THE TOTAL SYNTHESIS OF VERATRUM ALKALOIDS

by

John Cable

B.Phm., University of Sydney, Sydney, Australia, 1963
M.Phm., University of Sydney, Sydney, Australia, 1965

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Chemistry

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September, 1968

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and Study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of CHEMISTRY

The University of British Columbia
Vancouver 8, Canada

Date September 20th, 1968

Abstract

A synthetic approach to members of the Veratrum alkaloids and its application in the synthesis of verarine is described.

The condensation of optically active 3β-acetoxy-5α-etiojerv-12-en-17-one (76), a known compound available from the degradation of hecogenin acetate, with the lithium derivatives of various substituted 2-ethylpyridines is outlined as a general scheme for synthesising the carbon skeleton of members of the Veratrum alkaloids.

Condensation with the lithium derivative of 2-ethyl-5-methylpyridine (105) followed by acetylation of the product gave a mixture of four isomers possessing the verarine skeleton (106). The two major isomers designated "A" and "B" were separately converted to the ring D aromatic compounds (107) by heating with palladised charcoal and the products shown to be isomeric. Selective hydrogenation of the pyridine moiety in either ring D aromatised compound (107) gave a mixture of four isomers which contained the piperidine ring (108). These compounds were separated and then converted to the N-acetyl derivatives (110) via the 3-O,N-diacetyl derivatives (109).

Degradation of veratramine (2) by a known procedure gave 3-O,N-diacetylverarine (117). Hydrogenation of the 5,6-double bond employing Adams catalyst in acetic acid gave a 1:1 mixture of the 5α,6- and 5β,6-dihydro compounds which were separated as the N-acetyl derivatives (120, 121).

The N-acetyl derivative of one of the eight isomers obtained from hydrogenation of the isomeric aromatic compounds (107) has been identified as N-acetyl-5α,6-dihydroverarine (120). The conversion of this compound

to verarine (3) was carried out on a quantity of material obtained from veratramine. Oxidation with Jones reagent led to N-acetyl-3-keto-5α,6-dihydroverarine (111) which was converted to N-acetyl-Δ^4-3-keto-5,6-dihydroverarine (112). Treatment of this α,β-unsaturated ketone with isopropenyl acetate gave the enol acetate (113) which was converted to N-acetylverarine (114). Removal of N-acetyl group gave verarine (3) which was identified by comparison with an authentic sample. This completes in a formal sense the total synthesis of verarine since hecogenin has been totally synthesised.

The total synthesis of racemic 3β-acetoxy-5α-etiojervan-17-one from β-naphthol by other members of this laboratory is mentioned and its comparison with the natural (+) 3β-acetoxy-5α-etiojervan-17-one is noted.

Table of Contents

List of Figures

List of Figures (Cont'd)

Acknowledgements

I wish to express my thanks to Professor J. P. Kutney for his continual encouragement and excellent guidance throughout the course of my research.

I am grateful for scholarships from the MacMillan Bloedel and Powell River Co. and the National Research Council of Canada which I have received during my study.

Financial support of this project by Smith Miller and Patch Inc., The National Research Council of Canada and the Medical Research Council of Canada is greatly appreciated. In addition the receipt of generous gifts of hecogenin acetate from Smith, Miller and Patch Inc. and Syntex S.A., Mexico is Acknowledged.

INTRODUCTION

During the past decade significant contributions have been made regarding the chemistry and stereochemistry of several sub-groups among the Veratrum alkaloids. The general classification, Veratrum alkaloids includes those alkaloids from the tribe Veratreae belonging to the Liliaceae family and specifically from the genera Veratrum, Zygadenus, Stenanthium and Schoenocaulon. Alkaloids of similar structure have also been shown to occur in the Fritillaria genus of the Liliaceae.

Reviews on the various aspects of the chemistry of the Veratrum alkaloids have been written by Fieser and Fieser[1], Boit[2], Narayanan[3] and Kupchan[4]. In addition, the occurrence of the alkaloids in the plants of the Veratreae, and the implications of alkaloid occurrence and structure to the taxonomy of the Veratreae have been reviewed[5]. The division of the Veratrum alkaloids into the jerveratrum and cerveratrum groups as proposed by Feiser has been adopted by Kupchan in the most recent review.

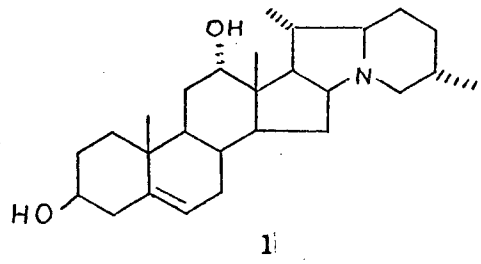
The jerveratrum alkamines contain only 1 to 3 atoms of oxygen and are found in the unhydrolysed plant extracts, in part as the free alkamines, and in part, in combination with one molecule of D-glucose as glucoalkaloids. The cerveratrum alkamines are highly hydroxylated and contain 7 to 9 atoms of oxygen. They usually occur esterified with various acids but are in some instances unconjugated although they do not occur as glycosides.

Crude extracts from Veratrum and related plants have been used for various medicinal purposes since the middle ages, and their use in the control of hypertension dates from a report by Baker[6] in 1859.

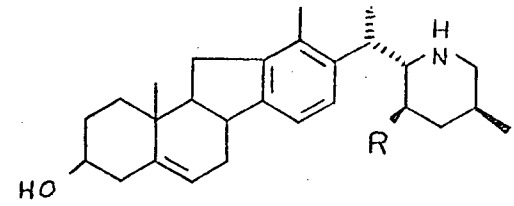
Treatments in the second half of the nineteenth century employed crude extracts which gave erratic results and their usage was discontinued. During the late 1930's purified alkaloidal preparations became available and improved techniques led to the first crystalline alkaloidal preparation, protoveratrine, which was shown to be a powerful hypotensive agent[7,8].

Following pharmacological investigation by Krayer[9] this preparation was introduced into clinical use in the treatment of certain types of hypertension[10]. One of the limiting factors in the use of this drug is the narrow dosage range between hypotensive and emetic effects. The advent of the superior hypotensive compound, reserpine, has led to the use of the protoveratrines being largely discarded.

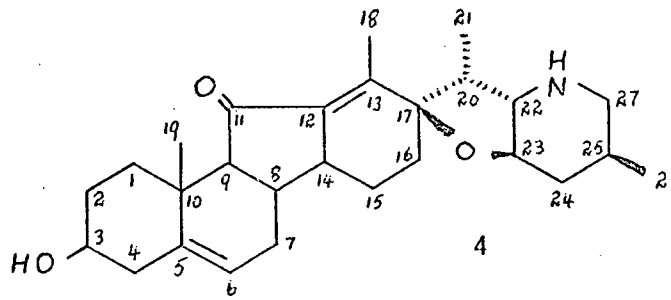
The jerveratrum alkamine rubijervine (1), so named because of its red colour with sulphuric acid rather than a similarity to jervine, may be regarded as the simplest of the veratrum alkaloids from a structural point of view. It possesses the normal C-27 steroid skeleton (e.g. cholesterol) and the E and F rings may formally be regarded as having been formed by folding the normal cholesterol side chain around the nitrogen atom. Other jerveratrum members veratramine (2), verarine (3) and jervine (4) are characterised by the C-nor-D-homo ring skeleton which may formally be regarded as having originated by migration of the C-13, C-14 bond of a normal steroid to the C-12, C-14 position. Since it was our aim to provide a synthetic entry into the C-nor,D-homo jerveratrum alkaloids the structural elucidation of veratramine, verarine and jervine will now be reviewed.



1



2 R = OH
3 R = H

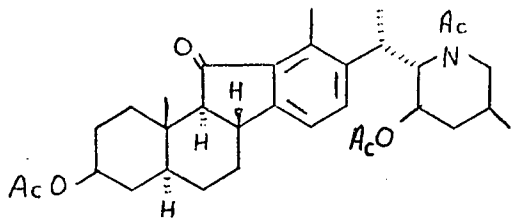


4

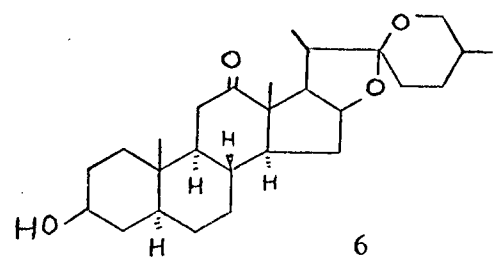
Figure I

Fried and Klingsberg[11] proposed the term "jervane" be adopted to represent the carbon skeleton of jervine (4) and the term "etiojervane" to represent the parent tetracyclic hydrocarbon. This proposal has been widely adopted and simplifies the nomenclature of these alkaloids. The systematic steroid nomenclature for the "etiojervane" portion is 17-methyl-C-nor-D-homo-18-nor-5,12 androstane indicating the obvious advantages of the above proposal. The numbering scheme for these compounds is as indicated for jervine (4) in fig 1. It has been proposed recently [11a] that the term "cholojervane" be adopted for the 24 carbon skeleton which arises by cleavage of the C-24, C-25 bond.

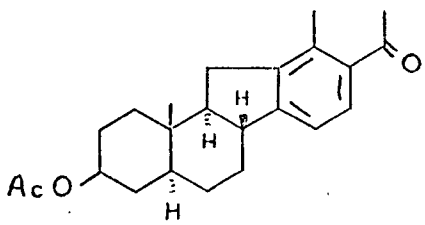
Both jervine (2) and veratramine (4) have been converted to the triacetyl derivative (5) and jervine has been interrelated with hecogenin[12] (6) via compound 7. This suggested the 9α configuration for veratramine and further support for this assignment was obtained by conversion of hecogenin[13] and veratramine[14] to compound 8. Additional



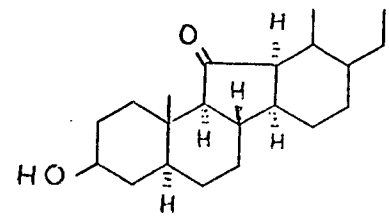
5



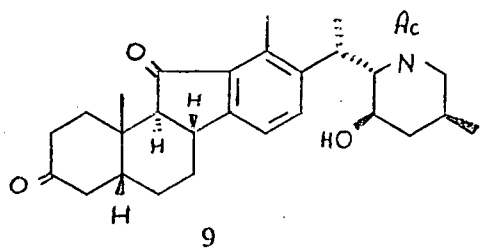
6



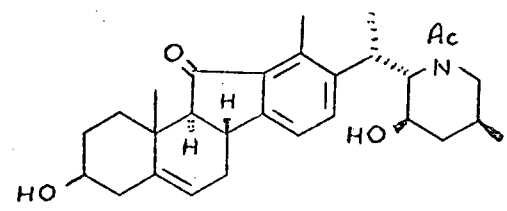
8



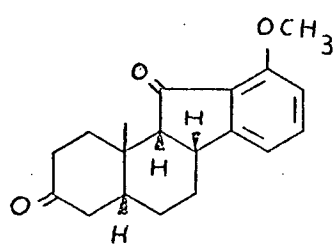
7



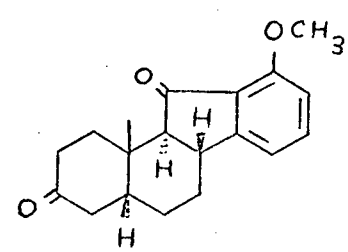
9



10



11



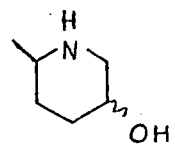
12

Figure 2

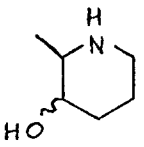
evidence for the 9α configuration was provided by Johnson[15] in a base catalysed equilibration study of compound 9 formed from N-acetyl-11-ketoveratramine (10). Treatment of the diketone 9 with methanolic potassium hydroxide effected partial conversion to a new compound. The n.m.r. spectrum of the diketone 9 showed a sharp signal attributed to the C-19 methyl at τ 8.81 which was superimposable upon the corresponding signal in the synthetic trans compound 12. The equilibration mixture exhibited a new sharp signal at τ8.45 which was superimposable on the corresponding C-19 methyl signal in the synthetic cis compound 11.

This work established the B/C trans junction for N-acetyl, 11-ketoveratramine (10) which was related to veratramine by conversion to N-ethyl veratramine under conditions which allowed no epimerisation at C 9.

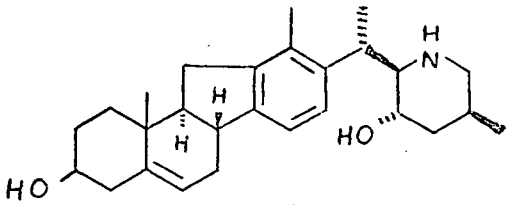
The configuration of the piperidine moiety was first investigated by Sicher and Tichy[16] by infrared comparison with two pairs of pipecolinol derivatives (13 and 14). They found that the cis isomers exhibited two hydroxyl bands at 3μ whilst the trans isomers showed a single hydroxyl band. They assumed that the ethyl group would occupy an equatorial position placing the hydroxyl group axial and so allow hydrogen bonding to the nitrogen. Consequently the cis isomers show a hydroxyl band due to the free hydroxyl as well as one due to the hydrogen bonded species. The trans isomers can exist with both groups equatorial and the infrared spectrum shows only the band due to free hydroxyl. The infrared spectrum of veratramine shows only one hydroxyl band supporting a trans diequatorial arrangement for the alkyl side chain at C-22 and the C-23 hydroxyl. It had been proposed[18] that the C-26 methyl group belongs to the (S)-series and occupied the β position. From these considerations the configuration of veratramine was assigned as in 15.



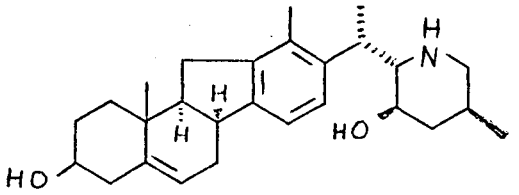
13



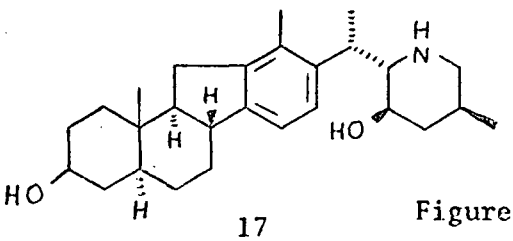
14



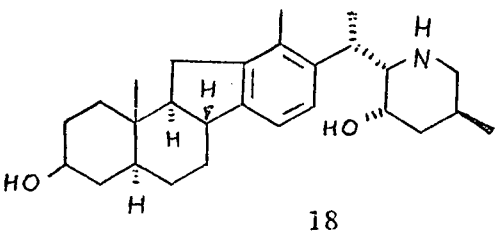
15



16



17



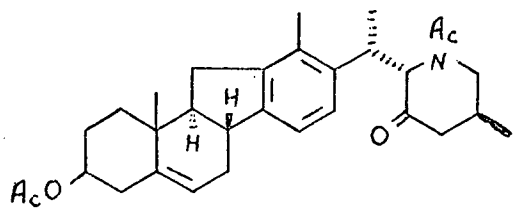
18

Figure 3

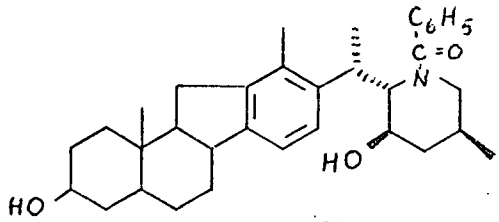
However, a recent investigation by Johnson[17] has indicated that veratramine is correctly represented by 16 in which the substituents on the piperidine are all equatorial. Johnson synthesised 5α,6-dihydro-veratramine (17) and 5α,6-dihydro-23-iso-veratramine (18) without epimerising the C-22 alkyl side chain. The iso compound must have the C-23 hydroxyl group in an axial orientation. This would be expected to cause a 12-13 cps shift to lower field of the C-26 methyl signal if this group is also axially oriented. The C-26 signal was shown to occur at τ 9.18 in both compounds 17 and 18 indicating that the C-26 methyl group is equatorial. Since this group occupies the β configuration and the substituents at C-22 and C-23 are trans and equatorial the correct assignment for veratramine is represented by 16. Earlier work by Augustine[19] and Masamune[20] involving equilibration studies on the keto amide of veratramine had supported the assignment of Sicher and Tichy[15]. Masamune studied the base catalyzed epimerisation of 3,N-

diacetyl-23-dehydro-veratramine (19), which in the original postulate has one of the alkyl groups on the piperidine moiety in an axial orientation. Treatment of 19 with methanolic potassium hydroxide gave a predominance of the C-22-isomeric compound in a ratio of 10:1. This result was explained on the basis that epimerisation at this position would lead to the piperidine ring being able to assume the more stable conformation with both groups equatorial.

Paulson and Todt [21,22] have shown that acylation of 2-methyl piperidine is accompanied by conformational inversion of the ring (methyl group axial) to relieve steric interaction between the equatorial methyl group and amide carbonyl.



19



20

The recent n.m.r. study by Johnson supports this inversion of the piperidine ring in the N-benzoyl derivative of veratramine (20). The C-23 proton appears as a relatively sharp unresolved multiplet indicative of an axial hydroxyl whilst the signal assigned to the C-26 methyl group now appears at τ 9.9 7. This downfield shift of the C-26 methyl group indicates a 1,3 diaxial relationship between the C-23 hydroxyl and the C-26 methyl groups.'

It therefore seems very probable that the ketoamide 19 exists preferentially in that conformation with the C-22 and C-25 substituents

both axial. Epimerisation to the C-22 iso compound occurs readily since this compound can exist in the conformation with the C-22 axial and the C-26 methyl equatorial. These studies indicate that veratramine is correctly represented as 16 since the configuration at all centres except C-20 has been investigated. The configuration at C-20 is assigned only on the basis of biogenetic analogy with other steroids.

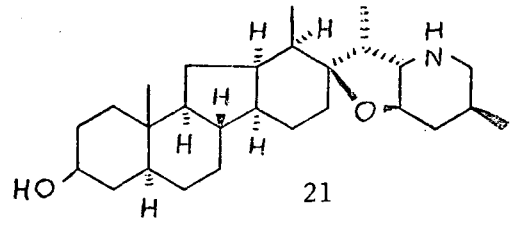
The configurations at C-22 and C-23 in jervine have been interrelated with those of veratramine and therefore the tetrahydrofuran ring in jervine must be attached to the piperidine ring in a trans manner. Degradation of jervine by Klinsberg[11] gave 7 which had been synthesised from hecogenin[12]. The interrelation with veratramine gave additional support for the 9α configuration whilst the configurations at all centres of 5, 6, 12-tetrahydrojervine (21) has been proposed by Wintersteiner and Moore[23]. In this investigation the configurations at C-17 and C-20 were assigned on the basis of biogenetic analogy with other steroids rather than by investigation.

Under fragmentation conditions N-methyl jervine gave 1, 5-dimethyl-3-piperidone which was then reduced to 1,3-dimethylpiperidine[17]. A synthesis of 1-(3R)-dimethylpiperidine from D-(+) citronellal was carried out and the product shown to be the antipode of the compound from N-methyljervine. Consequently the absolute configuration at C-25 in jervine belongs to the (S)-series and the C-26 methyl group has the β orientation. The recently revised configurations of the piperidine ring in veratramine also apply to jervine since the two compounds have been interrelated.

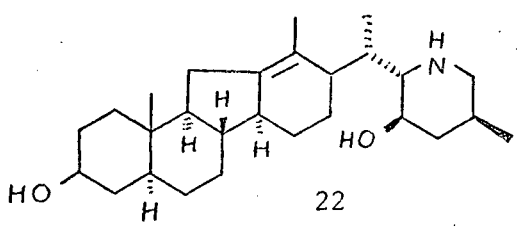
In view of the 11-keto group in jervine allowing for some

doubt about the actual configuration at C-9 in jervine and its derivatives, Masamune[24] has recently reexamined this problem and has provided conclusive evidence for the 9α configuration via the conversion of veratramine and "Jervine-11β-ol" to the 5α,6-dihydro derivative 22. At this time the configurations at C-17 and C-20 were assigned only by biogenetic analogy and not by any direct evidence. A recent X-ray diffraction determination on veratrobasine has revealed that this compound is probably jervin-11β-ol[25]. This study reveals that the configuration which had been assigned to C-17 in jervine might be incorrect. Kupchan[26] has investigated the relationship of jervine and veratrobasine and has shown that the latter compound is indeed jervin-11β-ol. In the light of the X-ray work on veratrobasine the structure of jervine is correctly represented by 23 in which the ether bridge is β at both C-17 and C-23.

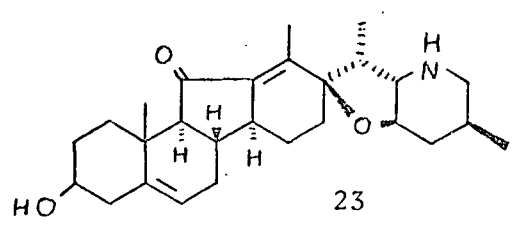
The jerveratrum alkaloid verarine (3) was first isolated by Tomko who proposed that the compound was probably 23-desoxy veratramine[27]. Masamune[20] has investigated this possibility by the removal of the 23-hydroxyl from veratramine and has shown that verarine is in fact 23 - desoxyveratramine. The reactions involved in this conversion will be discussed later since they are pertinent to the work of the thesis. From this interrelation the configurations assigned to veratramine must necessarily apply to verarine.



21

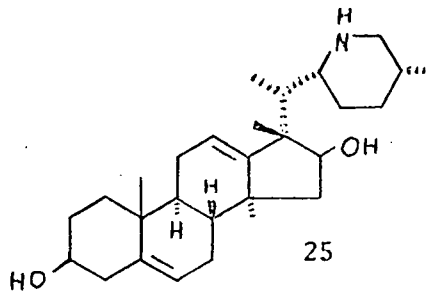


22

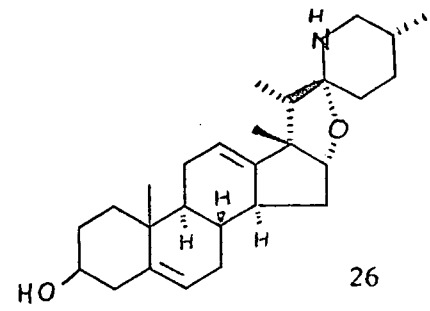


23

Two new veratrum alkaloids veralkamine[28] (25) and veramine[29] (26) have recently been isolated and investigated by Tomko and Schreiber. These compounds have been shown to possess the unusual 17β-methyl-18-nor-17-iso-cholestane carbon skeleton as indicated in fig. 4. These compounds are unusual when compared with the known jerveratrum alkaloids and closely resemble the spiroaminoketal alkaloids solasodine and tomatidine. The above workers have further investigated these types of alkaloids and the results will soon appear in print[30,31].



25

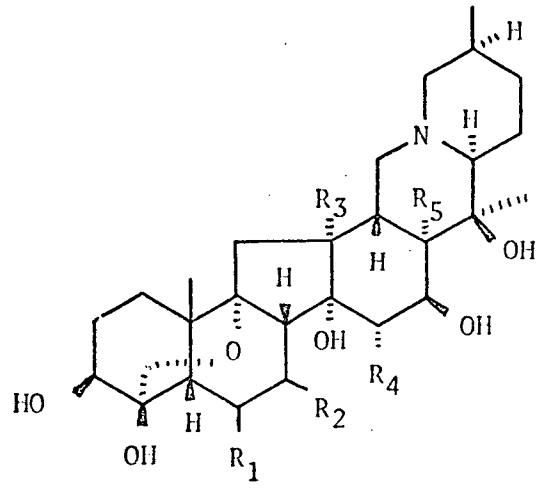


26

Figure 4

The cerveratrum alkaloids are highly oxygenated and generally occur as esters. All have the cevan nucleus which is characterised by the C-nor,D-homo nucleus with an alternate folding of the side chain around the nitrogen atom. The commonly occurring alkamines are veracevine (27), germine (28), zygadenine(29), protoverine (30) and sabine (31). (Fig. 5)

The protoverine esters A and B (32 and 33) have been investigated thoroughly. Structural studies on these compounds culminated in 1960 with the elucidation of the structure and configuration of the alkamine protoverine and the tetraesters protoveratrine A and protoveratrine B [32]. Protoveratrine A is a potent hypotensive agent with a narrow therapeutic dosage range whilst protoveratrine B is less active but the emetic side

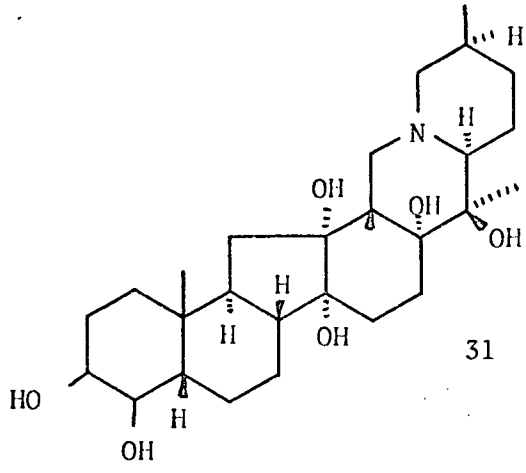


27 $R_1 = R_2 = R_4 = H$; $R_3 = R_5 = OH$

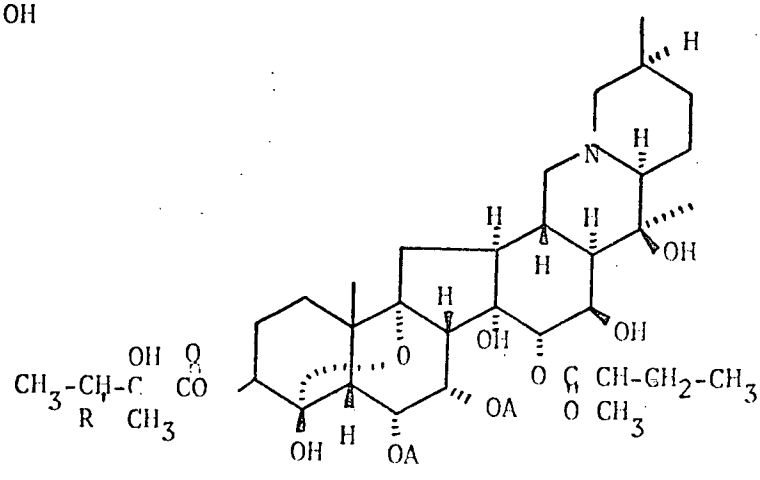
28 $R_1 = R_3 = R_5 = H$; $R_2 = R_4 = OH$

29 $R_1 = R_2 = R_3 = R_5 = H$; $R_4 = OH$

30 $R_3 = R_5 = H$; $R_1 = R_2 = R_4 = OH$



31



32 R = H

33 R = OH

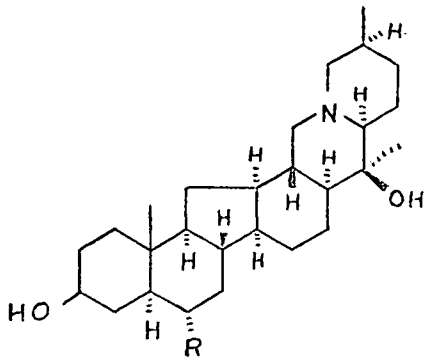
Figure 5

effects are not as pronounced. Structurally the difference between these two compounds is small and this prompted Kupchan to examine a number of protoveratrine derivatives with the aim of improving the therapeutic dosage range[33]. This study led to a number of generalisations concerning structure activity relationships for the protoveratrines but no radical improvement of the therapeutic dosage range.

The assignment of configurations at C-8, C-13, C-16, C-20 and C-22 in veracevine has been presented and a subsequent X-ray study of cevine hydroiodide has provided conclusive evidence for the configurational assignements of thirteen of the fourteen asymmetric centres[35] whilst evidence has been advanced to support the 20-β configuration.

Alkaloids of related structure have been isolated from plants of the Fritillaria genus of the Liliaceae family. The Fritillaria alkaloid verticine (34) was first isolated by Fukuda[36] from F. verticillata Willd. var. thunbergii Baker. The same alkaloid was isolated by Chou and Chen[37] from F. roylei Hook and named peimine. The identity of the two compounds was established by direct comparison. Structurally verticine represents the simplest example of a compound having the cevane nucleus as found in the cerveratrum alkaloids. A further alkaloid Fritillarine was isolated in the above studies and has since been characterised as verticinone (35) by mixed melting point and infrared spectra comparison[38].

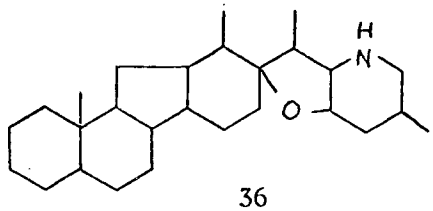
An extensive n.m.r. study of members of the cerveratrum group has been carried out[39]. The spectra of 37 alkaloids of this group were recorded and data for methyl group signals examined. The study provides additional support for the configuration of the C-27 methyl group and the effect of forming the D ring orthoacetate upon the stereochemistry of the D ring was examined.



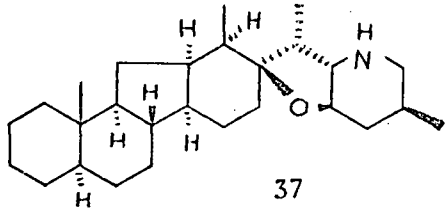
34 R = OH

35 R = =O

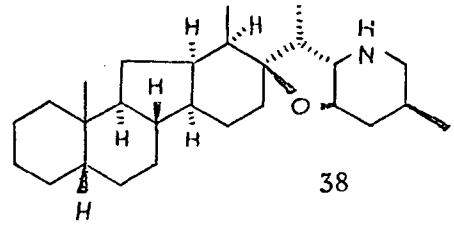
Masamune[40] has examined the n.m.r. spectra of 65 derivatives of the jerveratrum group of alkaloids. All the compounds contain the 22,27-imino-17,23-oxidojervane ring skeleton (36) and the data from the spectra is confined to the correlation of the chemical shifts of the methyl groups with changes in environment.



36



37



38

In the n.m.r. spectra of steroids[41] and triterpenoids[42] the long range shielding effects of various substituents on the chemical shift of the angular methyl protons have been shown to be additive.

In order to examine both additivity and shielding effects in the 22,27-imino-17, 23-oxidojervane skeleton,the compounds were divided into those derived from the A/B trans form (37) and those from the A/B

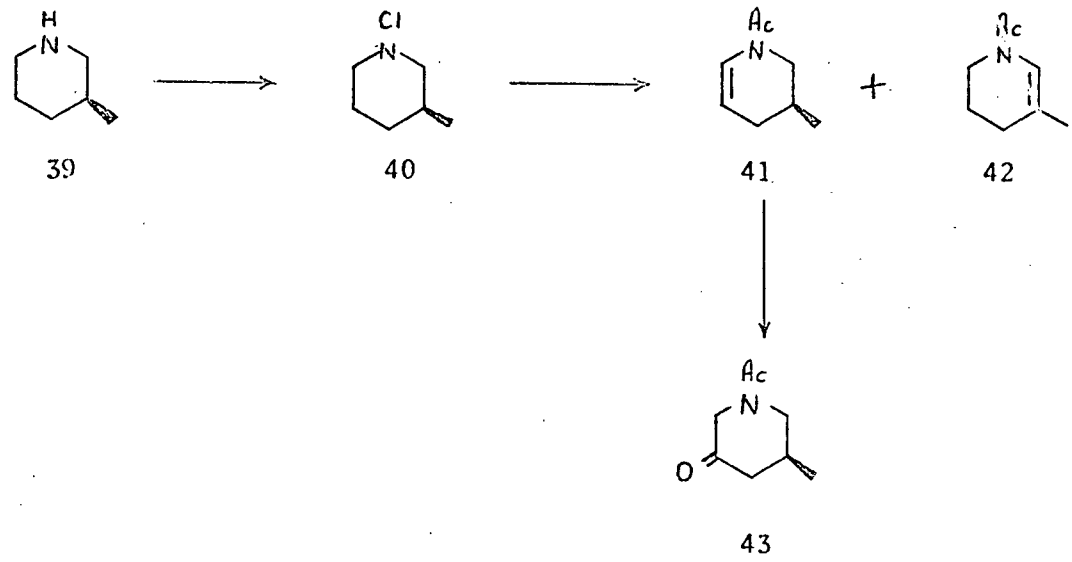
cis (38). The contributions due to various functional groups were obtained by pairing compounds which differ only by the group in question. Values calculated for the C-19 methyl protons using these contributions were in good agreement (within 0.02 τ) with the observed chemical shifts, indicating the "principle of additivity" holds satisfactorily for the 19-methyl protons of these C-nor-D-homosteroid alkaloids.

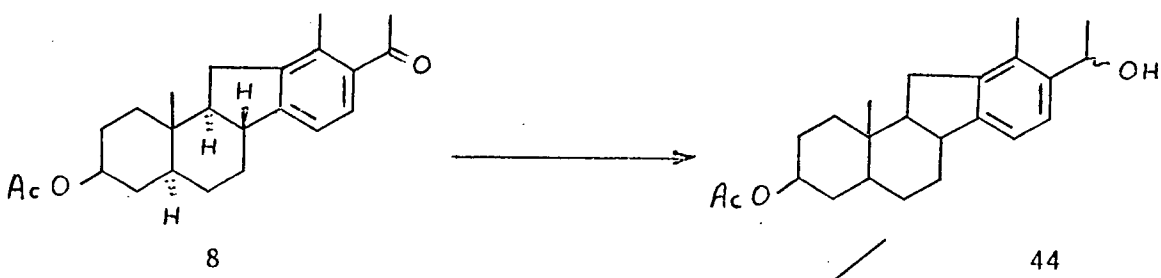
Masamune[43] and Johnson[44] have recently published the results of their independent investigations regarding the synthesis of some Veratrum alkaloids. Masamune has succeeded in synthesising veratramine and jervine whilst Johnson has synthesised veratramine. Both groups utilised 17-acetyl-5α-etiojerva-12,14,16-triene-3β-ol (8) as the source of the C-nor-D-homo skeleton followed by elaboration of the 17-acetyl side chain to provide the hetereocyclic portion to complete the jerveratrum skeleton.

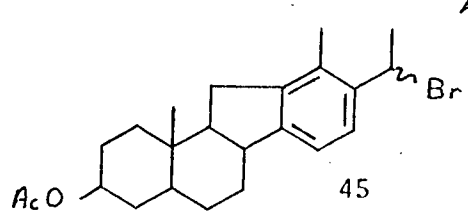
An outline of Masamunes' synthetic approach is given in Fig. 6. In this sequence compound 8 was obtained by degradation of hecogenin and the piperidine portion is attached via an alkylation of the pyrrolidine enamine 46 with 45. This leads directly to an isomeric mixture of 3,N-diacetyl-5α,6-dihydro-23-dehydro-veratramines (47) since the (S) configuration of the methyl group is established in 43. This isomeric mixture was compared with an authentic sample obtained from 5α,6-dihydroveratramine. The ether bridge between C-17 and C-23 was synthesised by a series of elegant steps including formation of the important compound 22,27-iminojervan-13(17)-ene-3β,23β-diol (49) which was identified by comparison with an authentic sample.

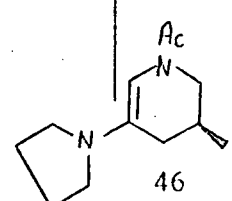
Epoxidation of this compound gives 50 which undergoes cleavage of the epoxide and concommitant attack of the C-23 hydroxyl to give the desired ether bridge. Dehydration with thionyl chloride gave 3,ON-diacetyl-

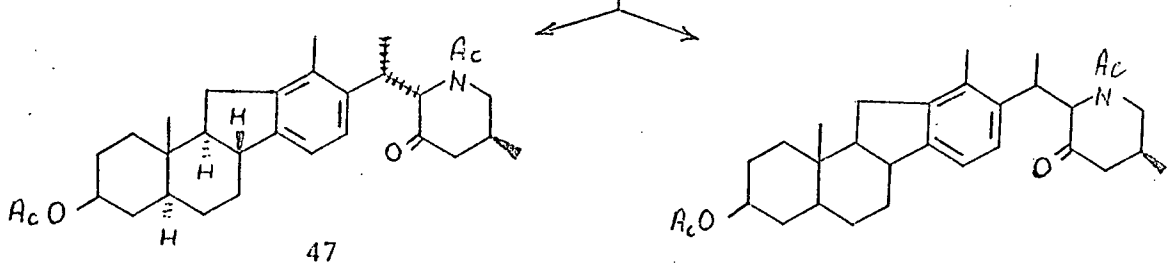
Figure 6


H
N
Cl
N
Ac
N
Ac
N
39
40
41
42
Ac
N
O
43


O
H
H
Ac O
H
OH
Ac O
8
44


Br
Ac O
45


Ac
N
N
46


Ac
N
H
H
O
Ac O
H
47
Ac
N
O
Ac O

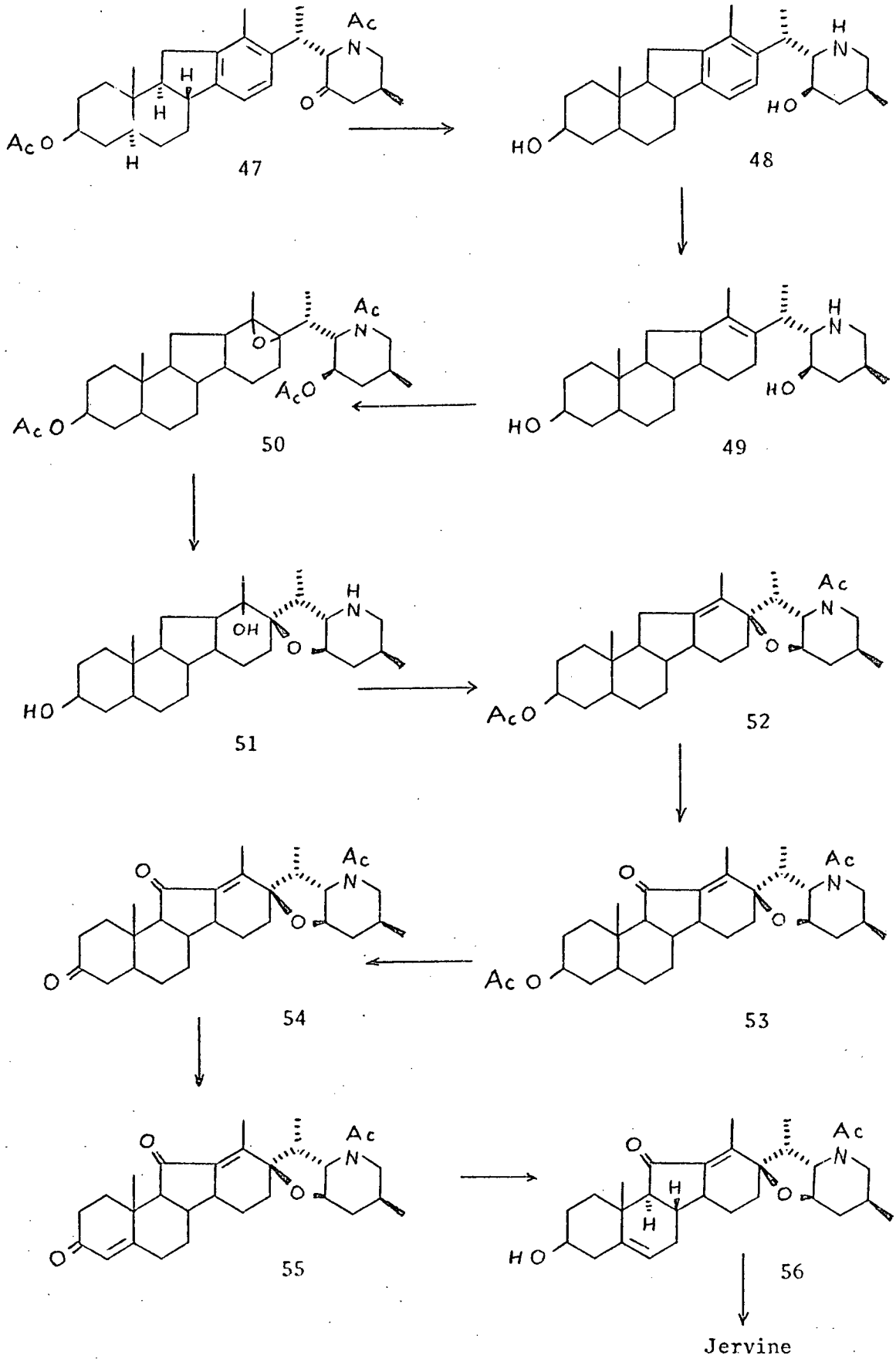


Figure 6 continued

11-deoxo-5α,6-dihydrojervine (52) which was compared with an authentic sample prepared from jervine in an unambiguous manner. The introduction of the 11-keto group unfortunately proceeds in a yield of 1% but the authors were able to isolate 53 and complete the sequence to jervine as shown. It should be noted that in view of the recent paper by Kupchan[26] the formulae 51-56 are indicated with the correct stereochemistry at C-17. Since hecogenin has been totally synthesised[45] this work represents in a formal sense a total synthesis of veratramine and jervine.

The W. S. Johnson group also employed 17-acetyl-5α-etiojerva-12,14,16-trien-3β-ol (8) as the source of the C-nor-D-homo portion but in their approach this compound was totally synthesised from Hagemann's ester (57) as outlined in Fig. 7 rather than obtained from the degradation of hecogenin. The synthesis involves the use of a relay compound from veratramine for the conversion of 66 to 8 . This same group of workers has recently published[46] an alternate synthesis of compound 8 by extension of the hydrochrysene approach which they had investigated earlier[47].

Fig. 8 outlines the synthesis of veratramine from 17-acetyl-5α-etiojerva-12,14,16-trien-3β-ol in which the piperidine ring is built up from the uncyclized compound (72) through 73 and 74 to yield a mixture of epimeric N-benzoyl 5β,6-dihydro-3,23-diketoveratramines (75) which was compared with an authentic sample prepared from veratramine. Introduction of the 5-6 double bond was achieved via formation of the Δ^4-3-ketone which was converted to the enol acetate. Reduction of this compound with sodium borohydride provided N-benzoyl veratramine which was debenzylated to give veratramine. This synthesis represents a direct total synthesis of veratramine which can be extended to jervine employing procedures developed

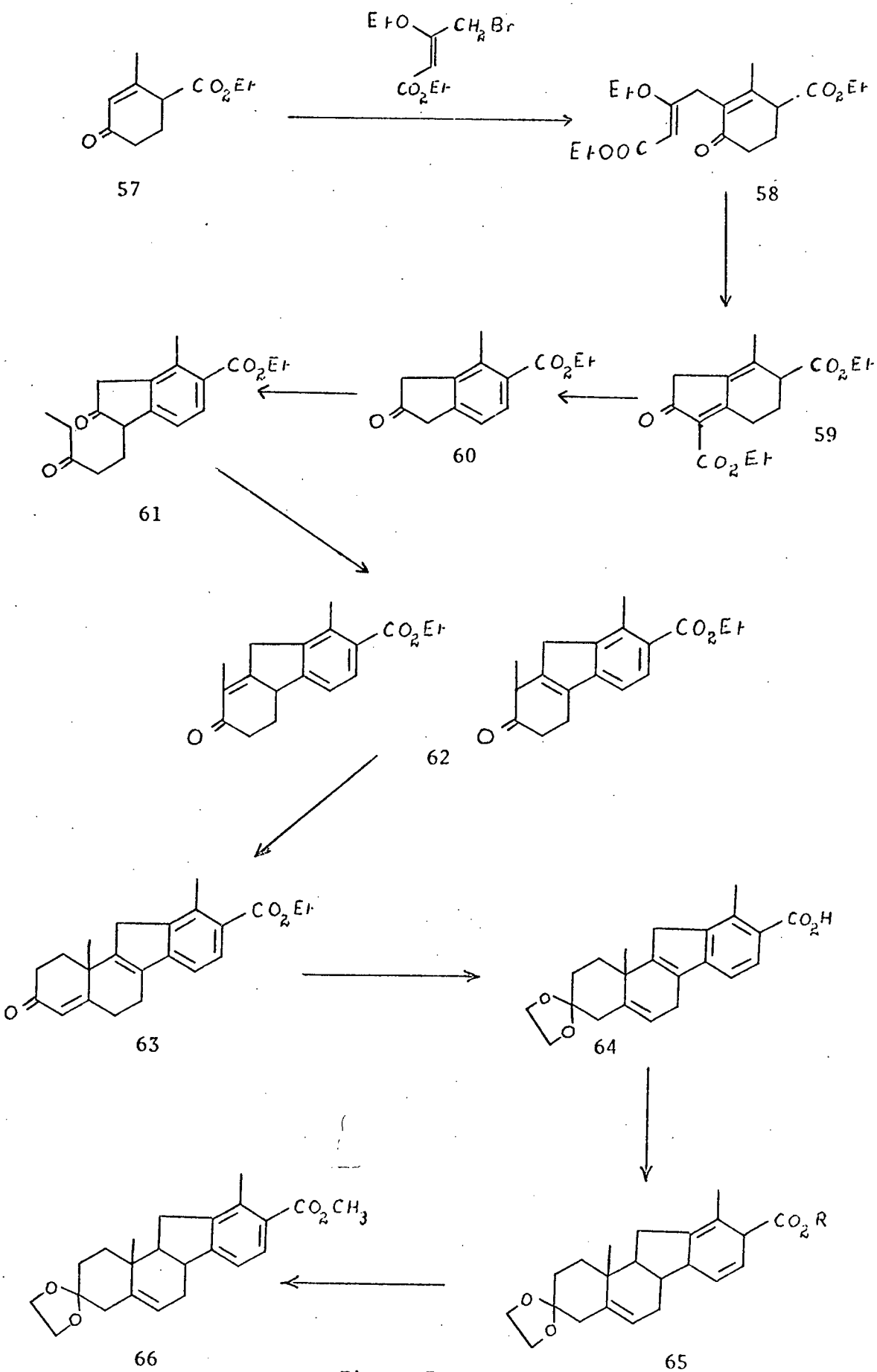

EtO
CH2Br
CO2Et
57
58
EtOOC
59
60
61
62
63
64
65
66
CO2H
CO2R
CO2CH3

Figure 7

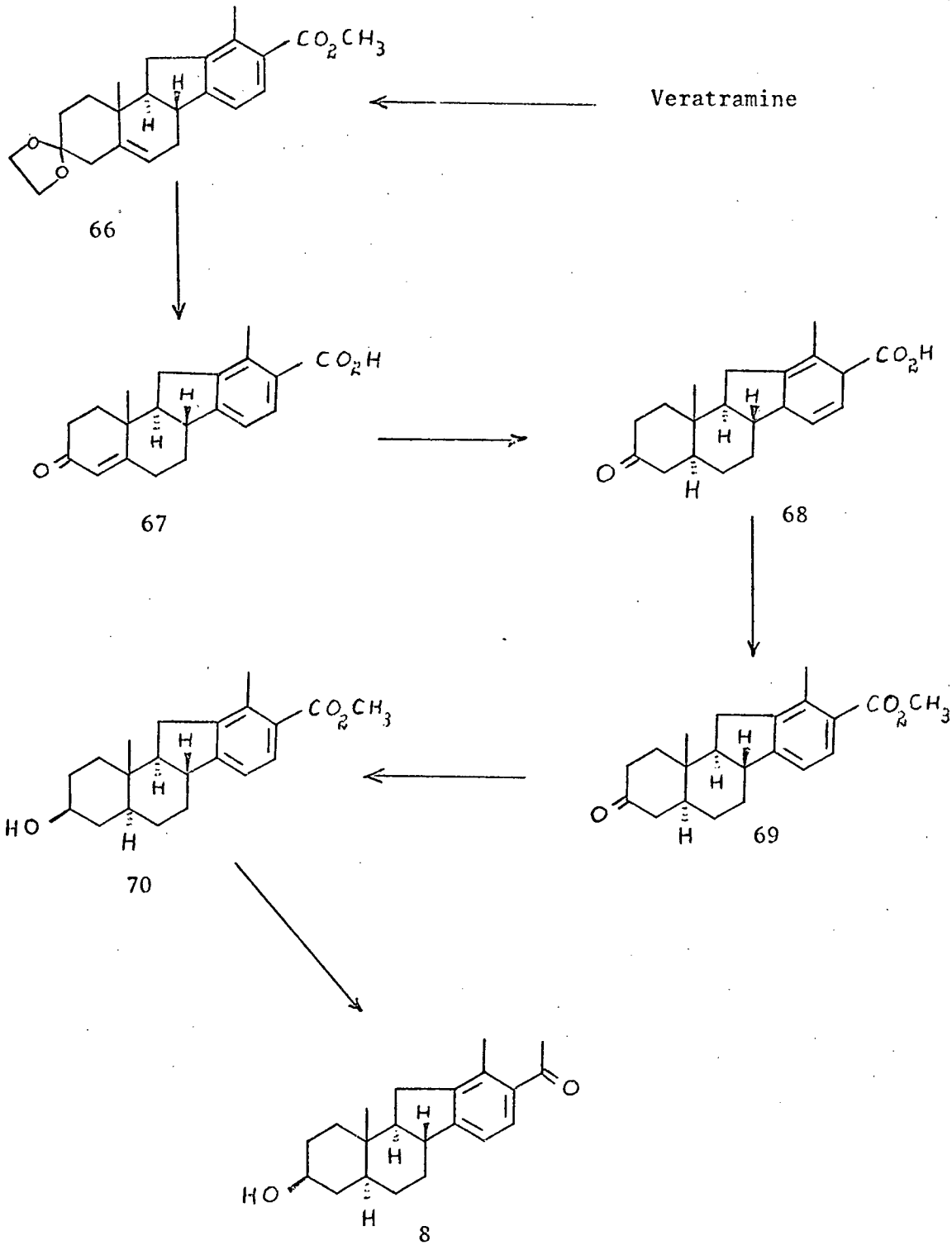


Figure 7 continued

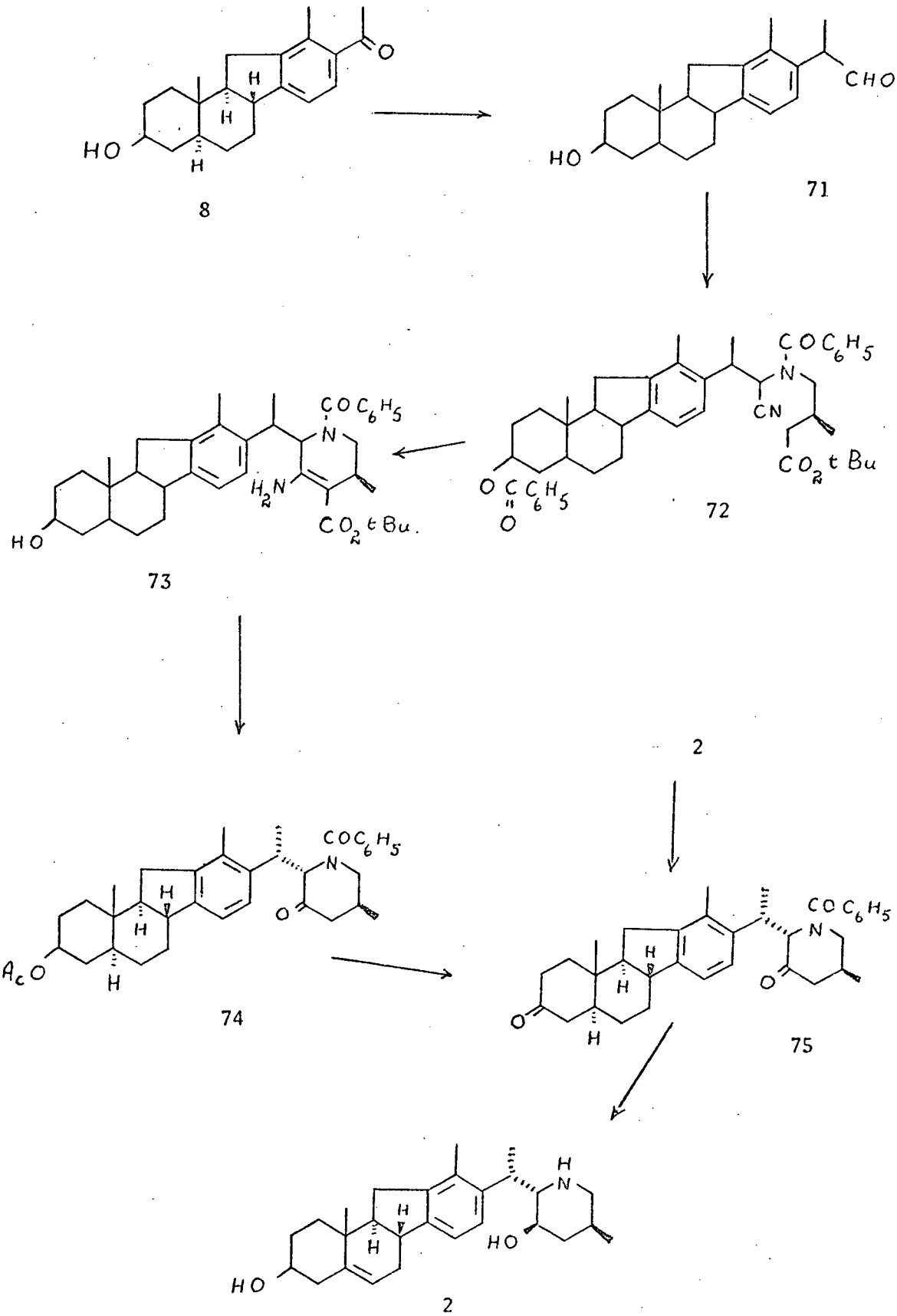

HO
O
H
H
H
8
CHO
HO
71
COC_6H_5
N
CN
CO_2tBu
OCC_6H_5
O
72
COC_6H_5
N
H_2N
CO_2tBu
HO
73
2
COC_6H_5
N
O
H
H
AcO
H
74
COC_6H_5
N
O
H
H
O
H
75
H
N
H
H
HO
HO
2

Figure 8

by the Masamune group.

During the period of time that these two groups have investigated the total synthesis of the jerveratrum alkaloids we have also directed our efforts, in a somewhat different manner, towards a totally synthetic entry into the veratrum alkaloids. The overall plan was to employ a method which would allow a general entry into these alkaloids so that slight modifications of procedure would lead to different alkaloids. This thesis represents the work done on the latter stages of this approach which I would now like to present.

DISCUSSION

During the past few years members of this laboratory have been actively engaged in research designed to provide a totally synthetic entry into the Veratrum alkaloids. The approach has been to consider the veratrum skeleton as consisting of an etiojervane portion and a substituted piperidine, coupled by the C-17, C-20 bond.

The aim was to provide a total synthesis of these two molecules and then couple them in such a manner as to provide the desired skeleton. The proposed sequence is outlined in fig. 9 in which 3β-acetoxy-5α-etiojerv-12-en-17-one(76) is reacted with the lithium derivative of the appropriately substituted 2-ethylpyridine (77) to provide a isomeric mixture of compounds possessing the structure 78. Aromatisation of ring D would provide compounds of type 79 differing only in configuration at C-20. A selective reduction of the pyridine to the piperidine system generates new asymmetric centres and would give rise to a mixture of isomers (80) with the number depending upon the nature of R'.

If all the possible isomers are obtained after the selective hydrogenation then one of these isomers should be identical with the 5 ,6-dihydro alkaloid for which the sequence is designed. If R' is hydrogen then the isomeric 5α,6-dihydroverarines will be obtained and in the instance where R' is hydroxyl the sequence leads to an isomeric mixture of the 5α,6-dihydroveratramines.

The reason for choosing 3β-acetoxy-5α-etiojerv-12-en-17-one (76) as the source of the etiojervane portion stems from earlier research carried out in our laboratory which has been directed towards the total

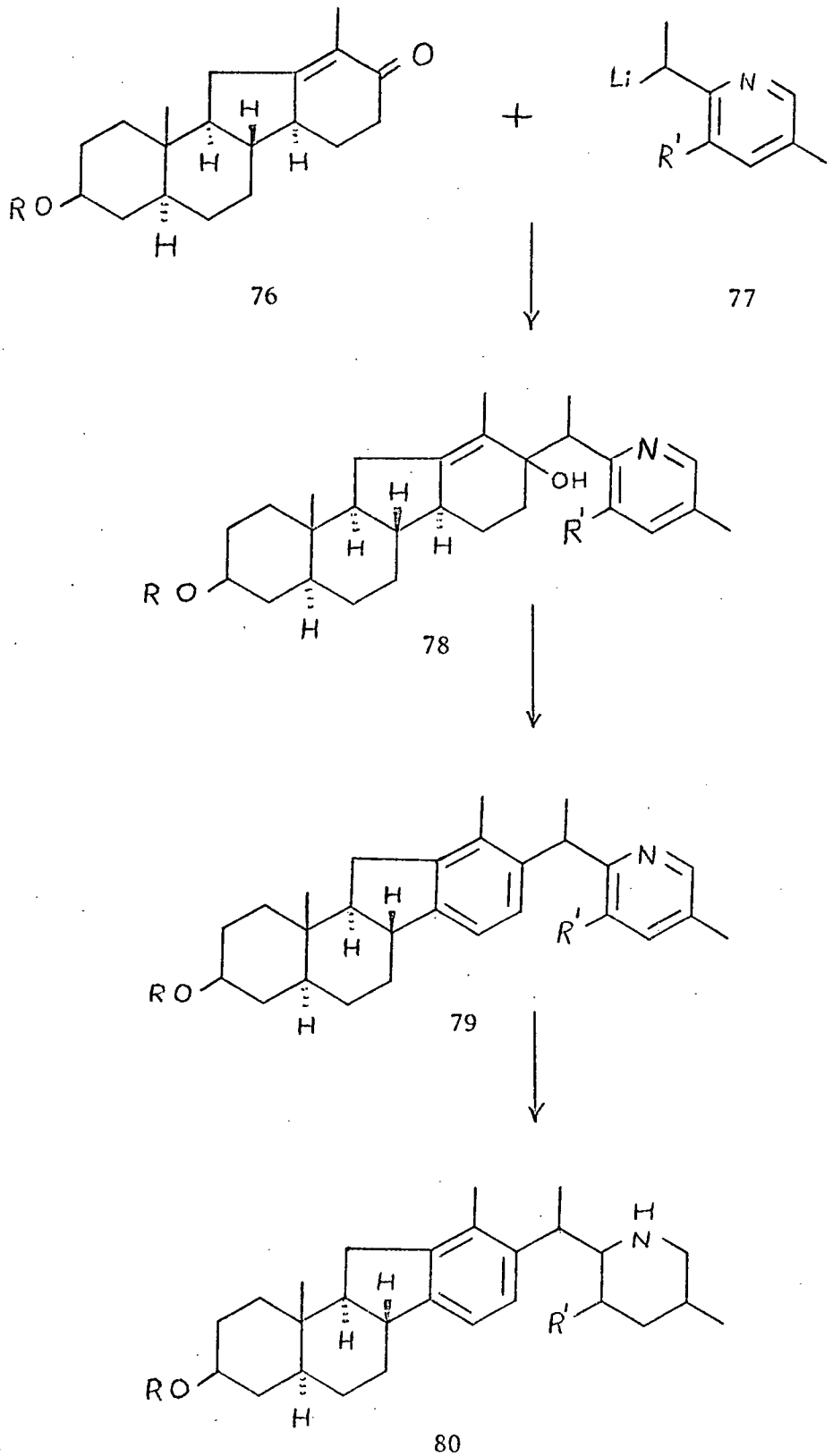


Figure 9

synthesis of this compound, which is readily available from the degradation of hecogenin by published procedures. It was felt that the quantity of 3 β-acetoxy-5 α-etiojerv-12-en-17-one which would result from the totally synthetic approach would be insufficient to complete the total synthesis of members of the veratrum alkaloids. Consequently we envisaged the use of 3β-acetoxy-5α-etiojerv-12-en-17-one obtained from degrading hecogenin, as a relay compound, and the total synthesis of this compound, as providing the totally synthetic approach to representatives of the veratrum alkaloids.

The procedure of W. F. Johns[48] was employed in the degradation of hecogenin as outlined in fig. 10. This sequence gives a reasonable yield of the desired 3β-acetoxy-5α-etiojerv-12-en-17-one although some twelve steps are involved in the degradation. A feature of this sequence which proved important in our totally synthetic approach is that the desired α, β-unsaturated ketone (76) is formed from the saturated ketone (87) by bromination and then dehydrobromination.

A synthesis of any naturally occurring alkaloid from 3β-acetoxy-5α-etiojerv-12-en-17-one obtained from hecogenin constitutes a formal total synthesis of that alkaloid since hecogenin has been totally synthesised from isoandrosterone[45]. A more notable achievement however is the direct total synthesis which would result if 3β-acetoxy-5α-etiojerv-12-en-17-one (76) could be prepared by a totally synthetic route. The total synthesis of this compound has recently been achieved in our laboratory[49] and the approach employed is outlined in fig. 11. The synthesis of the diol aldehyde (94) has appeared in print[50] and involves a ring contraction developed by W. S. Johnson in an isomeric series of compounds[51].

Attempts to prepare 3β-acetoxy-5α-etiojerv-12-en-17-one from

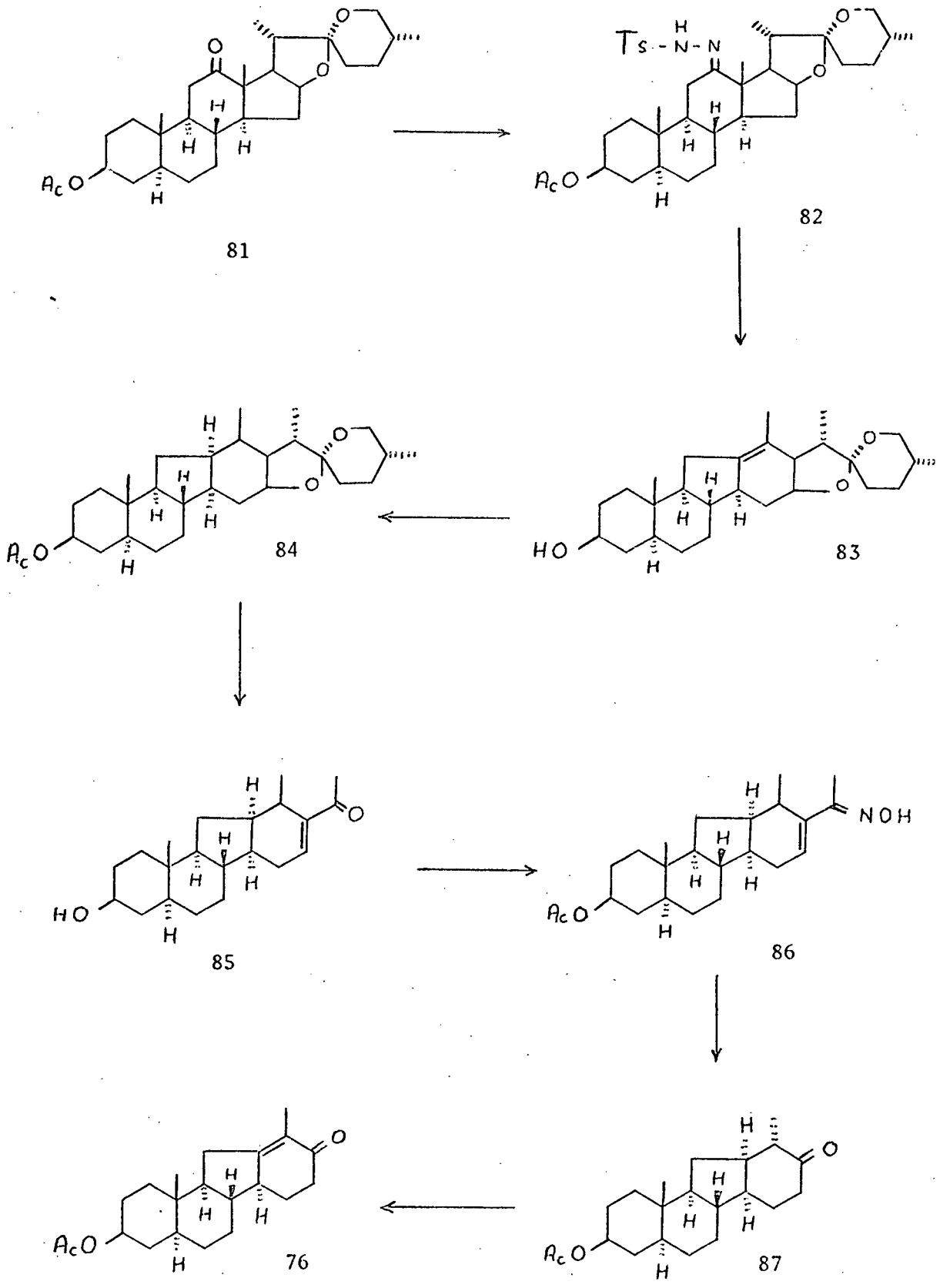
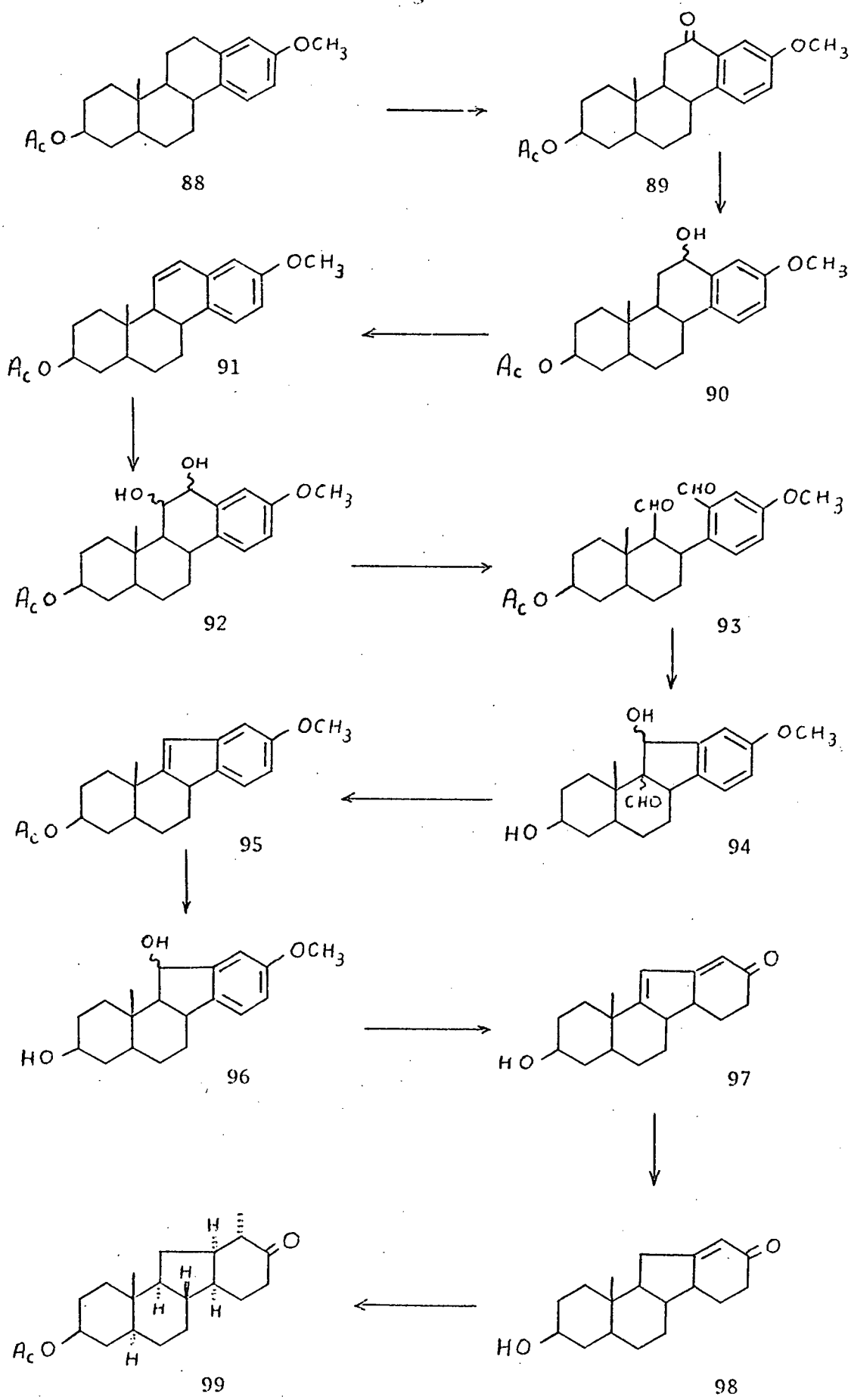


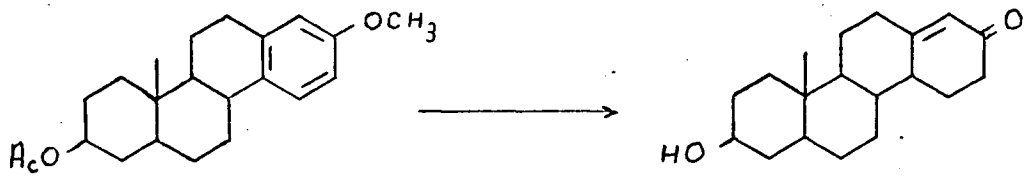
Figure 10

Figure 11



compound 98 via alkylation of the enamine were unsuccessful, However alkylation via enolate anion led to compound 99 which was shown to be structurally identical with the saturated ketone 87 obtained in the degradation of hecogenin. This compound provides the point of linkage between the use of a relay compound from hecogenin and the totally synthetic approach to the veratrum alkaloids. The compound (99) prepared by the totally synthetic approach was racemic and was not resolved, due to the small quantities available, whereas that obtained from hecogenin is a single enantiomer and has a rotation of + 122°.

To test the feasibility of the sequence in fig. 9 the reaction of compound (101) with 2-ethylpyridine was investigated. Earlier work (fig. 11) had made available the anisole compound (100) which was converted to the unsaturated ketone (101) via Birch reduction and acid hydrolysis of the resulting enol ether.

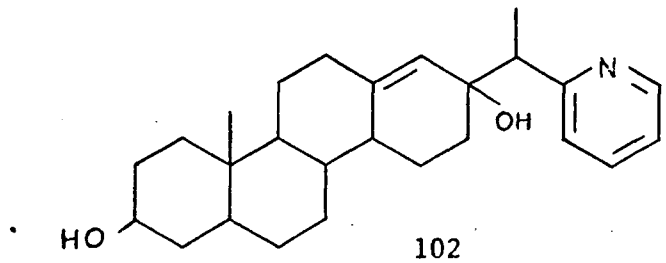


100 101

When 2-ethylpyridine is added to a tetrahydrofuran solution of methyl lithium a deep red colour rapidly develops. The lithium derivative of the 2-ethylpyridine is formed by the removal of a proton from the α position of the ethyl side chain. The resultant charge in the carbanion can be extensively delocalised by resonance into the pyridine ring. Other carbanions where similar delocalisation of the negative charge can occur (e.g. triphenylmethyl,[52] and fluorenyl) give rise to a deep red colour in solution. By analogy the deep red colour observed in this reaction is

probably indicative of the formation of the desired lithium salt.

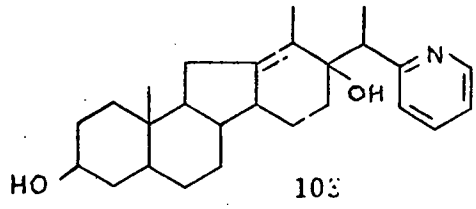
Addition of compound 101 to the red solution of 2-ethylpyridine and methyl lithium was continued until the colour faded and then the reaction quenched with water. Examination of the ether extract by t.l.c. indicated that one major compound had been formed. After purification by column chromatography this substance, in its ultraviolet spectrum, showed peaks at 257, 262 and 270 mμ corresponding to the pyridine chromophore whilst the mass spectrum exhibited a peak at m/e 395 corresponding to the parent ion for compound 102.



102

The absence of a carbonyl peak in the infrared supported the 1,2 addition of the organolithium derivative to the α, β-unsaturated carbonyl compound as opposed to any 1, 4 addition.

This procedure was then extended to the reaction of the lithium salt of 2-ethylpyridine with 3 β-acetoxy-5 α-etiojerv-12-en-17-one (76). Column chromatography of the products from this reaction primarily gave a material which appeared as one compound on t.l.c. The ultraviolet spectrum showed peaks at 257, 262 and 269 mμ indicative of the pyridine chromophore whilst the mass spectrum exhibited a peak at m/e 395 corresponding to the parent ion for compound 103.



103

The elemental analysis supported the assumption that the material obtained was the product of the desired coupling but the n.m.r. spectrum indicated that it was probably a mixture of two isomers. The spectrum showed two doublets in the region τ 8.84 and these were tentatively assigned to the C-21 methyl group in each of two compounds.

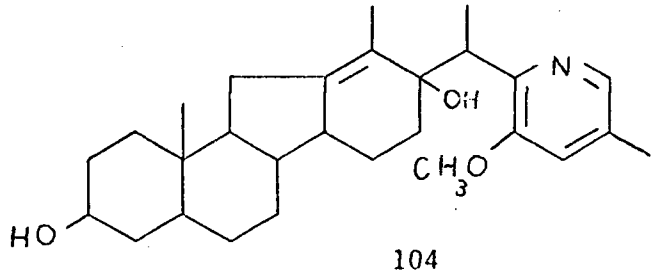
These reactions indicated that the desired coupling between 3β-acetoxy-5α-etiojerv-12-en-17-one (76) and 2-ethylpyridine was indeed possible and so our efforts were turned to considering substituted 2-ethylpyridines which would allow an entry to the skeleton of some of the naturally occurring veratrum alkaloids.

The initial aim was the synthesis of veratramine, which has as its heterocyclic portion, 2-ethyl-3-hydroxy-5-methylpiperidine. It was therefore necessary to prepare the corresponding 2-ethyl-3-hydroxy-5-methylpyridine for coupling with 3β-acetoxy-5α-etiojerv-12-en-17-one (76). This pyridine is not readily available but was synthesised by Dr. G.V. Nair of our laboratory. Treatment of this compound with methyl lithium would possibly lead to the formation of the lithium alkoxide which might hinder the formation of the carbanion at the α position on the adjacent 2-ethyl side chain. Of the protecting groups available we chose to prepare the benzyl ether since the benzyl group should be easily removed during the subsequent catalytic hydrogenation of the pyridine ring.

The treatment of 2-ethyl-3-benzyloxy-5-methylpyridine with methyl lithium gave only a faint transient pink colouration rather than the deep red colour which had been observed with 2-ethylpyridine. The

product after addition of 3 β-acetoxy-5 α-etiojerv-12-en-17-one (76) showed no coupled compounds. Repeated attempts to achieve this coupling under varying conditions of temperature and time were unsuccessful. The only compounds which could be isolated in any quantity from the column chromatography of the reaction products were the α,β-unsaturated ketone 76 and the original substituted pyridine.

In an attempt to investigate the effect of the benzyl group on the formation of the desired carbanion, the methyl ether was prepared. A deep red colour slowly developed when 2-ethyl-3-methoxy-5-methylpyridine was refluxed with methyl lithium in tetrahydrofuran. Compound 76 was added until the colour faded and the reaction worked up as usual. Examination of the ether extract by t.l.c. indicated one major compound besides recovered α,β-unsaturated ketone. This product was separated by preparative t.l.c. on silica gel and the resulting material obtained crystalline from ether. The ultraviolet spectrum showed a peak at 284 mμ indicative of the pyridine chromophore whilst the mass spectrum exhibited a peak at m/e = 439 corresponding to the parent ion for compound 104.



104

That the material obtained was a mixture of two compounds was indicated by the n.m.r. spectrum which exhibited two sharp singlets at 6.21 and 6.24 due to the presence of two methoxyl groups. Two doublets were also seen at 8.84 and 8.81 which were probably due to the C-21 methyl group in each

of the two compounds. A separation of these two compounds was not done at this time due to the relatively small quantities available.

Some preliminary investigations concerning the aromatisation of ring D were carried out on the mixtures of isomers obtained from the reaction of 3β-acetoxy-5α-etiojerv-12-en-17-one (76) with 2-ethylpyridine and 2-ethyl-3-methoxy-5-methylpyridine. Treatment of compound 103 with 10% Pd/C at 200° for 10 minutes gave a new compound which was separated by preparative t.l.c. The mass spectrum of this compound (fig. 17) exhibited a peak at m/e 375 corresponding to the molecular ion for the ring D aromatised compound. The n.m.r. spectrum supported the conclusion that this aromatisation had taken place as a new three proton singlet appeared at 7.85 and was assigned to the C-18 methyl group. A three proton singlet at 9.07 in the product was due to the C-19 methyl group which appeared at 9.26 in compound 100. This downfield shift would be expected upon aromatisation of the D ring.

Treatment of the mixture of isomers of structure 104 with Pd/C under the same conditions yielded a new compound which was separated from the product mixture by preparative t.l.c. The mass spectrum exhibited a peak at m/e 419 corresponding to the expected molecular ion for the ring D aromatised compound. The C-19 methyl resonance in the n.m.r. spectrum of this compound now appeared at 9.09 compared with 9.22 in compound 101 whilst a six proton singlet appeared at 7.75 corresponding to the C-18 and C-26 methyl groups.

A shortage of the desired 2-ethyl-3-methoxy-5-methylpyridine at that time prevented any further investigation and clarification of the isomers encountered in this sequence. However this work has since been further extended by Dr. G. V. Nair who has confirmed the existence

of the compounds as indicated above.

The naturally occurring jerveratrum alkaloid verarine has recently been confirmed to possess the 2-ethyl-5-methylpiperidine molecule as the hetereocyclic portion[20]. Since the pyridine precursor of this compound should be readily available via the methylation of 2,5-lutidine our efforts were turned towards the synthesis of verarine whilst a larger quantity of the appropriate pyridine for the synthesis of veratramine was being prepared.

The methylation of 2,5-lutidine was achieved by the addition of this compound to an ether solution of phenyl lithium followed by the addition of methyl iodide. The ether extract obtained after dilution of the reaction mixture with water was reduced to a small volume in vacuo and then the desired 2-ethyl-5-methyl pyridine was separated from the 2,5-lutidine by use of a spinning band distillation column. The n.m.r. spectrum of the fraction distilling at $62^{o}/10$ mm showed the replacement of $\overset{a}{\Lambda}$ methyl singlet present in the starting material by a quartet at τ 7.20 and a triplet at 8.80. The picrate derivative of this fraction, with a melting point of 144^{o}, was in good agreement with that reported in the literature[53].

The reaction sequence for the synthesis of verarine is shown in fig. 12 with the initial steps following the proposal of fig. 9 whilst the introduction of the 5,6-double bond represents an application of procedures employed by W. S. Johnson in his synthesis of veratramine[44].

Refluxing 2-ethyl-5-methyl pyridine with methyl lithium in anhydrous tetrahydrofuran led to the rapid development of a deep red colouration in the solution, 3 β-Acetoxy-5 α-etiojerv-12-en-17-one (76)

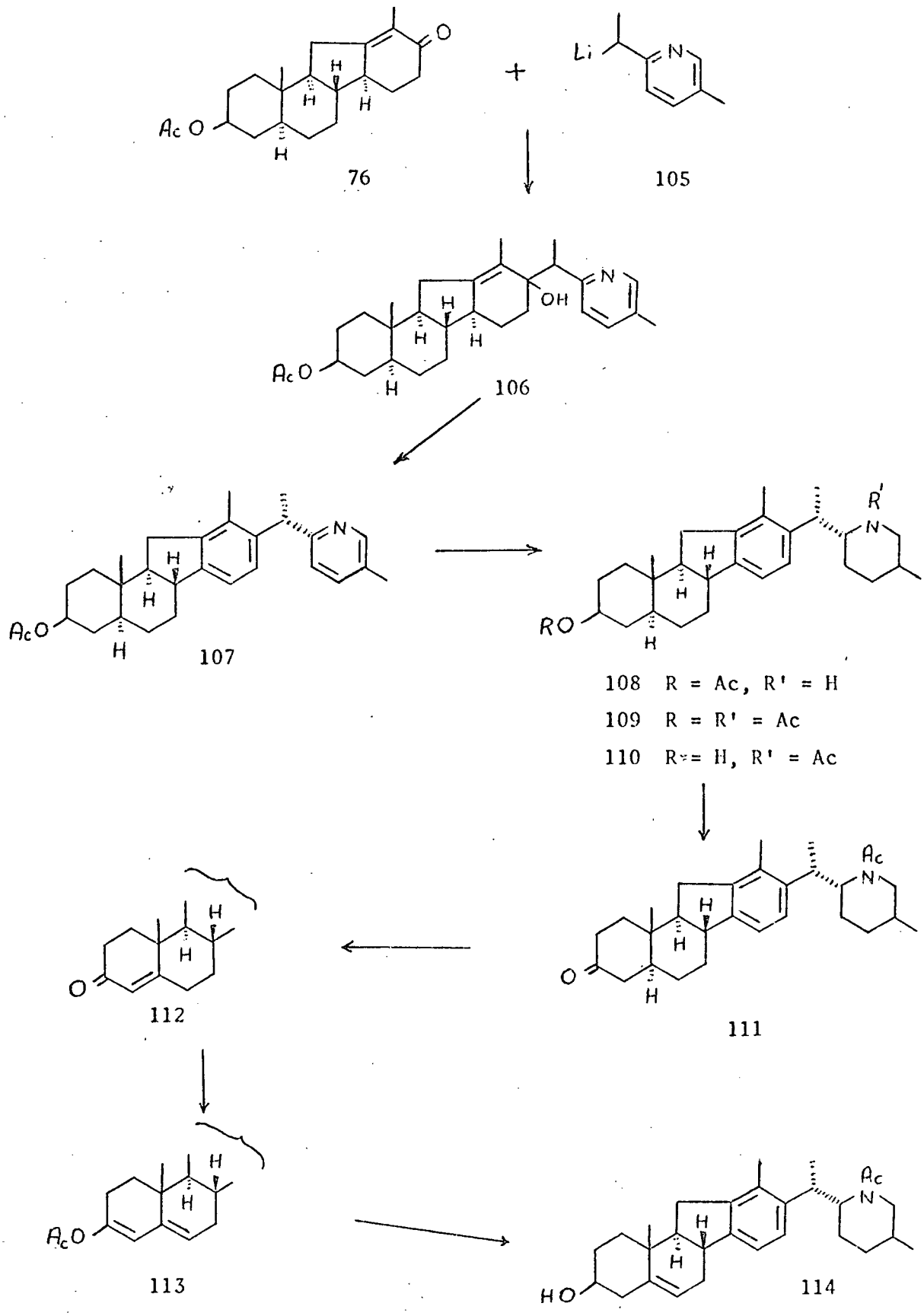

AcO
O
H
H
H
H
76
+
Li
N
105
OH
106
107
R'
RO
108 R = Ac, R' = H
109 R = R' = Ac
110 R = H, R' = Ac
Ac
111
112
113
HO
114

Figure 12

was added until the colour faded and then the reaction was diluted with water. Examination of the ether extract by t.l.c. showed that two new compounds with similar R_f values had been formed but the separation of these two compounds was very difficult. In an attempt to simplify this separation the compounds were converted to their 3-0 acetates by standing overnight in the presence of pyridine-acetic anhydride (1:1). The ether extract obtained after working up this mixture, deposited needle-like crystals, m.pt. 191-192°C, when the solution was allowed to evaporate slowly at room temperature. Examination of these crystals by t.l.c. indicated that only one compound was present and this was designated compound "A".

The ultraviolet spectrum of compound "A" showed λ_{max}^{a} peak at 270 mμ with shoulders at 265 and 276 indicative of the pyridine chromophore, whilst the mass spectrum exhibited a peak at m/e 451 corresponding to the molecular ion for the desired compound 106. The n.m.r. spectrum is reproduced in fig. 13 and is in accord with the proposed structure for compound A. A quartet occurring at τ 6.71 was assigned to the proton at C-20 and a decoupling experiment showed this signal to be coupled to a doublet at 8.79. This doublet was assigned to the C-21 methyl since the integral indicated three protons, whilst the C-19 methyl signal appeared as a sharp three-proton singlet at τ 9.21. The pyridine protons were readily discernible in the aromatic region of the spectrum with a one-proton doublet at τ 2.82, J = 8 cps, corresponding to the C-23 H. The C-24 proton gave rise to two doublets at τ 2.52 (J = 8 cps and J = 2 cps) since it is ortho coupled to C-23 H and meta to the C-25 H. The latter proton appeared as a broad singlet at τ 1.67. A broad unresolved one proton signal at 3.98 which disappears upon addition of deuterium oxide

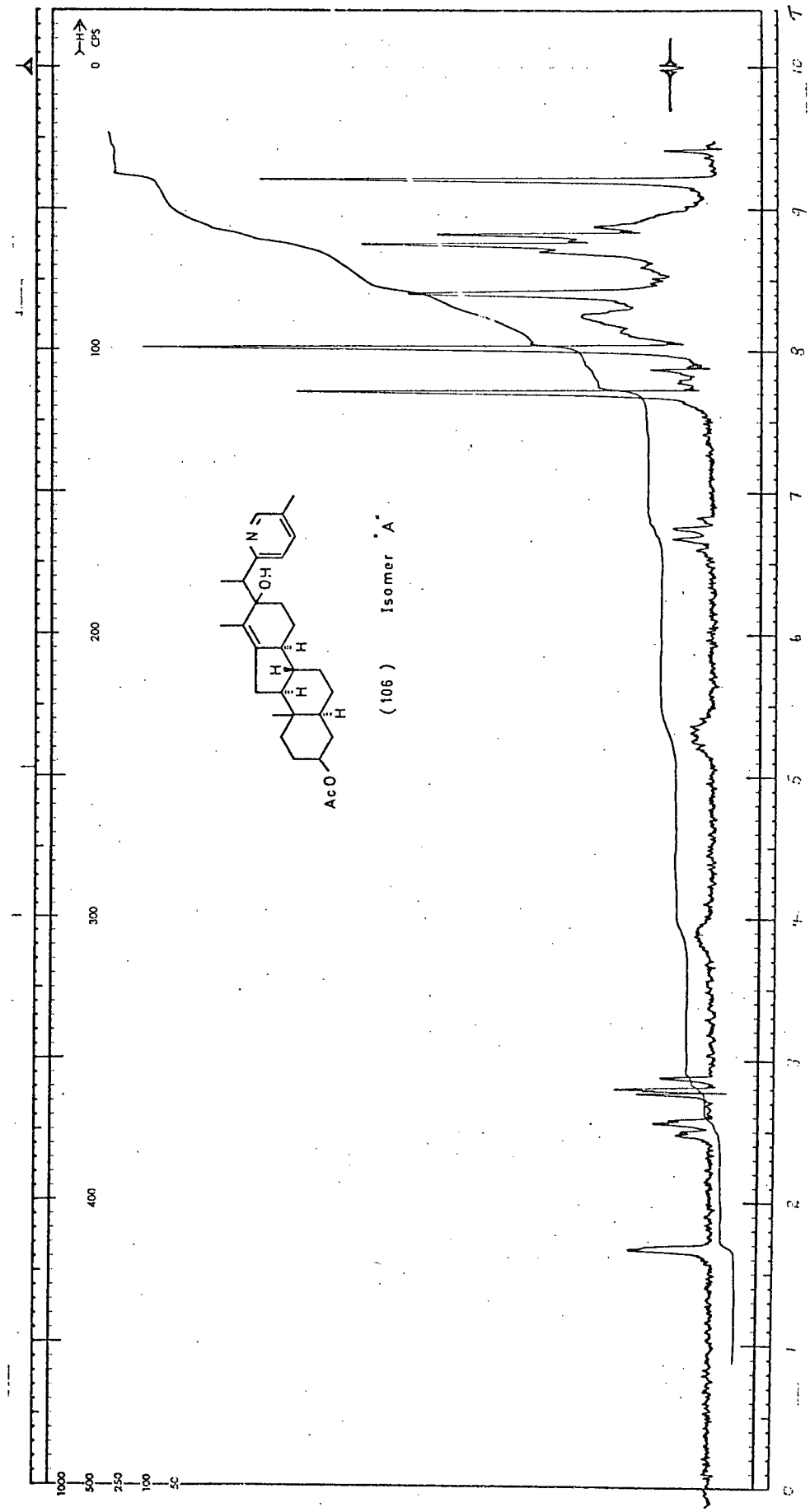

0 CPS
100
200
300
400
1000
500
250
100
50
0
1
2
3
4
5
6
7
8
9
10
τ
N
OH
H
H
H
H
AcO
(106) Isomer 'A'

Figure 13

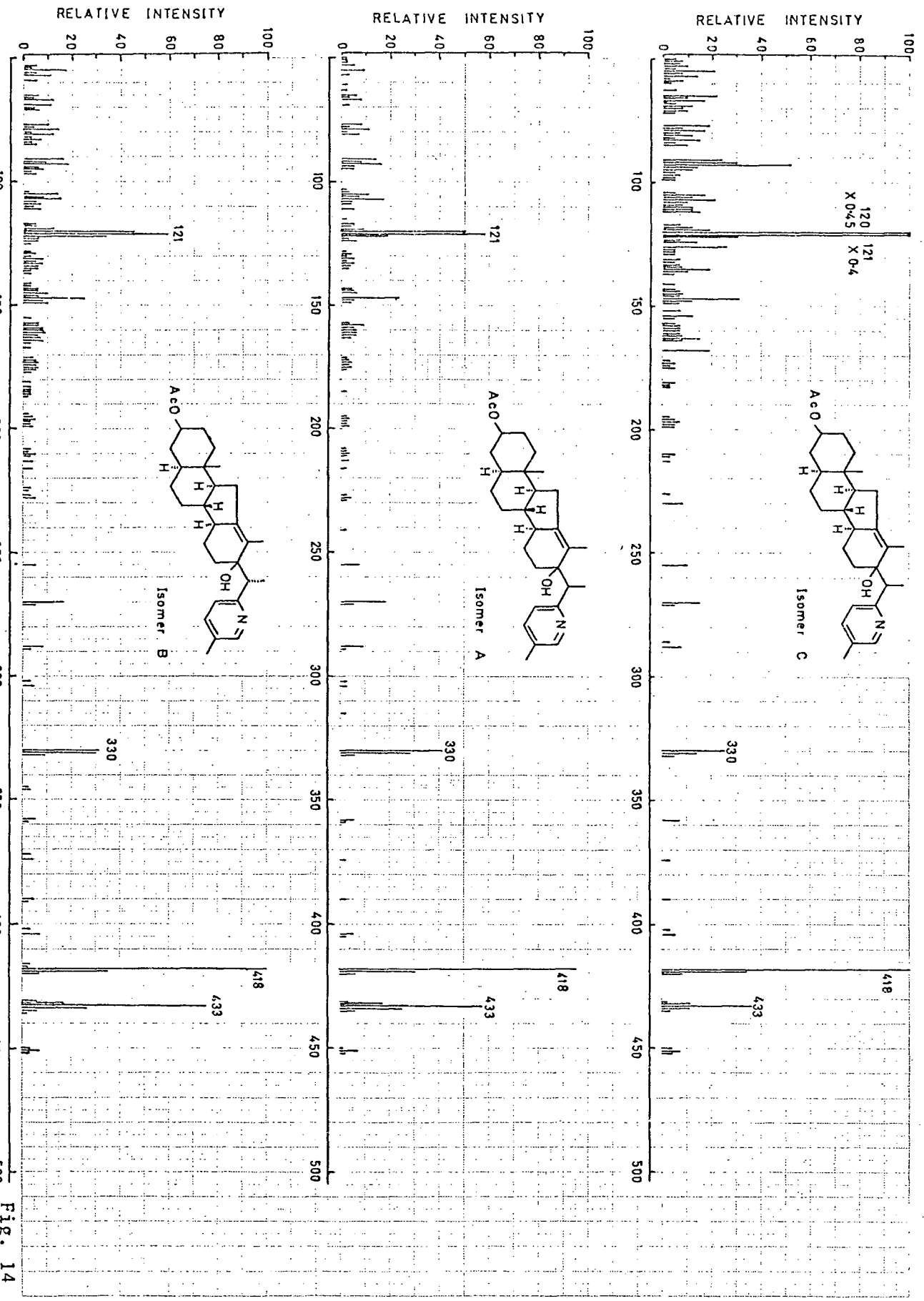

RELATIVE INTENSITY
120 X 0·45
121 X 0·4
AcO
OH
N
Isomer C
Isomer A
Isomer B
121
330
418
433
M/E

Fig. 14

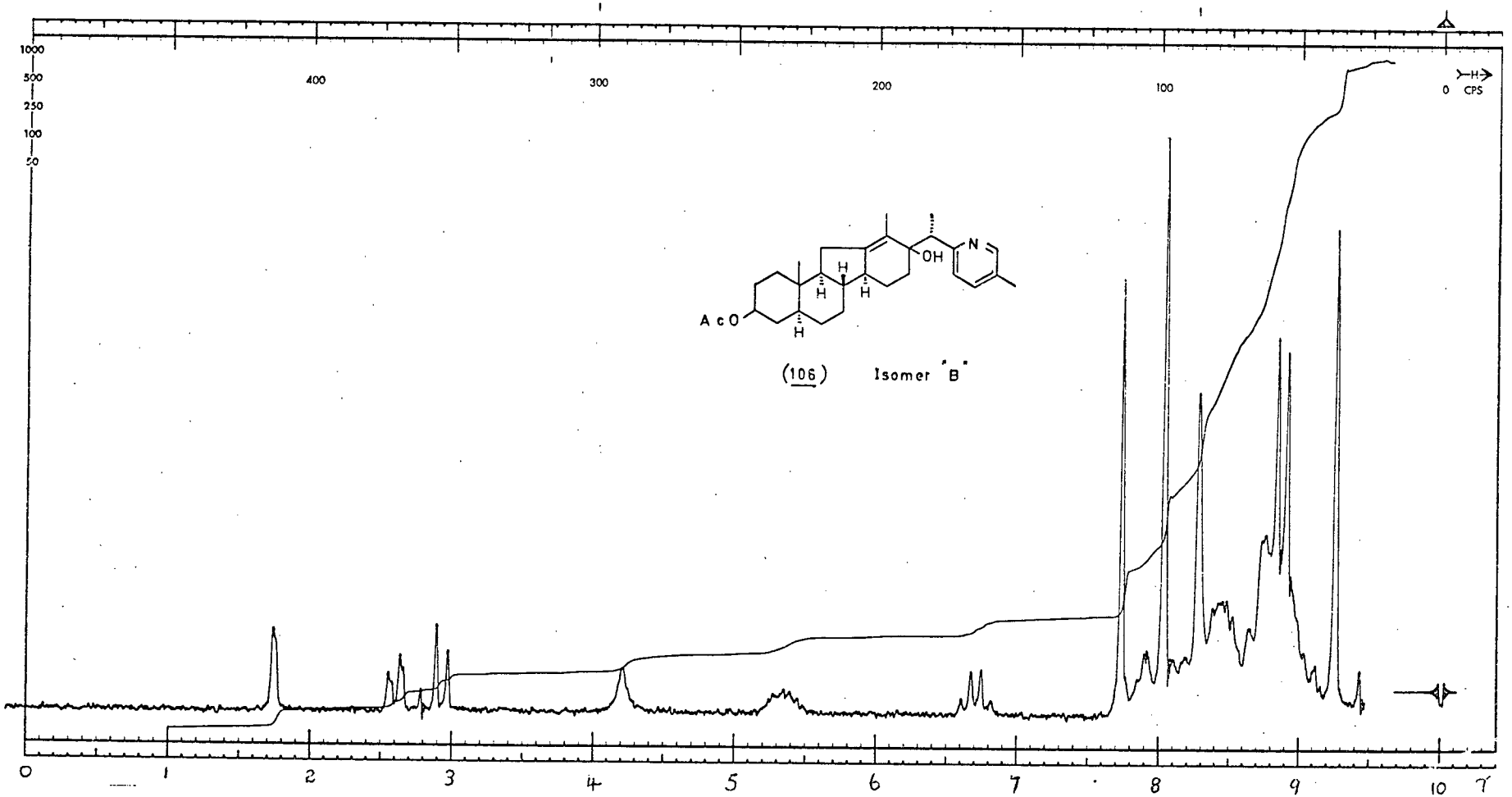

1000
500
250
100
50
400
300
200
100
0 CPS
AcO
OH
N
H
H
H
H
(106) Isomer "B"
0
1
2
3
4
5
6
7
8
9
10
τ

Figure 15

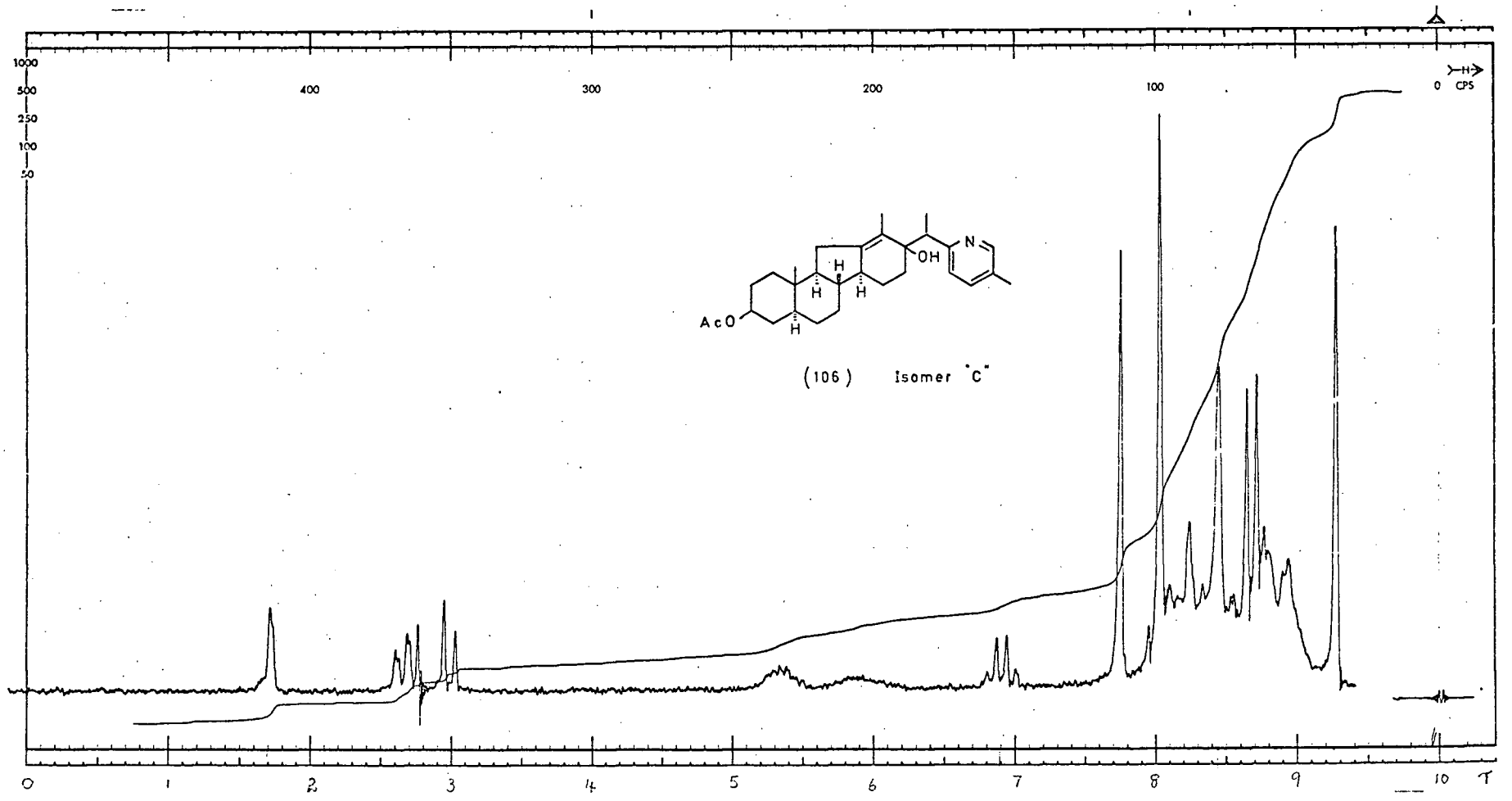

1000
500
250
100
50
400
300
200
100
0 CPS
AcO
OH
N
H
H
H
H
(106) Isomer "C"
0
1
2
3
4
5
6
7
8
9
10
τ

Figure 16

was assigned to the hydroxyl proton at C-17. Elemental analysis confirmed the molecular formula of compound "A" as corresponding to the desired compound 106.

Column chromatography of the mother liquor from the crystallisation of compound "A" gave a second compound which had a larger R_f value than compound "A" but the same colour reaction when sprayed with antimony pentachloride reagent. This compound gave rhomboid crystals m.pt. 189-190° from a small volume of ether and was designated compound "B". The ultraviolet spectrum showed a maximum at 270 mμ with shoulders at 265 and 276 mμ indicative of the pyridine chromophore whilst the mass spectrum exhibited a peak at m/e 451 corresponding to the parent ion for structure 106. The overall mass spectrum of this latter substance was virtually superimposable on that of compound "A" (fig. 14). Elemental analysis confirmed the isomeric nature of compounds "A" and "B". The n.m.r. spectrum of compound "B" is reproduced in fig. 15 and shows the same signals as are present in compound "A" with some differences in chemical shift. The largest differences are seen in the positions of the C-18 and C-21 methyl groups. A three-proton singlet for the C-18 methyl appears at τ 8.28 representing a shift of 0.12 to lower field whilst the three-proton doublet corresponding to the C-21 methyl appears at 8.88 and corresponds to an upfield shift of 0.09 when compared with isomer "A". The hydroxyl proton now appears as a one-proton signal at τ4.20 and is not quite as broad as the corresponding signal in isomer "A". The pyridine protons occur at higher field with the one proton doublet due to the C-23 H at 2.92 whilst C-24 H gives rise to two doublets at τ2.70. The broad signal corresponding to the C-27 proton appears at τ 1.75. It can be seen from fig. 12 that the coupling reaction creates two asymmetric centres and

and consequently there exists the possibility of obtaining four compounds possessing structure 106. In an attempt to determine whether the other two compounds are formed in this reaction, two of the fractions, from the column chromatographic separation of compound "B", were further investigated. T.l.c. examination of these consecutive fractions showed two compounds, which gave a similar colour reaction when sprayed with $SbCl_5$ as do compounds "A" and "B". However the R_f values were not identical with the two compounds already characterised and these two additional compounds were designated "C" and "D". Preparative t.l.c. yielded these compounds in a pure state although neither could be induced to crystallise.

Compound "C" was present in the product mixture to the extent of 4% whilst compound D only constitutes about 1% of the total product. The ultraviolet spectrum of compound C showed a maximum at 270 mμ with shoulders at 265 and 276 mμ indicating the pyridine chromophore whilst the mass spectrum (fig. 14) exhibited a peak at m/e 451 corresponding to the molecular ion for structure 106. Elemental analysis confirmed that compound "C" was isomeric with "A" and "B" and the n.m.r. spectrum which is reproduced in fig. 16 is entirely consistent with this conclusion. A three-proton singlet at τ 9.26 was assigned to the C-19 methyl whilst the C-21 methyl appears as a three-proton doublet at 8.77. The quartet corresponding to the C-20 proton is centred at τ 6.90 and the C-18 methyl peak appears as a broad three-proton singlet at 8.43. The pyridine protons give rise to the usual pattern of a doublet for the C-23 proton, two doublets for the C-24 proton and a broad singlet corresponding to the C-27 H in the aromatic region of the spectrum. A broad unresolved multiplet at about τ 5.8 which disappears on the addition of deuterium

oxide was assigned to the hydroxylic proton.

The ultraviolet spectrum of compound "D" also exhibited the characteristic maximum at 270 m μ with shoulders at 265 and 276 which was associated with the presence of the pyridine chromophore. A peak at m/e 451 corresponding to the parent ion for compounds of structure 106 was again evident in the mass spectrum. The n.m.r. of compound "D" indicated that it possessed the same functionalities that are present in the three compounds already isolated from the reaction. Elemental analysis was not done on compound D due to the relatively small amount available but the molecular formula was confirmed by high resolution mass spectrometry.

The further extension of this sequence to the ring D aromatised series only involved the compounds "A" and "B" as the small amounts of "C" and "D" which were available precluded any useful reactions.

Compound "B" was ground with 10% palladised charcoal until the two substances were thoroughly mixed and then the powder was heated at 200°C for 10 minutes. The resultant solid residue was washed several times with chloroform to remove the organic material. Examination of the filtered chloroform solution by t.l.c. indicated compound B had largely been converted to a new compound possessing a similar R_f value. Separation of the reaction mixture by preparative t.l.c. indicated this new compound, which was designated "aromatic II", had been produced in 80% yield. The ultraviolet spectrum showed no qualitative difference from that of compound B but the extinction coefficient at 270 m μ increased from 4,130 in the latter to 5,400 in this compound. The mass spectrum exhibited a peak at m/e 431 corresponding to the molecular ion for a compound of structure 107. The overall mass spectrum (fig. 17)

shows a completely different pattern to that observed with the series of compounds A to D. The n.m.r. spectrum reproduced in fig. 18 was particularly useful in confirming that the desired ring D aromatisation had occurred. The most significant feature is the appearance of a second pair of doublets in the downfield region of the spectrum corresponding to the expected AB system for the protons at C-15 and C-16. The one-proton quartet assigned to the C-20 H in compound B now appears at τ 5.58 since it is deshielded by the aromatic D ring and the pyridine, whilst the three-proton doublet due to the C-21 methyl has also suffered a downfield shift and now appears at τ 8.40. The broad three-proton singlet which corresponds to the C-18 methyl in the starting material has disappeared but a new sharp singlet appears at τ 7.89 in the product in accord with the aromatisation of ring D. One of the important features of the n.m.r. spectrum is that in the aromatic region (2.75 - 3.1 τ) all 8 lines expected for the two AB systems present in structure 107 are clearly resolved. The molecular formula was confirmed by high resolution mass spectrometry (calc: 431.2824 found 431.2809).

Isomer A was similarly treated with 10% Pd/C at 200°C for seven minutes and the residue washed with chloroform. Examination of this chloroform extract by t.l.c. indicated that three major compounds had resulted from the reaction. All three compounds were obtained in a pure state by preparative t.l.c. and two of these were identified as the α-β unsaturated ketone (76) and 2-ethyl-5-methypyridine. The third compound which was formed in 25% yield showed a colour reaction with $SbCl_5$ similar to that obtained with the aromatic compound from isomer B. This third component was designated "aromatic I" and the mass spectrum exhibited a peak at m/e 431 corresponding to the molecular ion for a compound of

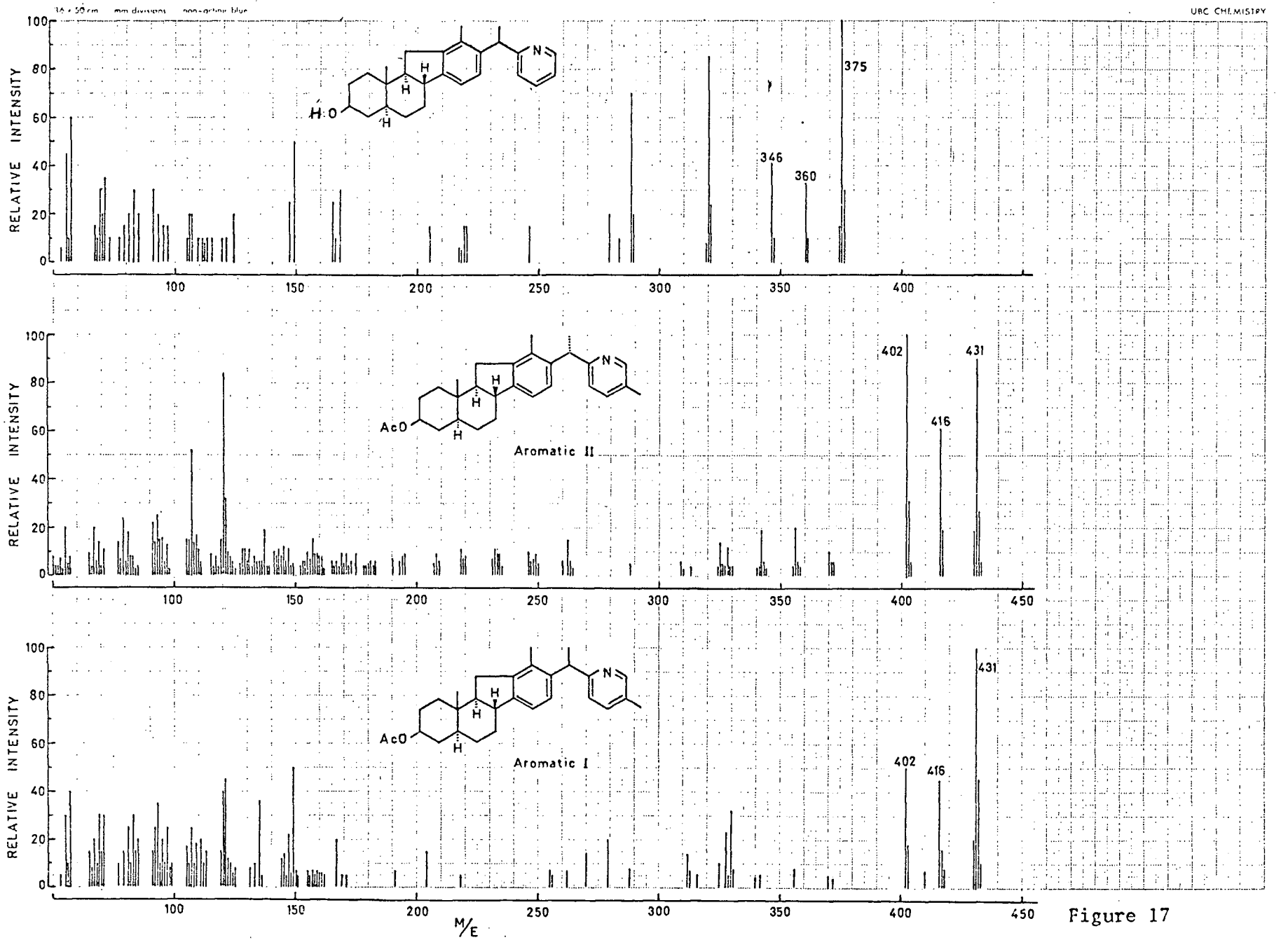

UBC CHEMISTRY
RELATIVE INTENSITY
100
80
60
40
20
0
100
150
200
250
300
350
400
450
M/E
HO
H
H
H
N
375
346
360
AcO
Aromatic II
402
431
416
Aromatic I

Figure 17

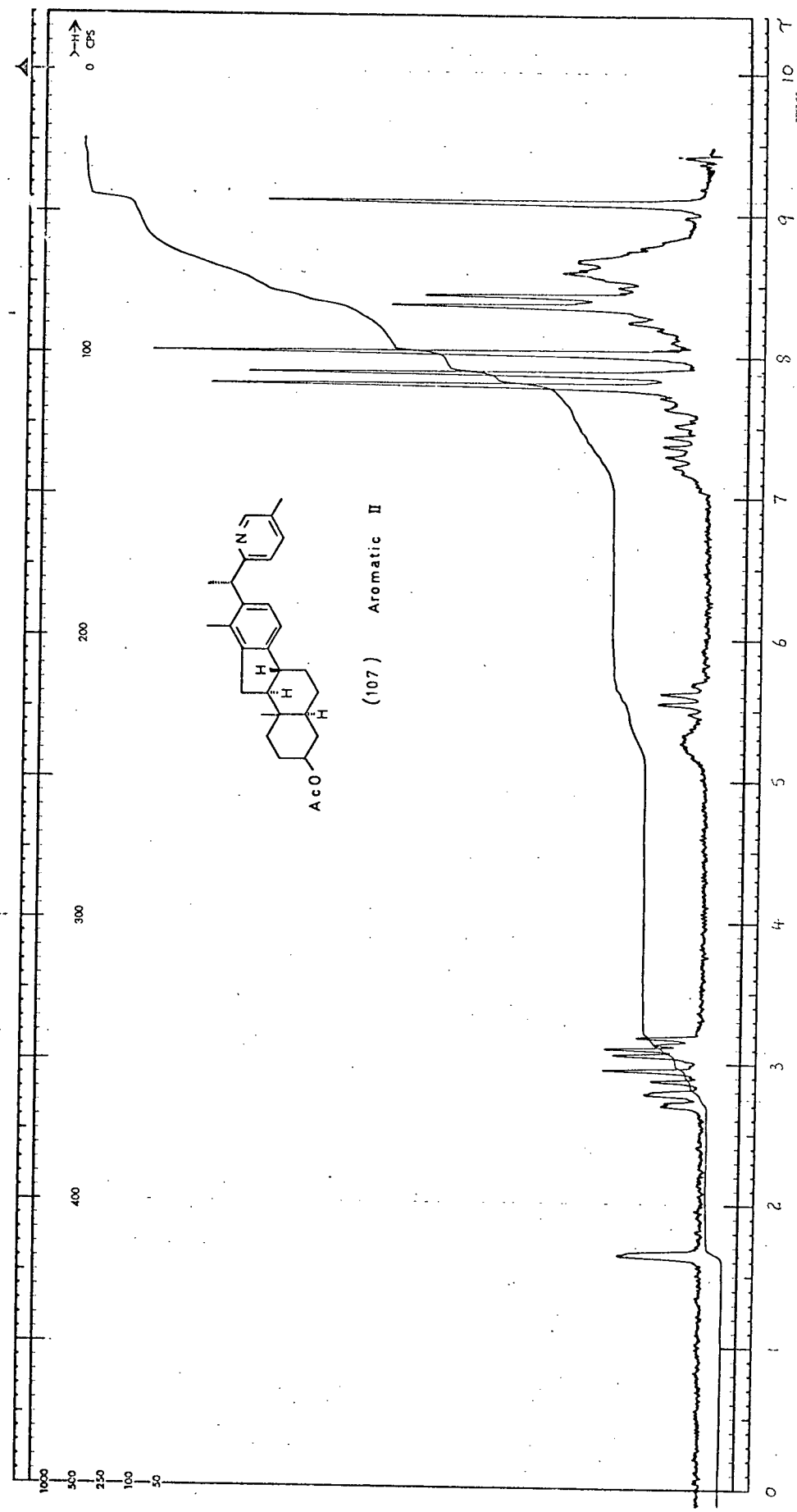

(107) Aromatic II
AcO
N
H
H
H
0 CPS
100
200
300
400
1000
500
250
100
50
0
1
2
3
4
5
6
7
8
9
10
τ

Figure 18

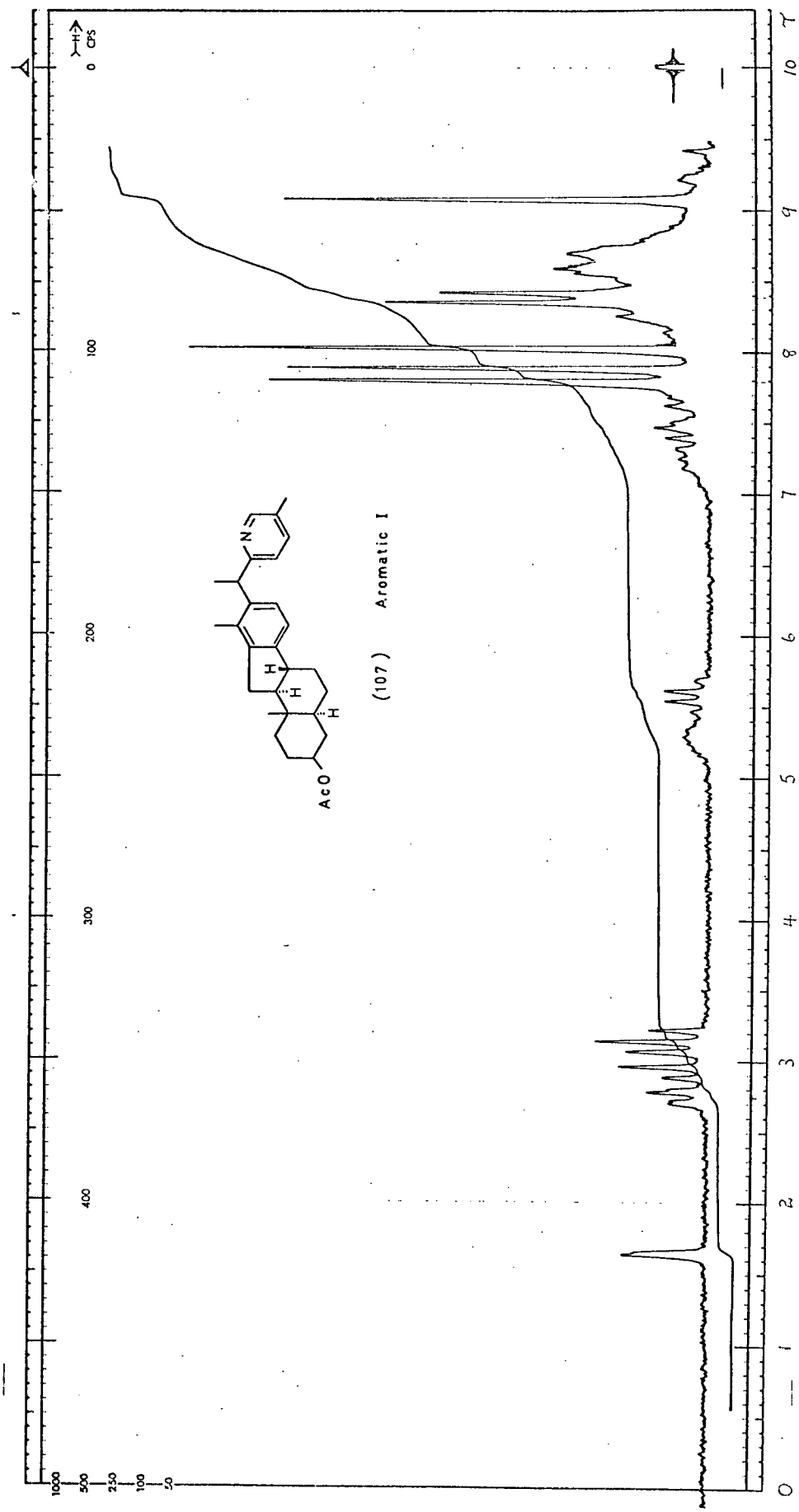

(107) Aromatic I
AcO
N
H
H
H
0 CPS
100
200
300
400
1000
500
250
100
50
0
1
2
3
4
5
6
7
8
9
10
τ

Figure 19

structure 107. The overall mass spectrum was very similar to that of aromatic compound "I" (fig. 17) whilst the n.m.r. spectrum shown in fig. 19 lent strong support to the presence of an aromatic ring D in the compound. The one-proton quartet due to the C-20 proton appears at τ 5.61 with the three-proton doublet assigned to the C-21 methyl occurring at 8.41. The three-proton singlet at 8.40 assigned to the C-18 methyl in compound A has now disappeared and a new singlet integrating for three protons appears at 7.90 in accord with the aromatisation of ring D. The interesting difference in the nature of the n.m.r. spectra of the aromatic compounds "I" and "II" was noted in the aromatic region (2.75 - 3.1). In "I" only seven of the theoretical eight lines were observed for the overlapping AB systems whilst as mentioned above all eight lines were clearly present in compound "II". The molecular formula of aromatic compound I was confirmed by high resolution mass spectrometry as being the same as that of aromatic II. These two compounds must be isomers since the n.m.r. spectra indicate they are not identical. These compounds contain only one additional asymmetric centre (C-20) when compared with 3β-acetoxy-5α-etiojerv-12-en-17-one. Since this latter substance was obtained from hecogenin it is not racemic, and in fact has a rotation of + 122°. The introduction of the asymmetric centre at C-20 would therefore be expected to give rise to two diastereomers. This may well represent the nature of the difference between the two aromatic compounds obtained in this work.

In view of the different extent of ring D aromatisation encountered with isomers "A" and "B", the relative stability of these two compounds was further investigated. The C-17, C-20 bond cleavage, which occurs with isomer "A" under the conditions for the D ring aromatisation, appears to be very facile since this cleavage also occurs slowly in

methanol or ethanol. The cleavage is slightly enhanced when the compound is dissolved in 0.1 N methanolic potassium hydroxide. Isomer A is stable in dimethylformamide or benzene but is converted to 3β-acetoxy-5α-etiojerv-12-en-17-one and 2-ethyl-5-methylpyridine when dissolved in aqueous dimethylformamide containing potassium hydroxide (pH = 9).

Isomer B is stable under the above conditions and shows no C-17, C-20 bond cleavage. Dehydration of the tertiary alcohol appears to be the main process for both isomers "A" and "B" in acidic methanol solution.

Thus two compounds of structure 107 had been prepared and the selective reduction of the pyridine to a piperidine ring was investigated. One of these aromatic compounds should lead to an isomeric mixture of 5α,6-dihydroverarines whilst the other would lead to a mixture of isomers of similar structure but differing in configuration at C-20 from the naturally occurring alkaloid verarine. The selective hydrogenation of a pyridine ring in the presence of a benzene ring is reported[54] to occur smoothly when PtO_2 is used as the catalyst in acetic acid at room temperature, and a hydrogen atmosphere at 45 psi is employed. These conditions were used in our work in the conversion of the aromatic compounds, to the piperidine containing compounds of structure 108.

Aromatic compound II was hydrogenated under these conditions for three hours and the filtrate was reduced to a small volume by evaporation in vacuo. The resulting mixture was diluted with water, basified with aqueous ammonia and then extracted with chloroform. Examination of the product by t.l.c. indicated that four new compounds possessing very similar R_f values had been formed. These compounds which were numbered 1 to 4 in order of decreasing R_f values on silica gel, were

separated by careful preparative t.l.c. These compounds were suspected to be isomers of 3-O-acetyl-5α,6-dihydroverarine. However preparation of a sample of this compound from verarine for comparison purposes is difficult since acetylation of verarine yields N-acetylverarine or 3-O,N-diacetylverarine.

None of the four compounds from the hydrogenation of aromatic compound II could be induced to crystallise but it was found from other investigations that N-acetyl-5α,6-dihydroverarine (110) crystallises readily from an ethereal solution. Consequently it was decided to convert the four compounds obtained above to the N-acetyl derivatives (110), via acetylation to the 3-O,N-diacetates (109) followed by selective hydrolysis of the 3-acetoxyl group. The conversion to the respective diacetates was accomplished upon standing overnight with acetic anhydride:pyridine (1:1) at room temperature, whilst hydrolysis of the 3-acetoxyl group was achieved with 0.1 M potassium hydroxide in methanol.

The other alternative open to us at this point, was the removal of the 3-acetate by saponification to give the 5α,6-dihydroverarine. This proposal was discarded in favour of the approach outlined above for two reasons. Firstly the amount of verarine available was relatively small and secondly Masamune[20] has shown that veratramine, which is readily available, can be converted to N-acetylverarine. Since the quantities of the isomeric compounds obtained from the hydrogenation were expected to be insufficient for the introduction of the 5,6-double bond which is necessary to complete the synthesis of verarine, it was desirable to compare the products with a compound which was readily available. Once the comparison had been made then this compound could be used for the further reactions involved in the synthesis of verarine.

The conversion of veratramine to N-acetylverarine as published by Masamune[20] is outlined in fig. 20. The procedure gave a reasonable yield of 3-O,N-diacetyl verarine (117) although some difficultly was experienced with the thioketal formation. The thioketal derivative (116) was obtained by dissolving the ketone (19) in glacial acetic acid and treating this solution with ethanedithiol and boron trifluoride etherate. This reaction was not accompanied by the loss of the 3-acetoxyl group as was the case when the compound was dissolved in methanol and then treated with ethanedithiol and hydrogen chloride. Consequently the procedure for the conversion as repeated here led to 3-O,N-diacetylverarine rather than N-acetylverarine previously obtained by Masamune.

The diacetate was identified by t.l.c. comparison with an authentic sample, melting point, rotation, mass spectrum and n.m.r., all of which were in accord with the published data[27]. Subsequent hydrolysis of the 3-acetoxyl gave N-acetylverarine with the same melting point as recorded in the literature.

Hydrogenation of the 5-6 double bond in veratramine employing Adams catalyst in acetic acid had been reported to give mainly the 5α,6-dihydroveratramine[55]. By analogy it was expected that the hydrogenation of the 5,6-double bond in 3-O,N-diacetylverarine (117) using these conditions would provide mainly the desired 3-O,N-diacetyl-5α,6-dihydroverarine. This hydrogenation was carried out and the compound was observed to consume approximately one mole of hydrogen after 4 hours. Examination of the product by t.l.c. employing both silica gel and alumina under several solvent systems indicated that only one compound had been formed. The n.m.r. spectrum indicated the unresolved one-proton multiplet at τ 4.5 present in 3-O,N-diacetylverarine which had been assigned to the vinylic proton, was no longer present. Thus the hydrogenation of the

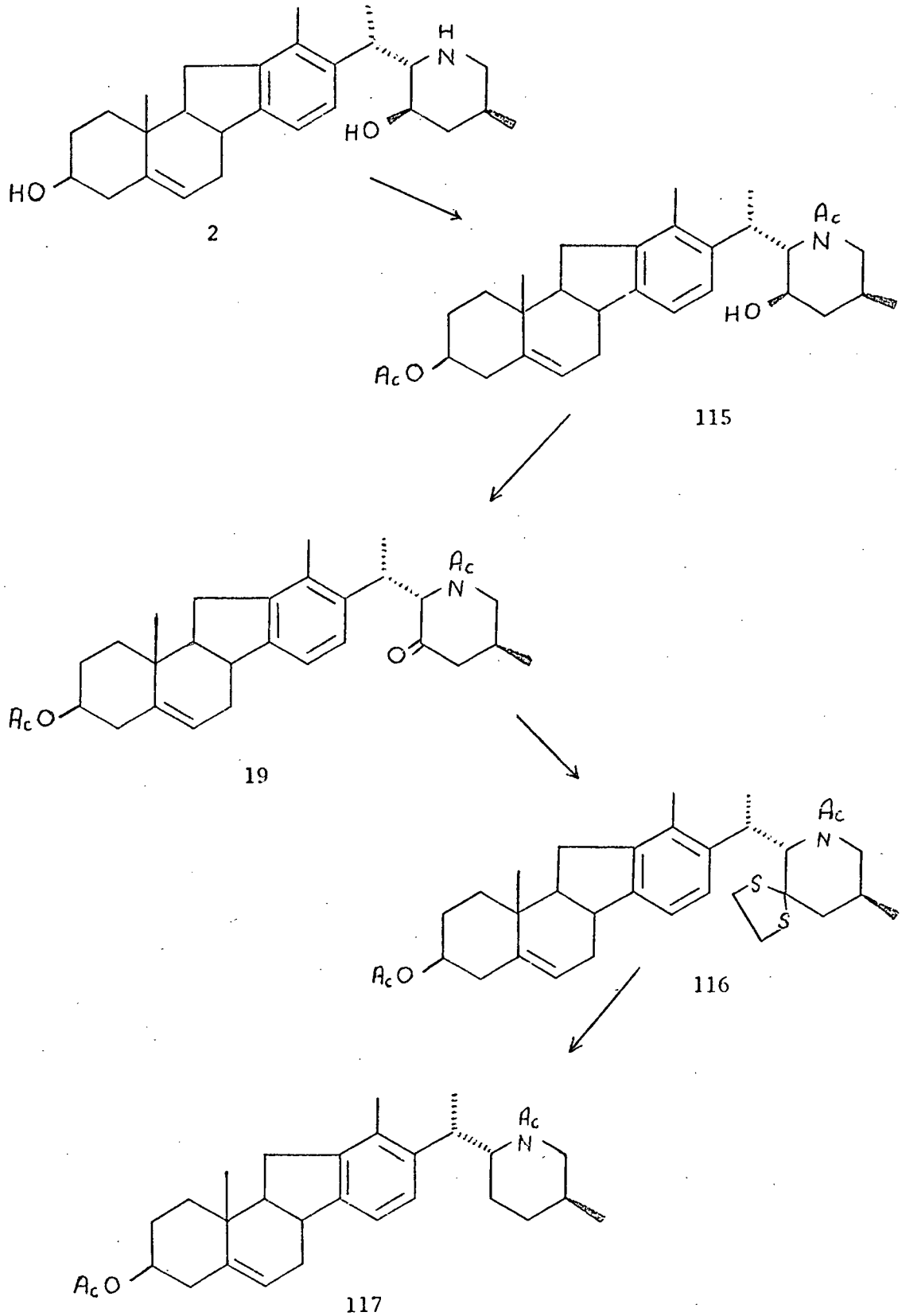
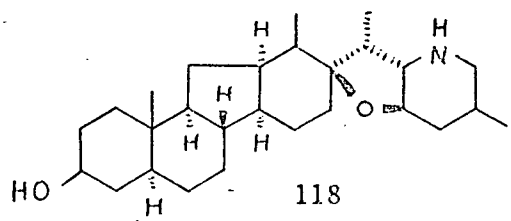


Figure 20

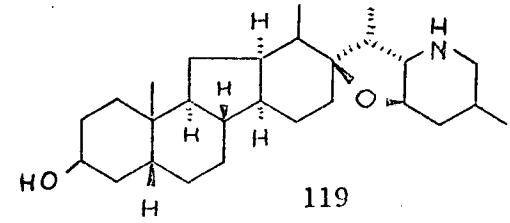
double bond, had indeed occurred. However, a closer examination of the spectrum particularly in the region τ 8.5 - 9.5 indicated that both the 5α and the 5β compounds had been formed.

The n.m.r. spectrum of 3-O,N-diacetylverarine 117 exhibited a three-proton doublet at τ 9.02 which was attributed to the C-26 methyl whilst the C-19 methyl appeared as a three-proton singlet at 8.85. The n.m.r. spectrum of the hydrogenation product exhibited a sharp singlet at 8.92 and a doublet at 9.03. The upfield half of the doublet centred at 9.03 was however very intense and the integral of this doublet corresponded to 4.5 protons. From the recent excellent n.m.r. study of 22,27-imino-17,23-oxidojervane derivatives by Masamune[40] one would expect the C-19 methyl signal for the 5α compound to be at a higher field position than in the 5β compound. This difference is due to the shielding of the C-19 methyl group by the ring A protons which are in close proximity in the 5α derivatives but removed to a large extent in the 5β derivatives. This effect has been noted in various steroids[41] where the stereochemistry of the molecule is fixed.

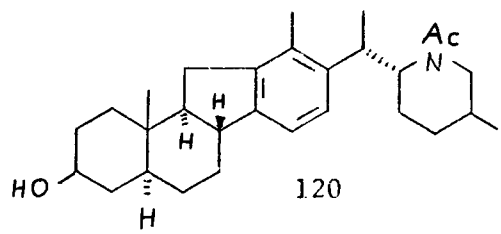
The difference in chemical shift between the singlet at 8.92 and the upfield half of the doublet due to the C-26 methyl group was 13 cps and it was informative to compare this difference with the C-19 methyl resonances in compounds 118 and 119.



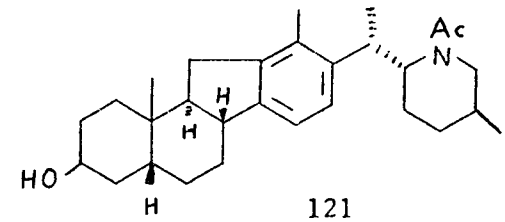
118



119



120



121

The n.m.r. spectra of these two compounds have been studied by Masamune who found the C-19 methyl resonance in compound 118 to occur at τ 9.19 whilst in compound 119 the three-proton singlet is observed at τ 9.07 - a separation of 12 cps.

It therefore appeared that the hydrogenation of 3-O, N-diacetyl verarine had given both the 5α and 5β compounds. In an attempt to obtain some separation of these two compounds and verify their existence the product was converted to the monoacetate by hydrolysis of the 3-acetoxyl group by means of 0.1M potassium hydroxide in methanol. A t.l.c. examination of the resulting N-acetyl-5,6-dihydroverarines showed clearly that two compounds (120, 121) were present. The n.m.r. spectra of the two compounds after separation by preparative t.l.c. on silica gel, supported the analysis of the C-19 methyl group positions outlined above. (see figs. 21, 22)

Additional support for the 5β configuration corresponding to the compound which showed the C-19 methyl resonance at τ 8.92 was gained from the presence of a relatively narrow multiplet at τ 5.97 assigned to the proton geminal to the C-3 hydroxyl supporting the equatorial position of this proton which should result in the conversion of Δ^{5-6} to the 5β,6 dihydro compound. The compound in which the C-19 methyl resonance overlies the upfield half of the C-26 methyl doublet exhibited a broad multiplet in the region τ 6.3 - 6.5 which was assigned to this proton and indicated its axial position supporting the 5α configuration.

It was vital to establish firmly the identity of these two compounds whose molecular formulae had been verified by their mass spectra and elemental analysis. The O.R.D. curves of 3-keto steroids with the 5α

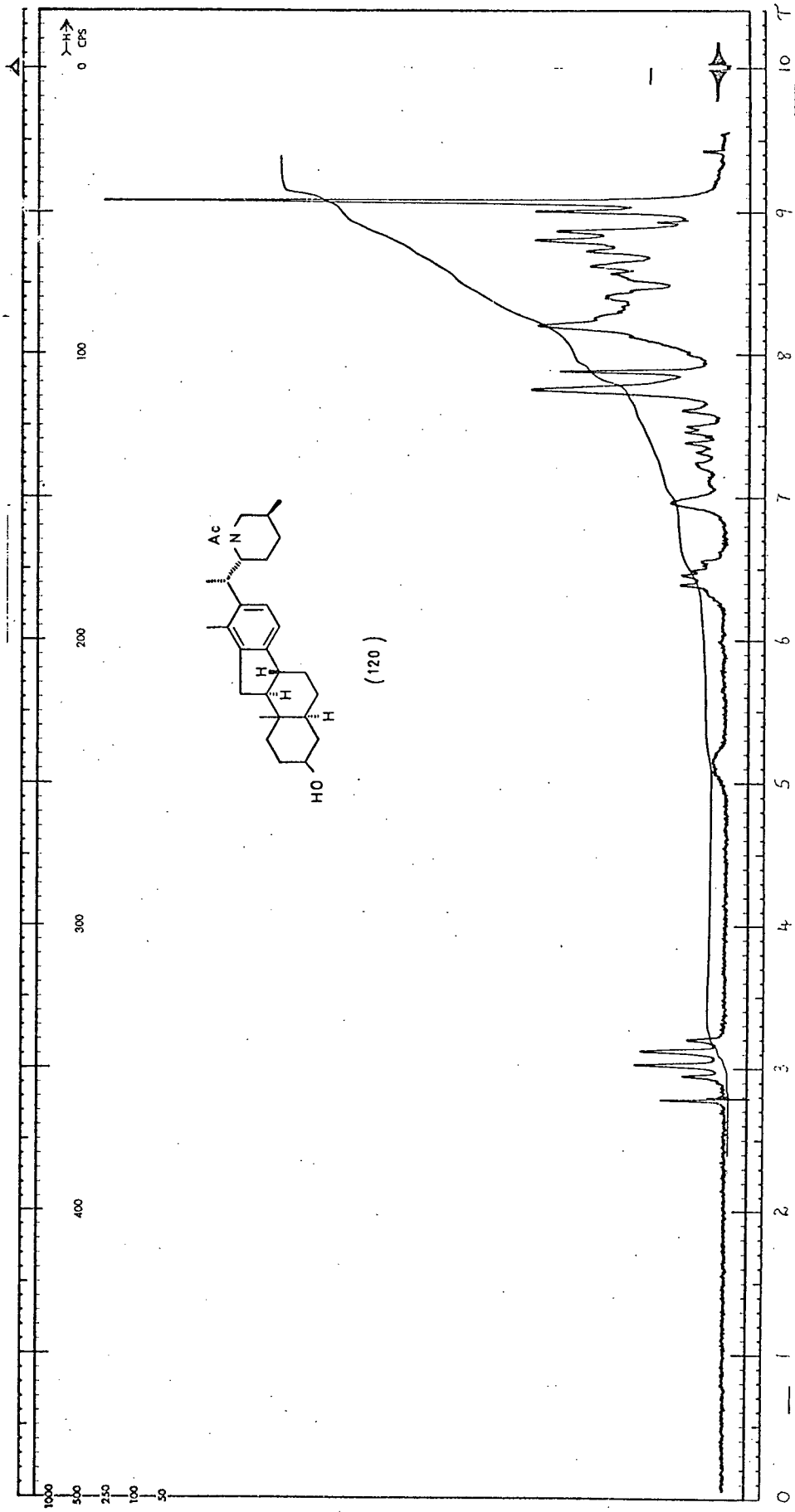

Ac
N
H
H
H
HO
(120)
0 CPS
100
200
300
400
1000
500
250
100
50
0
1
2
3
4
5
6
7
8
9
10
τ

Figure 21

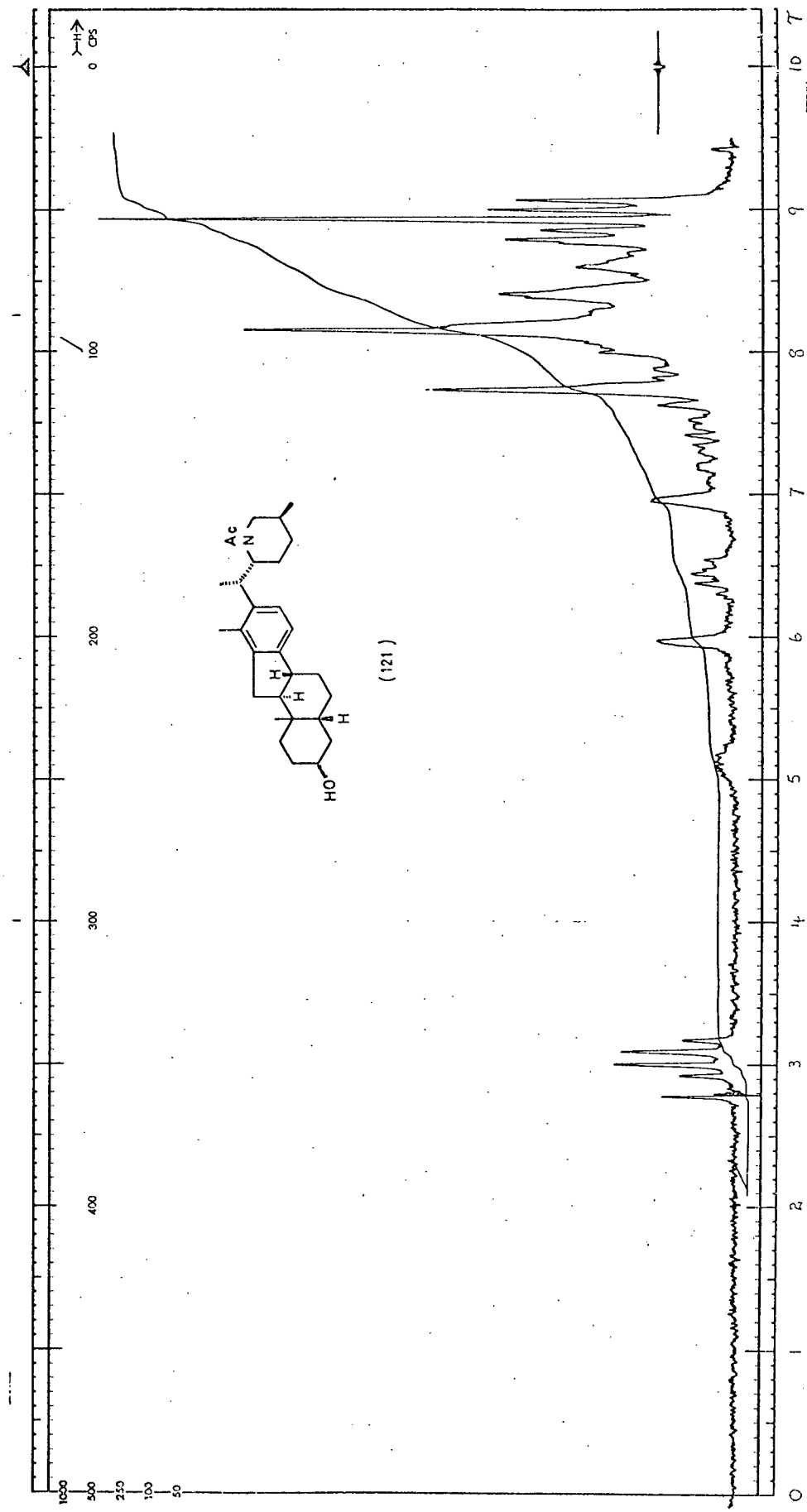
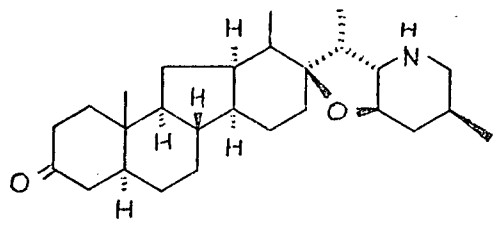
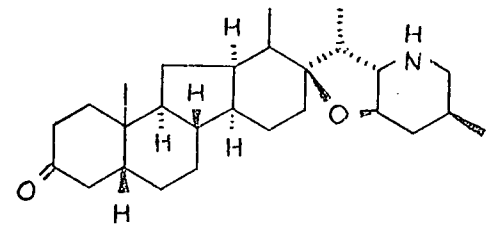

Ac
N
H
H
H
HO
(121)
0 CPS
100
200
300
400
1000
500
250
100
50
0
1
2
3
4
5
6
7
8
9
10
τ

Figure 22

and 5β configuration has been extensively studied and Masamune[40] has recorded the O.R.D. curves of compounds 122 and 123. These latter substances provide a very good analogy with the compounds under consideration.



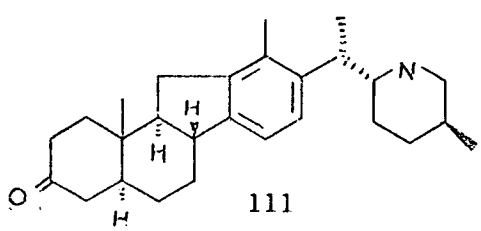
122



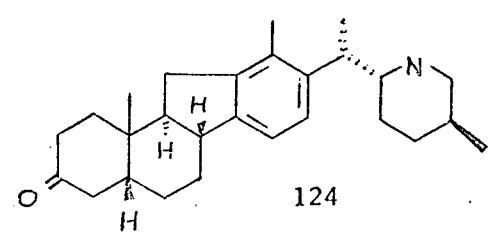
123

Compound 120 exhibits a strong +ve Cotton effect curve with values of $[\phi]^{\text{peak}}_{304\ m\mu}$ + 2740° and $[\phi]^{\text{trough}}_{265\ m\mu}$ - 1180° whilst compound 121 exhibits a negative Cotton effect curve with values of $[\phi]^{\text{trough}}_{304\ m\mu}$ + 265° and $[\phi]^{\text{peak}}_{265\ m\mu}$ + 2340°.

The hydrogenation products which had tentatively been assigned the 5α (120) and 5β (121) configurations were converted to the respective 3-keto compounds (111 and 124) using Jones reagent as the oxidising agent. The presence of the



111



124

N-acetyl group tended to complicate the O.R.D. curves to some extent since it had a strong absorption at 230 mμ but the absorption at 300 mμ was not sufficient to affect the curve in this region. Compound 111 which had

been assigned the 5α configuration on the basis of the n.m.r. analysis of its parent alcohol, showed a strong positive Cotton effect curve with a peak at 305 mμ. The position and intensity of the trough at 270 mμ was probably affected by the increasing absorption due to the N-acetyl group. Compound 124 on the other hand showed only a very weak trough at 305 mμ and the position of the peak at 275 mμ was probably influenced by the N-acetyl group. In view of these O.R.D. curves, however one can firmly assign the 5α and 5β configurations to the hydrogenation products as already noted above.

Since this work has been completed, a publication[24] has appeared in which the hydrogenation of veratramine employing Adams Catalyst in acetic acid has been more closely examined. Saito[55] had reported only the formation of the 5α,6-dihydroveratramine but the recent work indicates that a mixture of 5α,6-dihydroveratramine (41%) and 5β,6-dihydroveratramine (44%) is produced. It is interesting to note that these workers distinguished between the two compounds on the basis of the chemical shifts of the C-19 methyl resonances in the n.m.r. spectra.

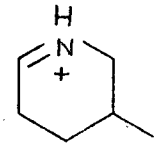
Having established the identity of the desired N-acetyl-5α,6-dihydroverarine (120) the comparison of this compound with the N-acetyl derivatives (110) of the four compounds obtained from the hydrogenation of aromatic compound II was now undertaken. Only hydrogenation products I and 2 had a similar R_f value to N-acetyl-5α,6-dihydroverarine when compared by t.l.c. on silica gel.

The melting points of the four compounds were as follows,

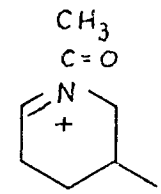
No	m.pt.
1	247 - 248°
2	263 - 264°
3	270 - 274°

No	m.pt.
4	260 - 261°

whereas the N-acetyl-5α,6-dihydroverarine prepared from veratramine had a melting point of 248-249°. A mixture of compound 1 with the authentic sample was observed to melt at 248-249° whilst a mixture of compound No. 2 and the authentic compound had a melting point of 230-240°. The infrared spectra of all five compounds were determined in chloroform and only that of compound No 1 and the authentic sample were completely superimposable. The mass spectra of all five compounds are essentially the same exhibiting a peak at m/e 437 corresponding to the molecular ion for N-acetyl-5α,6-dihydroverarine. The mass spectra of N-acetyl-5α,6-dihydroverarine, compound "No 1" and compound "No 2" are reproduced in fig. 23 and all show strong peaks at m/e 140 and 98. Budzkiewicz[56] has examined the mass spectra of veratramine, N-acetylveratramine and verarine. Cleavage of the benzylic C-20, C-22 bond is postulated to give rise to the strong peaks at m/e 114 and 98 in veratramine and verarine respectively. This cleavage gives rise to a strong peak at m/e 156 in N-acetylveratramine whilst a slightly smaller peak at m/e 114 is attributed to the m/e 156 fragment less the acetyl group.



125



126

Therefore the peak at m/e 98 in our mass spectra was assigned to structure 125 whilst the strong peak at m/e 140 was assigned to the N-acetyl derivative (126).

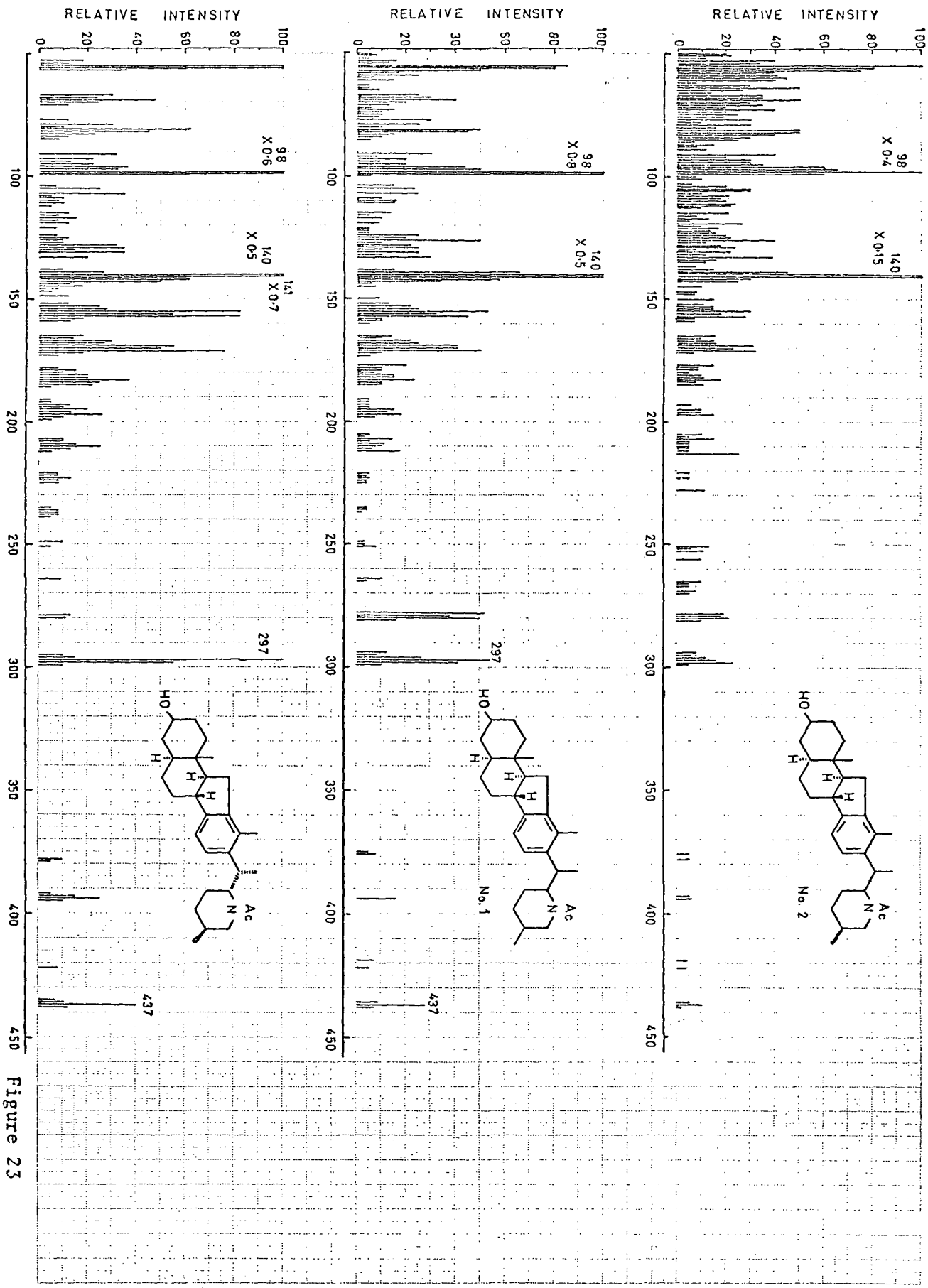

RELATIVE INTENSITY
98 X 0·4
140 X 0·15
No. 2
RELATIVE INTENSITY
98 X 0·8
140 X 0·5
297
No. 1
437
RELATIVE INTENSITY
98 X 0·6
140 X 0·5
141 X 0·7
297
437
HO
H
Ac
N

Figure 23

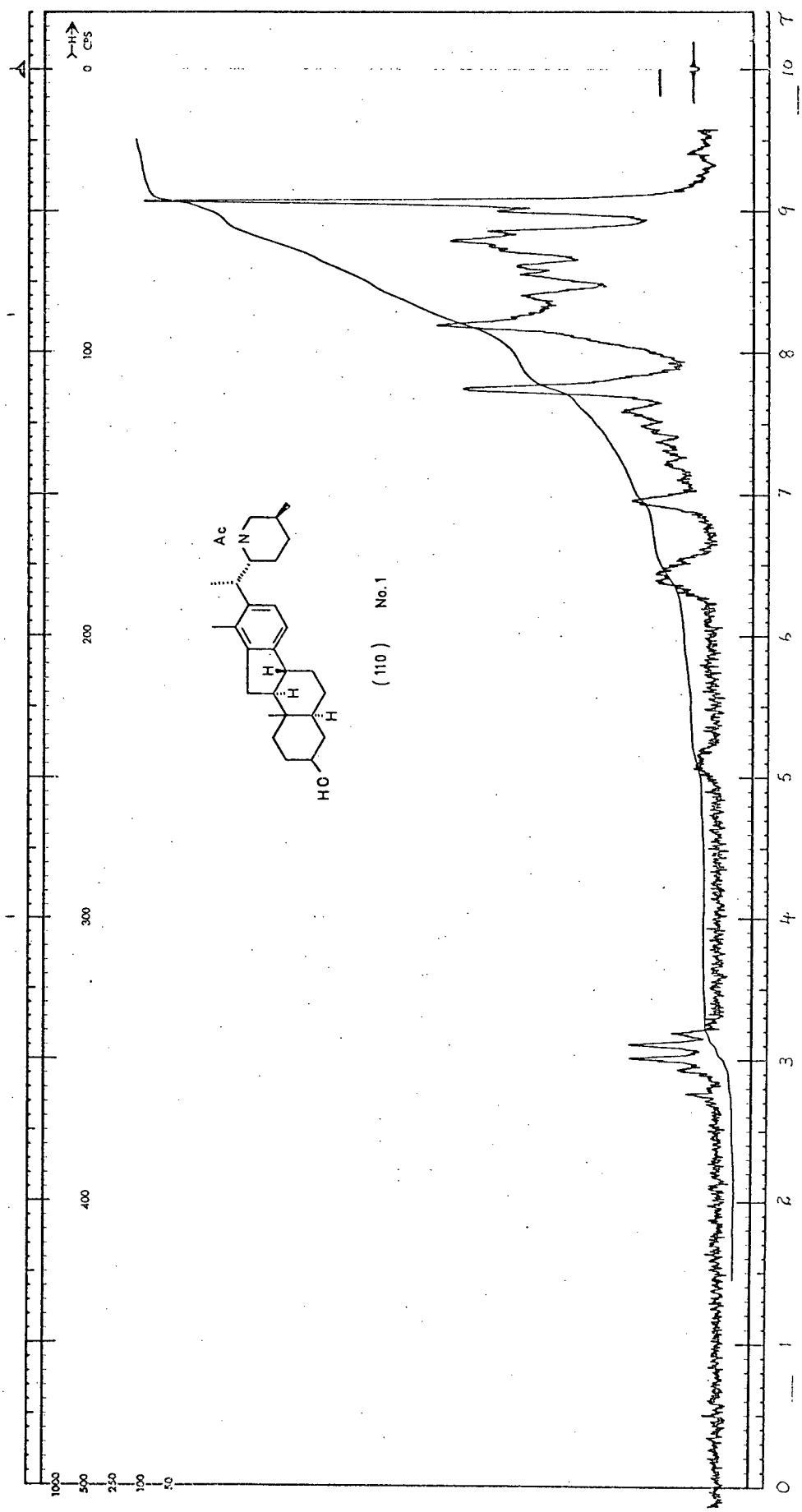

Ac
N
H
H
H
HO
(110) No.1
0 CPS
100
200
300
400
1000
500
250
100
50
0
1
2
3
4
5
6
7
8
9
10
τ

Figure 24

The n.m.r spectrum of compound "No 1" was virtually identical with that of the authentic compound (see Fig. 21) although the resolution was not good. (Fig. 24) The n.m.r. spectrum of compound "No 2" exhibited a doublet at τ 9.14 due to the C-26 or C-21 methyl group whilst a sharp singlet assigned to the C-19 methyl group appeared at τ 9.07.

These factors together constitute a firm basis for concluding that the N-acetyl derivative of the compound designated "No 1" obtained from the hydrogenation of aromatic compound "II" is identical with N-acetyl-5α,6-dihydroverarine.

Aromatic compound I which was obtained in 25% yield from isomer A was subjected to hydrogenation under the same conditions as applied to aromatic compound II. Examination of the product by t.l.c. indicated that four new compounds of similar R_f values had been formed. For the sake of convenience these were numbered from 5 to 8 in order of decreasing R_f values on a thin-layer chromatoplate. These compounds were separated by careful preparative t.l.c. and then converted via the diacetates to the respective N-acetyl derivatives. A t.l.c. comparison of the four N-acetates with N-acetyl-5α,6-dihydroverarine indicated that only compounds No's. 5 and 6 had a similar R_f value, whilst all showed the same colour reaction when sprayed with $SbCl_5$. Surprisingly none of the N-acetyl derivatives could be induced to crystallise from ether in contrast to the N-acetyl-5α,6-dihydroverarine and the N-acetates of compounds 1 - 4 which all crystallise very readily from this solvent. The mass spectra of the compounds 5 - 8 exhibited a peak at m/e 437 corresponding to the molecular ion for N-acetyl-5α,6-dihydroverarine as well as prominent peaks at m/e 98 and 140.

Since compound No. 1 obtained in these hydrogenation experiments has been shown to be identical with N-acetyl-5α,6-dihydroverarine, the remaining steps in the synthesis of verarine were carried out using a quantity of this compound which had been prepared from veratramine as already discussed. To complete the synthesis it was necessary to introduce the 5,6-double bond and remove the N-acetate function from N-acetyl-5α,6-dihydroverarine. The steps involved in achieving these goals are outlined in fig. 12 and are analogous to the reactions carried out by W. S. Johnson[44] in the conversion of 23-O,N-dibenzoyl-5α,6-dihydroveratramine to veratramine.

The formation of Δ^4-3-ketones from steroidal 3-ketones has been extensively studied by various groups of workers and a thorough investigation of the application of this reaction in the synthesis of cortisone has been published by Evans et al[57]. The steps involved in the introduction of the 4,5-double bond in 4,5 -dihydro cortisone acetate 127 are outlined in fig. 25. Treatment of 127 with 2 moles of bromine in acetic acid led to the 2,2-dibromo compound (128) which readily rearranged to the 2,4-dibromo compound (129) in the presence of hydrobromic acid.

When the 2,4-dibromo compound is refluxed with sodium iodide in acetone the bromine atom at C-2 tends to undergo halogen exchange rather than dehydrobromination whilst the C-4 bromine shows no replacement by iodine but undergoes dehydrobromination, if the refluxing is prolonged, giving rise to the Δ^4-2-iodo compound (131). Treatment of this compound with zinc in acetic acid effects dehalogenation to give the desired cortisone acetate (132).

Consequently N-acetyl-5α,6-dihydroverarine was oxidised with Jones reagent and a t.l.c. examination of the product was indicative of

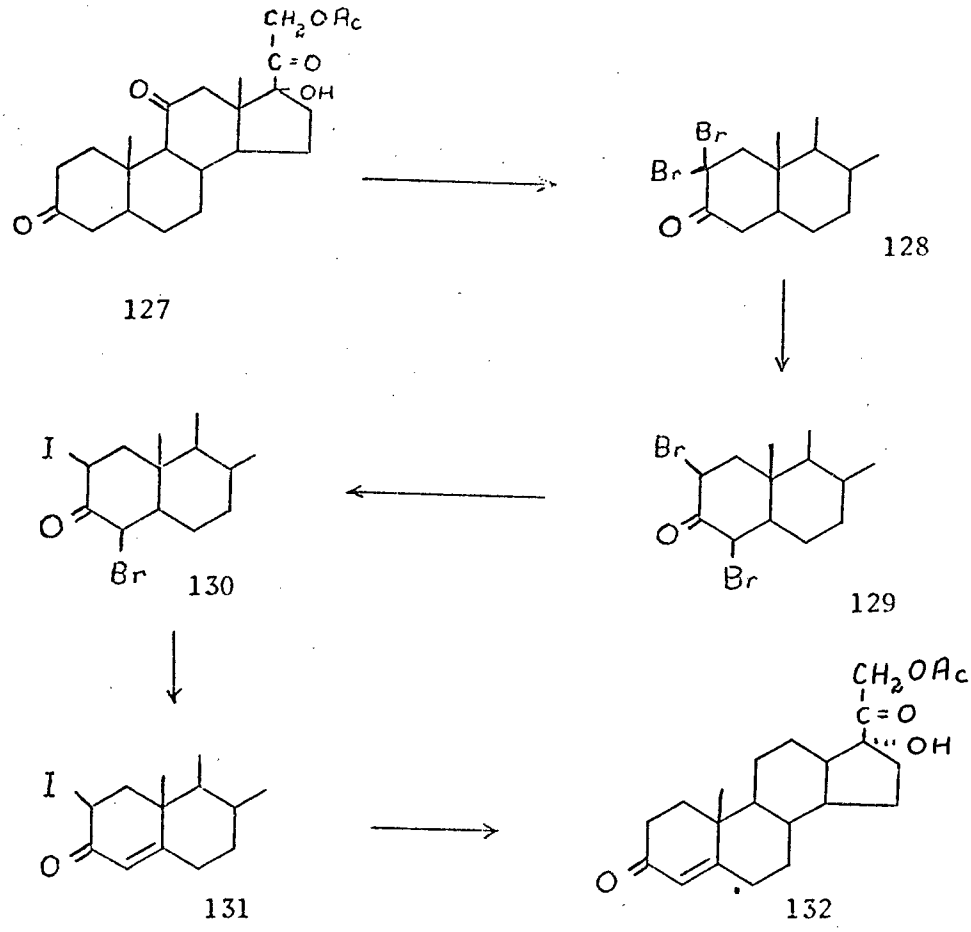


Figure 25

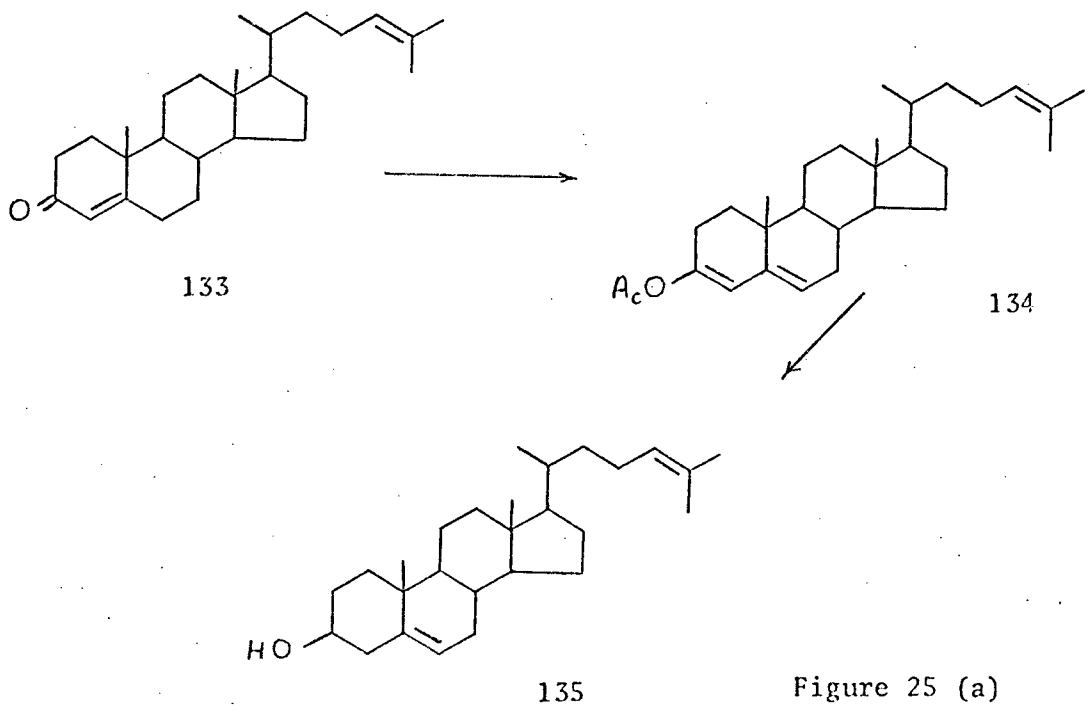
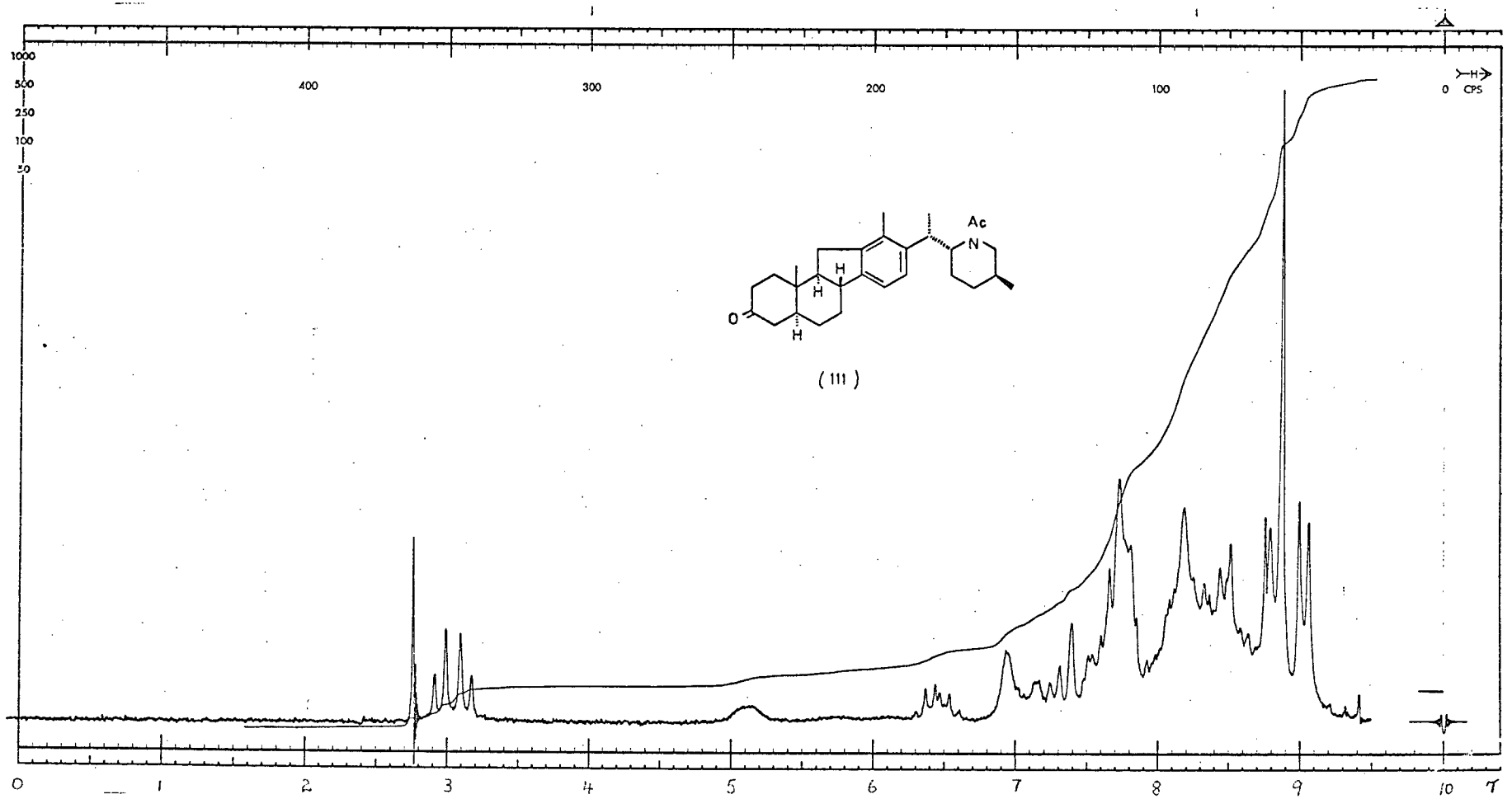


Figure 25 (a)

an almost quantitative conversion to a new compound. The infrared spectrum of this compound exhibited a peak at 5.89 μ corresponding to a saturated ketone. The O.R.D. curve exhibited as expected a strong + Cotton effect whilst the n.m.r. spectrum fig. 26 showed a three-proton singlet at τ 8.88 readily assigned to the C-19 methyl group. This value represented a shift of 0.18 τ when compared with the parent compound and was in fair agreement with the value of 0.21 expected from the excellent n.m.r. study of Masamune[40] mentioned previously.

The 3-keto-N-acetyl-5α,6-dihydroverarine (111) obtained from the Jones oxidation was then subjected to the conditions as outlined by Evans for the introduction of the 4,5-double bond. The reaction mixture after the treatment with zinc dust in acetic acid was seen to contain one major compound and several minor compounds when examined by t.l.c. The infrared spectrum, of the major compound, which was obtained in a pure state by preparative t.l.c., exhibited a band at 6.02 μ whilst the carbonyl band present at 5.89 μ in the 3-keto compound (111) had disappeared. This band at 6.02 indicated that the α,β-unsaturated carbonyl system was probably present in the molecule. This compound, which was obtained in 50% yield from the 3-keto-N-acetyl-5α,6-dihydroverarine, exhibited a three-proton singlet at τ 8.72, assigned to the C-19 methyl group, in its n.m.r. spectrum (fig. 27). This methyl resonance showed a downfield shift of 0.34 when compared with N-acetyl-5α,6-dihydroverarine and this value corresponded closely to the -0.38 contribution due to a Δ^4-3-keto in the iminojervane series. In addition the n.m.r. spectrum showed a one-proton singlet at 4.22 which was in the region expected in N-acetyl-Δ^4-3-keto-5,6-dihydroverarine (112).

Dauben[58] has studied the conversion of Δ^4-cholestenone (133)



(III)

Figure 26

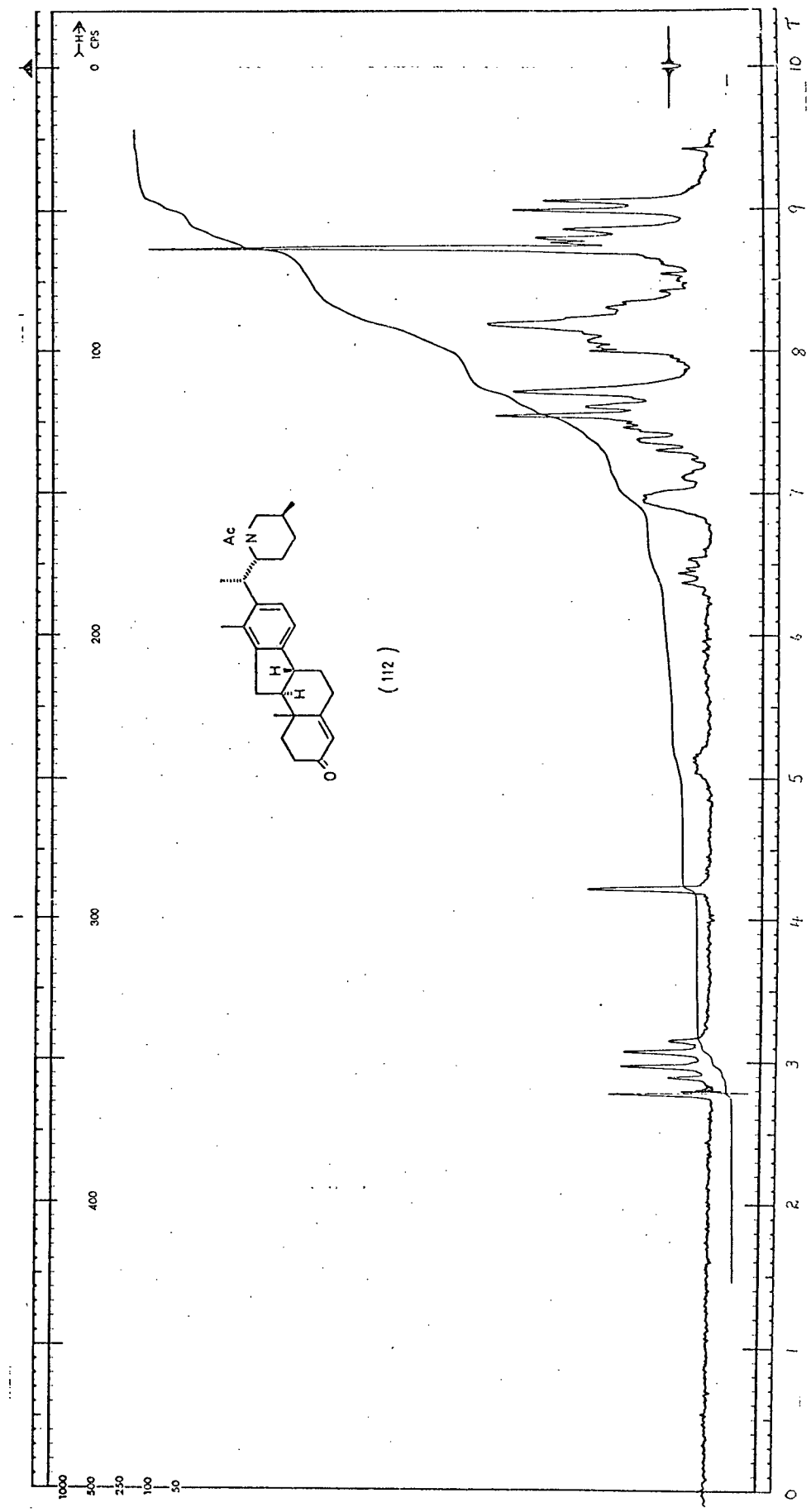


Figure 27

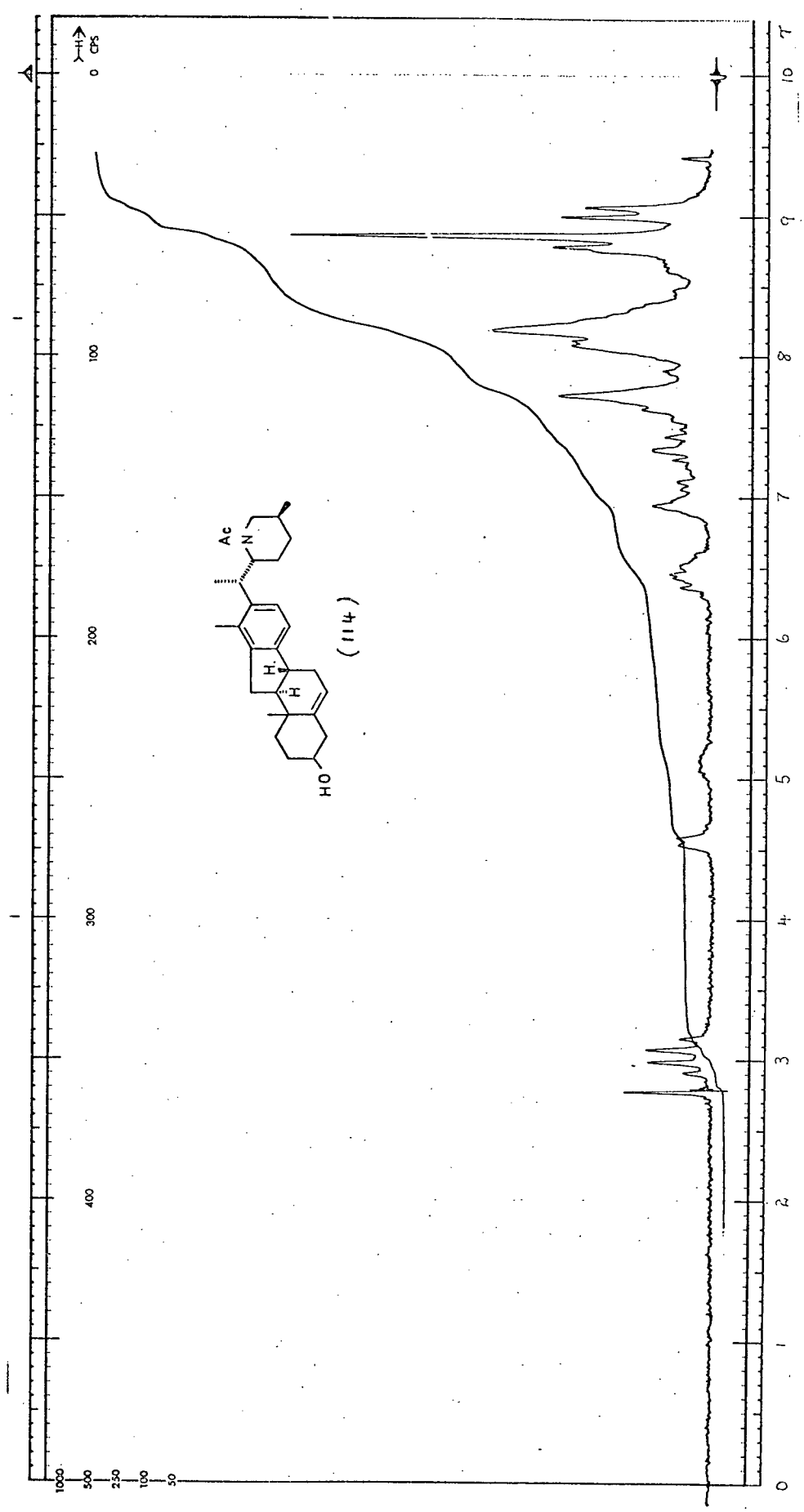

Ac
N
H
H
HO
(114)
0 CPS
100
200
300
400
1000
500
250
100
50
0
1
2
3
4
5
6
7
8
9
10
τ

Figure 28

to cholesterol (135) via the enol acetate (134) as outlined in fig. 25(a) This procedure was applied to N-acetyl-Δ^4-3-keto-5,6-dihydroverarine (112) with the aim of obtaining N-acetylverarine.

Compound (112) was refluxed with isopropenyl acetate containing 1% sulphuric acid for one hour after which time t.l.c. indicated conversion to a new compound. The infrared spectrum of the product showed that the carbonyl at 6.02μ in the the starting material was no longer present but a new band appeared at 5.75μ which was very characteristic of an enol acetate. This product was not purified but subjected to the sodium borohydride reduction in methanol under conditions outlined by Dauben.

Examination of the product from the sodium borohydride reaction by t.l.c indicated that the major product was a compound of identical R_f value and having the same colour reaction to $SbCl_5$ as N-acetylverarine. The reaction mixture was separated by preparative t.l.c. and the identity of the major component with N-acetylverarine (114) was verified by comparison of the infrared, n.m.r.(Fig. 28) and mass spectra.

The final step in the conversion of N-acetyl-5α,6-dihydroverarine to verarine involved the removal of the N-acetate group. N-acetylveratramine was used as a model compound in the search for optimum conditions for the hydrolysis of such an N-acetate by potassium hydroxide in aqueous dimethylsulfoxide which had been reported by Masamune[43]. Some hydrolysis was encountered during these experiments but the procedure was finally abandoned in favour of refluxing with 10% potassium hydroxide in ethylene glycol for 16 hours. This procedure gave a reasonable yield of veratramine with N-acetyl veratramine being the other major component in the product mixture.

When these conditions were applied to N-acetylverarine (114) a compound of similar R_f value and colour reaction to that of verarine

was observed upon t.l.c. examination of the product. Preparative t.l.c. indicated this compound had been formed in 60% yield and its identity with verarine was established by its infrared and mass spectra. The infrared spectrum was superimposable upon that of an authentic sample of verarine obtained from Professor Tomko[59], whilst the mass spectrum showed the characteristic m/e 98 and a more intense m-1 than m^+ peak in accord with the literature.

Compound No. 1 from the hydrogenation of aromatic compound II had been shown to be identical with N-acetyl-5α,6-dihydroverarine in this last step represented the completion of the total synthesis of the naturally occurring alkaloid verarine from hecogenin. The synthetic approach carried out by other workers in this laboratory had provided racemic 3β-acetoxy-5α,etiojerv-17-one (99) which was not resolved due to the quantity available whereas here (+) 3β-acetoxy-5α-etiojerv-17-one (87) from hecogenin was utilised in the synthesis of verarine.

Very recently an account of a synthetic approach to 5,6-dihydroverarine has appeared in the literature[60] and reports problems similar to those of our work in the separation of isomers. In this recent publication four compounds possessing the dihydroverarine skeleton were obtained but the authors were unable to establish the identity of any of the compounds with 5α,6-dihydroverarine. The approach involved methods similar to those employed by Schreiber[61] in the synthesis of the Solanum alkaloids.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (U.V.) spectra were measured in 95% ethanol on a Cary 11 recording spectrophotometer. Infrared (I.R.) spectra were obtained on a Perkin Elmer model 21 or 457 spectrophotometer. Nuclear magnetic resonance (n.m.r.) spectra were determined at 100 megacycles per second on a Varian Associates HA 100 spectrometer using deuteriochloroform solutions with tetramethylsilane as internal standard. The chemical shifts are recorded in the Tiers τ scale. The types of protons, integrated area, multiplicity and spin coupling constant J (in cps) are indicated in parentheses. Double irradiation experiments were performed with a Hewlett Packard 200 CD oscillator and calibration was checked with an electronic frequency counter. Optical rotatory dispersion (O.R.D.) curves were taken in chloroform solution on a Jasco UV/ORD/CD-5 spectropolarimeter. Specific rotations ($[\alpha]_D$) were determined in chloroform solution at 20^o on an O.C. Rudolph and Sons No. 219 polarimeter using a one decimeter cell. Mass spectra were recorded on an Atlas CH-4 or Associated Electrical Industries MS-9 spectrometer, high resolution measurements being determined on the latter instrument.

Unless otherwise specified silica gel G containing 1% electronic phosphor was used in preparing thin-layer chromatoplates and Woelm neutral alumina, or Shawinigan alumina deactivated by addition of 3% of 10% aqueous acetic acid solution, was used for column chromatography. Elemental analyses were performed by Mr. P. Borda of the Microanalytical Laboratory, University of British Columbia.

Synthesis of 3β-Acetoxy-5α-etiojerv-12(13)-en-17-one (76)

Throughout the entire project this compound occupied a position of prime importance since it provided the etiojervane portion of most of the compounds synthesised. It is not a commercially available compound but was prepared from hecogenin acetate[62] employing degradation procedures well documented by W. F. Johns[48]. Details of the quantities used and the yields obtained in this work, for the various steps are given below. Wherever modifications in the experimental procedure or additional data was obtained, for characterisation of the intermediates in the degradation, this is included.

Hecogenin p-toluenesulfonylhydrazone (82)

A solution of hecogenin acetate (400 g) in glacial acetic acid yielded hecogenin p-toluenesulfonylhydrazone (560 g) when treated with p-toluenesulfonylhydrazine hydrochloride as outlined by Hirschmann et al[63]. The product was not further purified but subjected to the next reaction.

13-Methyl-C-nor-D-homo-18-nor-5α,22α-spirost-12(13)-en-3β-ol. (83)

A solution of hecogenin p-toluenesulfonylhydrazone (560 g) in potassium hydroxide/ethylene glycol was heated under nitrogen as outlined by W. F. Johns. The product was obtained crystalline from ethanol/water as colourless needles. M.pt. 108-111° (lit. m.pt. 108-118°). N.m.r. signals: 9.28 (singlet, 3H, C-19 CH_3), 9.24 (doublet, J_{25-26} = 6, 3H, C-26 CH_3), 8.91 (doublet, J_{21-20} = 6, 3H, C-21 CH_3), 8.40 (broad singlet, 3H, C-18 CH_3). Mass spectrum: M.W. 414; base peak m/e 300; main peaks, m/e 414, 263, 271.

13-Methyl-C-nor-D-homo-18-nor-5α,22α-spirostan-3β-ol. Acetate

13-Methyl-C-nor-D-homo-18-nor-5α,22α-spirost-12(13)-en-3β-ol Acetate (280 g) which had been prepared by treatment of the olefine-3β-ol(83) with pyridine-acetic anhydride overnight was hydrogenated in acetic acid over 5% rhodium on alumina as outlined by W. F. Johns. The product (84) was readily obtained crystalline from methanol.. Needles m.pt. 168-173° (220 g) (lit. m.pt. 173-177°). N.m.r. signals: 9.20 (singlet, 3H, C-19 CH_3), 8.01 (singlet, 3H, $\underline{CH_3}$-C-). Mass spectrum: M.W. 458; base peak; m/e 342.

17-Acetyl-5α,13β-etiojerv-16-en-3β-ol (85)

The hydrogenation product (110 g) was subjected to ring opening by octanoic anhydride, oxidation with chromic acid and elimination employing potassium hydroxide in t-butanol, according to the procedures outlined by W. F. Johns. Chromatography of the product on alumina (2 Kg, Act. III[64]) eluting with 20% chloroform in benzene gave 17-acetyl-5α,13β-etiojerv-16-en-3β-ol (34 g) which was crystallised from acetone/petroleum ether. M.pt. 162-165° (lit. m.pt. 164-168°). N.m.r. signals: 9.22 (singlet 3H, C-19 CH_3), 9.19 (doublet, J_{18-13} = 7.0, 3H, C-18 CH_3), 7.0 (quintet, 1H, C-13H), 3.1 (two doublets, J_{16-15} = 3, J_{16-15} = 7.5, 1H, C-16 H).

Conversion to 17-acetyl-5α-13β-etiojerv-16-en-3β-ol acetate was accomplished by dissolving the product in acetic anhydride/pyridine (1:1) and allowing the solution to stand at 20° for 12 hours. Recrystallisation of the product from acetone/petroleum ether gave needles (34.2 g) m.pt. 143-144°.

17-Acetyl-5α 13β-etiojerv-16-en-3β-ol-3-Acetate 20-Oxime (86)

A solution of 17-acetyl-5α,13β-etiojerv-16-en-3β-ol acetate (33 g)

in pyridine was treated with hydroxylamine hydrochloride according to the procedure of W. F. Johns. The product was obtained crystalline from acetone as needles (33.5 g) m.pt. 198-202° (lit. 205-210). N.m.r. signals: 9.18 (singlet, 3H, C-19 CH_3) 9.07 (doublet J_{18-13} = 7, 3H, C-18 CH_3), 8.0 (singlet, 6H, $\underline{CH_3}$ -C(=O)- and $\underline{CH_3}$ -C(=N)-), 7.03 (quintet, 1H, C-13H), 3.82 (two doublets $J_{16-15} = J_{16-15}$ = 7, 1H, C-16 H). Mass spectrum: M.W. 373; base peak m/e 358; main peaks m/e 373, 296, 255.

3β-Acetoxy-5α-etiojervan-17-one (87)

A solution of 17-acetyl-5α,13β-etiojerv-16-en-3β-ol acetate, 20-oxime (33.0 g) in pyridine (400 ml) was treated with phosphorous oxychloride following the procedure of W. F. Johns. The product, obtained by filtration after pouring the reaction mixture into aqueous hydrochloric acid at 60°, was chromatographed on alumina (700 g, act III). Elution with 15% chloroform in benzene gave pure 3β-acetoxy-5α-etiojervan-17-one (24.8 g) which was crystallised from methanol/water giving colourless needles. M.pt. 168-169° (lit 175-177°); $[\alpha]_D$ + 122° (lit + 123°); N.m.r. signals: 9.21 (singlet, 3H, C-19 CH_3), 9.05 (doublet J_{18-13} = 6, 3H, C-18 CH_3), 8.06 (singlet, 3H, $\underline{CH_3}$ C(=O)-). Mass spectrum: M.W. 332; base peak, m/e 272; main peaks m/e 332, 257, 200.

3β-Acetoxyetiojerv-12(13)-en-17-one (76)

Bromination of 3β-acetoxy-5α-etiojervan-17-one (87) using an equimolar quantity of bromine followed by dehydrobromination as outlined by W. F. Johns gave a mixture of compounds. Chromatography on alumina (400 g, act III) eluting with 15% chloroform/benzene gave 3β-acetoxyetiojervan-17-one whilst further elution with the same solvent provided

3β-acetoxyetiojerv-12(13)-en-17-one (76) m.pt. 160-162°. N.m.r. signals: 9.18 (singlet, 3H, C-19 CH_3), 8.31 (singlet, 3H, C 18 CH_3), 8.01 (singlet, 3H, CH_3 -C-). Mass spectrum: M.W. 330; base peak, m/e 330; main peaks, m/e 288, 272, 270, 255.

18-Nor-C-homo-26-nor-22,27-iminojerva-12α(13),22,24,27-tetraene-3β,17-diol (102)

An ether solution of 1.88 M methyl lithium (1.0 ml) was added to anhydrous tetrahydrofuran (4 ml) in a round bottom flask which had been flame dried and flushed with dry nitrogen. A tetrahydrofuran solution of 2.0 M 2-ethylpyridine (1 ml) was added immediately and the mixture was refluxed under nitrogen for thirty minutes. During this time the solution developed a deep red colour. After the period of reflux the α,β-unsaturated ketone (101) in the form of a fine powder was added slowly until the colour faded (120 mg) and the reaction mixture refluxed for a further thirty minutes whilst the flask was still under nitrogen. Water was then added cautiously to destroy any alkyl lithium remaining and the resulting mixture diluted with ether (10 ml). The organic phase was separated and washed with water (4 x 10 ml), prior to drying over anhydrous sodium sulfate. Evaporation of the ether gave a light oil (100 mg) which appeared as one major and several minor components when examined by thin layer chromatography. The major component was obtained pure by preparative thin-layer chromatography on Woelm neutral alumina (20 x 20 cm., 0.4 mm, 2% methanol in chloroform). Spectral data indicated this was the desired condensation product (102) although the n.m.r. indicates the material is probably a mixture of two isomers. Infrared ($CHCl_3$): 6.3, 6.4 (pyridine), 3.1 μ (hydroxyl), Ultraviolet λ max (log ε): 257 (3.55), 262 (3.58), 270

(3.45) mμ. N.m.r. signals: 9.26 (singlet, 3H, C-19 CH_3), 8.8, 8.75 (two overlapping doublets due to the presence of two isomers in the product, J = 7, 3H, $\underline{CH_3}$ - Ç - H), 6.53 (quartet J = 7, 1H, -CH-CH_3), 5.1 (broad singlet, 1H, C-13H), 2.0 3.0 (multiplet, 3H, pyridine), 1.5 (multiplet, 1H, pyridine).

26-nor-22,27-iminojerva-12(13),22,24,27-tetraene-3β,17-diol. (103)

An ether solution of 1.88 M methyl lithium (1.0 ml) was added to anhydrous tetrahydrofuran in a dry round bottom flask under nitrogen. A solution of 2-ethylpyridine (1.0 ml of 2.0 M) in tetrahydrofuran was added immediately and the mixture refluxed for 30 minutes during which time a deep red colour developed. Addition of 3β-acetoxy-5α-etiojerv-12(13) en-17-one (76) in the form of a finely ground dry solid was continued until the colour faded (90 mg added) and the mixture stirred for a further thirty minutes, whilst the flask was still under nitrogen. Water was added to quench the reaction, and the mixture diluted with ether (10 ml). The organic phase was separated and washed with water (4 x 10 ml) prior to drying over anhydrous sodium sulfate. Examination of the ethereal extract by thin-layer chromatography indicated one major component which appeared as a bright yellow spot when the chromatoplate was developed with 1:1 antimony pentachloride/carbon tetrachloride (1:1) spray reagent. Column chromatography on alumina (Shawinigan, act III, 25 g) eluting with benzene/chloroform (4:1) enabled purification of this component which was crystallised from benzene/ether as needles. (42 mg) M.pt. 204-206°. Infrared ($CHCl_3$): 6.30, 6.41 μ (pyridine). Ultraviolet λmax (log ε): 257 (sh), 262.5 (3.58), 269 (sh) mμ; N.m.r. signals: 9.26 (singlet, 3H, C-19 CH_3), 8.84 (doublet J_{21-20} = 7.0, 3H, C-21 CH_3), 8.26 (singlet, 3H,

C-18 CH_3), 2.8 (multiplet, 2H, pyridine), 2.34 (multiplet, 1H, pyridine), 1.5 (multiplet, 1H, pyridine). Mass spectrum: M.W. 395; main peaks: m/e 377, 362, 288. Found: C, 78.85; H, 9.50; N, 3.50. Cald. for $C_{26}H_{37}O_2N$: C, 78.94; H, 9.43; N, 3.54.

23-Methoxy-22,27-iminojerva-12(13),22,24,27-tetraene-3β,17-diol (104)

An ether solution (1.0 ml) of 1.88 M methyl lithium was added to anhydrous tetrahydrofuran (5.0 ml) in a dry round bottom flask which had been flushed with nitrogen. A solution of 1.9 M 2-ethyl-3-methoxy-5-methylpyridine (1 ml) in tetrahydrofuran was added immediately, and the mixture refluxed for one hour, during which time a deep red colour slowly developed in the solution. The solution was cooled and 3β-acetoxy-5α-etiojerv-12(13)-en-17-one (76) was added as a fine powder until the colour faded (116 mg) and the mixture stirred for a further thirty minutes. Water (5.0 ml) was added cautiously whilst the mixture was still under nitrogen and the resulting solution diluted with ether (10 ml). The organic phase was washed with water (4 x 10 ml) prior to drying over anhydrous sodium sulfate. Evaporation of the solvent in vacuo gave a light oil (120 mg) which appeared as primarily one compound when examined by thin-layer chromatography (yellow spot when the chromatoplate is developed with antimony pentachloride spray reagent). Column chromatography on alumina (40 g, act III) eluting with benzene removed the unreacted pyridine whilst the desired compound was only partially purified. Preparative thin-layer chromatography on silica gel (20 x 20 cm., 0.4 mm, 2% methanol in chloroform plate developed 2 x) gave a pure sample of 23-methoxy-22,27-iminojerva-12(13),22,24,27-tetraene-3β,17-diol (104) as a light oil which crystallised from ether. M.pt. 209-213°. Infrared

($CHCl_3$); 6.3, 9.7 μ. Ultraviolet, λ max (log ε): 284.5 (3.86) mμ. N.m.r. signals: 9.22 (singlet, 3H, C19 CH_3) 8.84, 8.81 (two overlapping doublets J_{21-26} = 7.0, 3H, C-21 CH_3), 6.24, 6.21 (two singlets, 3H, $-OCH_3$), 3.10 (singlet, 1H, pyridine), 2.04 (broad singlet, 1H, pyridine). Mass spectrum: M.W. 439; base peak m/e 406; main peaks m/e 421, 288. Found: 439.3076. Calcd. for $C_{28}H_{41}O_3N$: 439.3086.

26-Nor-22,27-iminojerv-12(13),14(15),16,22,24,27-hexaene-3β-ol(103a)

Condensation compound 103 (20 mg) was ground with 10% palladised charcoal (10 mg) until a homogeneous powder was obtained. This powder was heated at 200° in an inert atmosphere for ten minutes. The resulting solid residue was washed several times with chloroform and the combined washings examined by thin-layer chromatography which indicated a slightly more polar compound had been formed. Preparative thin-layer chromatography (20 x 20 cm., 0.3 mm. 2% methanol in chloroform, silica gel G) afforded this component as a colourless oil (12 mg) which could not be induced to crystallise. The spectral data is in accord with this compound possessing structure 103(a). Infrared, ($CHCl_3$): 6.3, 6.4, 6.65. Ultraviolet, λ max (log ε). 263 (3.67) mμ. N.m.r. signals: 9.07 (singlet, 3H, C-19 CH_3), 8.35 (doublet, J_{21-20} =7, 3H, C-21 CH_3), 7.85 (singlet, 3H, C-18 CH_3). Mass spectrum: M.W. 375; base peak m/e 375; main peaks m/e 360, 346, 320, 288.

23-methoxy-22,27-iminojerv-12(13),14(15),15,22,24,27-hexaene-3β-ol (104a)

Condensation compound 104 (25 mg) was ground with 10% palladised charcoal until a homogeneous powder was obtained. This powder was heated at 200°C for ten minutes. The chloroform extract obtained by washing the solid residue several times with chloroform appeared to contain one major

new compound when examined by thin-layer chromatography. This component was separated by preparative thin-layer chromatography (20 x 20 cm., 0.4 mm, 2% MeOH/$CHCl_3$) as a light oil (10 mg) which could not be induced to crystallise. The spectral properties are in accord with this product having structure 104(a). Infrared (K Br): 6.29, 11.6 μ. Ultraviolet, λ max, (log ε); 283 (4.03) mμ. N.m.r. signals: 9.09 (singlet, 3H, C-19 CH_3), 7.75 (singlet, 6H, C-26 and C-18 CH_3), 6.33 (singlet, 3H, $-OCH_3$). Mass spectrum: M.W. 419; main peaks, m/e 419, 404, 390, 372 288.

2-ethyl-5-methylpyridine (105)

An anhydrous ether solution (250 ml) of bromobenzene (31.4 g, 0.2 mole) was added slowly to a dry round bottom flask which had been flushed with nitrogen and which contained lithium wire (2.8 g. 0.4 mole). When the lithium had largely dissolved an ethereal solution (100 ml) of 2,5-lutidine (21 g, 0.2 mole) was added and the mixture refluxed for thirty minutes, during which time a deep red colour developed in the solution. Methyl iodide (14.2 g, 0.1 mole) in ether (40 ml) was added slowly to this red solution over a period of fifteen minutes and the mixture stirred for ten minutes. The excess lithium and phenyl lithium was removed by the careful addition of water (100 ml) to the reaction mixture whilst the flask was still under nitrogen. A further 200 ml of water was then added, the organic phase separated and washed with water (2 x 50 ml). The ethereal solution was reduced in volume to 40 ml by use of a rotary evaporator. This 40 ml was then carefully distilled at a pressure of 10 mm, employing a spinning band column. The first fraction, distilling at 50-55° was shown by n.m.r. to be recovered 2.5-lutidine whilst that distilling at 62-63° was shown to be 2-ethyl-

5-methylpyridine (105). Infrared (film): 6.3, 6.4, 6.8, 7.02 μ. Ultraviolet, λ max (log ε): 265 sh (3.54), 269 (3.60), 276 (3.48) mμ. N.m.r. signals; 8.70 (triplet, J = 7.5, 3H, $\underline{CH_3}$-CH_2), 7.72 (singlet, 3H, $\underline{CH_3}$-C), 7.20 (quartet = 7.5, 2H, -$\underline{CH_2}$ - CH_3), 2.93 (doublet, J_{3-4} = 8.0, 1H, C-3H), 2.59 (two doublets J_{4-3} = 8.0, J_{4-6} = 2.0, 1H, C-4 H), 1.63 (doublet J_{6-4} = 2.0, 1H, C 6-H). Picrate derivative from actone, m.pt. 143-144° (lit. 144° [53]).

3β-Acetoxy-22,27-iminojerva-12(13),22,24,27-tetraene-17-ol (106)

A 2.05 M ethereal solution of methyl lithium (25 ml, 0.0512 moles) was added to a flame dried round bottom flask containing anhydrous tetrahydrofuran (40 ml) under nitrogen. A mixture of anhydrous tetrahydrofuran (10 ml) and 2-ethyl-5-methylpyridine (6 g, 0.05 moles) was added immediately and the reaction mixture refluxed for one and a half hours. During this period a deep red colour developed in the solution. A faint pink colour persisted after 3β-acetoxy-5α-etiojerv-12-en-17-one (76) (2.0 g. 0.006 moles) was added as a fine powder to the cooled solution, and the mixture stirred for ten minutes. Water (50 ml) was added cautiously to the reaction mixture whilst still under nitrogen, followed by ether (50 ml) prior to separation of the organic and aqueous phases. The organic phase was washed with water (3 x 25 ml) and then dried over anhydrous sodium sulfate. Removal of the solvent in vacuo gave a light oil (2.3 g) which appeared to consist of two new components when examined by thin-layer chromatography. Attempts to obtain these compounds in a pure state were unsuccessful. However a small scale investigation indicated that the compounds could be separated more readily as the C-3 acetates rather than as the C-3 alcohols which were formed in the reaction.

Conversion to the C-3 acetates was achieved by dissolving the

crude product from the reaction in acetic anhydride-pyridine (1:1, 20 ml) and allowing the solution to stand at room temperature for twelve hours. The solution was then poured onto crushed ice and the resultant flocculant solid extracted into ether. The combined ethereal extracts were washed with saturated aqueous sodium hydrogen carbonate solution and then several times with water before drying over anhydrous sodium sulfate. The volume of the ethereal solution was reduced in vacuo to 20 ml and then allowed to stand whereupon colourless needles were deposited. M.pt. 187-189° (370 mg). Examination of the crystals by thin-layer chromatography indicated only one compound was present and recrystallisation from ether gave an analytical sample. m.pt. 189-190°. This compound was designated "A" and the physical and spectral data are in accord with structure 106. Infrared, ($CHCl_3$): 5.83 (OAc) 6.25, 6.37 (pyridine) μ. Ultraviolet, λmax (log ε): 270 (3.61) mμ . $[\alpha]_D$ + 83° (c. 124). N.m.r. signals: 9.21 (singlet, 3H, C-19 CH_3), 8.79 (doublet, J_{21-20} = 7.0, 3H, C-21 CH_3) 8.40 (singlet, 3H, C-18 CH_3), 8.01 (singlet, 3H, O-C-CH_3), 7.70 (singlet, 3H, C-26 CH_3), 6.71 (quartet J_{20-21}= 7.0, 1H, C-20 H) 3.89 (broad multiplet - disappears on D_2O addition, 1H, -OH), 2.82 (doublet J_{23-24} = 8, 1H, C-23 H), 2.52 (two doublets, J_{24-23} = 8 J_{24-27}= 2, 1H, C-24 H), 1.67 (doublet J_{27-24} = 2, 1H, C-27 H). Mass spectrum: M.W. 451; base peak m/e 418; main peaks m/e 433, 330, 147, 121. Found: C,76.92; H, 8.95; O, 10.46; N, 3.21. Calcd.for $C_{29}H_{41}O_3N$: C, 77.12; H, 9.15; O, 10.63; N, 3.10.

Examination by thin-layer chromatography of the mother liquours from the crystallisation of compound A indicated the presence of a further compound of similar colour reaction when sprayed with antimony pentachloride-carbon tetrachloride reagent. These mother liquours were combined and chromatographed on alumina (60 g. act III). Elution with benzene yielded,

unreacted 2-ethyl-5-methylpyridine (105) initially, whilst further elution provided an additional compound (350 mg) which was obtained crystalline from a small volume of ether as prisms, m.pt. 190-192°. Recrystallisation from acetone-petroleum ether gave an analytical sample (250 mg) m.pt. 190-192°. This compound was designated compound "B" and was slightly less polar than compound "A" on a silica gel chromatoplate developed with 2% methanol in chloroform. Infrared, ($CHCl_3$): 5.83 (OAc), 6.25, 6.37 (pyridine)μ. Ultraviolet, λ max (log ε): 265 sh (3.57), 270 (3.61), 276 sh (3.51) mμ. $[\alpha]_D$ - 126° (c, 1.26). N.m.r. signals: 9.23 (singlet, 3H, C-19 CH_3), 8.88 doublet, J_{20-21} = 7.0 3H, C-21 CH_3), 8.28 (singlet, 3H, C-18 CH_3), 8.02 (singlet, 3H, $\underline{CH_3}$-C(=O)-), 7.72 (singlet, 3H, C-26 CH_3), 6.71 (quartet J_{20-21} = 7.0, 1H, C-20 H) 4.20 (broad signal, disappears on D_2O addition, 1H; -OH), 2.92 (doublet, J_{23-24} = 8.0, 1H, C-23 H), 2.70 (two doublets J_{24-23} = 8.0, J_{24-27} = 2.0, 1H, C-24 H), 1.75 (doublet J_{27-24}, 1H, C-27 H). Mass spectrum: M.W. 451; base peak m/e 418, main peaks; m/e 433, 330 147, 121. Found: C, 77.19; H, 9.21; N, 3.19. Cald. for $C_{29}H_{41}O_3N$: C, 77.12; H, 9.15; N, 3.10. Elution with 20% ether in benzene gave additional compound A (150 mg) whilst two fractions appeared to contain two further compounds of similar reaction to the spray reagent when examined by thin-layer chromatography. Separation by preparative thin-layer chromatography (20 x 20 cm., 0.3 mm, 1% methanol in chloroform, -chromatoplate developed twice) yielded 35 mg and 8 mg of these compounds which were designated "C" and "D" respectively. Compound "C" could not be induced to crystallise and an analytical sample was prepared by sublimation at 190° / 0.1 mm which gave a clear glass. The spectral data was consistent with this compound being isomeric with "A" and "B". Infrared (KBr): 5.8 (-OAc), 6.25, 6.39 (pyridine) μ. Ultraviolet, λ max (log ε): 270 (3.60) mμ. N.m.r. signals:

9.26 (singlet, 3H, C-19 CH_3), 8.77 (doublet, J_{21-20} = 7.0, 3H, C-21 CH_3), 8.43 (broad singlet, 3H, C-18 CH_3), 8.01 (singlet, 3H, $\underline{CH_3}$-C(=O)-), 7.73 (singlet, 3H, C-26 CH_3), 6.90 (quartet J_{20-21} = 7.0, 1H, C-20H), 2.97 (doublet, J_{23-24} = 8.0, 1H, C-23H), 2.65 (two doublets, J_{24-23} = 8.0, J_{24-27} = 2.0, 1H, C-24H), 1.72 (doublet J_{27-24} = 2.0, 1H, C-27H). Mass spectrum: M.W. 451; base peak m/e 120; main peaks m/e 433, 418, 330. Found: C, 77.05; H, 9.33, Calcd. for $C_{29}H_{41}O_3N$: C, 77.12, H, 9.15.

Compound D could not be induced to crystallise but the n.m.r. and mass spectrum indicates this compound is probably isomeric with the three characterised above. Infrared ($CHCl_3$): 5.83 (OAc), 6.25, 6.37 (pyridine) μ Ultraviolet λ max (log ε): 270 (3.62) mμ. N.m.r. signals 9.21 (singlet, 3H, C-19 CH_3), 8.63 (doublet J_{21-20} = 7.0, 3H, C-21 CH_3), 8.27 (singlet, 3H, C-18 CH_3), 8.01 (singlet, 3H, $\underline{CH_3}$ C(=O)-), 7.74 (singlet, 3H, C-26 CH_3), 6.89 (quartet J_{20-21} = 7.0, 1H, C-20 H), 3.08 (doublet J_{23-24} = 8.0, 1H, C-23H), 2.62 (two doublets J_{24-23} = 8.0, J_{24-27} = 2.0, 1H, C-24H), 1.70 (doublet J_{27-24} = 2.0, 1H, C-27H). Mass spectrum: M.W. 451; base peak, m/e 121, main peaks, m/e 433, 418.

3β-Acetoxy-22,27-iminojerv-12(13),14(15),16,22,24,27-hexaene. (107)

"Aromatic I"

Compound "A" (118 mg) was thoroughly ground with 10% palladised charcoal (30 mg) until a homogeneous powder was obtained. This powder was heated at 200° for seven minutes and the resultant solid residue washed several times with chloroform. Thin-layer chromatography of the chloroform extract indicated the product contained three major components and these were separated by preparative thin-layer chromatography (20 x 20 cm., 0.4 mm, 2% methanol in chloroform). One of these three

components was shown to be 2-ethyl-5-methylpyridine (105) by n.m.r. The band which exhibited a strong fluorescence when the chromatoplate was viewed under ultraviolet light was extracted and yielded a light oil which crystallised from acetone-petroleum ether as needles m.pt. 167°. This compound was identified as 3β-acetoxy-5α-etiojerv-12(13)-en-17-one (76) by its ultraviolet spectrum and by mixed melting point with an authentic sample. The third compound which appeared as a light orange spot when the chromatoplate was sprayed with antimony pentachloride-carbon tetrachloride reagent was extracted to yield a light oil which could not be induced to crystallise. An analytical sample of this compound which was designated "Aromatic I" was obtained as a clear glass after sublimation at 185° / 0.1 mm. Spectral data indicated this compound possessed structure 107. Infrared (KBr): 5.82, 6.26, 6.39 μ. Ultraviolet, λ max (log ε): 265 (sh), 269 (3.73) 277 (3.62) mμ. $[\alpha]_D$ + 13° (c, 0.84). N.m.r. signals: 9.10 (singlet, 3H, C-19 CH_3), 8.41 (doublet J_{21-20} = 7.0, 3H, C-21 CH_3), 8.03 (singlet, 3H, $\underline{CH_3}$ C-), 7.90 (singlet, 3H, C-18 CH_3), 7.80 (singlet, 3H, C-26 CH_3), 5.61 (quartet J_{20-21} = 7.0, 1H, C-20H), 3.22 - 3.0 (two doublets overlapping J= 8, 2H, C-15 or C-16 H and C-23 or C-24 H), 2.94 (doublet J_{15-16} = 8, 1H, C-15 or C-16 H), 2.72 (two doublets J_{24-23} = 8, J_{24-27} = 2, 1H, C-24 H), 1.7 (doublet, J_{27-24} = 2, 1H, C-27 H). Mass spectrum: M.W. 431; base peak, m/e 431; main peaks m/e 416, 402. Found: 431.2809. Calcd. for $C_{29}H_{37}O_2N$: 431.2824.

3β-Acetoxy-22,27-iminojerv-12(13),14,(15,16,22,24,27-hexaene. (107) "Aromatic II"

Compound B (80 mg) was ground with 10% palladised charcoal (20 mg) until a homogeneous powder was formed. This powder was heated

at 200° for seven minutes and the solid residue washed several times with chloroform. Thin-layer chromatographic examination of the chloroform extract indicated the major component was a new compound which appeared as a light orange spot when the chromatoplate was sprayed with antimony pentachloride/carbon tetrachloride reagent. This compound was separated from the reaction mixture by preparative thin-layer chromatography (20 x 20 cm., 0.4 mm, 1% methanol in chloroform - developed twice) as a light oil (60 mg) which could not be induced to crystallise. An analytical sample of the compound, which was designated "Aromatic II", was obtained as a clear glass after sublimation at 185° / 0.01 mm. Infrared (KBr): 5.82, 6.26, 6.39 μ. Ultraviolet, λ max (log ε): 265 sh, 269 (3.73), 277 (3.62), mμ. N.m.r. signals: 9.10 (singlet, 3H, C-19 CH_3), 8.40 (doublet J_{21-20} = 7 , 3H, C-21 CH_3), 8.02 (singlet, 3H, $\underline{CH_3}$-C-), 7.89 (singlet, 3H, C-18 CH_3), 7.80 (singlet, 3H, C-26 CH_3), 5.58 (quartet J_{20-21} = 7, 1H, C-20 H), 3.14 (doublet J_{23-24} = 8, 1H, C-23 H), 3.09 (doublet, J_{15-16} = 8, 1H, C-15 H or C-16 H), 2.91 (doublet, J_{15-16} = 8, 1H, C-15 H or C-16 H), 2.75 (two doublets, J_{24-23} = 8, J_{24-27} = 2, 1H, C-24 H), 1.73 (doublet J_{27-24} = 2, 1H, C-27 H). Mass spectrum: M.W. base peak, 431, main peaks m/e 416, 402. Found: 431.2809. Calcd. for $C_{29}H_{37}O_2N$: 431.2824.

N-acetyl-22,27-iminojerva-12(13),14(15),16-triene-3β-ol. (110) Isomers 1-4

Aromatic compound II (50 mg) was dissolved in glacial acetic acid (10 ml) and the solution hydrogenated at 20° and 45 p.s.i. over Adams catalyst (PtO_2, 20 mg) for three hours. The mixture was filtered to remove the catalyst which was carefully washed with additional acetic acid (5 ml). The combined filtrates were reduced in volume to 2 ml

in vacuo, then diluted with water (20 ml) and made basic with ammonia solution. The resultant suspension was extracted with methylene chloride (3 x 10 ml) prior to drying over anhydrous sodium sulfate. Thin-layer chromatography revealed the presence of four compounds of similar R_f value and colour reaction with various spray reagents. These compounds were designated 1-4 in order of decreasing R_f value, (silica gel G, 5% methanol in chloroform) and were separated by careful preparative thin-layer chromatography (20 x 20 cm., 0.3 mm, 1% methanol in chloroform, plates developed three times). The bands corresponding to the four compounds were delineated by inspection of the developed plates under ultraviolet light (chromatovue, C-3). Extraction of the various bands and removal of the solvent (methanol / chloroform - 1:1) gave each of the compounds as a clear oil. None of the compounds could be induced to crystallise.

All four compounds were separately converted via the 3-O, N-diacetates (109) to the N-acetyl derivatives (since a sample of N-acetyl-5α,6-dihydroverarine was available for comparison) as outlined for one of the isomers below.

Compound No. 2 was dissolved in pyridine-acetic anhydride (2 ml, 1:1) and allowed to stand for twelve hours at 20°. The solution was then poured into ice water (5 ml) and the resulting suspension extracted with methylene chloride (3 x 3 ml). The combined extracts were washed with saturated aqueous sodium hydrogen carbonate (10 ml) and then with water (2 x 5 ml), prior to drying over anhydrous sodium sulfate. Removal of the methylene chloride in vacuo gave a light oil which crystallised from ether (the diacetates of compounds 1, 3 and 4 were not obtained crystalline) as needles m.pt. 203-205°. Infrared ($CHCl_3$) 5.80 (OAc), 6.19 (NAc) μ. Ultraviolet: λ max (log ε): 268 (2.7) 277 (2.7) mμ.

The diacetate (20 mg) was refluxed with 0.1 M potassium hydroxide in methanol (5 ml) for one hour, the solution cooled and diluted with water (20 ml). The aqueous suspension was extracted with methylene chloride (3 x 5 ml) and the combined extracts washed with water (2 x 5 ml) prior to drying over anhydrous sodium sulfate. Evaporation of the solvent in vacuo gave a light oil which was readily crystallised from anhydrous ether. (All four N-acetyl derivatives were obtained crystalline via this procedure).

The N-acetyl derivatives of compounds 1 and 2 had an R_f value comparable with that of N-acetyl-5α,6-dihydroverarine whilst those of the N-acetyl derivatives of compounds 3 and 4 were slightly smaller. All compounds showed similar colour reactions when the chromatoplate was sprayed with various reagents. The N-acetyl derivatives were characterised as follows.

Compound No. 1 N-acetate; prisms m.pt. 249-250°, (6 mg). Infrared ($CHCl_3$): 6.20, 9.70 μ. Ultraviolet, λ max (log ε): 268 (2.68), 277 (2.66) mμ. $[\alpha]_D$+ 36° (c, 0.605). N.m.r. signals: 9.07 (singlet, C-19 CH_3), 8.19 (singlet, 3H, $\underline{CH_3}$ C-N), 7.74 (singlet, 3H, C-18 CH_3), 3.15 (doublet J_{15-16} = 8, 1H, C-15H or C-16 H), 2.96 (doublet J_{15-16} = 8, 1H, C-15H or C-16 H). Mass spectrum: M.W. 437; base peak m/e 140; main peaks m/e 297, 98. Found: 437.325. Calcd. for $C_{29}H_{43}O_2N$: 437.329.

Compound No. 2 N-acetate; prisms m.pt. 263-264° (17 mg). Infrared ($CHCl_3$): 6.19, 9.70 μ. Ultraviolet λ max (log ε): 268 (2.65), 277 (2.61) mμ. $[\alpha]_D$ + 26°, (c, 1.71). Mass spectrum: M.W. 437; base peak m/e 140; main peaks m/e, 298, 297, 98.

Compound No. 3 N-acetate; rosettes m.pt. 270-274°. (5 mg) Infrared ($CHCl_3$): 6.20, 9.70 μ. Ultraviolet λ max: 268, 277 mμ. Mass spectrum:

M.W. 437; base peak, m/e 140; main peaks m/e 297,98.

Compound No. 4 N-acetate; long needles m.pt. 260-261° ₹ (10 mg).

Infrared ($CHCl_3$): 6.20, 9.70, 9.80 μ. Ultraviolet, λ max: 268, 277 mμ.

Mass spectrum: M.W. 437; base peak, m/e 140; main peaks, m/e 298, 297, 98.

N-acetyl-22,27-iminojerva-12(13),14(15),16-triene-3β-ol. (110) Isomers 5-8

Aromatic compound I (50 mg) was dissolved in glacial acetic acid (10 ml) and the solution shaken with Adams catalyst (PtO_2, 20 mg) in an atmosphere of hydrogen at 45 p.s.i. and 20° for a period of three hours. The mixture was filtered to remove the catalyst which was washed with a further 10 ml of glacial acetic acid and the filtrates combined. The volume of the acetic acid was reduced to 2 ml by use of a rotary evaporator prior to dilution with water (20 ml). The aqueous acid solution was made basic with dilute ammonia and the resulting suspension extracted several times with methylene chloride (3 x 10 ml) prior to drying over anhydrous sodium sulfate. Thin-layer chromatographic investigation of the light oil (45 mg) obtained by removal of the methylene chloride indicated the presence of four compounds, of similar R_f value and colour reaction to the spray reagent. These compounds were numbered 5-8 in order of decreasing R_f values and were separated by careful preparative thin-layer chromatography (silica gel G. 20 x 20 cm., 0.3 mm, 1% methanol in chloroform, plates developed three times). The compounds were obtained as colourless oils which could not be crystallised. Conversion of these compounds via the 3-O,N-diacetates to the N-acetyl derivatives was accomplished as outlined above for the 3β-acetoxy-22,27-iminojerva-12(13), 14(15),16-triene isomers No. 1-4 obtained from Aromatic compound II. None of the N-acetyl derivatives of compounds 5-8 could be induced to

crystallise although compounds 5 and 6 had comparable R_f values with N-acetyl-5,6-dihydroverarine. These compounds were not fully characterised but their spectral properties were in accord with structure 110. Compound No. 5 N-acetate: Infrared (KBr): 6.19, 9.28 μ. Ultraviolet λ max: 267, 276 mμ. Mass spectrum: M.W. 437; base peak, m/e 140; main peaks 298, 297, 98.

Spectral properties for the N-acetyl derivative of compounds 6-8 are virtually the same as for No. 5-acetate and no additional data was obtained for any of these compounds.

Hydrogenation of 3-O,N-diacetylverarine (117)

The 3-O,N-diacetylverarine utilised in this reaction was prepared from veratramine[20] and had the following physical constants. M.pt. 189-190° (lit. m.pt. 189-190°), $[\alpha]_D$ -23° ± 2° (ethanol).

A solution of 3 O, N-diacetylverarine (190 mg) in glacial acetic acid (5 ml) was stirred with Adams catalyst (PtO_2, 60 mg) in an atmosphere of hydrogen at 20°. After 14 hours 9.1 ml of hydrogen had been consumed and the mixture was filtered to remove the catalyst which was washed with a further 10 ml of acetic acid. The combined filtrates were reduced in volume (to 2 ml) in vacuo and diluted with water (30 ml) to give a white precipitate which was taken up in ether. The ethereal phase was washed with water (3 x 10 ml) prior to drying over anhydrous sodium sulfate. Thin-layer chromatographic examination on both silica gel and alumina using a variety of solvents indicated that a single new compound had been formed.

Removal of the ether in vacuo gave an oil which could not be induced to crystallise. A small scale investigation revealed that after selective hydrolysis of the 3-O-acetate the product appeared as two

components of similar R_f value on a silica gel chromatoplate. Consequently 3-O,N-diacetyl-5,6-dihydroverarine (170 mg) was refluxed with 0.1 N methanolic potassium hydroxide (5 ml) for one hour, the solution cooled, and then diluted with water (20 ml). The resulting aqueous suspension was extracted with methylene chloride (3 x 10 ml) and the combined extracts washed with water (3 x 10 ml) prior to drying over anhydrous sodium sulfate. Removal of the solvent in vacuo gave a light oil which on trituration with ether gave crystals. M.pt. 190-225°. Thin-layer chromatography showed the crystalline product to consist of two compounds which were separated by preparative thin-layer chromatography on silica gel. (20 x 20 cm., 0.4 mm, 2% methanol in chloroform). The least polar compound (75 mg) was tentatively assigned the 5β configuration on the basis of its n.m.r. spectrum. Recrystallisation from ether gave an analytical sample of N-acetyl-5β,6-dihydroverarine (121) m.pt. 198-199°. Infrared ($CHCl_3$): 6.21 (-NC-CH) μ. Ultraviolet, λ max (log ε): 267 (2.74), 276 (2.74) mμ. N.m.r. signals: 9.02 (doublet J_{26-25} = 7, 3H, C-26 CH_3), 8.94 (singlet, 3H, C-19 CH_3), 8.82 (doublet J_{21-20} = 8, 3H, C-21 CH_3), 8.14 (singlet, 3H, $\underline{CH_3}$ C-N), 7.71 (singlet, 3H, C-18 CH_3), 6.45 (doublet of quartets J_{20-21} = 8, J_{20-22} = 10, 1H, C-20 H), 5.97 (narrow multiplet, 1H, H-C-O-Ac), 3.12 (doublet J_{15-16} = 8, 1H, C-15 H or C-16 H), 2.96 (doublet J_{15-16} = 8, 1H, C-15 or C-16 H). Mass spectrum: M.W. 437; base peaks, m/e 140; main peaks, m/e 297, 98. Found: C, 79.51; H, 10.02; N, 3.25, Calcd. $C_{29}H_{43}O_2N$: C, 79.58; H, 9.90; N, 3.20.

The more polar compound was crystallised from ether as prisms m.pt. 249-250°. This compound was tentatively assigned the "5α" configuration on the basis of its n.m.r. spectrum, and an analytical sample of N-acetyl-5α,6-dihydroverarine (120) was prepared by sublimation

at 220° / 0.01 mm. as prisms m.pt. 249-250°. Infrared (KBr): 6.22 (N-$\overset{O}{\overset{\|}{C}}$-$CH_3$), 12.23 μ. Ultraviolet, λ max (log ε): 267 (2.69), 276 (2.68) mμ. $[\alpha]_D$ + 41° (c, 0.961). N.m.r. signals: 9.06, 9.02 (a singlet and a doublet, 6H, C-19 and C-26 CH_3 groups), 8.19 (singlet, 3H, $\underline{CH_3}$ $\overset{O}{\overset{\|}{C}}$-N), 7.72 (singlet, 3H, C-18 CH_3), 3.13 (doublet J_{15-16} = 8, 1H, C-15 or C-16 H), 2.99 (doublet J_{15-16} = 8, 1H, C-15 or C-16 H). Mass spectrum: M.W. 437; base peak m/e 140; main peaks m/e 437, 394, 297. Found: C. 79.32; H, 9.99. Calcd. for $C_{29}H_{43}O_2N$: C, 79.58; H, 9.90.

N-acetyl-3-keto-5α,6-dihydroverarine (III)

A solution of N-acetyl-5α,6-dihydroverarine (120 ,212 mg) in acetone (75ml) was treated with Jones reagent[65] (0.3 ml) and allowed to stand at 20° for two minutes. Isopropanol (2 ml) was added to remove the excess oxidant and the mixture diluted with water (600 ml). The aqueous acetone solution was extracted with methylene chloride (3 x 30 ml) and the combined extracts washed with water (3 x 30 ml) prior to drying over anhydrous sodium sulfate. Evaporation of the methylene chloride gave a colourless oil (191 mg) which could not be crystallised but appeared homogeneous when examined by thin-layer chromatography. An analytical sample was obtained as a clear glass after sublimation at 185° / 0.01 mm. Infrared (KBr): 5.89 (saturated carbonyl), 6.14 (N-$\overset{O}{\overset{\|}{C}}$-$CH_3$) μ. Ultraviolet, λ max (log ε): 267 (2.73), 276 (2.71) mμ. $[\alpha]_D$ + 66° (c, 1.15). O.R.D. $[\phi]_{305\ m\mu}^{peak}$ + 1090°, $[\phi]_{270\ m\mu}^{trough}$ + 115°, $[\phi]_{230\ m\mu}^{peak}$ + 3910°. N.m.r. signals: 9.03 (doublet J_{26-25} = 7.0, 3H, C-26 CH_3), 8.88 (singlet, 3H, C-19 CH_3), 6.45 (doublet of quartets J_{20-21} = 7.5, J_{20-22} = 9.0, 1H, C-20 H). 3.13 (doublet J_{15-16} = 8, 1H, C-15 H or C-16 H), 2.95 (doublet J_{15-16} = 8, 1H, C-15 H or C-16 H). Mass spectrum: M.W. 435; base peak m/e 140;

main peaks m/e 98, 295, 392. Found: 435,312. Calcd. for $C_{29}H_{41}O_2N$: 435.313.

N-Acetyl-Δ^4-3-keto-5,6-dihydroverarine (112)

A solution of N-acetyl-3-keto-5α,6-dihydroverarine (162 mg, 0.372 m. mole) in acetic acid (6 ml) containing hydrogen bromide (60 mg, 0.744 m. moles) was placed in a dry round bottom flask. Bromine (130 mg, 0.8 m. moles) in acetic acid (5 ml) was added slowly and the mixture stirred at 20° for twenty minutes and then diluted with water (25 ml). The resulting heavy white precipitate was taken up in methylene chloride and the extract washed with water (3 x 10 ml) prior to drying over anhydrous sodium sulfate. Removal of the solvent in vacuo gave a colourless gum (225 mg) which was shown by thin-layer chromatography to consist of one major compound. This crude product was immediately subjected to the conditions of Evans[57] for introduction of the 4,5-double bond as outlined below. Bromine (0.26 ml) was added to acetone (7.5 ml) at 0° and the solution stirred until the colour disappeared. Sodium carbonate (0.7 g) was then added and the mixture, stirred for a further thirty minutes, and then the mixture was filtered directly into hot acetone containing sodium iodide (7.0 g). This solution was refluxed for fifteen minutes during which time a precipitate of sodium bromide was formed.

A portion (6 ml) of the supernatant of this hot solution was added to a round bottom flask containing 2,4-dibromo ketone (220 mg) and the solution refluxed for two and a half hours. During this time more sodium bromide precipitated, indicative of the exchange of the 2-bromine for an iodine atom. Oxalic acid (220 mg) was added as a fine

powder and the solution refluxed for a further hour before cooling. The reaction mixture was diluted with ethyl acetate (20 ml) and filtered. This filtrate was washed with water (2 x 10 ml), saturated aqueous sodium hydrogen carbonate (2 x 10 ml) and water (2 x 10 ml). The ethyl acetate was decolourised by adding zinc dust (600 mg) and acetic acid (0.2 ml) and then shaking. When the solution had attained a light yellow colour the zinc dust was removed by filtration and the ethyl acetate filtrate washed with water (2 x 10 ml), sodium hydrogen carbonate solution (2 x 10 ml) and water (2 x 10 ml) prior to drying over anhydrous sodium sulfate.

Removal of the ethyl acetate in vacuo gave a light yellow gum (158 mg) which was examined by thin-layer chromatography. The spot on the chromatoplate corresponding to the major product exhibited an intense fluorescence under ultraviolet light, and appeared purple when the plate was sprayed with antimony pentachloride-carbon tetrachloride reagent. Preparative thin-layer chromatography on silica gel (20 x 60 cm, 0.4 mm developed twice with 1% methanol in chloroform) gave this compound as a light gum (110 mg, 50% yield) which was not crystallised. An analytical sample was prepared by sublimation at 180° / 0.01 mm which gave a light yellow glass. Infrared (KBr): 6.03 (α,β-unsaturated carbonyl), 6.14 (N-Ac) μ. $[\alpha]_D$ + 74° (c, 1.26). N.m.r. signals: 9.02 (doublet J_{26-25} = 7, 3H, C-26 CH_3), 8.72 (singlet, 3H, C-19 CH_3), 8.18 (singlet, 3H, $\underline{CH_3}$ C-N), 6.45 (doublet of quartets J_{20-21} = 7.5, J_{20-22} = 10, 1H, C-20 H), 4.22 (singlet, 1H, C-4 H), 3.10 (doublet J_{15-16} = 8, 1H, C-15 or C-16 H), 2.94 (doublet J_{15-16} = 8, 1H, C-15 or C-16 H). Mass spectrum: M.W. 433 ; base peak, m/e 140; main peaks, m/e 433, 390, 293, 98. Found: 433.2974, Calcd. for $C_{29}H_{39}O_2N$: 433.2980.

N-acetylverarine (114)

A solution of N-acetyl-Δ^4-3-keto-5,6-dihydroverarine (100 mg) in isopropenyl acetate (3 ml) containing 0.5% sulfuric acid was refluxed for one hour. Anhydrous sodium acetate (60 mg) was then added and most of the isopropenyl acetate removed by use of a rotary evaporator. The residue was diluted with methylene chloride (10 ml) and then filtered. Thin-layer chromatography of the filtrate indicated the complete absence of (112) and the formation of a less polar compound. Evaporation of the methylene chloride gave an oil (100 mg) the infrared spectrum of which exhibited a carbonyl band at 5.75 μ indicative of an enol acetate (113).

This oil was dissolved in a mixture of methanol (10 ml) and ether (3 ml) and heated to reflux. During the refluxing a methanolic solution of sodium borohydride (150 mg in 5 ml) was added slowly over a period of fifteen minutes. The solution was refluxed for a further one hour and then concentrated hydrochloric acid (1 ml) was added and the refluxing continued with stirring for thirty minutes. During this final period vigorous stirring was necessary to overcome severe bumping.

The reaction mixture was cooled and then diluted with ether (50 ml). The ethereal solution was washed with water and then dried over anhydrous sodium sulfate. Removal of the solvent gave a light oil (70 mg) which was shown to contain a compound of identical R_f value to that of N-acetyl verarine. Separation by preparative thin-layer chromatography (20 x 20 cm., 0.3 mm, 2% methanol in chloroform) and extraction of the band corresponding to this product yielded an oil (30 mg). This compound was shown to be identical with N-acetylverarine by thin-layer chromatography, superimposable infrared and n.m.r. spectra. Infrared ($CHCl_3$): 6.19

(NC-CH_3) μ. Ultraviolet, λ max (log ε): 268 (2.76), 277 (2.65) mμ. N.m.r. signals: 9.03 (doublet J_{26-25} = 7, 3H, C-26 CH_3), 8.87 (singlet, 3H, C-19 CH_3), 8.18 (singlet, 3H, CH_3 C-N-), 4.56 (multiplet, 1H, C-6 H), 3.12 (doublet J_{15-16} = 8, 1H, C-15 H or C-16 H), 2.94 (doublet J_{15-16} = 8, 1H, C-15 H or C-16 H). Mass spectrum: M.W. 435; base peak m/e 140; main peaks m/e 98, 295.

Verarine (3)

A solution of N-acetylverarine (20 mg) in ethylene glycol (5 ml) containing 10% potassium hydroxide was refluxed for twelve hours. The reaction mixture was cooled, diluted with water (20 ml) and extracted with methylene chloride (3 x 5 ml). Examination of the extract by thin-layer chromatography indicated the presence of N-acetylverarine and a more polar compound which had the same R_f value and colour reaction to the spray reagent, as an authentic sample of verarine. The more polar compound was separated by preparative thin-layer chromatography (20 x 20 cm., 0.3 mm, 5% methanol in chloroform) as a light oil (8 mg) which crystallised from ether. This compound was shown to be identical with verarine by thin-layer chromatography, infrared and n.m.r. comparison. Infrared ($CHCl_3$): 9.57 μ. Ultraviolet, λ max (log ε): 267 (2.74) 276 (2.73) mμ. N.m.r. signals: 9.15 (doublet J_{26-25} = 6, 3H, C-26 CH_3) 8.88 (singlet, C-19 CH_3), 7.73 (singlet 3H, C-18 CH_3), 4.55 (broad doublet, 1H, C-6 H), 3.02 (singlet, 2H, C-15 H and C-16 H). Mass spectrum: M.W 393; base peak m/e 98, main peaks, m/e 392, 376,295, 284, 256. High resolution on m/e (m^+-1) 392. Found: 392.2922. Calcd. for $C_{27}H_{39}O$ N: 392.2953.

BIBLIOGRAPHY

L. L. F. Feiser and M. Feiser. "Steroids", pp. 867-895, Reinhold Publishing Corp., New York, 1959.

2. H. G. Boit. "Ergebnisse der Alkaloid-Chemie bis 1960" pp 798-832, Akademie-Verlag, Berlin 1961.

3. C. R. Narayanan in "Progress in the Chemistry of Organic Natural Products" (L. Zeichmeister ed.) Vol. 20, p. 298 Springer-Verlag Vienna 1962.

4. S. M. Kupchan and A. W. By in "The Alkaloids" (R. H. Manske ed.) Vol. X, pp. 193-280, Academic Press, New York 1968.

5. S. M. Kupchan, J. H. Zimmerman and A. Afonso, Lloydia, 24, 1 (1961).

6. P. D. Baker, Southern Med. and Surg., 15, 4, (1859).

7. W. Poethke, Arch. Pharm., 275, 571 (1937).

8. L. C. Craig and W. A. Jacobs, J. Biol. Chem., 143 427 (1942).

9. O. Krayer and G. A. Acheson, Physiol. Rev. 26, 383 (1946).

10. J. H. Currens, G. S. Myers, and P. D. White, Am. Heart. J., 46, 576 (1953).

11. J. Fried and A. Klingsberg, J. Am. Chem. Soc., 75, 4929 (1953).

11a. F. C. Chang and R. C. Ebersole, Tetrahedron Letters, 3521 (1968).

12. H. Mitsuhashi and Y. Shimizu, Tetrahedron, 19, 1027 (1963).

13. H. Mitsuhashi and K. Shibata, Tetrahedron Letters, 2281 (1964).

14. T. Masamune, M. Takasugi, and Y. Mori, Tetrahedron Letters, 489 (1965).

15. D. M. Bailey, D.P.G. Hamon and W. S. Johnson, Tetrahedron Letters, 555 (1963).

16. J. Sicher and M. Tichy, Tetrahedron Letters, 6 (1959).

17. J. W. Scott, L. J. Durham, H.A.P. de Jongh, U. Burckhardt and W. S. Johnson, Tetrahedron Letters, 2381 (1967).

18. S. Okuda, K. Tsuda, and H. Kataoka, Chem. and Ind.(London) 512 (1961).

19. R. L. Augustine, Chem. Ind. (London), 1448 (1961).

20. T. Masamune, I. Yamazaki and M. Takasugi, Bull. Chem. Soc. Japan 39,1090 (1966).

21. H. Paulsen and K. Todt, Angew. Chem. Int. Ed. 5., 899 (1966).

22. H. Paulsen and K. Todt, Chem. Ber., 100,3385 (1967).

23. O. Wintersteiner and M. Moore, Tetrahedron, 20,1947 (1964).

24. T. Masamune, K. Kobayashi, M. Takasugi, Y. Mori and A. Murai, Tetrahedron 24, 3461, (1968).

25. G. N. Reeke, R. L. Vincent and W. N. Lipscomb, J. Am. Chem. Soc., 90, 1663 (1968).

26. S. M. Kupchan and M. I. Suffness, J. Am. Chem. Soc., 90 2730 (1968).

27. J. Tomko and S. Bauer, Collection Czech. Chem. Commun., 29, 2570 (1964).

28. J. Tomko, A. Vassova, G. Adam, K. Schreiber and E. Hohne, Tetrahedron Letters, 3907 (1957).

29. G. Adam, K. Schreiber, J. Tomko, Z. Voticky and A. Vassova, Tetrahedron Letters, 2815 (1968).

30. J. Tomko, A. Vassova, G. Adam and K. Schreiber, Tetrahedron (in press).

31. E. Höhne, G. Adam, K. Schreiber and J. Tomko, Tetrahedron (in press).

32. S. M. Kupchan and C. I. Ayres, J. Am. Chem. Soc., 82, 2252 (1960).

33. L. C. Weaver, W. R. Jones and S. M. Kupchan, J. Pharm. Sci., 51, 1144 (1962).

34. S. M. Kupchan, W. S. Johnson and S. Rajagopalan, Tetrahedron, 7, 47 (1959).

35. W. T. Eeles, Tetrahedron Letters, 24 (1960).

36. M. Fukuda, Nippon Kaguku Zasshi, 69, 165 (1948): C.A. 46, 4555 (1952).

37. T. Q. Chou and K. K. Chen, Chinese J. Physiol., 6, 265, (1932); C.A. 26, 5703, (1932).

38. S. Ito, M. Kato, K. Shibata and T. Nazoe, Chem. Pharm. Bull. (Tokyo) 8, 871 (1960).

39. S. Ito, M. Kato, K. Shibata and T. Nazoe, Chem. Pharm. Bull (Tokyo), 9, 253 (1961).

40. T. Masamune, N. Sato, K. Kobayashi, I. Yamazaki and Y. Mori, Tetrahedron, 23, 1591 (1967).

41. N. S. Bhacca and D. H. Williams, "Application of N.M.R. Spectroscopy in Organic Chemistry" p. 14. Holden-Day, San Francisco (1964).

42. A. T. Cohen, D. Rosenthal, G. W. Karkower and J. Fried, Tetrahedron, 21, 3171 (1965).

43. T. Masamune, M. Takasugi, A. Murai and K. Kobayshi, J. Am. Chem. Soc., 89, 4521 (1967).

44. W. S. Johnson, H.A.P. de Jongh, C. E. Coverdale, J. W. Scott and U. Burckhardt, J. Am. Chem. Soc., 89, 4523, (1967).

45 Y. Mazur, N. Danieli and F. Sondheimer, J. Am. Chem. Soc., 82, 5889, (1960).

46. W. S. Johnson, N. Cohen, E. R. Habicht, D.P.G. Hamon, G. P. Rizzi and D. J. Faulkner, Tetrahedron Letters, 2829 (1968).

47. P. W. Schiess, D. M. Bailey and W. S. Bailey and W. S. Johnson, Tetrahedron Letters, 549 (1963).

48. W. F. Johns and I. Laos, J. Org. Chem., 30, 123 (1965).

49. J. P. Kutney, J. Cable, W.A.F. Gladstone, H. W. Hansen, E. J. Torupka and W.D.C. Warnock, J. Am. Chem. Soc., (in print).

50. J. P. Kutney, A. By, T. Inaba and S. Y. Leong, Tetrahedron Letters, 2911 (1965).

51. Reference 47.

52. C. R. Hauser and B. E. Hudson, Organic Reactions, 1, 266, (1942)

53. W. A. Jacobs and L. C. Craig, J. Biol. Chem., 124, 659 (1938)

54. R. L. Augustine in "Catalytic Hydrogenation - Techniques and Applications in Organic Synthesis. pp 104-105, Marcel Dekker Inc. New York 1965

55. K. Saito, Bull. Chem. Soc. Japan, 15, 22 (1940)

56. H. Budzikiewicz, Tetrahedron, 20, 2267 (1964)

57. R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones, A. G. Long, J. F. Oughton, L. Stephenson, T. Walker and B. M. Wilson, J. Am. Chem. Soc., 4356 (1965)

58. W. G. Dauben and J. F. Eastham, J. Amer. Chem. Soc., 73, 4463 (1951)

59. We are grateful to Professor J. Tomko, Institute of Chemistry Slovak Academy of Sciences, Bratislava, Czechoslovakia for a sample of verarine.

60. H. Mitsuhashi, Chem, Pharm. Bull. (Tokyo) in print

61. K. Schreiber and G. Adam, Tetrahedron, 20, 1707 (1964)

62. Donated by Smith,Miller and Patch Inc. and Syntex S. A. Mexico

63. R. Hirschmann, C. S. Snoddy, C. F. Hiskey and N. L. Wendler, J. Am. Chem. Soc., 76, 4013 (1954)

64. H. Brockmann and H. Schroeder, Ber., 74 73 (1941)

65. A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, J. Chem. Soc., 2548 (1953)