, Carbon Tetrachloride

To a solution of 18-carbomethoxy-4β-dihydrocleavamine (∿ 30% 18α, 70% 18β) (41 mg) in methylene chloride, carbon tetrachloride (1:1)

(7 ml) maintained at a reflux temperature of 53° by immersion in an oil bath at 55°C was added t-butyl hypochlorite (20 mg) all at once under nitrogen. The reflux was allowed to continue for a further 5 minutes. The reaction mixture was then cooled to room temperature washed with ice-water (2 x 10 ml) dried over anhydrous sodium sulfate and the solvent removed in vacuo to yield a golden coloured foam.

This material was dimerized under standard conditions with vindoline (28.7 mg) in methanolic 1.5% hydrogen chloride to yield an estimated 0.5% of dimeric material (i.e. not visible either under uv light or upon spraying with antimony pentachloride on an alumina plate).

Experiment 8

Experiment 7 with 2:1 Mixture of Methylene Chloride to Carbon Tetrachloride

Experiment 7 above was repeated exactly using 43.4 mg of 18-carbomethoxy-4β-dihydrocleavamine and 7 ml of methylene chloride, carbon tetrachloride (2:1). Reflux temperature here was 50°C.

The dimerization was performed under standard conditions using 29.0 mg of vindoline and 2.0 ml of methanolic 1.5% hydrogen chloride. The yield of dimer was estimated as above to be 10.0%.

Experiment 9

Standard Dimerization with Prior Reaction of the Chloroindolenine with Methanol

To a solution of 18-carbomethoxy-4β-dihydrocleavamine (41.9 mg) in methylene chloride (4 ml) cooled in an ice-water bath, was added a

solution of t-butyl hypochlorite in carbon tetrachloride (2.6 ml of a 0.05 M solution) over a period of 30 minutes. The solution was washed with ice cold saturated brine (2 x 20 ml), dried over anhydrous sodium sulfate and the solvent removed in vacuo to yield a light foam. This was treated with chloroform and methanol at 0°C followed by evaporation of the solvent at room temperature. No reaction was apparent by tlc.

Dimerization in methanolic 1.5% hydrogen chloride (2 ml) with 26.5 mg of vindoline resulted in a 75% yield of dimer isolated after column chromatography in the usual way on 10 g of Woelm III alumina (isolated 49.8 mg from 66.5 mg of crude product). This material was found to be identical to dimer 94 by nmr.

Experiment 10

Standard Dimerization with Prior Reaction of the Chloroindolenine with 1.5% Methanolic Hydrogen Chloride

To a solution of 18-carbomethoxy-4β-dihydrocleavamine (42.2 mg) in methylene chloride (4 ml) cooled in an ice-water bath, was added a solution of t-butyl hypochlorite in carbon tetrachloride (2 ml of a 0.05 M solution) over a period of 30 minutes. The usual workup as above yielded a light yellow foam.

To this foam was added, at room temperature, methanolic 1.5% hydrogen chloride and the resulting solution stirred for 15 minutes. A check by tlc at this point revealed the complete disappearance of starting chloroindolenine and the appearance of new more polar spots, one of which was clearly the major one and could be ascribed to an 18-OMe compound by correlation with some other work (vide supra).

At this point solid vindoline was added (32.1 mg) and the solution stirred at room temperature for a further 15 minutes and then plunged into a preheated oil bath at 65°C. Within 30 minutes of heating, the reaction was complete and normal workup and column chromatography yielded 55% (32.8 mg from 72.8 mg crude product) of a dimeric compound which was identified by nmr as dimer 94.

Experiment 11

Experiment 10 with an NMR of the Crude Intermediate after Methanolic HCl Reaction on the Chloroindolenine

Experiment 10 was repeated exactly as above with the single exception that the reaction product from the reaction of the chloroindolenine with methanolic hydrogen chloride was isolated by basification with dilute ammonium hydroxide and extraction with methylene chloride. The resulting organic phase was dried over anhydrous sodium sulfate and the solvent removed in vacuo to yield a glass (82 mg from 87 mg of starting chloroindolenine). This material was analyzed by nmr and showed a clear incorporation of a methoxyl group into the molecule.

Subsequent standard dimerization followed by isolation of the dimeric material yielded 40.5% yield (34 mg from 84 mg of crude reaction mixture obtained from 44 mg of the above OMe containing compound with 40 mg of vindoline) of dimer identified by nmr as dimer 94.

Experiment 12

Dimerization at 140°C

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (55 mg) in methylene chloride (4.5 ml) cooled in an ice water bath, was added a

solution of t-butyl hypochlorite in carbon tetrachloride (2.5 ml of a 0.05 M solution) over 30 minutes. Evaporation of the solvent in vacuo yielded the desired chloroindolenine (60 mg) as a light brown foam.

The above material was combined with vindoline (110 mg) in methylene chloride (1 ml) and the resulting reddish oily solution was transferred to a tube which was then evacuated to yield a white foam. The tube was flushed with dry nitrogen and methanolic 1.5% hydrogen chloride (30 ml) was added at 0°C. The tube was sealed and plunged in an oil bath which had been preheated to 140°C. After 30 minutes the reaction mixture was cooled to room temperature and worked up in the usual way to yield no dimer at all.

Experiment 13

Experiment 12 at 100°C

The chloroindolenine of 18β-carbomethoxy-4β-dihydrochleavamine (160 mg) was made as above in experiment 12.

This material was combined with 200 mg of vindoline in methylene chloride (1 ml) and transferred to a tube which was then evacuated to yield a white foam. To this was added, at 0°C, methanolic 1.5% hydrogen chloride (2 ml). The tube was sealed and immersed in an oil bath which had been preheated to 100°C. After 15 minutes the reaction mixture was cooled to room temperature and worked up in the usual way to yield dimeric product which was identified by nmr as dimer 94 (∿ 30% yield).

Experiment 14

Dimerization using Benzene as the Solvent and BF_3 Etherate as the Catalyst

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (31.2 mg) was made as in experiment 13 above.

This material was combined with vindoline (32.5 mg) in benzene (4 ml) and the solution was treated with BF_3 etherate (five drops). The mixture was stirred at room temperature overnight and then worked up in the usual way to yield no dimeric material at all.

Experiment 15

Dimerization in N,N-Dimethylformamide with HCl Catalyst

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (47.5 mg) in methylene chloride (5 ml) cooled in an ice-water bath, was added a solution of t-butyl hypochlorite in carbon tetrachloride (2.5 ml of a 0.05 M solution) over 30 minutes. Evaporation of the solvent in vacuo resulted in a light yellow foam.

To this foam was added vindoline (103.2 mg) and methylene chloride (1 ml). The resulting solution was evaporated to yield an intimate mixture of vindoline and the chloroindolenine as a crisp white foam. This material was dissolved in 3 ml of N,N-dimethylformamide and dry HCl gas was bubbled in for 15 seconds at a brisk rate, care being taken to ensure that the reaction vessel remained at room temperature. After stirring for 19 hours under nitrogen at room temperature the reaction mixture was poured onto ice-cold aqueous 10% sodium bicarbonate and extracted several times with methylene chloride (3 x 25 ml). The combined reddish-brown organic phase was dried over anhydrous sodium

sulfate and the solvent was evaporated to yield a reddish oil which could be converted to a foam by extended evacuation. The crude reaction mixture (∿ 150 mg) was subjected to column chromatography on 30 g of Woelm III alumina and yielded 60 mg of dimeric material (∿ 60% based on the fact that only 50 mg of vindoline are expected to react) which was identified by nmr as dimer 94.

Experiment 16

Dimerization in Tetrahydrofuran (THF) with HCl Catalyst

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (50.0 mg) was formed under standard conditions. This material was dissolved with vindoline (50 mg) in anhydrous THF containing 1.5% hydrogen chloride. The resulting solution was refluxed for 3 hours under nitrogen. The solvent was removed under reduced pressure and the residue partitioned between methylene chloride and aqueous 10% sodium bicarbonate. The aqueous phase was washed with further portions of methylene chloride and the combined organic phase was dried over anhydrous sodium sulfate. Evaporation of the solvent *in vacuo* resulted in a gum (102 mg) which upon chromatography yielded dimeric material (26 mg or roughly 25%) which was identified by nmr to be dimer 94.

Experiment 17

Dimerization in Methanolic 1.5% Hydrogen Chloride Prepared from Wet Methanol

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (50 mg) in methylene chloride (5 ml) was added *t*-butyl hypochlorite (2.5 ml of a 0.05 M solution) over 30 minutes at 0°C. Evaporation of the solvent

in vacuo resulted in a light brown foam (52 mg).

Reagent grade methanol (400 ml) was cooled in an ice bath and treated dropwise with dry acetyl chloride (9.25 ml). This solution (3 ml) was used to dissolve the above chloroindolenine and vindoline (100 mg) together. The resulting solution was stirred at room temperature for 4 hours and then briefly heated (45 minutes) at reflux. The usual workup procedure yielded crude material (171.9 mg) as a foam. This was chromatographed on Woelm III alumina (20 g) to yield dimeric material in roughly 30% yield which was identified by nmr to be dimer 94.

Experiment 18

Standard Dimerization with Acetic Acid Added as a Cocatalyst

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (40 mg) in methylene chloride (4 ml) was added *t*-butyl hypochlorite (2 ml of a 0.05 M solution in carbon tetrachloride) at 0°C over 30 minutes. Evaporation of the solvent *in vacuo* yielded a brownish foam (43 mg).

To a solution of the above chloroindolenine and vindoline (40 mg) in methanolic 1.5% hydrogen chloride (5 ml) was added glacial acetic acid (2 ml). The whole solution was refluxed under nitrogen for 3 hours and then worked up in the usual way to yield some small quantity of dimeric material (less than 5%).

The reaction was repeated two more times with the quantity of acetic acid being 3.0 and 4.0 millilitres respectively. It was found that the yield of dimeric material decreased sharply being just detectable

in the former and completely absent in the latter case. The combined dimeric fractions from the first two attempts were analyzed by nmr and found to be dimer 94.

Experiment 19

Dimerization in Trifluoroacetic Acid as Solvent and Catalyst

To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (50 mg) in benzene (10 ml) was added a solution of 1-chlorobenzotriazole (31.2 mg) in benzene (10 ml) at room temperature over 30 minutes. After an additional 30 minutes the solvent was removed in vacuo and the resulting material partitioned between ice cold brine and methylene chloride. The aqueous phase was washed with further portions of methylene chloride (2 x 10 ml) and the combined organic phase was dried over anhydrous sodium sulfate. Filtration and removal of the solvent in vacuo yielded a light brown foam.

A solution of the above material and vindoline (10 mg) in trifluoroacetic acid (10 ml) was stirred at room temperature for 50 hours and then worked up as follows. The contents of the reaction flask was poured gradually onto ice cold ammonium hydroxide (dilute) and the resulting milky suspension was extracted with methylene chloride (3 x 50 ml). The combined organic phase was dried over sodium sulfate and filtered. Evaporation of the solvent in vacuo yielded crude material (110 mg) from which dimeric material could be isolated in minute quantities by column chromatography on Woelm III alumina.

This dimeric material was analyzed by Fourier Transform nmr spectroscopy and found to be dimer 94.

Experiment 20

Dimerization in Dioxane with Perchloric Acid as Catalyst

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (50.0 mg) was formed under standard conditions. This material was dissolved with vindoline (50 mg) in dioxane (10 ml) and a few drops (five drops) of perchloric acid were added. The resulting bluish solution was stirred under nitrogen at room temperature for 48 hours. At the end of this period, it was poured into an aqueous solution of 10% sodium bicarbonate and extracted several times with ethyl acetate (4 x 30 ml). The combined organic phase was dried over anhydrous sodium sulfate, filtered, and the solvent removed under reduced pressure to yield a brownish-red foam. Column chromatography yielded dimer 94 (5 mg) which could be identified as such by nmr spectroscopy.

Experiment 21

Dimerization in Methanol Containing HBr as Catalyst

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (50.0 mg) was formed in the standard way. To this material, vindoline (50.0 mg) was added and the whole was dissolved in methanol (10 ml) which had been freshly distilled from magnesium turnings into the reaction flask. Hydrogen bromide was bubbled into this reaction mixture, after passage through a drying tower, for a short period (15 seconds). After this the reaction vessel was connected to a positive pressure of nitrogen and stirred at room temperature for 48 hours.

Workup and chromatography in the usual way afforded dimer 94 as the only dimeric product (50 mg, 50% yield).

Experiment 22

An Investigation of the Effect of Hydrogen Chloride Concentration on the Dimerization Reaction

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (200 mg) was made in the standard way and was then divided into 4 equal parts and treated as follows:

A. 50 mg of vindoline with 5 ml of methanolic 1.5% HCl

B. 50 mg of vindoline with 5 ml of methanolic 1.6% HCl

C. 50 mg of vindoline with 5 ml of methanolic 2.0% HCl

D. 50 mg of vindoline with 5 ml of methanolic 5.0% HCl

These were all simultaneously lowered into a preheated oil bath at 60°C. Reaction rates as evidenced by colour changes and tlc monitoring could be clearly compared and were found to roughly vary in linear fashion with acid concentration. The reactions were all worked up in 2 hours and after column chromatography the following results were obtained by nmr analysis.

A. 30 mg dimer 94 30% yield

B. 36.4 mg dimer 94 36% yield

C. 45.0 mg dimer 94 45% yield

D. 56.4 mg dimer 94 56% yield.

No nmr was taken in case A since this was the standard dimerization which has yielded consistently only dimer 94.

Experiment 23

An Investigation into the Effects of HCl Concentration at Higher Concentrations of Acid

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (200 mg) was made in the standard way and was then divided into 4 equal parts and treated as follows:

A. 50 mg of vindoline with 5 ml of methanolic 15% HCl

B. 50 mg of vindoline with 5 ml of methanolic 30% HCl

C. 50 mg of vindoline with 5 ml of methanolic 60% HCl

D. 50 mg of vindoline with 5 ml of acetyl chloride.

These were all simultaneously lowered into a preheated oil bath at 67°C. After 3.0 hours these were all worked up. Column chromatography of each reaction mixture in the usual way afforded the following results by nmr analysis.

A. 40 mg of dimeric material (94) containing a trace of some of the other material. Yield 40%.

B. 26.8 mg of dimeric material containing mostly dimer 94. Yield 27%.

C. 25 mg of dimeric material containing mostly dimer 94. Yield 25%.

D. 97 mg of dimer 94. Yield 97%.

Experiment 24

Dimerization under "Cleavage" Conditions

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (50 mg) was prepared in the usual way and then solid vindoline (50 mg) was added to it. This mixture was dissolved in 10 ml of freshly prepared 7% methanolic hydrogen chloride under a positive pressure of

dry nitrogen. The resulting reddish-purple solution was refluxed for 3.5 hours. After this period it was cooled to room temperature and worked up in the usual way.

Column chromatography yielded the dimer 94 (47 mg) upon elution with benzene containing 20% ethyl acetate. Gradual increases in the polarity of the solvent to pure ethyl acetate afforded a mixture of dimer 94 and another dimer (∿ 10 mg). This mixture was resolved by high pressure liquid chromatography on deactivated alumina with ethyl acetate containing 2.5% methanol as solvent. New compound pure: λ_{max}^{EtOH} nm: 305, 295, 285, 264, 226(sh), 215 (log ε 3.8, 3.9, 3.8, 3.9, 4.5, 4.5, respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3680, 3620, 3450, 2940, 1730, 1620, 1470, 1380; mass spectrum: peaks at m/e = 822, 808, 794(M^+).

15-Bromovindoline (114)

To a solution of vindoline (1.0249 g, 2.18 x 10^{-3} moles) in dichloromethane (10 ml) contained in a 100 ml round-bottomed flask was added 1.0 mole equivalents (390.62 mg) of N-bromosuccinimide dissolved in dichloromethane (15 ml) at room temperature over a period of 15 minutes. The reaction mixture was stirred for a further 15 minutes. After this period it was poured into ice-cold water and the lower organic phase was separated and washed with 10% sodium bicarbonate (3 x 50 ml), brine (1 x 50 ml) and finally, water. The combined organic phase was dried over anhydrous sodium sulfate, filtered and solvent removed under reduced pressure to afford a white powder consisting solely of the desired product (1.2350 g or 97% yield). This material was one spot on alumina tlc developed in chloroform, chloroform-benzene

(1:1) or benzene. It could be recrystallized from ethanol, mp 267-269°C. λ_{max}^{EtOH} nm: 312, 255, 212 (log ε 3.7, 3.9, 4.5 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 2995, 2950, 2870, 1735, 1600, 1490, 1429, 1370, 1040; Nmr: τ 0.5-0.9 (broad diffuse singlet, 1H, C_3-O<u>H</u>), 2.86 (singlet, 1H, C_{14}-<u>H</u>), 3.87 (singlet, 1H, C_{17}-<u>H</u>), 4.10 (doublet of doublets of doublets, J = 11, 6, 2 cps, 1H, C_7-<u>H</u>), 4.54 (singlet, C_4-<u>H</u>), 4.74 (broad doublet, J ∿ 11 cps, 1H, C_6-<u>H</u>), 6.11 (singlet, 3H, C_{16}-OM<u>e</u>), 6.20 (singlet, 4H, C_3-$CO_2C\underline{H}_3$, and C_2-<u>H</u>), 6.48 (multiplet, 2H, C_8-$C\underline{H}_2$), 7.30 (singlet, 3H, N_1-$C\underline{H}_3$), 7.93 (singlet, 3H, C_4-OAc), 9.47 (triplet, J ∿ 7 cps, 3H, C_5-$CH_2C\underline{H}_3$); mass spectrum: main peaks at m/e = 537, 535, 478, 476, 377, 376, 375, 374, 282, 269, 268, 267, 266, 255, 254, 253, 252, 241, 240, 239, 222, 188, 187, 172, 136, 135, 122, 121, 107.

<u>Anal</u>. Found: C, 56.05%; H, 5.92%; N, 5.26%; Br, 14.71%; Calcd. for $C_{25}H_{31}N_2O_6Br$: C, 56.10%; H, 5.80%; N, 5.25%; Br, 14.71%.

<u>Anion of Vindoline by Halogen-Metal Interchange</u>

Bromovindoline (50 mg) was dissolved in freshly distilled anhydrous tetrahydrofuran (20 ml) and cooled to -78°C by immersion into a dry ice-acetone bath. Exactly 2.0 mole equivalents of a 2.02 M solution of <u>n</u>-butyl lithium in hexane was added slowly (20 minutes) with stirring under an inert atmosphere of anhydrous nitrogen. The solution became clear and turned to a light yellow colour. After the addition was complete, the reaction mixture was slowly warmed to room temperature after 0.5 hours; and quenched by the injection of water into the system. Nmr spectroscopy together with tlc comparisons (alumina, benzene as developer) with authentic material showed that the crude

product contained less than 5 to 10% of unreacted bromovindoline and consisted almost entirely of vindoline and deacetyl vindoline together with a small amount of unidentifiable baseline material.

Dimer 94 from the Reaction of the Anion of Vindoline on the Appropriate Chloroindolenine

The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (250 mg) was dissolved in anhydrous, distilled, tetrahydrofuran and added slowly (45 minutes) to the anion of vindoline (400 mg in 40 ml of tetrahydrofuran) prepared as above, at -78°C. After 4 hours, the reaction was allowed to come to room temperature and then poured into saturated aqueous ammonium chloride. The organic phase was collected and the reddish aqueous phase was washed (2 x 25 ml) with ethyl acetate. The combined organic extract was dried (anhydrous sodium sulfate), filtered and taken to dryness *in vacuo* to yield a light brown foam (712.2 mg). This material was submitted to two sequential counter current distributions over 72 tubes at a pH gradient of 2.3 to 3.95 and 3.5 to 3.75 respectively (citric acid/ammonium hydroxide buffer as the stationary lower phase and benzene as the moving upper phase). The earliest tubes (1-16) afforded dimer 94 (22.1 mg) and deacetyl dimer 94 (8.5 mg) as the only dimeric products. The identity of the former was deduced on the basis of nmr and ir being superimposable with those of authentic material.

Catharanthine N-Oxide

Catharanthine (250 mg) as the free base, was dissolved in dichloromethane (35 ml). To this clear colorless solution was added, at room

temperature, 1.1 mole equivalents of m-chloroperbenzoic acid (150 mg) in solid form. After stirring for 1 hour at room temperature, the reaction was worked up. The clear colorless solution was shaken with an ice cold aqueous 10% sodium bicarbonate solution (25 ml). The organic phase was separated, dried over anhydrous sodium sulfate, filtered and taken to dryness under vacuum to yield a white glass (262 mg). This material was quickly chromatographed on deactivated alumina (neutral Woelm III, 20 g) by elution with dichloromethane (300 ml, 2 fractions) and methanol (300 ml, 1 fraction). The first two fractions contained a rearrangement product of catharanthine N-oxide (35 mg). λ_{max}^{EtOH} nm: 293, 285, 276(sh), 226, (log ε 3.8, 3.9, 3.8, 4.5 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 2950, 2920, 1725, 1465, 1435, 1340, 1265, 1160, 1120, 1055, 1030, 1015, 915, 905(sh), 890; Nmr: τ 1.20 (broad singlet, 1H), 2.30-3.00 (multiplet, 4H, aromatic), 3.75 (singlet, 1H), 5.42 (broad doublet, J ∿ 9 cps, 1H), 6.21 (singlet, 3H, $CO_2C\underline{H}_3$), 8.80 (triplet, J ∿ 7 cps, 3H, $CH_2C\underline{H}_3$); mass spectrum: main peaks at m/e = 352 (M^+), 254, 248, 222, 204, 135, 121, 119.

The major compound isolated was, however, the desired catharanthine N-oxide; λ_{max}^{EtOH} nm: 291, 283, 275(sh), 224 (log ε 3.8, 3.9, 3.8, 4.5 respectively); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 2960, 1730, 1740, 1465, 1440, 1305, 1270, 1185, 1100, 925; Nmr: τ 1.39 (broad singlet, 1H, N-H), 2.28-3.00 (multiplet, 4H, aromatic), 3.84 (broad doublet, J ∿ 6 cps, 1H, C_3-olefinic), 5.24 (broad singlet, 1H, C_5-$\underline{H}$), 6.26 (singlet, 3H, C_{18}-$CO_2C\underline{H}_3$), 8.83 (triplet, J ∿ 7 cps, $-CH_2C\underline{H}_3$); mass spectrum: main peaks at m/e = 336 (M^+-16), 334, 135, 122, 121, 107. The structure of the latter compound was proved by submitting it immediately to hydrogenation

in ethanol over Adam's catalyst. The sole product was identified by ir and mass spectroscopy as dihydrocatharanthine. The N-oxide underwent rearrangement even when dried to a white powder and stored in an evacuated dessicator, for 48 hours, to yield a 1:1 mixture of the starting N-oxide and the rearrangement product mentioned above. The latter compound once formed was stable to chromatography.

Reaction of Catharanthine Hydrochloride with m-Chloroperbenzoic Acid

Catharanthine hydrochloride (62.2 mg) was dissolved in dichloromethane (7 ml). m-Chloroperbenzoic acid (34.4 mg, 1.1 mole equivalent) was added in solution in dichloromethane (5 ml) over 15 minutes with stirring and then washed in with a further portion of solvent (2 ml) and the reaction mixture was allowed to stir at room temperature for 32 hours. After this time the clear colourless solution was washed with an aqueous 10% solution of sodium bicarbonate (2 x 10 ml) and the organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to yield the crude reaction mixture as a white foam (64.4 mg). This material was purified by preparative tlc on alumina (2 plates 20 cm x 20 cm, 0.3 cm thickness) using chloroform, methanol (20:1) as the developer. The major compound was catharanthine (36.9 mg) identical by nmr, ir, uv, and mass spectroscopy to authentic material. Minor products included catharanthine N-oxide (9.9 mg) and baseline material (4.6 mg). The total weight recovery was 83.5%.

"Biomemetic" Dimerization Reaction

Catharanthine hydrochloride (710 mg) was dissolved in dichloromethane (30 ml) and cooled to 0°C. A solution of excess m-chloroperbenzoic acid (2.2 g) in dichloromethane (30 ml) was added dropwise over 40 minutes. The reaction mixture was warmed to room temperature and stirred for 39.5 hours. After this period, solid sodium sulfite was added and the resulting suspension was stirred for a further 10 minutes, after which it was partitioned between ice-cold water and dichloromethane. The organic phase was washed with 10% aqueous sodium bicarbonate (2 x 25 ml) and then collected, dried over anhydrous sodium sulfate, filtered and the solvent removed under reduced pressure to yield a whitish-yellow foam (702 mg). This material was chromatographed on alumina (neutral Woelm III, 100 g). Benzene elution furnished a compound (31 mg) in fractions 4, 5, and 6 which was different from the starting material or its N-oxide. Nmr (T-60): τ 2.2-3.0 (multiplet, 4H, aromatic), 4.0 (broad unresolved doublet, 1H, olefinic), 5.22 (broad singlet, 1H, C_5-H), 6.38 (singlet, 3H, CO_2CH_3), 8.97 (triplet, J $\sim$ 8 cps, 3H, CH_2CH_3).

This material was dissolved in methanol (5 ml) containing vindoline (35 mg) and 1 M hydrochloric acid (7 ml) was added. The reaction flask was sealed under a nitrogen atmosphere and stirred at room temperature for 10 days. During this time it changed from a light yellow colour to a deep, almost opaque, emerald green. After this time the reaction mixture was poured into a beaker containing water (10 ml) and dichloromethane (30 ml) with stirring. All of the material remained in the aqueous phase. The addition of small amounts of sodium borohydride

(0.5 g in small portions over 20 minutes) with rapid blending of the two phases resulted in the sudden loss of colour and concomitant extraction into the organic phase. The organic phase was separated and the aqueous phase washed with further portions (2 x 25 ml) of dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, filtered, and the solvent removed under reduced pressure to yield a yellowish glass-like material (60 mg) which fluoresced either in solution (methanol) or as a solid when exposed to uv light. Column chromatography on alumina (neutral Woelm III, 20 g) yielded, upon elution with benzene, ethyl acetate (4:1) a dimeric material in fraction 7 to 10 which proved to be the fluorescent principle in the reaction mixture (20 mg, ∿ 30% yield). λ_{max}^{EtOH} nm: 296, 285, 265, 215 (log ε 3.8, 3.9, 4.0, 4.5 respectively); Nmr: τ 2.0 (broad singlet, 1H), 2.30-3.00 (multiplet, 5H, aromatic + C_{14}-H), 3.94 (singlet, 1H, C_{17}-H), 4.00 (broad doublet, J ∿ 7 cps, 1H), 4.18 (doublet of doublets, J∿10,4 cps, 1H, C_7-H), 4.52 (singlet, 1H, C_4-H), 4.78 (doublet, J ∿ 10 cps, 1H, C_6-H), 5.24 (broad singlet, 1H), 5.72 (multiplet, 1H), 6.20 (singlet, 3H, $-CO_2CH_3$), 6.24 (singlet, 3H, $-CO_2CH_3$), 6.42 (singlet, 3H, C_{16}-OCH_3), 7.35 (singlet, 3H, N-CH_3), 7.95 (singlet, 3H, C_4-OAc), 8.79 (triplet, J ∿ 7 cps, 3H, $-CH_2CH_3$), 9.29 (triplet, J ∿ 7 cps, 3H, $-CH_2CH_3$); mass spectrum: main peaks at m/e = 792, 791, 683, 624, 617, 521, 523, 469, 457, 415, 309, 296, 282, 266, 222, 188, 174, 158, 136, 135, 122, 121.

Anal. High resolution mass spectrometry: Found, 792.4118; Required for $C_{46}H_{56}O_8N_4$, 792.4098.

BIBLIOGRAPHY

1. A.A. Gorman, M. Hesse, H. Schmid, P.G. Waser and W.H. Hopff, "Bisindole Alkaloids", in The Alkaloids, Vol. I, Specialist Periodical Reports, of The Chemical Society, 1971.
2. D.H.K. Lee and W.R.M. Drew, Med. J. Australia, 1, 699 (1929).
3. R.A. Paris and H. Moyse-Mignon, Compt. Rend., 236, 1993 (1953).
4. A. Quevauviller, J. Le Men and M.M. Janot, Ann. Pharm. Franc., 12, 799 (1954).
5. C.T. Beer, British Empire Cancer Campaign, 33rd. Annual Report, 487 (1955).
6. G.H. Svoboda, J. Am. Pharm. Assoc., 47, 834 (1958).
7. M. Gorman, N. Neuss and G.H. Svoboda, J. Amer. Chem. Soc., 81, 4745 (1959).
8. N. Neuss and M. Gorman, Tetrahedron Letters, 206 (1961).
9. M. Gorman, N. Neuss and K. Biemann, J. Amer. Chem. Soc., 84, 1058 (1962).
10. N. Neuss, M. Gorman, H.E. Boaz and N.J. Cone, J. Amer. Chem. Soc., 84, 1509 (1962).
11. J.W. Moncrief and William N. Lipscomb, Acta Cryst., 21, 322 (1966).
12. J.W. Moncrief and W.N. Lipscomb, J. Amer. Chem. Soc., 87, 4963 (1965).
13. N. Neuss, M. Gorman, W. Hargrove, N.J. Cone, K. Biemann, G. Buchi, and R. Manning, J. Amer. Chem. Soc., 86, 1440 (1964).
14. J.P. Kutney, J. Trotter, T. Tabata, A. Kerigan and N. Camerman, Chem. and Ind., 648 (1963).
15. N. Camerman and J. Trotter, Acta Cryst., 17, 384 (1964).

16. J.H. Cutts, C.T. Beer and R.L. Noble, Rev. Canad. Biol., 16, 476 (1957).

17. R.L. Noble, C.T. Beer and J.H. Cutts, Ann. N.Y. Acad. Sci., 76, 882 (1958) and Biochem. Pharmacol., 1, 347 (1958).

18. J.H. Cutts, C.T. Beer and R.L. Noble, Cancer Res., 20, 1023 (1960).

19. N. Neuss, M. Gorman, G.H. Svoboda, G. Maciak and C.T. Beer, J. Amer. Chem. Soc., 81, 4754 (1959).

20. G.H. Svoboda, M. Gorman, N. Neuss and A.J. Barnes, Jr., J. Pharm. Sci., 50, 409 (1961).

21. G.H. Svoboda, Lloydia, 24, 173 (1961).

22. G.H. Svoboda, M. Gorman, A.J. Barnes, Jr., and A.T. Oliver, J. Pharm. Sci., 51, 518 (1962).

23. G.H. Svoboda, I.S. Johnson, M. Gorman and N. Neuss, J. Pharm. Sci., 51, 707 (1962).

24. G.H. Svoboda, Lloydia, 25, 334 (1962).

25. G.H. Svoboda, A.T. Oliver and D.R. Bedwell, Lloydia, 26, 141 (1963).

26. K. Jovanovics, G. Mark, L. Horompo and K. Szasz, Acta Pharm. Hung., 34, 36 (1964).

27. G.H. Svoboda and A.J. Barnes, Jr., J. Pharm. Sci., 53, 1227 (1964).

28. G.H. Svoboda, Lloydia, 27, 299 (1964).

29. N.R. Farnsworth and I.M. Hilinski, J. Chromatog., 18, 184 (1965).

30. H.P. Gregor, G.K. Hoeschele, J. Potenza, A.G. Tsuk, R. Feinland, M. Shida and Ph. Teyssie, J. Amer. Chem. Soc., 87, 5525 (1965).

31. A.G. Tsuk and H.P. Gregor, J. Amer. Chem. Soc., 87, 5534 (1965).

32. A.G. Tsuk and H.P. Gregor, J. Amer. Chem. Soc., 87, 5538 (1965).

33. J. Walden and H.P. Gregor, Principles Appl.: Water Chem., Proc. Rudolfs Res. Conf., 4th, Rutgers, State Univ., 491 (1965),(Published 1967)

34. H.P. Gregor, Preparation of an oleophilic ion-exchange resin: application in biological separations, Ion Exch. Process Ind., Pap. Conf. (1969) (published 1970).

35. I.M. Jakovljevic, J. Pharm. Sci., 51, 187 (1962).

36. N.J. Cone, R. Miller and N. Neuss, J. Pharm. Sci., 52, 688 (1963).

37. D. Groeger and K. Stolle, Arch. Pharm., 298, 246 (1965).

38. L.N. Prista, M.A. Ferreira and A.S. Roque, Garcia Orta, 12, 277 (1964).

39. A.N. Mosoud, N.R. Farnsworth, L.A. Sciuchetti, R.N. Blomster and W.A. Meer, Lloydia, 31, 202 (1968).

40. A.C. Sartorelli and W.A. Creasy, A. Rev. Pharmac., 9, 51 (1969).

41. "Symposium on vincristine", Cancer Chemother. Rep., 52, 453 (1968).

42. R.E. McMahon, Experientia, 19(8), 434 (1963).

43. R.J. Owellen and D.W. Donigian, J. Med. Chem., 15, 894 (1972).

44. H.F. Hebden, J.R. Hadfield and C.T. Beer, Cancer Res., 30, 1417 (1970).

45. H.F. Greenius, R.W. McIntyre and C.T. Beer, J. Med. Chem., 11, 254 (1968).

46. G.E. Calf, J.L. Garnett and W.A. Sollich-Baumgartner, Advan. Tracer Methodol., 4, 11 (1968).

47. G.E. Calf and J.L. Garnett, Chem. Comm., 373 (1969).

48. W.W. Hargrove, Lloydia, 27, 340 (1964).

49. Deacetylated polynuclear indoles. Eli Lilly & Co., (By W.W. Hargrove). Belg. 660, 843, Sept. 9, 1965, U.S. Appl. March 9, 1964.

50. Polynuclear deacetylindoles. Eli Lilly & Co., Belg. 659, 112, Aug. 2, 1965, Appl. Feb. 1, 1965.

51. Indole alkaloids. Eli Lilly & Co. Neth. Appl. 6,501,121 (Cl. C 07d), July 29, 1966, Appl. Jan. 28, 1965.

52. Vincaleukoblastine polynuclear indole derivatives. Eli Lilly & Co. (by W.W. Hargrove). Brit. 1,037,379 (Cl. C 07d), July 27, 1966, Appl. Jan. 11, 1965.

53. Novel aminoacyl esters of deacetylvincaleukoblastine. W.W. Hargrove. (Eli Lilly & Co.). U.S. 3,387,001 (Cl. 260-287), 4 Jun. 1968, Appl. 19 Oct. 1964.

54. G.H. Svoboda, "Proceedings of the First Symposium of the European Cancer Chemotherapy Group", Excerpta Medica Foundation, New York, 1966, p. 9-27 and references therein.

55. E. Wenkert, J. Amer. Chem. Soc., 84, 98 (1962).

56. M. Gorman, N. Neuss and N.J. Cone, J. Amer. Chem. Soc., 87, 93 (1965).

57. J.P. Kutney and E. Piers, J. Amer. Chem. Soc., 86, 953 (1964).

58. J.P. Kutney, R.T. Brown and E. Piers, J. Amer. Chem. Soc., 86, 2286 (1964).

59. A. Camerman, N. Camerman, J.P. Kutney, E. Piers and J. Trotter, Tetrahedron Letters, 637 (1965).

60. J.P. Kutney, N. Abdurahman, P. Le Quesne, E. Piers and I. Vlattas, J. Amer. Chem. Soc., 88, 3656 (1966).

61. J.P. Kutney, W.J. Cretney, P. Le Quesne, B. McKague and E. Piers, J. Amer. Chem. Soc., 88, 4756 (1966).

62. J.P. Kutney, K.K. Chan, A. Failli, J.M. Fromson, C. Gletsos and V.R. Nelson, J. Amer. Chem. Soc., 90, 3891 (1968).

63. G. Buchi and R.E. Manning, J. Amer. Chem. Soc., 88, 2532 (1966).

64. J.P. Kutney and F. Bylsma, J. Amer. Chem. Soc., 92, 6090 (1970).

65. M. Gorman, N. Neuss and N.J. Cone, J. Amer. Chem. Soc., 87, 93 (1965).

66. J.P. Kutney, R.T. Brown, E. Piers and J.R. Hadfield, J. Amer. Chem. Soc., 92, 1708 (1970).

67. G. Buchi, P. Kulsa and R.L. Rosati, J. Amer. Chem. Soc., 90, 2448 (1968).

68. G. Buchi, P. Kulsa, K. Ogasawara and R.L. Rosati, J. Amer. Chem. Soc., 92, 999 (1970).

69. J.P. Kutney, W.J. Cretney, J.R. Hadfield, E.S. Hall and V.R. Nelson, J. Amer. Chem. Soc., 92, 1704 (1970).

70. J.P. Kutney, J. Beck, F. Bylsma and W.J. Cretney, J. Amer. Chem. Soc., 90, 4504 (1968).

71. N. Neuss, M. Gorman, N.J. Cone and L.L. Huckstep, Tetrahedron Letters, 783 (1968).

72. H.O. House, Modern Synthetic Reactions, Benjamin, Menlo Park, California, 2nd Edition, 1972, p. 438.

73. J.P. Kutney, R.T. Brown and E. Piers, Can. J. Chem., 43, 1545 (1965).

74. P.S. Ellington, D.G. Hey and G.D. Meakins, J. Chem. Soc. (C), 1327 (1966).

75. J. Chatt, Chem. Rev., 48, 7 (1951).

76. D.G. Hey, G.D. Meakins and M.W. Pemberton, J. Chem. Soc. (C), 1331 (1966).

77. L. Merlini, G. Nasini and J.D. Phillipson, Tetrahedron, 28, 5971 (1972).

78. A. Patchornik, W.B. Lawson and B. Witkop, J. Amer. Chem. Soc., 80, 4747 (1958).

79. W.I. Taylor, Proc. Chem. Soc., 247 (1962).

80. J.C. Powers, J. Org. Chem., 31, 2627 (1966).

81. W.O. Godtfredson and S. Vangedal, Acta Chim Scand., 10, 1414 (1956).

82. P.G. Gassman, G.A. Campbell and G. Mehta, Tetrahedron, 28, 2749 (1972).

83. S.A. Monti and R.R. Schmidt, Tetrahedron, 27, 3331 (1971).

84. F. Bylsma, Ph.D. Thesis, University of British Columbia (1970).

85. H.C. Brown, Hydroboration, Benjamin, New York, N.Y., 1962.

86. J. Mokry and I. Kompis, Lloydia, 27, 428 (1964).

87. J.P. Kutney, W.J. Cretney, P. Le Quesne, B. McKague and E. Piers, J. Amer. Chem. Soc., 92, 1712 (1970).

88. N. Neuss, L.L. Huckstep and N.J. Cone, Tetrahedron Letters, 811 (1967).

89. J.D. Albright and L. Goldman, J. Amer. Chem. Soc., 89, 2416 (1967).

90. J.R. Parikh and W. von E. Doering, J. Amer. Chem. Soc., 89, 5505 (1967).

91. T.J. Brocksom, University of British Columbia (1971-1972).

92. K.E. Pfitzner and J.G. Moffatt, J. Amer. Chem. Soc., 87, 5661 (1965).

93. I. Itoh, University of British Columbia (1972-).

94. P. Bommer, W. McMurray and K. Biemann, J. Amer. Chem. Soc., 86, 1439 (1964).

95. N. Finch and W.I. Taylor, J. Amer. Chem. Soc., 84, 3871 (1962).

96. J.B. Patrick and B. Witkop, J. Amer. Chem. Soc., 72, 633 (1950).

97. L.S. Dolby and G.W. Gribble, J. Org. Chem., 32, 1391 (1967).

98. G. Buchi and R.E. Manning, J. Amer. Chem. Soc., 88, 2532 (1966).

99. W. Winkler, Naturwiss, 48, 694 (1961).

100. G. Buchi, R.E. Manning and S.A. Monti, J. Amer. Chem. Soc., 86, 4631 (1964).

101. S.G.P. Plant, R. Robinson and M. Tomlinson, Nature, 165, 928 (1950).

102. G. Buchi, R.E. Manning and S.A. Monti, J. Amer. Chem. Soc., 85, 1893 (1963)

103. J. Harley-Mason and A. Rahman, Chem. Comm., 1048 (1967).

104. We are indebted to Prof. J.C. Clardy, Iowa State University for these X-ray studies.

105. Dihydrovinblastine. Eli Lilly & Co. (By M. Gorman and N. Neuss). Belg. 661,948, Oct. 1, 1965, U.S. Appl. April 1, 1964.

106. R.L. Noble, C.T. Beer and R.W. McIntyre, Cancer, 20, 885 (1967).

107. K.V. Lichman, J. Chem. Soc. (C), 2539 (1971).

108. A. Rahman, Pakistan. J. Sci. Ind. Res., 14, 487 (1971).

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110. B.K. Moza, J. Trojanek, V. Hanus and L. Dolejs, Collection Czechoslov. Chem. Commun., 29, 1913 (1964).

111. W.J. Cretney, Ph.D. Thesis, University of British Columbia (1968).

112. H. Gilman and S.M. Spatz, J. Amer. Chem. Soc., 63, 1553 (1941).

113. R.G. Jones and H. Gilman, "The Halogen-Metal Interconversion Reaction with Organolithium Compounds", in Organic Reactions, Vol. VI, John Wiley & Sons, New York, 1951.

114. F.G. Bordwell and T.G. Mecca, J. Amer. Chem. Soc., 94, 5829 (1972).