THE TOTAL SYNTHESIS OF VERATRUM ALKALOIDS

BY

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ABSTRACT

The initial work toward the construction of the basic skeleton of verticine (88) and related Veratrum alkaloids is described.

Hecogenin (acetate) (115) was converted by a known method to rockogenin 12-methanesulfonate 3-pivalate (118), which gave in excellent yield 3β-pivaloyloxy-C-nor-D-homo-(25R)-5α,12α-spirst-13(18)-en (119b). Hydroboration of the 13(18) double bond is discussed with respect to the stereochemistry of the major product, namely 13β-hydroxymethyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirstan-3β-ol (121). The configuration at C-13 of (121) was reversed by means of epimerization of the aldehyde intermediate (148). 3β-Acetoxy-13α-acetoxymethyl-C-nor-D-homo-18-nor-5α,12α-spirostan (153) was prepared from (148) and the performic acid degradation of the spiroketal side chain was investigated The C_{21} pregnajervane ketone, namely 3β-acetoxy-13α-acetoxymethyl-18-nor-12α-pregnajervan-20-one (196) was obtained from the diacetate (153) with considerable difficulty. The difficulty was chiefly associated with selective hydrolysis of the performic acid oxidation product. Finally the ketone (196) was coupled with 2-lithio-5-methylpyridine. The coupling product was characterized after acetylation by n.m.r. and mass spectroscopy as 3β,18-diacetoxy-20-hydroxy-22,23,24,25,26-N-hexadehydro-5α,13β(H),17α(H)-veratranine (201).

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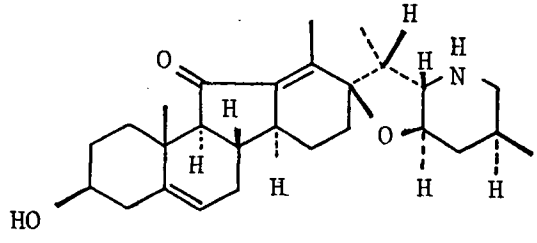
I wish to express my sincere thanks to Professor James P. Kutney for his unfailing help and constant encouragement during the course of my research. Thanks are also due to my colleagues for their invaluable suggestions and stimulating discussions.

INTRODUCTION

The first reported chemical investigations of a plant of the tribe Veratreae (Veratrum album) appeared as early as 1820.[1] One of the component alkaloids, jervine, was first obtained crystalline in 1837 by Simon.[2] Development of various analytical techniques, particularly that of chromatography and liquid-liquid countercurrent extraction, has resulted in the isolation of a number of steroid alkaloids in the form of glycosides, esters and free alkamines, and the genera which have been submitted to investigations are Veratrum, Zygadenus, Stenanthium, Amianthium, Melanthium, Fritillaria, etc. These steroid alkaloids are now recognized as the Veratrum alkaloids.

Extensive examination of the products of selenium dehydrogenation and correlative chemical evidence led Fried, Wintersteiner et al.[3] in 1951 to propose the structure of jervine ($C_{27}H_{39}O_3N$) (1). This postulate has been subsequently established through a series of investigations.[4-15] The unusual feature of this compound is characterized by the C-nor-D-homo steroid skeleton which had not been described previously. In 1953, Jacobs and Pelletier[16] demonstrated that the same steroid ring system is present in other members of the Veratrum alkaloids, cevine ($C_{27}H_{43}O_8N$: now known to be an artifact of the true

alkaloid veracevine), germine and protoverine. The first complete structure for veracevine (2) was proposed in 1954[17] and it was further elaborated to the true structure in 1959.[18] The latter has since been confirmed by X-ray diffraction studies of the hydriodide.[19] Jervine and veracevine represent the two distinctly different groups of the Veratrum alkaloids. On the basis of structural characteristics Fieser and Fieser[20]



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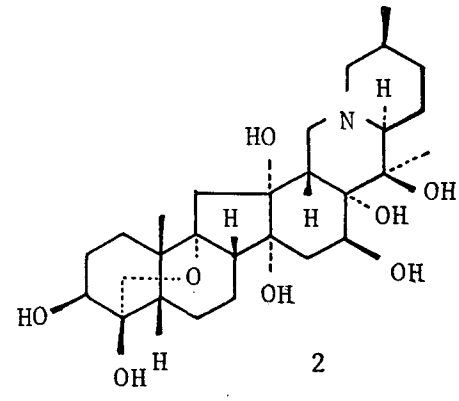

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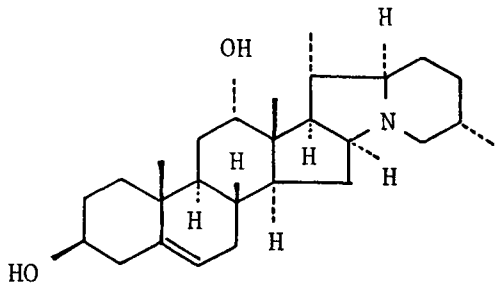
proposed the subdivision of the Veratrum C_{27} bases into the Jerveratrum and Ceveratrum groups. The Jerveratrum alkamines contain only 2 or 3 atoms of oxygen and are found to occur in part as unconjugated free alkamines and in part in combination with one molecule of D-glucose as glucoalkaloids. Rubijervine (3), jervine (1), veratramine (4), verarine (5) are cited as examples of the Jerveratrum alkamines. The Ceveratrum alkamines are highly hydroxylic, usually containing 7 to 9 oxygen atoms, and are found as ester alkaloids but have never been found as glycosides. They all contain the cevane nucleus (6), as proposed by Jacobs and Pelletier,[16] characterized by a hexacyclic ring system with the C-nor-D-homo steroid skeleton and folding of the normal



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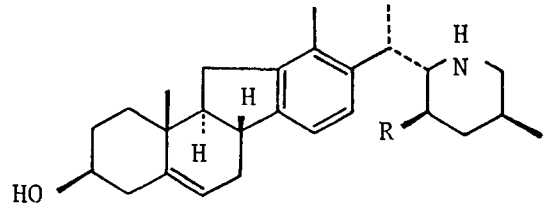
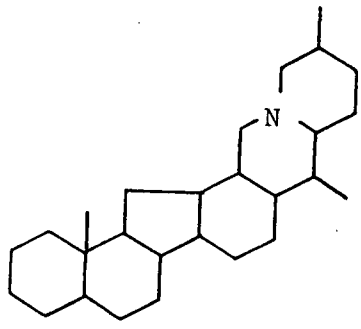


4 R = OH

5 R = H

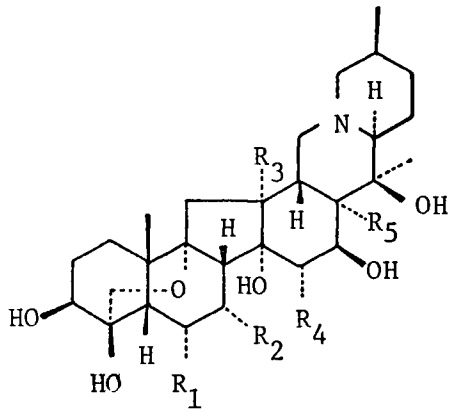


6

cholesterol side chain around a nitrogen atom. There are presently five naturally occurring Ceveratrum alkamines, namely zygadenine (7), veracevine (8), protoverine (9), germine (10) and sabine (11).

Kupchan and By[21] in their recent review on the Veratrum family recognized an emerging group of alkaloids now normally referred to as the Fritillaria alkaloids since they are obtained from plants of the genus Fritillaria. Verticine ($C_{27}H_{45}O_3N$) (12), being a representative and most thoroughly investigated member of this group, contains the cevane nucleus (6) but is much less hydroxylic than the Ceveratrum alkaloids.

As an increasing number of Veratrum alkaloids has been isolated and their structures determined during the last decade, a somewhat new aspect has become obvious, namely a closer phytochemical relationship with the alkaloids from plants of the genus Solanum. Among the recently characterized alkaloids from Veratrum album subsp. lobelianum are veralobine (13),[22-24] veralkamine (14),[25-28] veralinine (15),[28-30] veramine (16),[27-29,31,32] verazine (17),[33] and veracintine (18).[34]

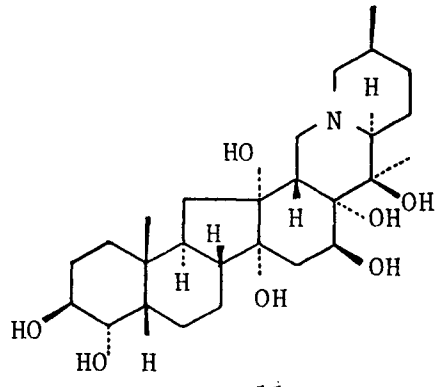


7 $R_1=R_2=R_3=R_5=H$; $R_4=OH$

8 $R_1=R_2=R_4=H$; $R_3=R_5=OH$

9 $R_3=R_5=H$; $R_1=R_2=R_4=OH$

10 $R_1=R_3=R_5=H$; $R_2=R_4=OH$



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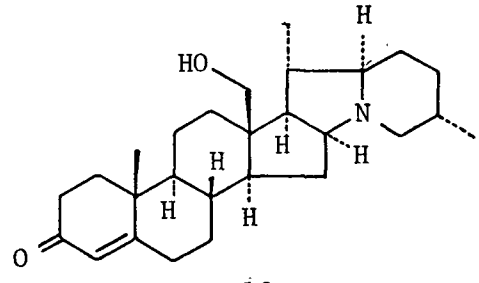

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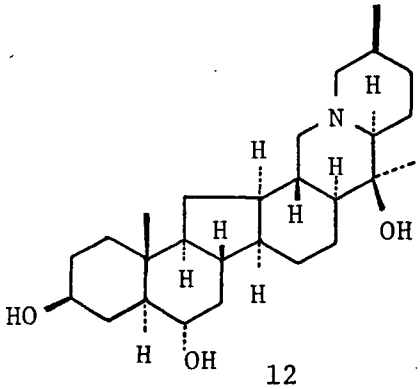

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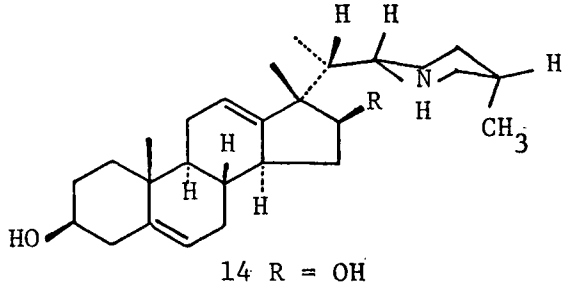
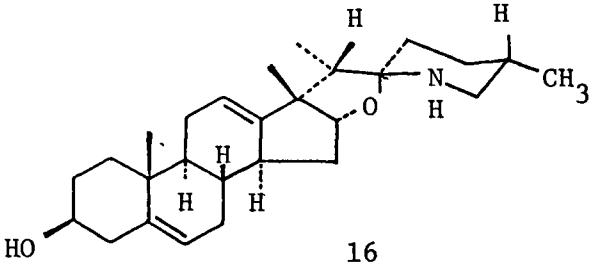


14 R = OH

15 R = H



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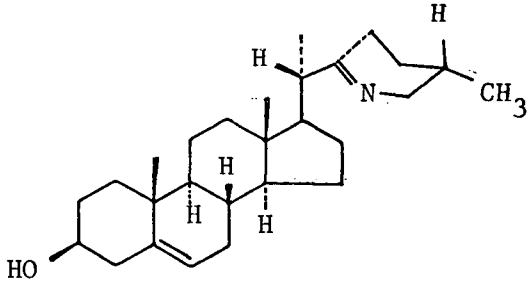

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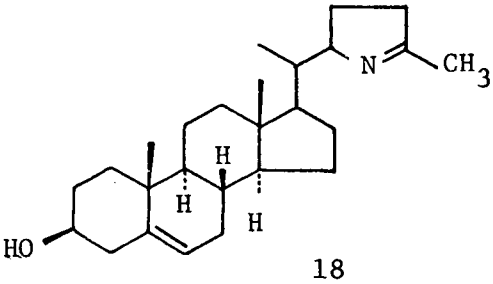
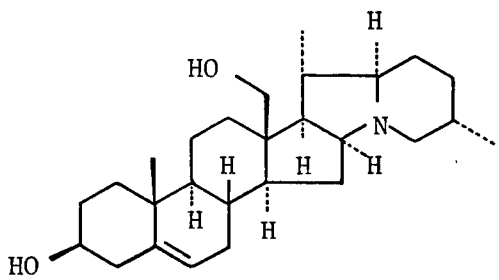


18

Figure 1. Examples of Veratrum alkaloids.

Veralobine (13) is closely related to isorubijervine (19) and is of the solanidane type. Veralkamine (14), veralinine (15) and veramine (16) not only represent a totally new family of steroid alkaloids which possess the 17β-methyl-18-nor-cholestane skeleton, but also bear close similarities to some of the Solanum alkaloids. Verazine (17) also finds its counterpart in a Solanum alkaloid solacongestidine (19a).[35]



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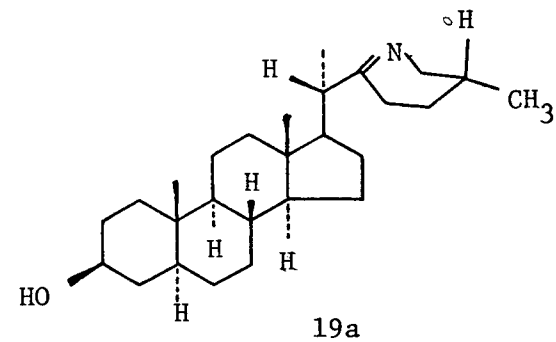


19a

Veracintine (18) is quite unique in that it is the first C_{26} alkamine and has a pyrroline ring. Thus, it seems that there is an increasing demand for a new, systematic reclassification of the Veratrum alkaloids, especially within the Jerveratrum series, at least from the structural point of view.

Reviews on various aspects of the chemistry of Veratrum alkaloids have been published by McKenna,[36] Prelog and Jeger,[37] Stoll,[38] Morgan and Barltrop,[39] Fieser and Fieser,[20] Jeger and Prelog,[40] Boit,[41] Narayanan,[42] Kupchan and By,[21] Schreiber,[43] and Brown.[44]

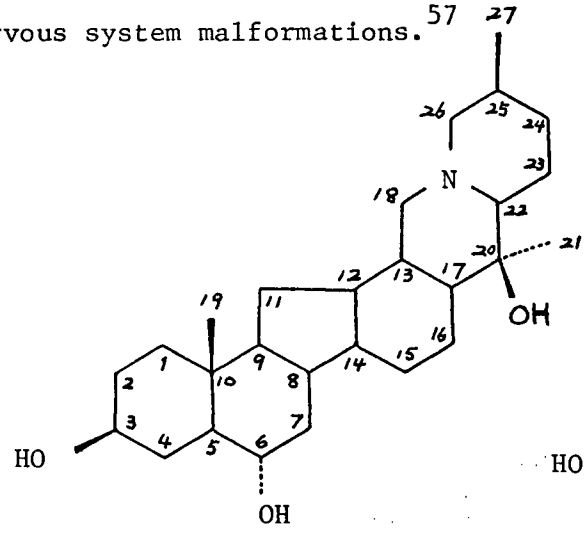
The use of Veratrum and related plants as natural insecticides and in control of certain types of hypertension has been reported since the middle of the nineteenth century. An insecticidal alkaloid mixture

"veratrine" prepared by E. Merck and Co. from the dried ripe seeds of Veratrum sabadilla has been in practical use for a long time[45] along with the pyrethrum extract from the flower heads of Chrysanthemum cinerariaefolium. The principal constituent of "veratrine" is cevadine (veracevine 3-angelate), first isolated in 1855 as "crystalline veratrine".[46]

Crude extracts of Veratrum and related plants have also been used since the middle ages in the treatment of circulatory disorders, fevers and tachycardia. Their first recorded use in controlling hypertension dates back to 1859.[47] Use of crude extracts, however, gave erratic results and their usage was eventually discontinued. The limiting factor in the employment of this drug is that the therapeutic dosage is dangerously close to the emetic level, and the margin decreases upon continued exposure. During the late 1930's pure ester alkaloids of protoverine (9) became available and some of them were shown to have a potentially antihypertensive activity.[48,49] Several laboratories then became engaged in extensive isolation and pharmacological characterization of esters of Ceveratrum alkaloids. The pharmacology of the Veratrum alkaloids has been reviewed in detail.[50-54] Since the therapeutic dosage range could not be improved, interest dropped off on the advent of a superior antihypertensive agent namely reserpine.

A noteworthy feature of the alkaloids of Veratrum californicum is their teratogenic activity toward sheep.[55,56] An alkaloid cyclopamine, the structure of which seems to resemble 11-deoxojervine, has

been found responsible for epidemic cyclopia and related central nervous system malformations.[57]



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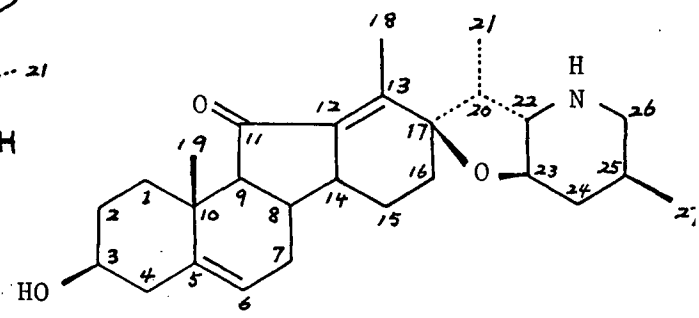
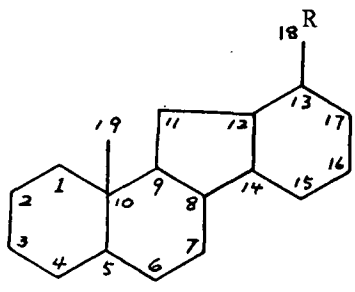


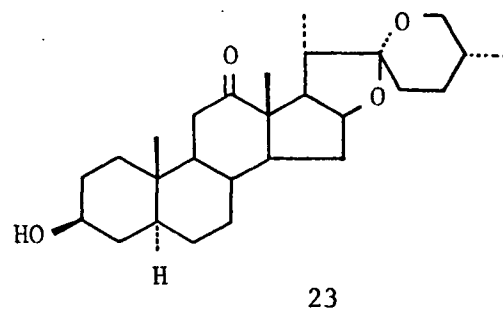
21

The nomenclature and the basic numbering system to designate the parent hydrocarbons of the Veratrum alkaloids have undergone a series of changes as new products were characterized and new families were recognized. The numbering scheme presently accepted for the C_{27} C-nor-D-homo steroid skeleton is depicted for verticine (20) and jervine (21). Fried and Klingsberg[58] proposed the terms "jervane" and "etio-jervane" to represent the carbon skeleton of jervine (21) and the parent tetracyclic C_{19} hydrocarbon (22, R = CH_3) respectively. On this basis the fundamental skeleton for many of the compounds discussed in this thesis, which lacks the methyl group at C-18, must be named



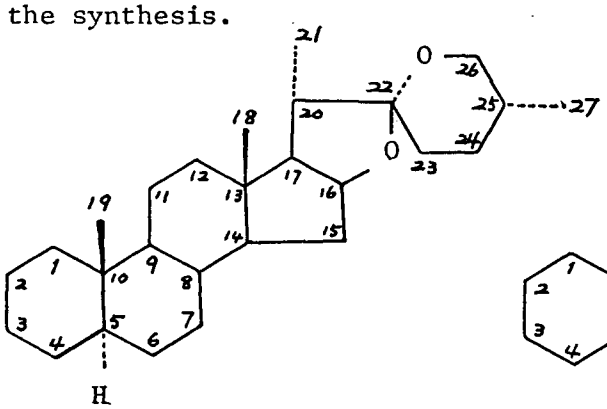
22 R = CH_3

22a R = H

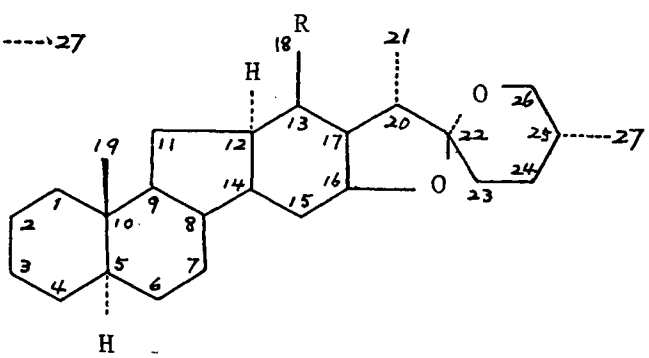


23

18-nor-etiojervane (22a, R = H). Since the earlier stages of this work are concerned with transformations of hecogenin (23), the spirostan nomenclature[59] will be used for compounds containing the intact spiroketal side chain, unless there exists a conventional name for a compound. Thus the hypothetical spiroketal steroid (23a) is (25R)-5α-spirostan and the C-nor-D-homo derivative (24, R = CH_3) is C-nor-D-homo-(25R)-5α,12α-spirostan and (24a, R = H) is C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan. The numbering system here differs from that of Hirschmann et al.[110] and Johns,[148] but it is much preferable for the sake of clarity and consistency with the subsequent etiojervane derivatives obtained in the later stages of the synthesis.



23a



24 R = CH_3

24a R = H

For the past decade several groups have been engaged in the synthesis of some Veratrum alkaloids. Masamune[60,61] and Johnson[62] have recently published the results of their successful synthetic schemes. Masamune has synthesized veratramine[60,61] and jervine,[60] while Johnson[62] has succeeded in a total synthesis of veratramine. Both groups have employed 17-acetyl-5α-etiojerva-12,14,16-trien-3β-ol (3-acetate) (25)[63-65]

as the source of the C_{21} C-nor-D-homo steroid portion upon which attachment of the appropriately substituted piperidine was performed to provide the jervane skeleton.

An outline of Masamune's synthesis of jervine is given in Figure 2. The relay compound (25), obtained from degradation of hecogenin (23)

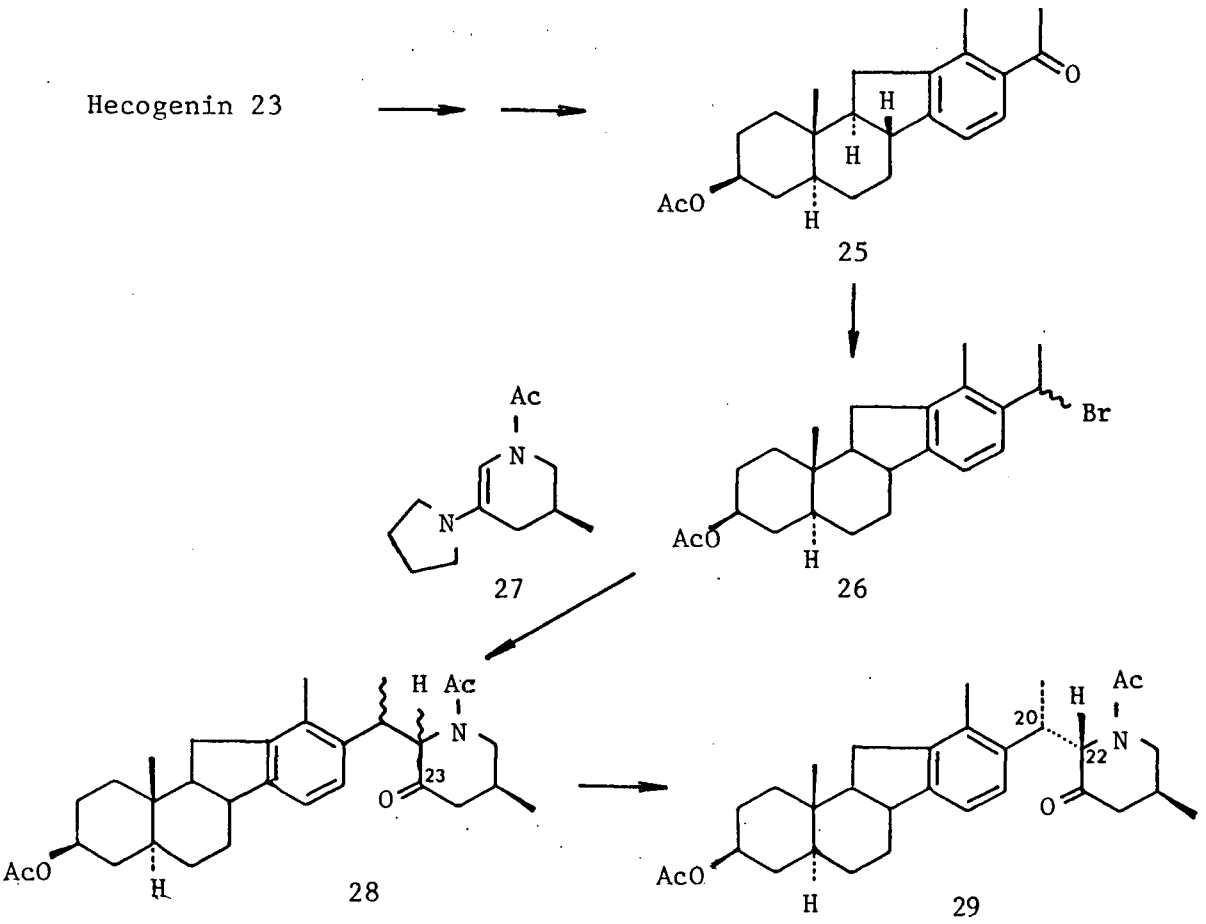


Figure 2. Masamune's synthesis of Jervine (1).

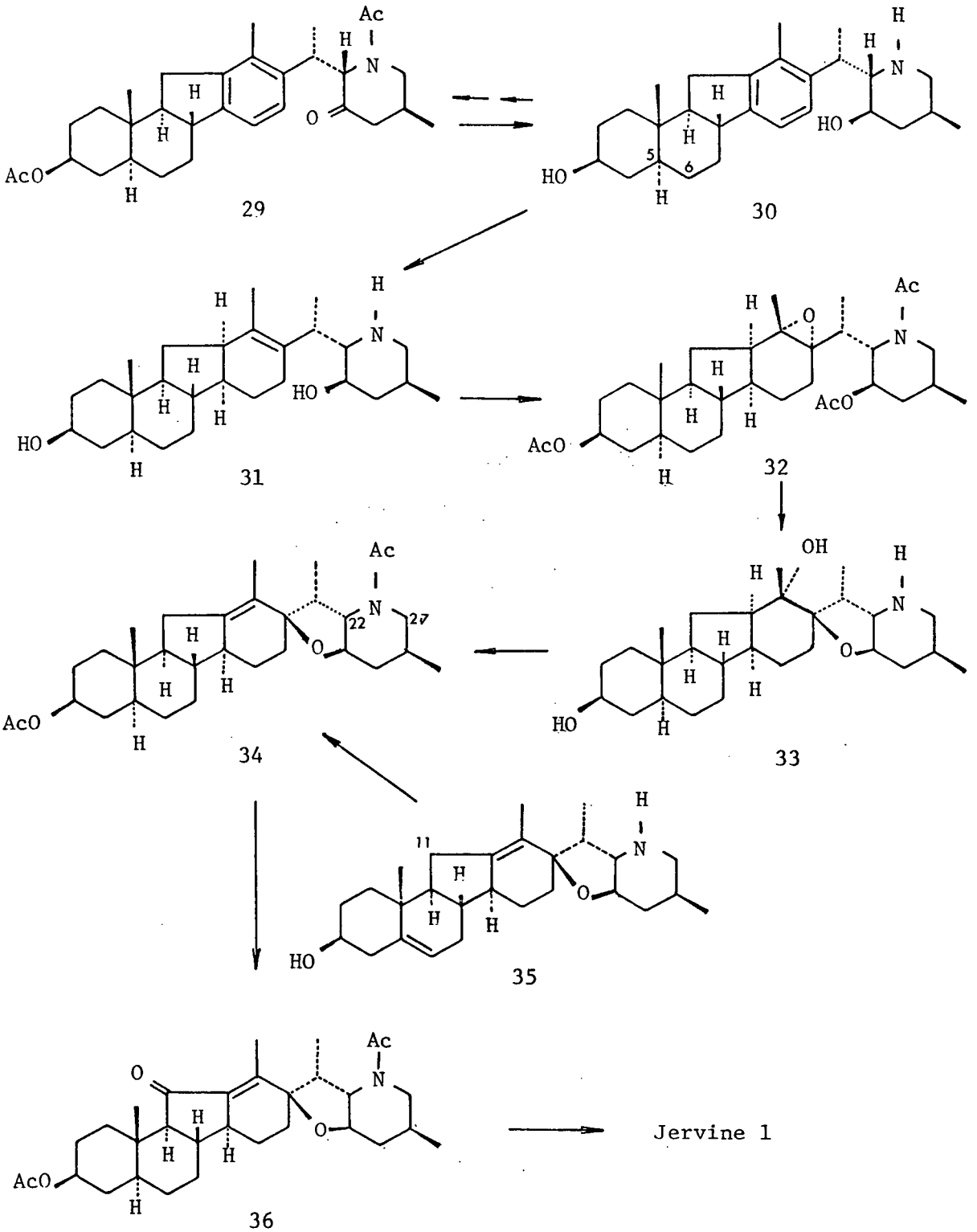


Figure 2 (continued). Masamune's synthesis of Jervine (1).

via a known sequence, was converted to the C-20 bromo derivative (26) which was then reacted with an excess of the pyrrolidine enamine of optically active 1-acetyl-3-(S)-methyl-5-piperidone (27) to given an isomeric mixture of 3,N-diacetyl-5α,6-dihydro-23-dehydroveratramines (28). The isomer (29) with the desired configurations at C-20 and C-22 was identified by comparison with an authentic sample prepared from 5α,6-dihydroveratramine (30). The second relay compound (29) was then transformed to dihydroveratramine (30), and the latter after being reduced with lithium in ethylamine containing 2-propanol was immediately hydrogenated to the third relay, 22,27-iminojervan-13(17)-ene-3β,23β-diol (31). The compound (31) had previously been obtained[66] from 11-deoxojervine (35), and the structure was confirmed through this total synthesis.

The formation of the 17β,23β-ether bridge was achieved by an elegant series of stereospecific reactions, namely epoxidation of (31) followed by intramolecular cyclization with concomitant cleavage of the α-epoxide ring. Low temperature dehydration with thionyl chloride in pyridine yielded 3,N-diacetyl-11-deoxo-5α,6-dihydrojervine (34) which was compared with an authentic sample prepared[67] from 11-deoxo-jervine (35) in an unambiguous manner. The introduction of the C-11 keto group with chromic anhydride and pyridine gave in poor yield (1%) the α,β-unsaturated ketone (36) which was then converted in a 2% yield to jervine (1).

W.S. Johnson and his co-workers in their total synthesis of veratramine (4) first synthesized[62] the relay compound 17-acetyl-5α-etiojerva-12,14,16-trien-3β-ol (25) from Hagemann's ester (37) via a

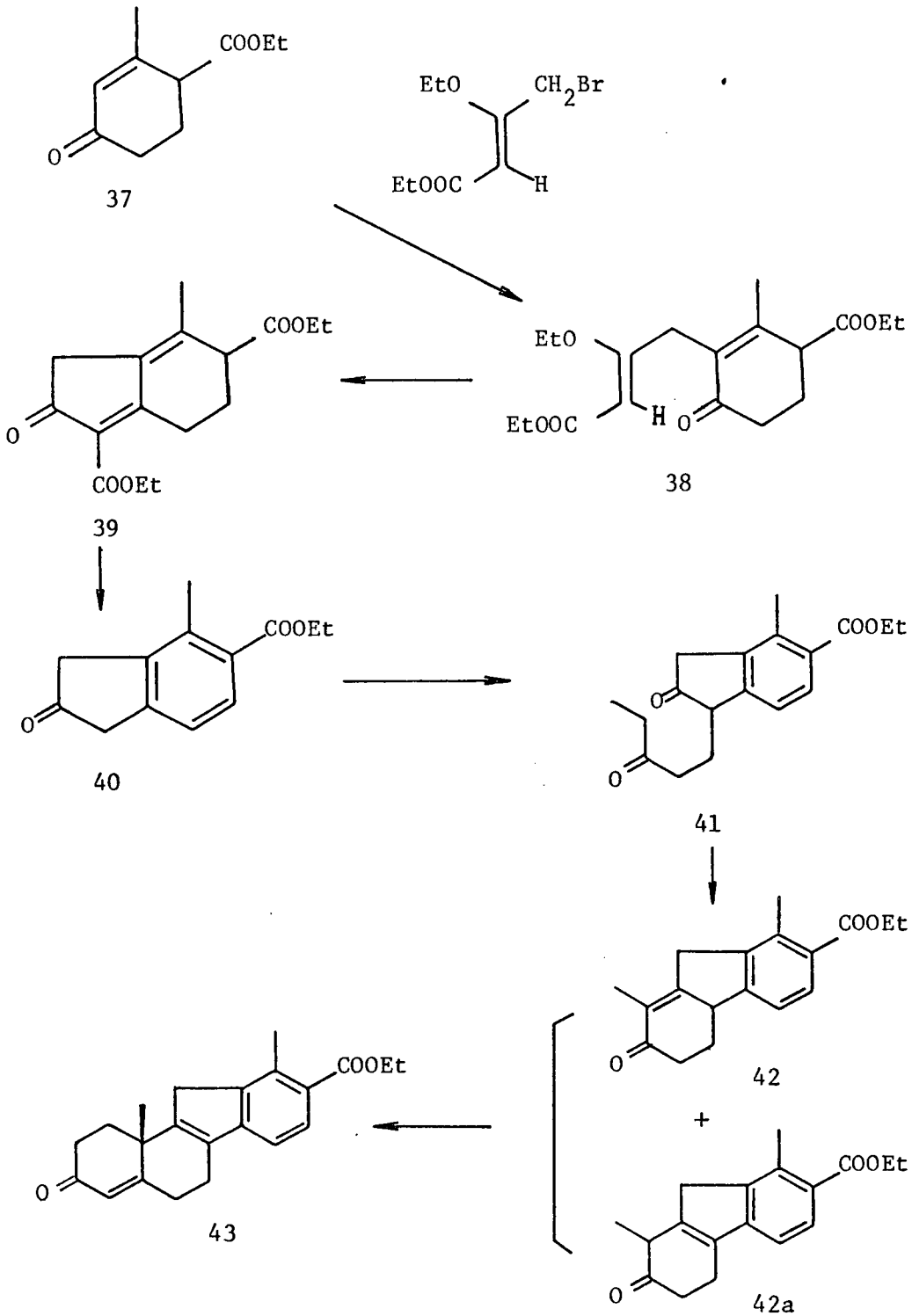


Figure 3. Johnson's synthesis of Veratramine (4) - Part 1.

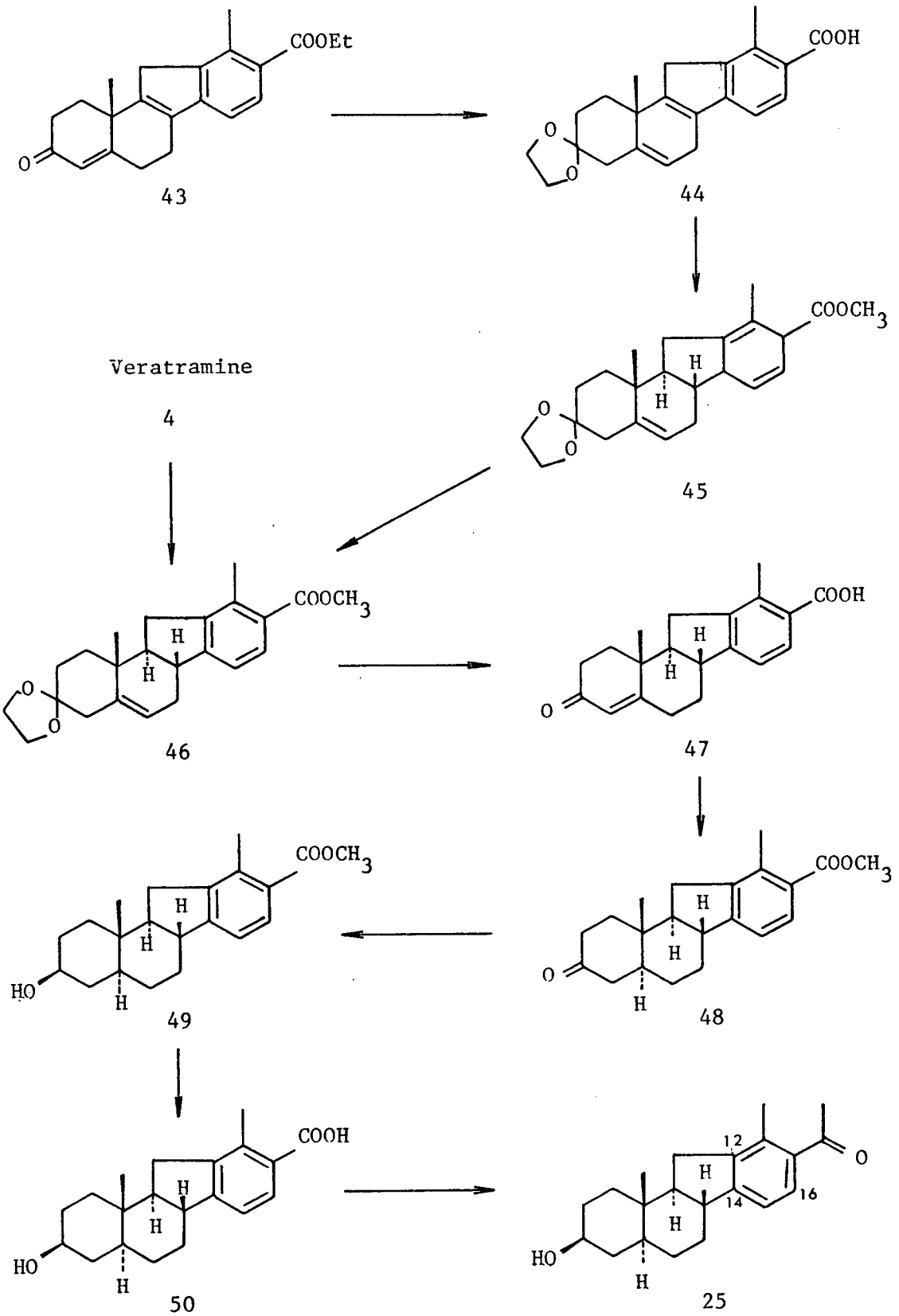


Figure 3. Johnson's synthesis of Veratramine (4) - Part 2.

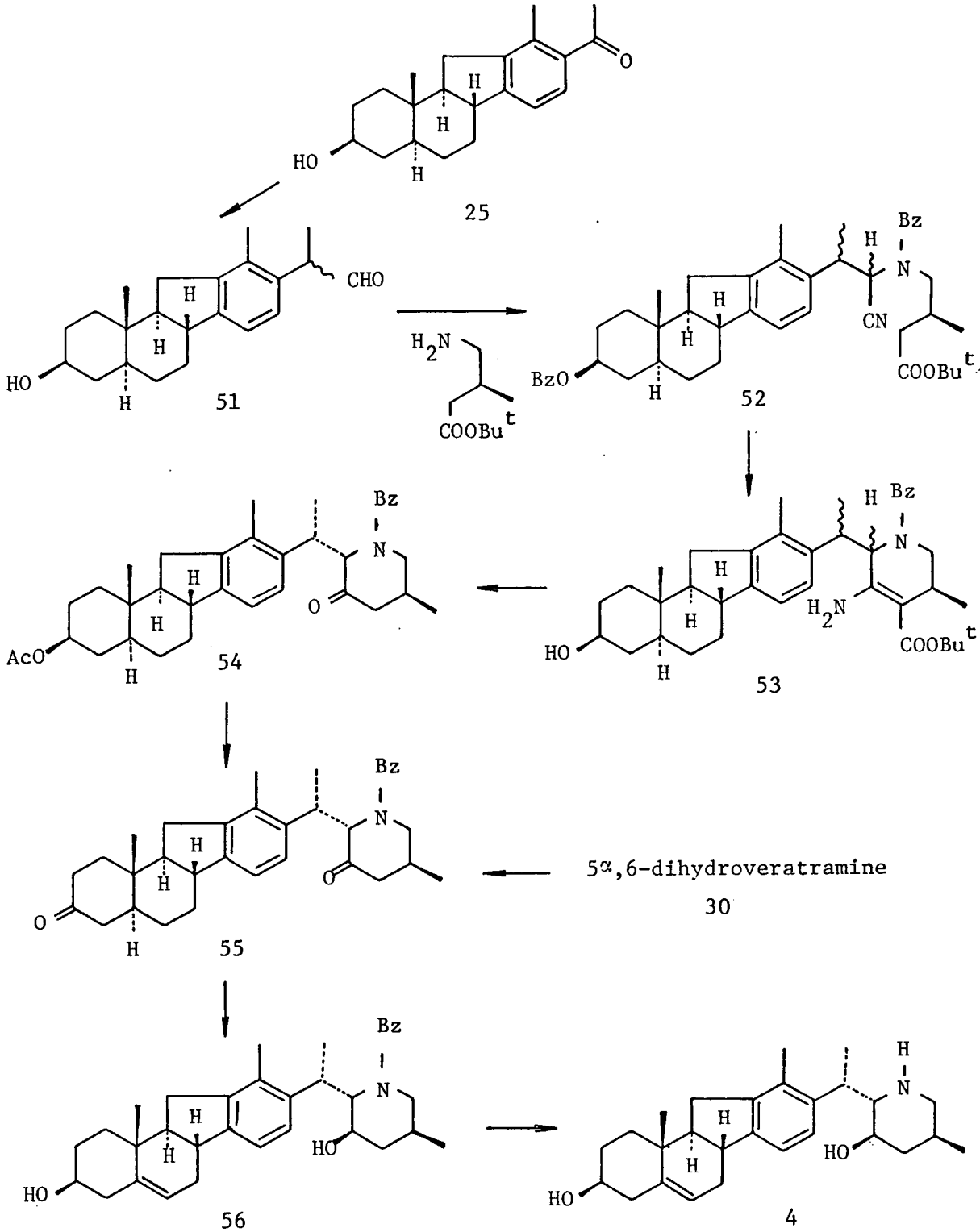


Figure 3. Johnson's synthesis of Veratramine (4) - Part 3.

sequence outlined in Figure 3. This compound (25) has also been prepared[64] from hecogenin (23), which has, in turn, been synthesized from the totally synthetic isoandrosterone.[68] The same group has recently developed an alternative synthetic route[69] to (25) by an extension of the hydrochrysene method.

Johnson's synthesis of veratramine is characterized by the ingenious method of building up the substituted piperidine ring. After conversion of the methyl ketone (25) to the aldehyde (51), a Strecker reaction with t-butyl ℓ-3-methyl-4-aminobutyrate gave, after benzoylation, the cyano ester (52) as a mixture of diastereoisomers. The cyclization of (52) to the enamino ester (53) was effected by treatment with excess methyl sulfinylcarbanion in dimethylsulfoxide. The enamino ester (53), on acid treatment, yielded the ketone (54) which was further converted to the diketone (55) and was identified by comparison with an authentic specimen produced from 5α,6-dihydroveratramine (30). The diketone (55) was then successively submitted to reduction, benzoylation, and partial hydrolysis, and the resulting 3β-ol derivative was converted *via* established procedures[70,71] to the Δ^5-3β-ol derivative, which, upon hydrolysis, produced a sample of veratramine (4) identical with the material from the natural source.

Our own research group has for some years now been engaged in the development of a general synthetic route leading to the Veratrum alkaloids. Part of this work has been published in several communications which describe the total synthesis of verarine (5)[72] and veratramine (4).[73]

In this approach 3β-acetoxy-13α-etiojervan-17-one (69) was chosen as the common relay intermediate. This compound (69) is also readily available by degradation of hecogenin (23).[63,64] β-Naphthol (57) was first converted to the diol aldehyde (66) via a multistep sequence reproduced in Figure 4. The diol aldehyde (66), on treatment with sodium acetate in refluxing acetic acid, gave the olefin (67). This olefin was then transformed by a series of reactions to the racemic relay compound (69) which was shown to be identical with 3β-acetoxy-13α-etiojervan-17-one obtained from hecogenin. The 12,13-double bond was reintroduced, and the heterocyclic portion was attached by means of a coupling reaction of the anion of 2-ethyl-5-methyl pyridine with the α,β-unsaturated ketone (70) to afford a mixture of diastereoisomers from which the desired isomer (71) could be isolated. Aromatization of ring D and selective hydrogenation of the pyridine ring led to a mixture of isomers from which 3-O-acetyl-5α,6-dihydroverarine (72) was isolated. This compound was converted to N-acetyl-5α,6-dihydroverarine (73) into which the 5,6-double bond was introduced by established procedures.[70,71] The final hydrolysis of N-acetyl group completed the total synthesis of verarine (5).

The synthesis of veratramine (4)[73] was also achieved in a similar fashion along the general scheme described above using 2-ethyl-5-methyl-3-methoxypyridine (74) and the relay compound (70).

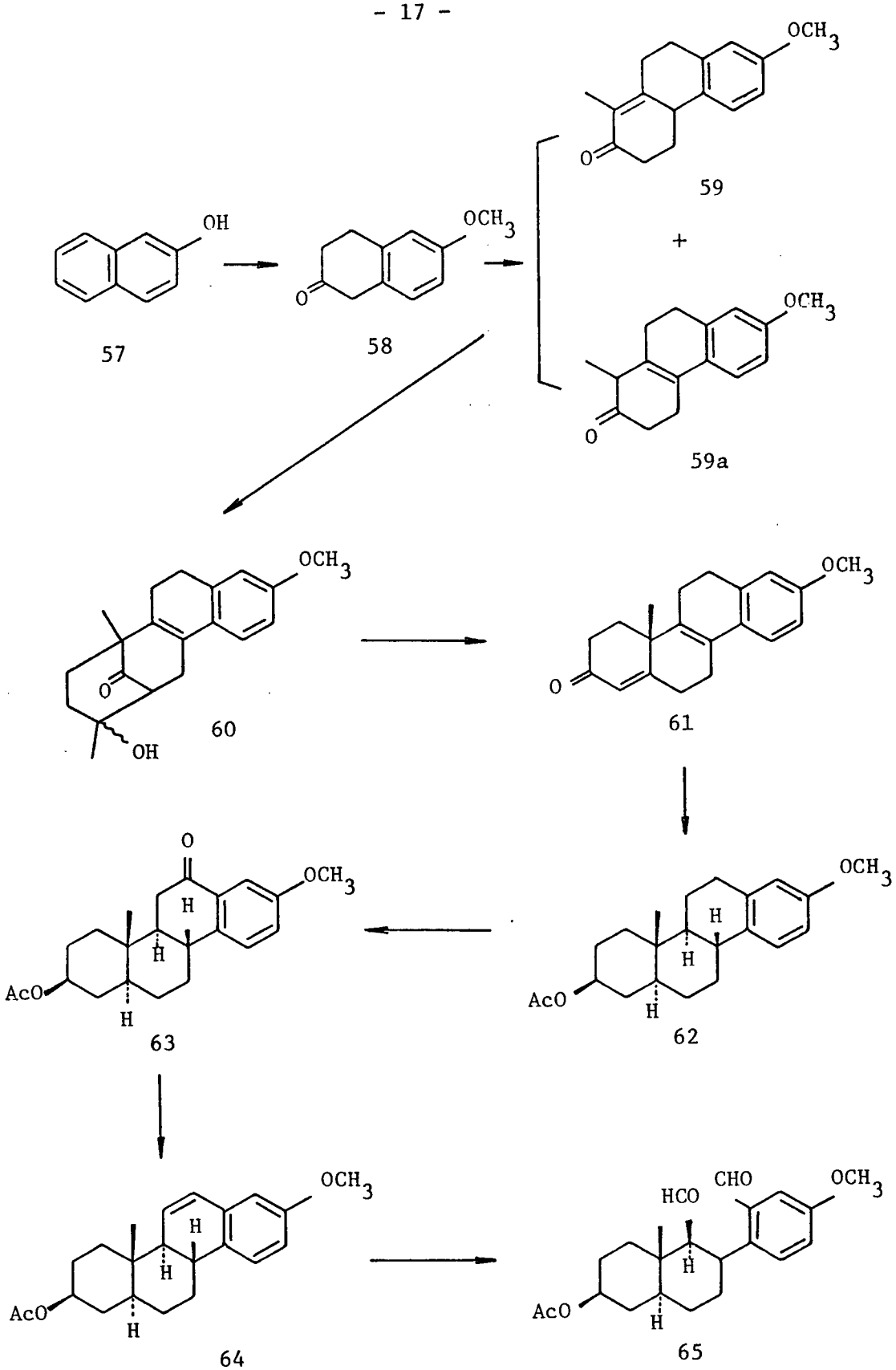


Figure 4. Kutney's synthesis of Veratramine (4) and Verarine (5) - Part 1.

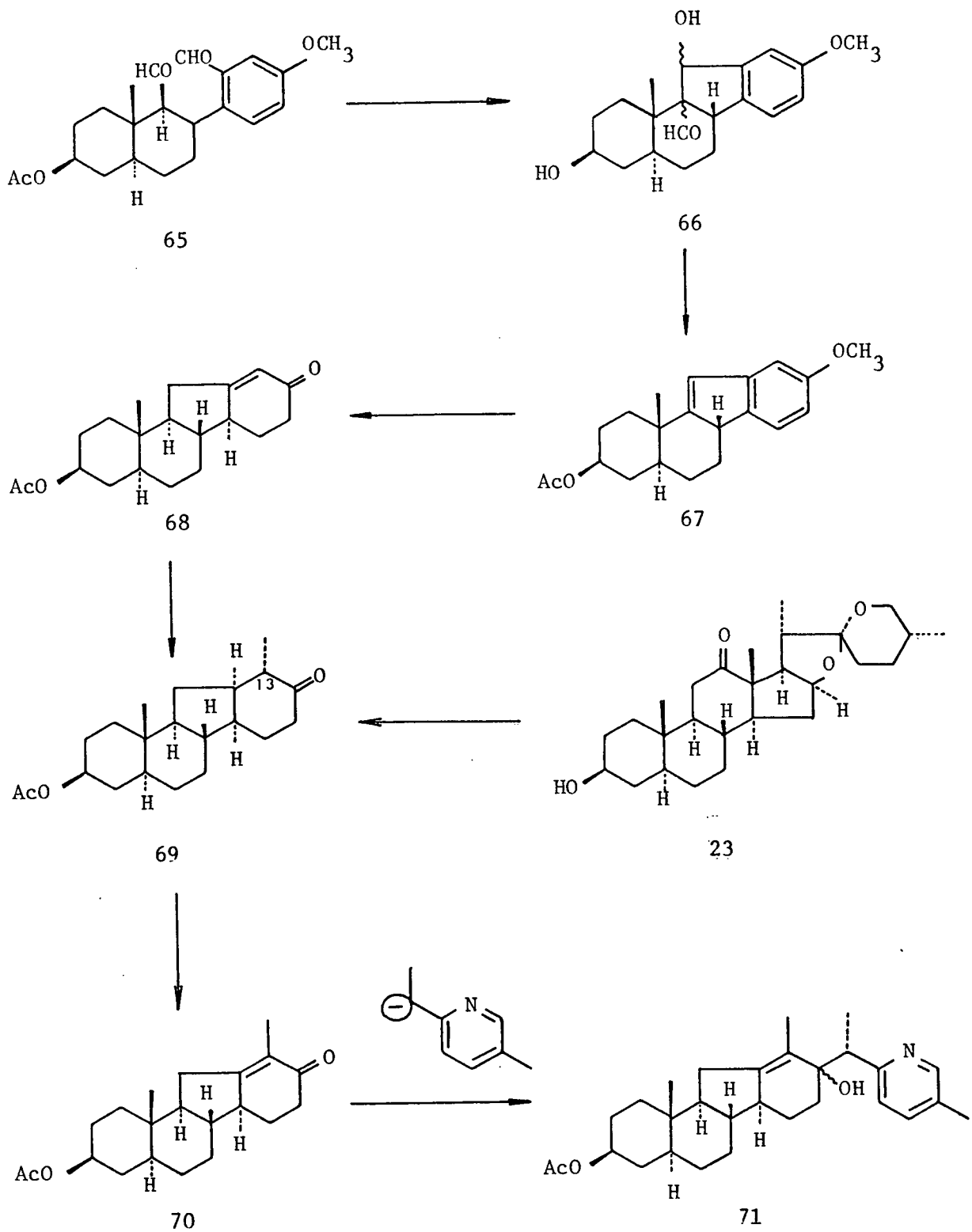


Figure 4. Kutney's synthesis of Veratramine (4) and Verarine (5) - Part 2.

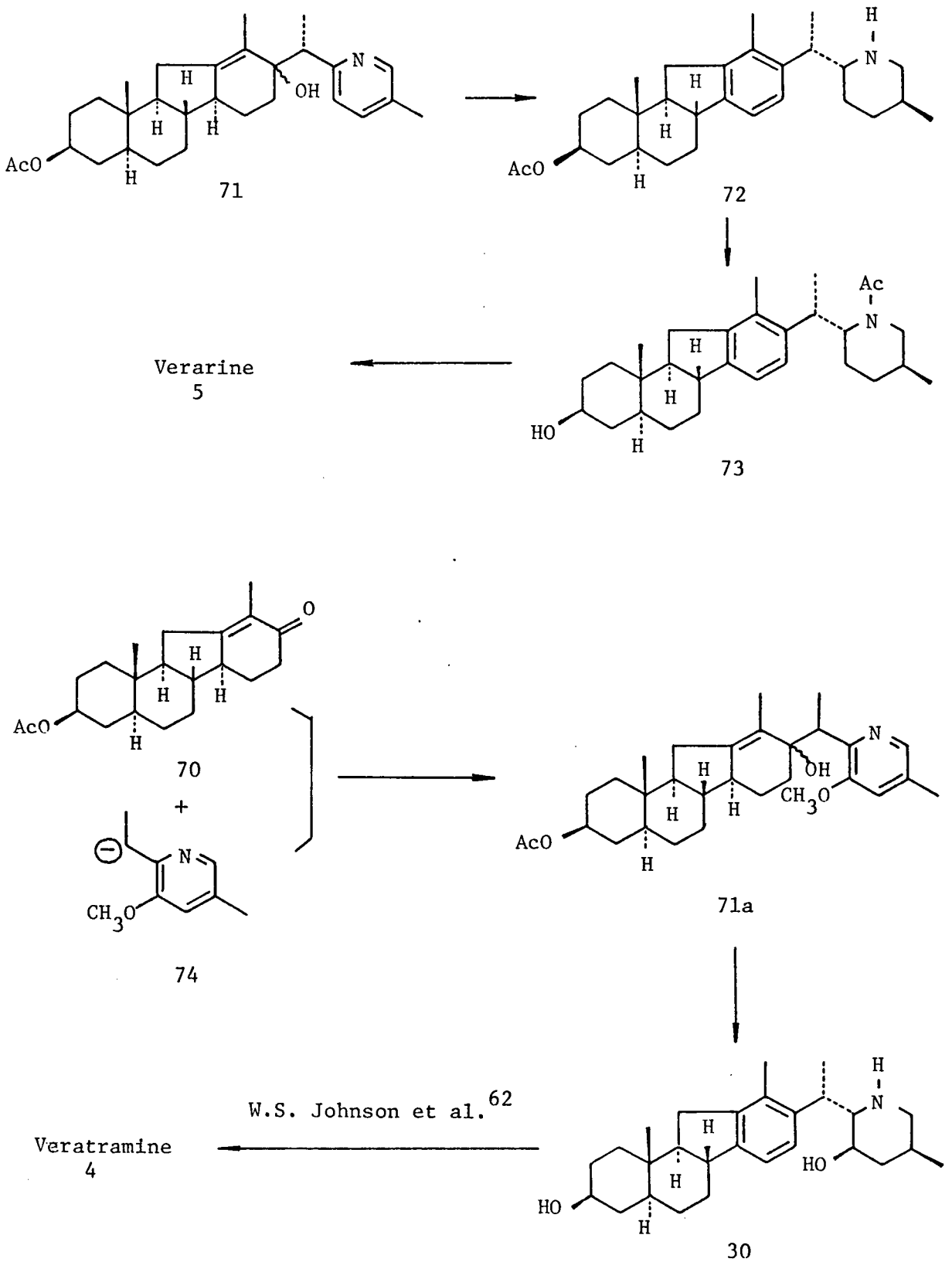
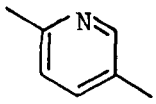


Figure 4. Kutney's synthesis of Veratramine (4) and Verarine (5) - Part 3.



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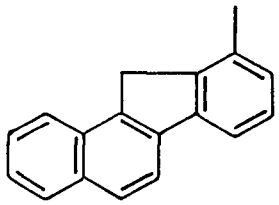

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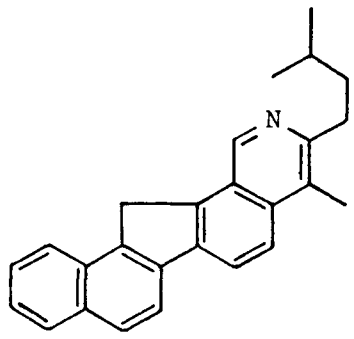

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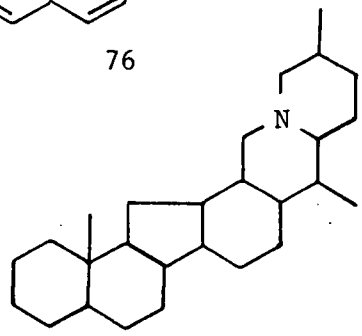

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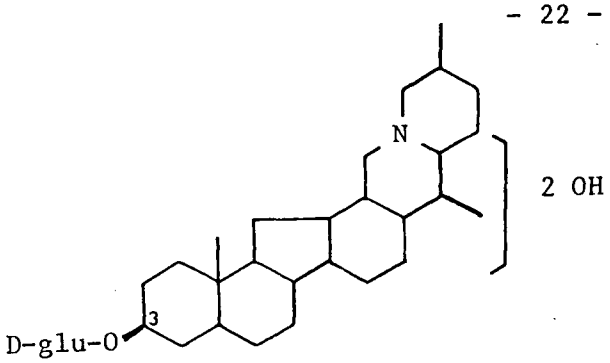
Since this thesis is concerned with development of a synthetic route leading to the hexacyclic cevane skeleton (78), it appears appropriate to outline the work which has been done on verticine (peimine) and verticinone (fritillarine, peiminine).

In 1929 Fukuda[75,76] reported the isolation of four alkaloids, namely verticine, fritillarine, verticilline and another amorphous base, from the corms of Fritillaria verticillata Willd. var. Thunbergii Baker. Another investigation by Chou and Chen[77,78] on the Chinese drug pei-mu (extracts of the dried corms of Fritillaria roylei Hook) has resulted in isolation of three physiologically active, crystalline alkaloids, peimine, peiminine and fritimine. Subsequently peimine and peiminine have been shown to be interconvertible via chromic acid oxidation and sodium-ethanol reduction.[79,80] Peimine, as was first suggested by Chi et al.[81] has been established to be identical with verticine by direct comparison.[82,83] Formation of 2,5-lutidine (75),[80,84] 8-methyl-1,2-benzofluorene (76)[80,84] and veranthridine (77)[16,85,86] has

been demonstrated by examination of the dehydrogenation products of verticine. This result indicated that verticine, which was shown to be a C_{27} alkamine,[87] contains the hexacyclic cevane nucleus (78), since these three compounds had also been found in the selenium dehydrogenation products of cevine, which as already mentioned earlier, is an artifact of the true alkaloid veracevine present in Veratrum.[16,88,89] The molecular formula for verticine has been established as $C_{27}H_{45}O_3N$.[82,87] Chou and Chu[90] showed the presence of two easily acylable hydroxyl groups in verticine, whereas the third oxygen atom was shown to be a tertiary hydroxyl group.[82,86,97] Since verticine affords a methiodide[87,92] with methyl iodide and a nitrite salt[93] with nitrous acid, the nitrogen atom is present as a saturated tertiary amine. Verticine shows a negative result in the Liebermann-Burchard reaction[93] and does not form a sparingly soluble digitonide.[87,93]

Morimoto and Kimata[93] isolated verticine and a D-glucoside of verticine from Fritillaria Thunbergii MIQ. and assigned the partial structure (79) to the new glucoalkaloid by assuming the cevane skeleton for verticine. They placed a hydroxyl group at the C-3 position on the basis of biogenetic considerations. In a later paper[94] the same authors confirmed that verticine has no ketonic group[90,92] and demonstrated that the two acylable hydroxyl groups are located on six-membered rings. Oxidation of verticine with chromic acid afforded a diketone derivative,[85,94] verticinedione, which did not exhibit any properties characteristic of α,β-diketones or amides.

Ito and co-workers[82] reported in their initial paper that two ketones, verticinone (81) and verticinedione (82), were obtained from



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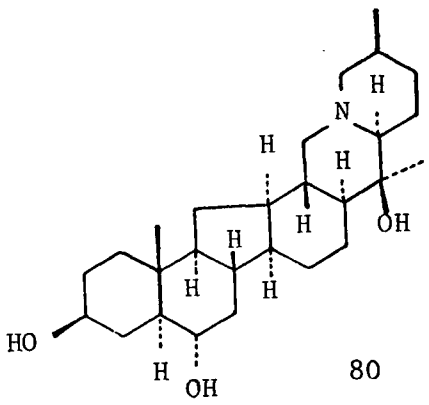

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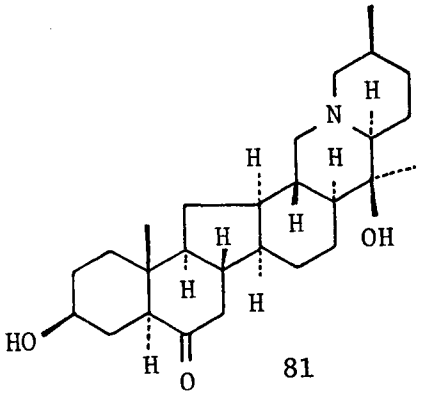

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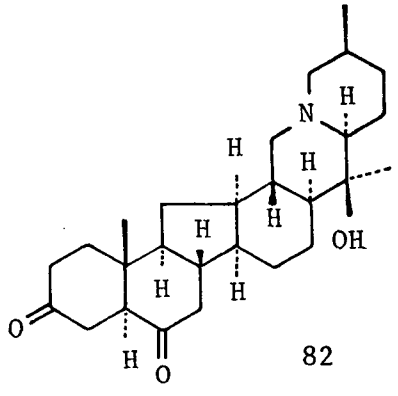

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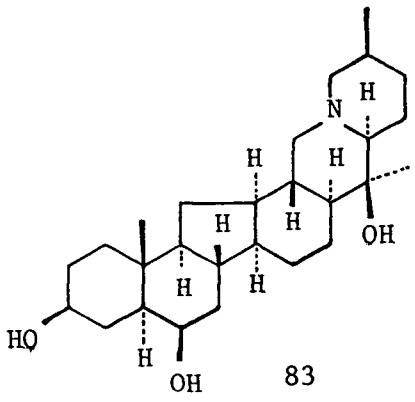

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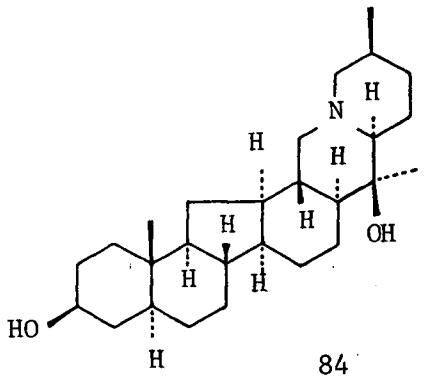

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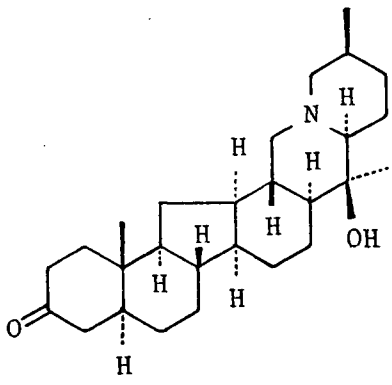

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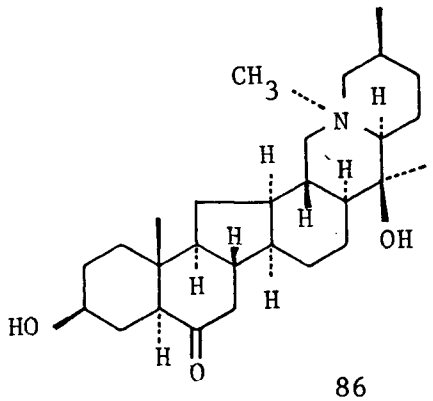


86

the chromic acid oxidation of verticine (80). Verticinone (81) was shown[82] to be identical with purified fritillarine[75,76] which is presumably also identical with verticilline[75,76] and peiminine[77] (melting point behavior). Verticinone (81) gave, on chromic acid oxidation, the dione (82) which was resistant to further oxidation. Both (81) and (82) could be converted to verticine (80) by reduction with sodium-ethanol or lithium in liquid ammonia in the presence of methanol.[83] However, reduction of both ketones with sodium borohydride, lithium aluminum hydride, aluminum isopropoxide, or by catalytic reduction (platinum in ethanol), resulted in formation of isoverticine (83).[83] Since both verticine and isoverticine gave verticinedione (82) on chromic acid oxidation, these compounds were considered to be epimeric at one of the secondary hydroxyl groups. On the basis of reactivity toward N-bromosuccinimide and relative stability in the sodium butoxide-butanol system, both secondary hydroxyl groups in verticine (80) were considered to be equatorial. On Huang-Minlon reduction verticinone (81) afforded deoxoverticinone (84) which was, in turn, converted by chromic acid to the corresponding ketone, dehydrodeoxoverticinone (85). The ORD curve of the ketone (85) showed a positive Cotton effect, in accord with that of cholestan-3-one. The other secondary hydroxyl was first tentatively placed at C-7[82] on the basis of the sign and shape of the ORD curve of verticinone (81) and its negative Zimmermann test.[95] However, additional chemical evidence led Ito and co-workers to place the second hydroxyl group at C-6. A small but clear band at 2760 cm^{-1} in the infrared spectra of verticine and its derivatives was advanced in support of the presence of a trans-quinolizidine system in these compounds. This postulate was

supported by the fact that the same band was absent in the spectrum of verticine N-oxide diacetate.

The NMR spectra of verticine derivatives[83] exhibited the presence of three methyl groups one of these appearing as a doublet and the other two as singlets. The chemical shift of one of the singlets varied with the nature of the substituents at C-3 and C-6 and these characteristic changes formed the basis for assignment of partial stereochemistry of verticine (80) as a 5α-cevane-3β,6α-diol. The site and configuration of the tertiary hydroxyl group was determined on the basis of the basicity of verticine, the intramolecular hydrogen bonding, and the base peak at m/e 112 in the mass spectrum. The configuration of the C-27 methyl group was indicated to be axial from the chemical shifts and coupling constants of the aforementioned doublet, which were qualitatively in accord with those of cevine derivatives.[96] The stereochemistry at positions C-8, C-9, C-12, C-14, C-16, and C-17 was undetermined. However, by analogy with the steroid series and from biogenetic considerations the authors proposed that the stereochemistry at these positions is as depicted in (80).[83] The correctness of this proposed structure was more recently confirmed by an X-ray analysis of verticinone methobromide (86).[97]

Partial characterization and degradation studies of verticine and verticinone have been described by Chi et al.,[98] and Wu.[99]

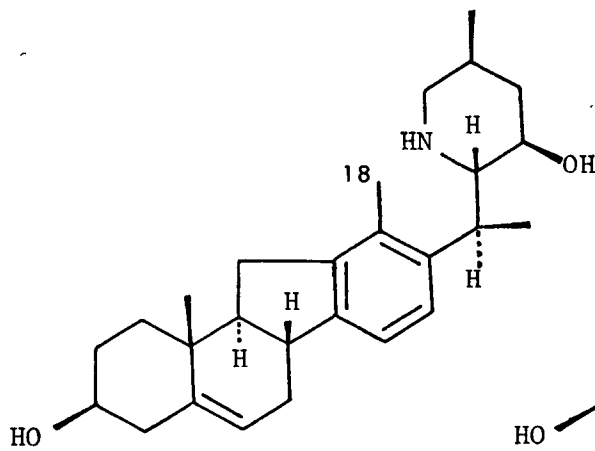
Although a number of alkaloids have been isolated from the plants belonging to the genus Fritillaria, only partial characterization studies have been accomplished except for verticine and verticinone described above. It is interesting to note that imperialine (sipeimine)[100-106] seems to have a similar structural feature as verticine, since it

gave veranthridine (77) among other dehydrogenation products.[104]

To date there have been described several attempts for synthesizing the alkamines containing the cevane nucleus without much success. I would like to present in the following discussion our initial efforts toward the construction of this nitrogen containing hexacyclic system, and more specifically, toward the total synthesis of verticine and related alkaloids.

DISCUSSION

A comparison of the structures of veratramine (87) and verticine (88) immediately reveals remarkable similarities as well as some significant differences, among which the most notable is the lack of a carbon-nitrogen bond between C-18 and the nitrogen atom of the piperidine unit in veratramine (87).



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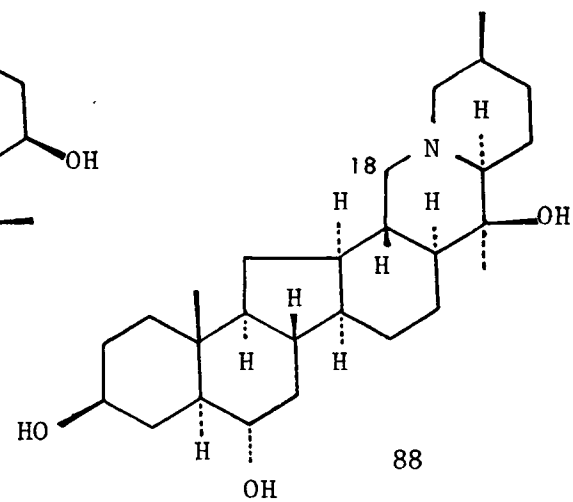

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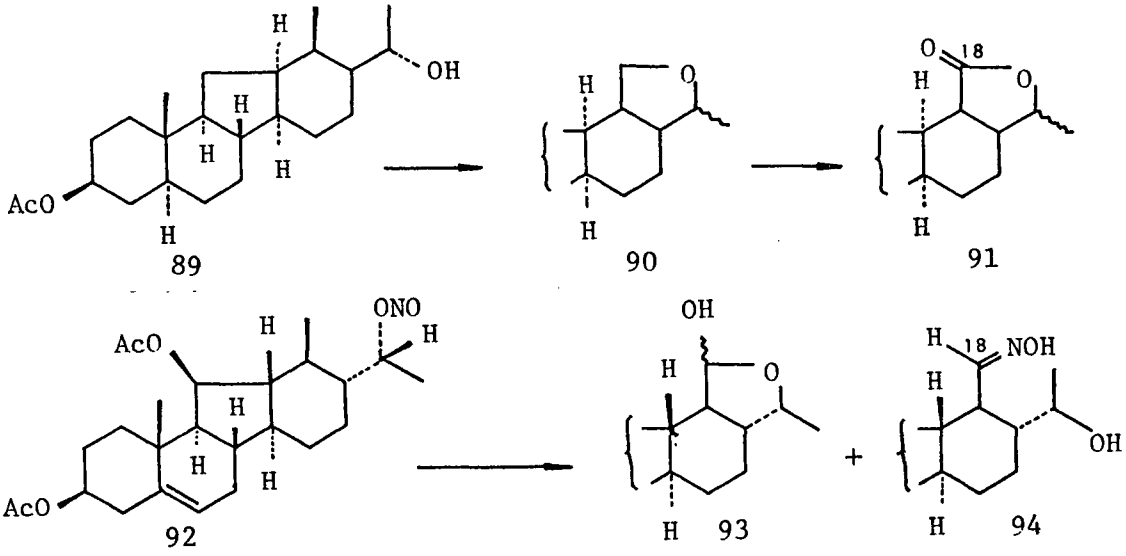
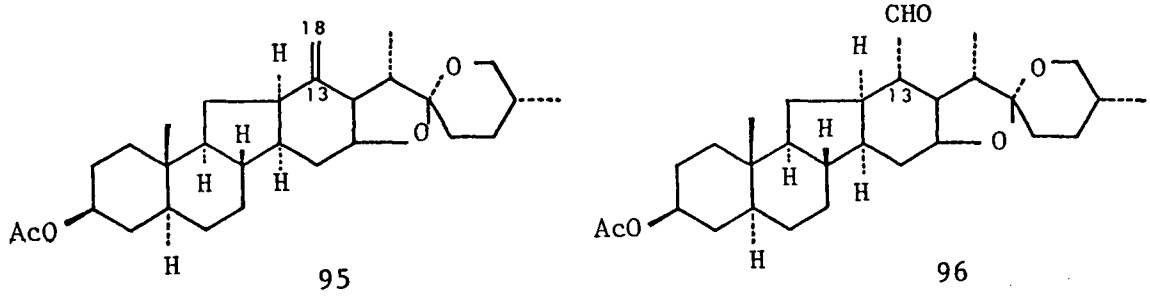
The previously developed synthetic approach to veratramine (87) and verarine (5) in our laboratory involves a coupling reaction of a suitably functionalized pyridine derivative with an etiojervane unit, in order to build up the jervane skeleton. If the potentially general applicability of this method were to be extended to verticine (88) and related alkaloids, the etiojervane system would have to accommodate

an appropriate functionality at C-18 so that the ring E of the cevane skeleton could be formed.



Compared with normal steroids little success has been achieved in the functionalization of the C-18 methyl group in C-nor-D-homo steroids. One such attempt has been described by Masamune and his co-workers.[107,108] A solution of the alcohol (89), iodine, sodium carbonate and lead tetraacetate in cyclohexane was irradiated to give the cyclic ether (90) in 16% yield. The ether (90) was oxidized to the corresponding γ-lactone (91) (19% yield) with chromium trioxide in acetic acid. A similar attempt with the nitrite (92) afforded the hemiacetal (93) and the oxime alcohol (94) in yields of 5% and 17% respectively.



Another possible approach to C-18 functionalized etiojervane derivatives is to utilize the sapogenin exocyclic olefin (95) which can be derived from hecogenin (23) via either a Wagner-Meerwein type reaction[109,110] or the Bamford-Stevens reaction.[111] This exocyclic olefin (95) affords two epimeric epoxides[110,112] and the minor epimer has been shown to give the aldehyde (96) upon brief treatment with perchloric acid.[113] Furthermore, a number of methods have been developed to convert the spiroketal system of sapogenins into Δ^{16}-20-one or 16,20-diol derivatives.[114,115,129,132-139]

Thus, in order to obtain a cevane derivative there are five main phases to the synthetic pathway. They are (A) establishment of the C-nor-D-homo steroid skeleton (B) functionalization of the C-18 methyl group (C) modification of the spiroketal system (D) attachment of a substituted pyridine to an etiojervane derivative (E) intramolecular cyclization of the product obtained in D and further elaborations, to the natural systems. The first three stages are closely interrelated.

A survey of the literature revealed that a variety of C-12 oxygenated steroid derivatives have been submitted to C-D ring rearrangement reactions.[110,111,116,128] Some examples are illustrated in Figure 5. As these examples indicate, a change in the substituent on ring D has less effect on the course of the reaction than a change in the reaction conditions employed in the rearrangement reaction. However, in a publication directly relevant to the present discussion,[129] W.F. Johns stated that "...the classical pseudomerization and chromic acid degradation failed in the 18-substituted C-nor-D-homo sapogenins." Although no specific details were given, it was clear that the well

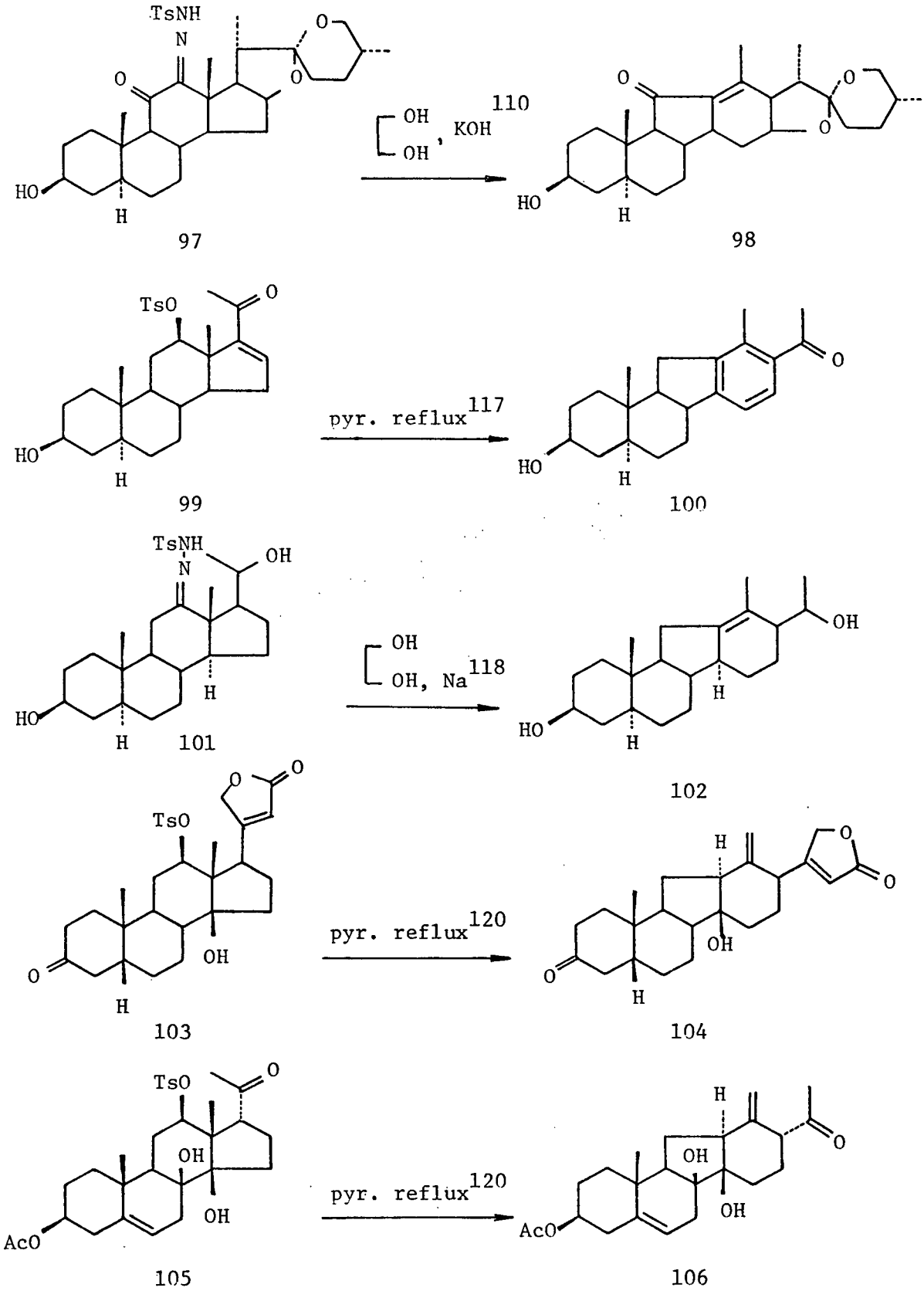


Figure 5. Examples of the C-D ring rearrangement reaction.

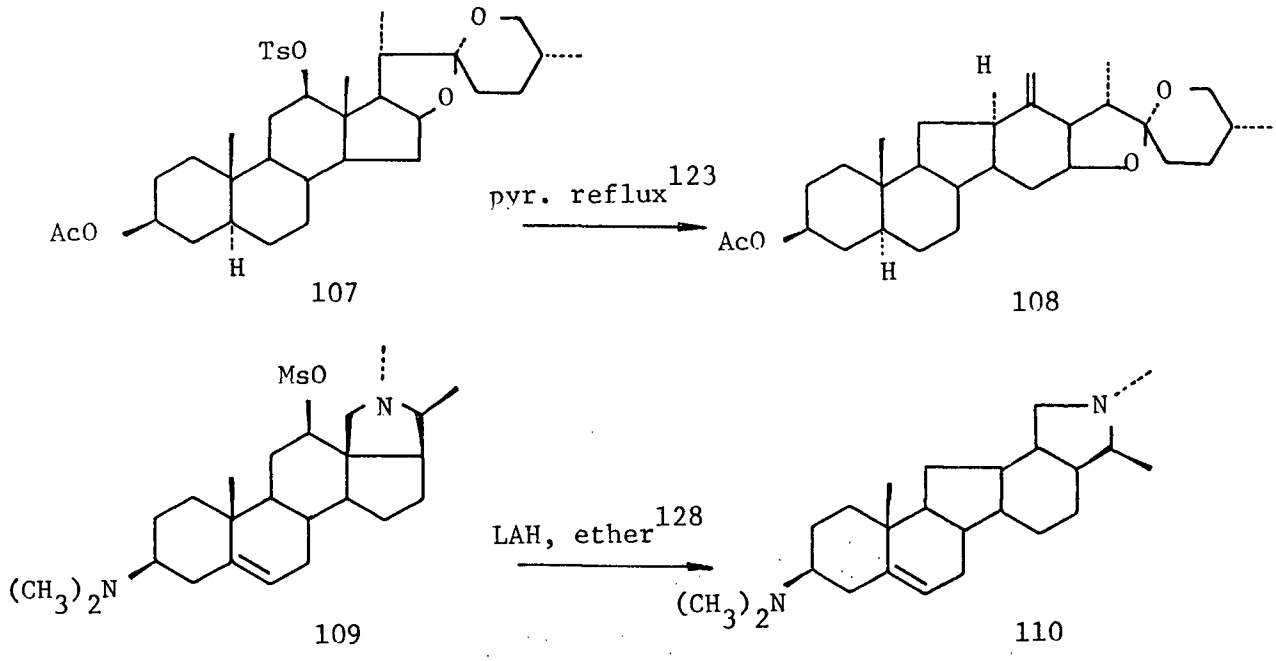


Figure 5 (continued). Examples of the C-D ring rearrangement reaction.

known degradation of the spiroketal side chain could not be employed in our case.

The classical pseudomerization utilized in the degradation of the spogenin spiroketal system was originally developed by Marker and Rohrmann[130,131] and has offered an important method of synthesizing cortisone and pregnane derivatives. Various methods have since been reported to effect the necessary conversion. These procedures have employed, for example, a carboxylic acid anhydride[132,133] with or without additional Lewis acid[134] or a salt of a weak base.[133,135,136] A general outline of this method is shown in Figure 6. The spiroketal system of the sapogenin (111) is opened by treatment with an acid anhydride at an elevated temperature to give the pseudosapogenin (112) which is oxidized with chromium trioxide in acetic acid to yield the intermediate keto-ester (113). Subsequent base-catalyzed elimination

of the side chain yields the α,β-unsaturated ketone (114). The yield in the conversion of (111) to (114) averages 35-50% depending upon the starting sapogenin (111). There are also other methods[129,137-147] by which this system can be opened to an intermediate which allows further elaborations.

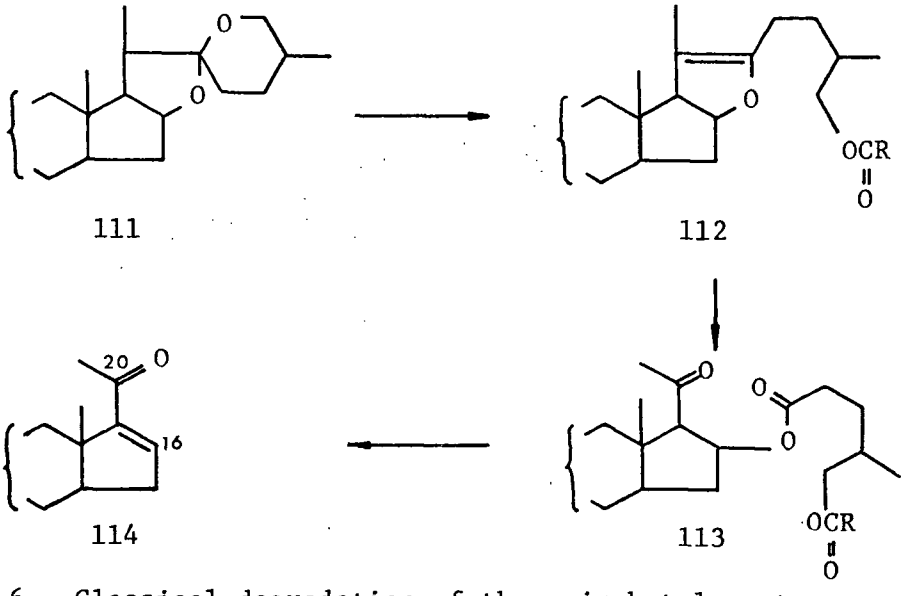
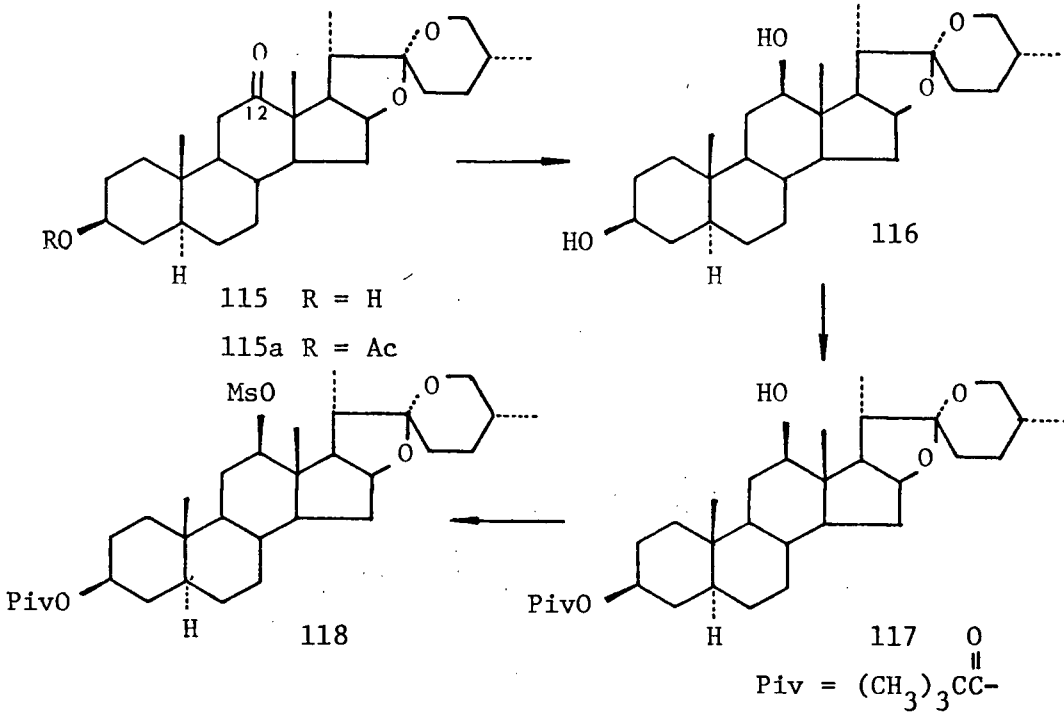


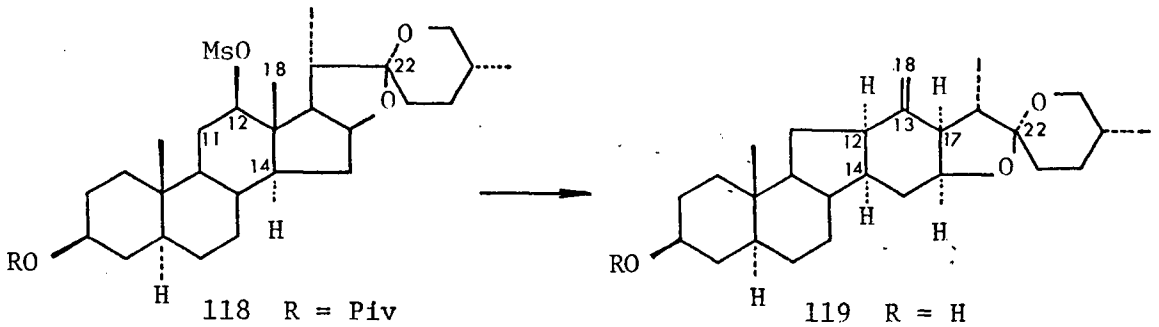
Figure 6. Classical degradation of the spiroketal system.

Our first objective in the present synthesis was to obtain a C-nor-D-homo spirostan derivative with an appropriate functionality at C-18 and possessing the desired α stereochemistry at C-13.

W.F. Johns[148] has employed a modified procedure for converting hecogenin (115), a readily available 12-oxosapogenin, into the 12β-methanesulfonate (118) which is a useful intermediate for the following solvolytic rearrangement. This procedure was carried out in our laboratory, essentially following his method but with minor modifications.



To facilitate large scale reactions, reduction of hecogenin (115) or hecogenin acetate (115a) to rockogenin (116) was done with potassium and 2-propanol, although W.F. Johns[148] found that, of several methods attempted, Birch reduction gave the best selectivity (95-98%) for the desirable 12β-hydroxyl isomer. Selective esterification, with pivaloyl chloride, of the 3β-hydroxyl group in rockogenin (116) was found to proceed smoothly at room temperature in a 1:2 mixture of pyridine and benzene, and at the end of the reaction the undesirable epirockogenin 3-pivalate formed a crystalline precipitate and was easily removed by filtration. Purified rockogenin 3-pivalate (117) gave, in quantitative yield, the methansulfonate (118) as a grayish granular solid.



118 R = Piv
118a R = $CH_3OCO(CH_2)_2CO$-

119 R = H
119a R = Ac
119b R = Piv

The conversion of the methanesulfonate (118a) into the C-nor-D-homo exocyclic olefin (119a) was first accomplished by Hirschmann et al.[172,173,110] in 1952. In an attempt to prepare the corresponding Δ^{11} derivative they treated the methanesulfonate (118a) with potassium t-butoxide in t-butanol, and contrary to their expectation they obtained, after acetylation of the product mixture, two olefinic products neither of which was the desired Δ^{11} derivative. They assigned the $\Delta^{13(18)}$ (119a) and $\Delta^{13(17)}$ structures to the olefins, but the latter was subsequently demonstrated to have the $\Delta^{12(13)}$ structure.[123,171] Following the procedure of Hirschmann et al.[110] rockogenin 12β-methanesulfonate 3-pivalate (118) was converted to the exocyclic olefin (119a) in 26% yield, although much better yields, 54%[110] and 73%,[111] had previously been reported. Since about 50% of the crude solvolysis mixture was shown by nuclear magnetic resonance (n.m.r.) spectroscopy, to be the exocyclic olefin (119), isomerization took place in part during the subsequent acetylation step. This entails refluxing with acetic anhydride for 30 min. In this way only

(119a) crystallizes upon cooling of the mixture. It is generally accepted[151] that an endocyclic double bond is thermodynamically more stable than the corresponding exocyclic one. In fact, with this particular exocyclic olefin (119a)[110,173] it was demonstrated that treatment with formic acid at room temperature effects the isomerization to the corresponding endocyclic system. Therefore, although Hirschmann's method afforded the C-nor-D-homo exocyclic olefin (119a) uncontaminated with other isomeric olefins, improvement with respect to the selectivity of reaction was desirable.

In 1969 Coxon et al.[123] described the rearrangement of rockogenin 3-acetate 12-p-toluenesulfonate in refluxing anhydrous pyridine. The 12-p-toluenesulfonate was converted to the exocyclic olefin (119a) with 90% selectivity. This method was promptly applied to rockogenin 12-methanesulfonate 3-pivalate (118) and the exocyclic olefin (119b) was obtained in 82% yield. The n.m.r. and the mass spectra of (119b) are shown in Figure 7 and Figure 8 respectively.

Infrared (i.r.) spectroscopy of steroidal sapogenins has been studied extensively in the early 1950's.[154-158] Wall et al.[154] reported that in the region, 850-1,000 cm^{-1}, four characteristic bands associated with the spiroketal side chain were observed and that these are distinctive for the spirostan derivatives of the (22S) and (22R) series. (22R)-Spirostans, such as our starting material, hecogenin (115), show absorption bands with maxima occurring near 982, 922, 900 and 866 cm^{-1} (in carbon disulfide), while (22S)-spirostans exhibit corresponding absorptions near 987, 922, 900 and 852 cm^{-1}. The order of intensity of these four bands in (22R) spirostans is as follows,

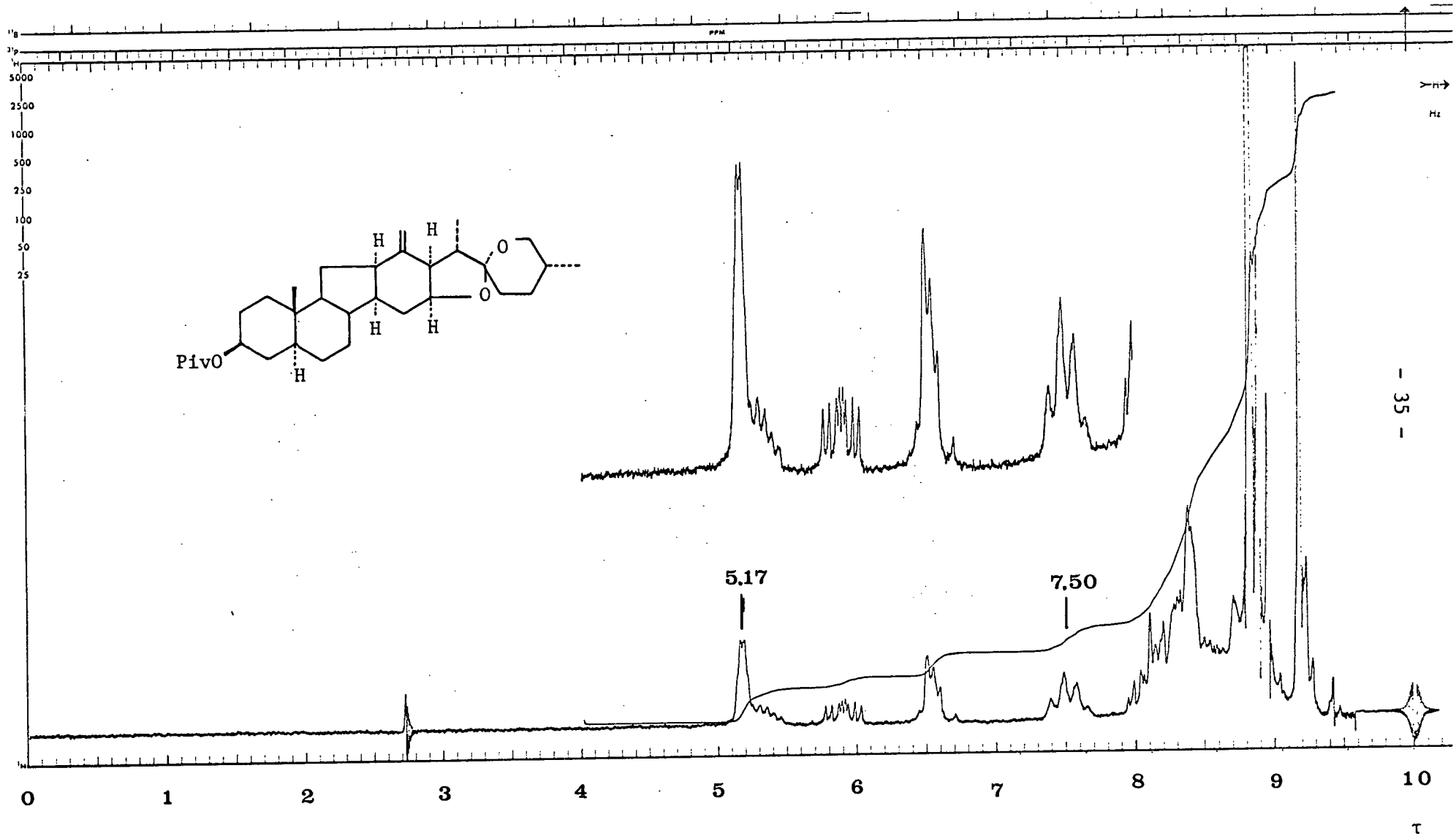


Figure 7: N.m.r. spectrum of compound (119b).

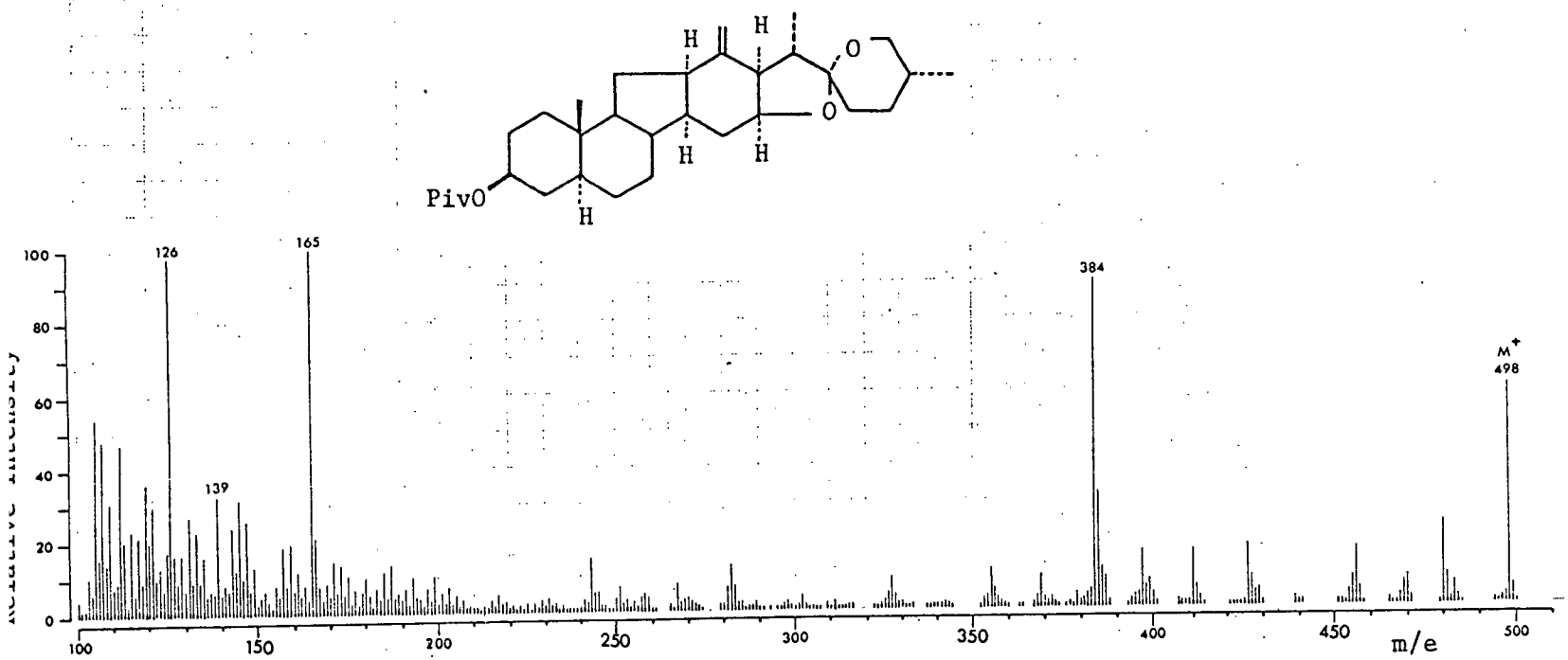
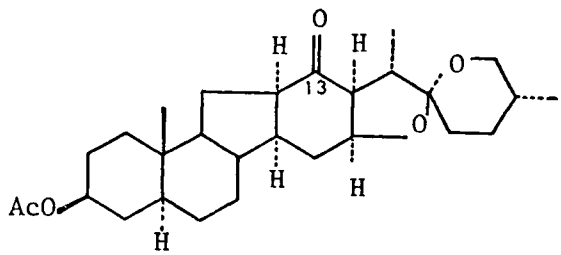


Figure 8: Mass spectrum of compound (119b).

$$\varepsilon_{982\ cm^{-1}} > \varepsilon_{900} > \varepsilon_{922} > \varepsilon_{866}$$

Studies of the i.r. spectra of compounds (115), (115a), (116), (117), and (118), though taken as potassium bromide pellets, confirmed these findings. However, while the exocyclic olefins (119a) and (119b) with the modified C-nor-D-homo skeleton showed the above four bands at approximately their predicted positions, the intensity of the 922 cm^{-1} band was found to be considerably diminished. That this particular band became hardly recognizable in some C-nor-D-homo-spirostan derivatives will be discussed in the following part.

The stereochemistry associated with the solvolytic reaction of the 12β-methanesulfonate (118a) to (119) was discussed in terms of the Wagner-Meerwein rearrangement.[110] The migrating center C_{14}, which is situated in an anti-parallel relationship to the 12β-mesyloxy group, approaches the migrating terminus C_{12} from the backside, resulting in retention of configuration at C_{14} and inversion at C_{12}. This mode of 1,2-rearrangement is widely found, for example in the pinacol rearrangement,[152] and the Demjanov deamination reaction.[150,152] Furthermore, the 12α-H configuration in the exocyclic olefin (119a) was demonstrated[112] by studies of the optical rotatory dispersion (O.R.D.) curve of the corresponding 18-nor ketone (120) derived from (119a) by the reaction with



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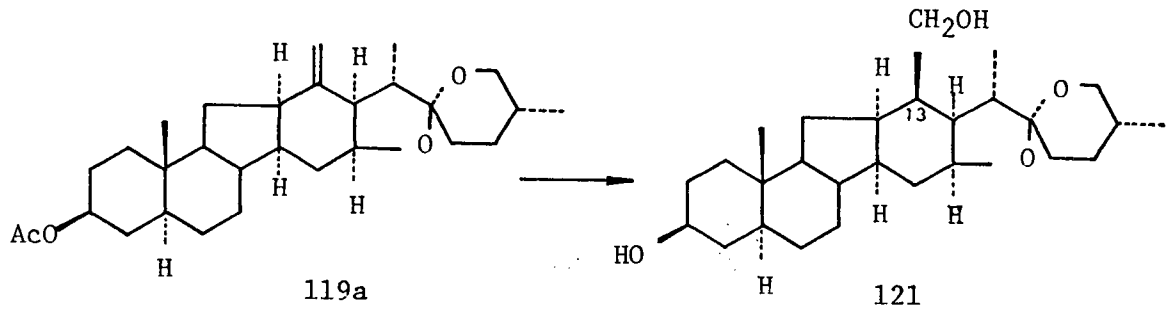
osmium tetroxide followed by sodium metaperiodate oxidation.[109,112] The negative Cotton curve (a = -29°) of the ketone (120) supports the 12α-H configuration as the 12β-H configuration would be expected to give rise to a strongly positive Cotton curve.

The exocyclic olefins (119a) and (119b) represent the intermediates which have the C-nor-D-homo steroid skeleton with the potential functionality at the C-18 position. Since a subsequent degradation of the spiroketal side chain[129,131,133-143] requires quite drastic conditions, it was necessary to alter the exocyclic double bond to a more suitable functionality in order to preserve not only the functionality itself at C-18 but also the stereochemical integrity at C-13.

Hydroboration of the exocyclic olefin (119a) followed by acetylation of the resulting primary alcohol was first considered as a method for fulfilling this aim. It was found, however, that an attempted hydroboration of the C-3 tetrahydropyranyl ether derivative of this particular olefin (119)[149] gave negative results. Diborane generated in situ or externally gave complex mixtures of products with the spiroketal side chain being apparently reduced. Since the successful hydroboration of a steroidal sapogenin had been reported,[153] it was not immediately apparent why this hydroboration should fail. Thus, the exocyclic olefin 3-acetate (119a) was treated with commercially available diborane in tetrahydrofuran (2.3 fold excess) under a nitrogen atmosphere for three hours at room temperature. Usual oxidation with hydrogen peroxide and base gave a reasonably clean reaction product which contained, by thin layer chromatography (t.l.c.), one quite polar material accompanied

by a second, slightly less polar component. Chromatography on alumina led to the isolation of the major product in a 82% yield. The n.m.r. and the mass spectra of this material are shown in Figure 9 and Figure 10, respectively. The i.r. spectrum of the material showed that the



two bands at 1636 cm^{-1} and 882 cm^{-1}, which were found in the starting olefin (119a), had disappeared, and that the set of four bands associated with the (22R)-spirostan system was still present with maxima at 985, 926, 900 and 863 cm^{-1} indicating the intact spiroketal side chain. The n.m.r. spectrum (Figure 9) further confirmed the successful hydroboration with concomitant hydrolysis of the C-3 acetate group. It shows that the acetate signal at τ 8.00 and a 2-proton broad singlet at τ 5.17 due to the exocyclic methylene protons in the olefin (119a) are absent, and that a 2-proton doublet at τ 6.35 ($C\underline{H}_2OH$) has appeared.

The mass spectrometric fragmentations of steroidal sapogenins have been studied during the last decade by Djerrasi and his co-workers.[159-161] With recourse to the use of deuterium labelled sapogenins coupled with low voltage and high resolution measurements, they have elucidated the characteristic fragmentation patterns of the basic structure of the steroidal sapogenin, (25R)-5α-spirostan. Their results are illustrated in Figure 11. Three important fragments occur

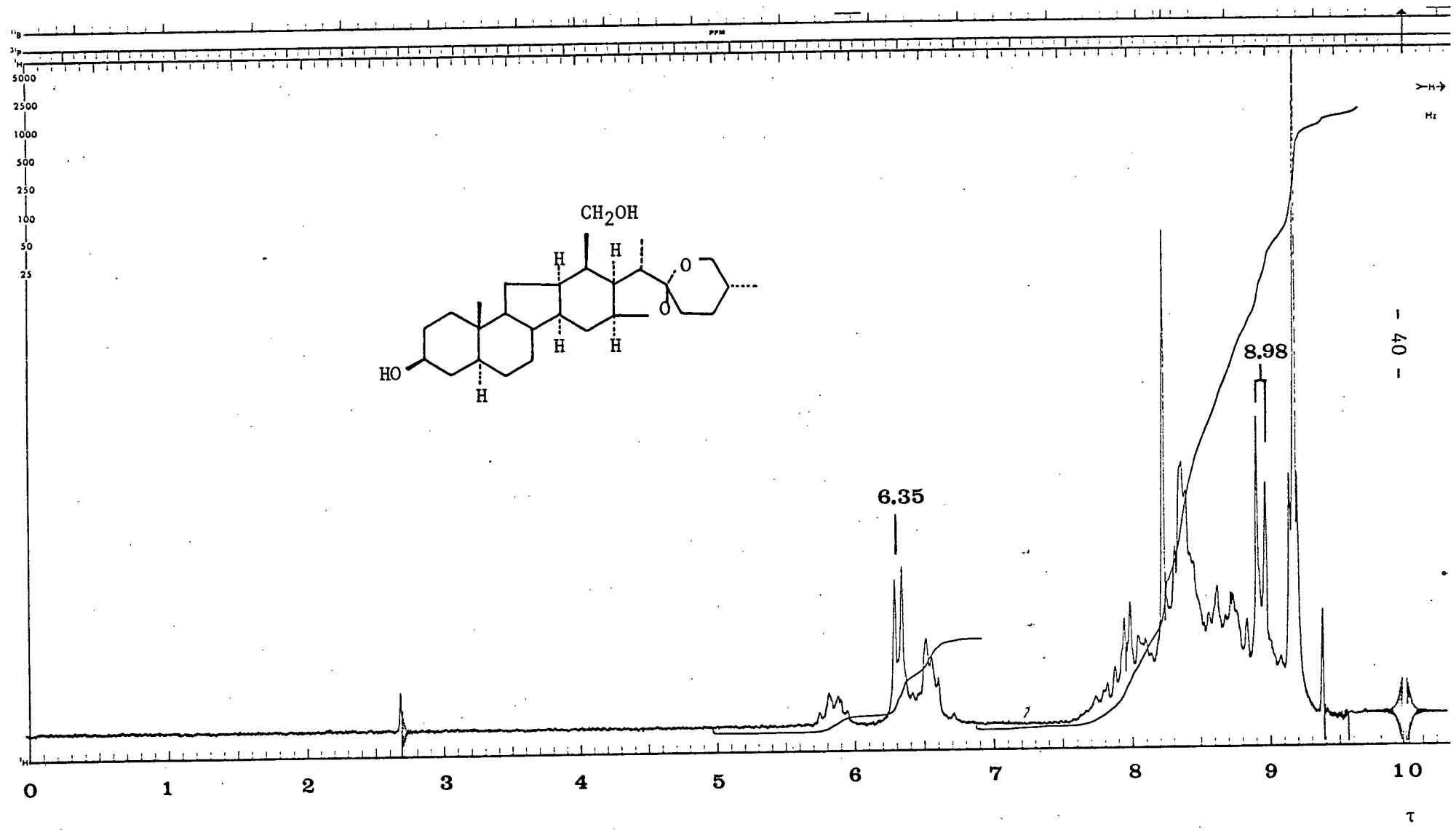


Figure 9: N.m.r. spectrum of compound (121).

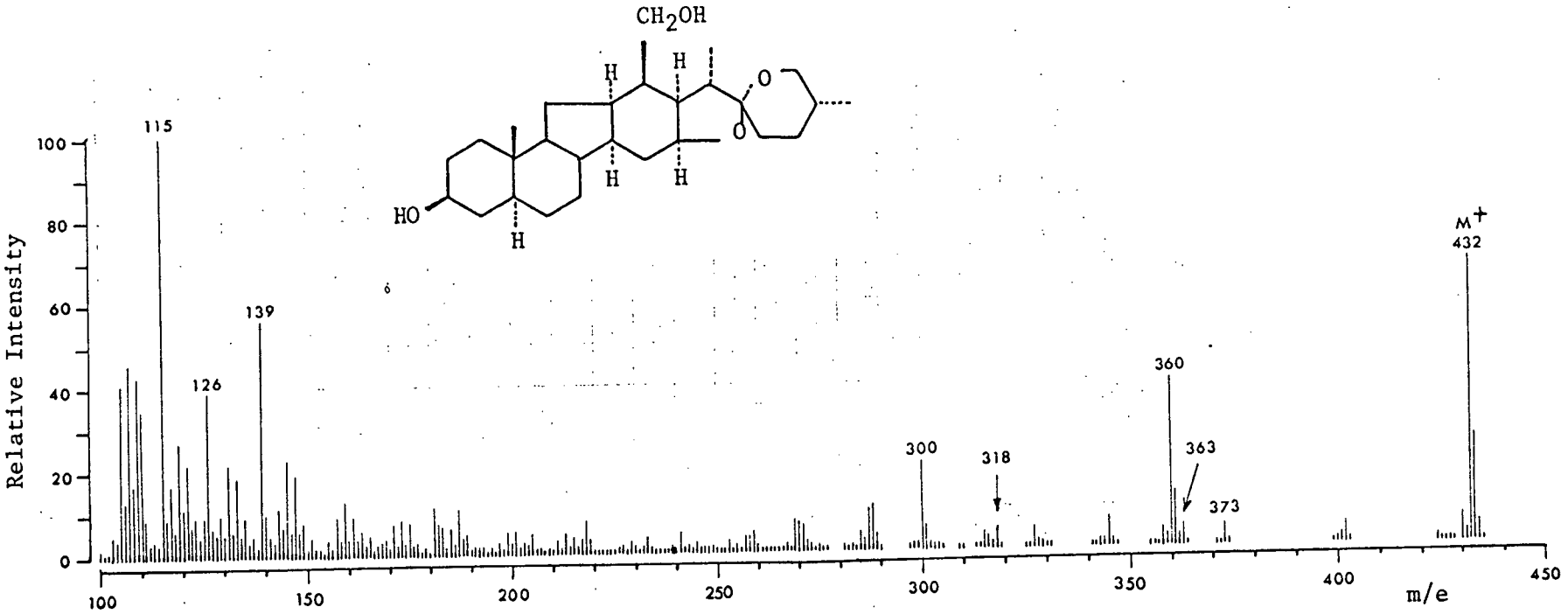


Figure 10: Mass spectrum of compound (121).

in the low mass range giving rise to peaks at m/e 139 (A), m/e 115 (B), and m/e 126 (C). The higher mass range, however, is much more diagnostically important in that six fragments have been associated with spiroketal systems. These characteristic fragments are found at M-59 (D), M-69 (F), M-72 (E), M-114 (G), M-129 (H), and M-143 (J).

In the mass spectrum (see Figure 10) of the hydroboration product of the olefin (119a) the base peak, above m/e 100, occurred at m/e 115 and this corresponds to the fragment (B). The other expected fragments in the low mass range corresponding to (A) and (C) were also found with intermediate intensities. The intense peak at m/e 432 (68%) corresponds to the mass number of the expected hydroboration product in which the acetate group has been lost. The peaks at m/e 373, 363, 360 and 318 can be attributed to the fragments (D), (F), (E) and (G) respectively. The peaks corresponding to (H) and (J) are, however, hardly distinguishable from the contiguous peaks. Furthermore, while the fragment (G) (M-114) gives rise to an intense peak in compounds (116), (117), (119a) and (119b), the intensity is very much reduced in the hydroboration product. Another noticeable change is that the peak at m/e 300 (M-132), which is also found in rockogenin (116) and its derivatives, (117) and (143), becomes prominent suggesting that a loss of a water molecule may be involved in a stage either preceding or following the formation of the fragment (G) (M-114).

On the basis of the data presented above it appears reasonable that the structure of the hydroboration product is represented by (121). The only remaining question with regard to this structure is the stereochemistry at C-13.

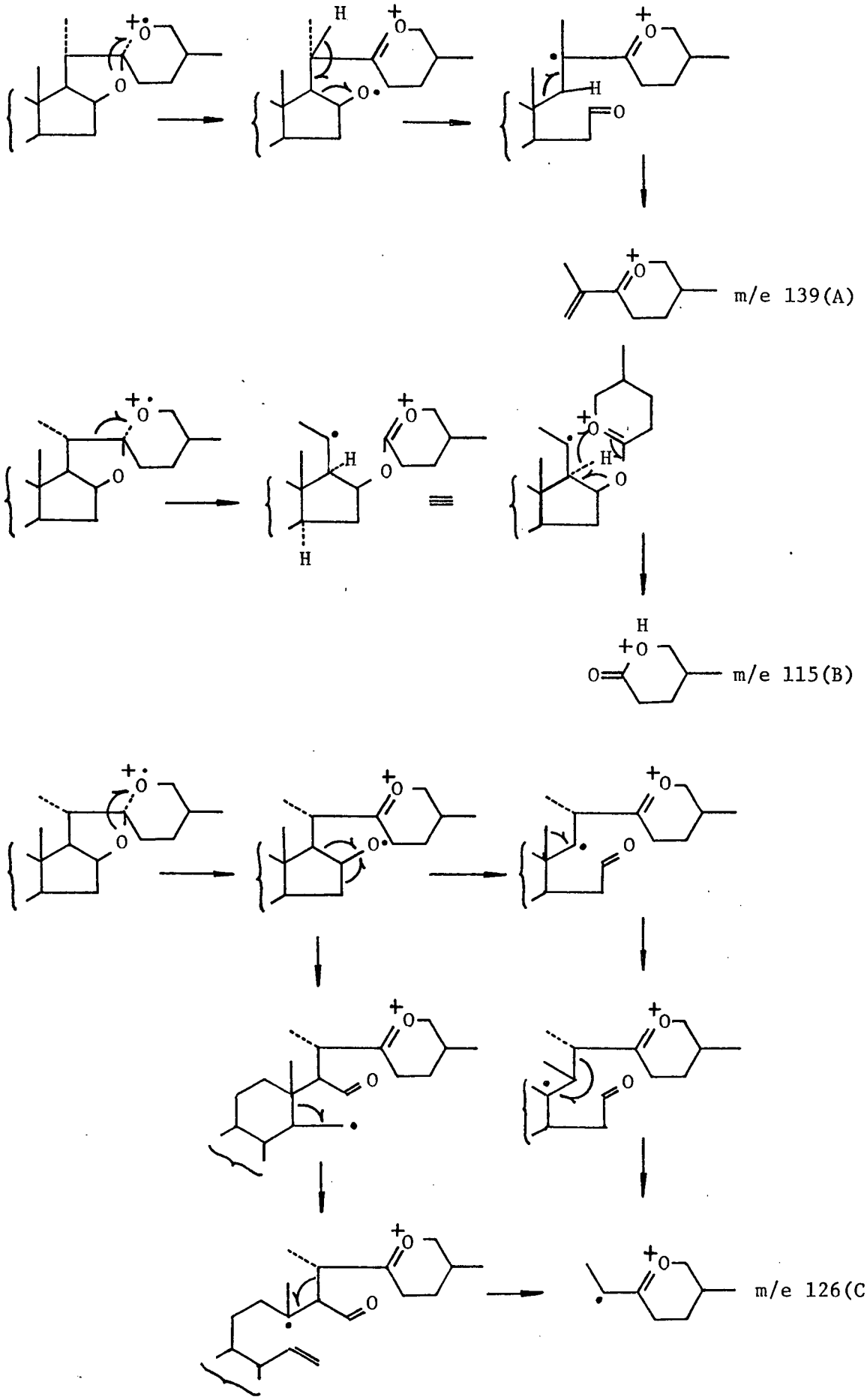


Figure 11: Mass spectrometric fragmentation of the spiroketal system.

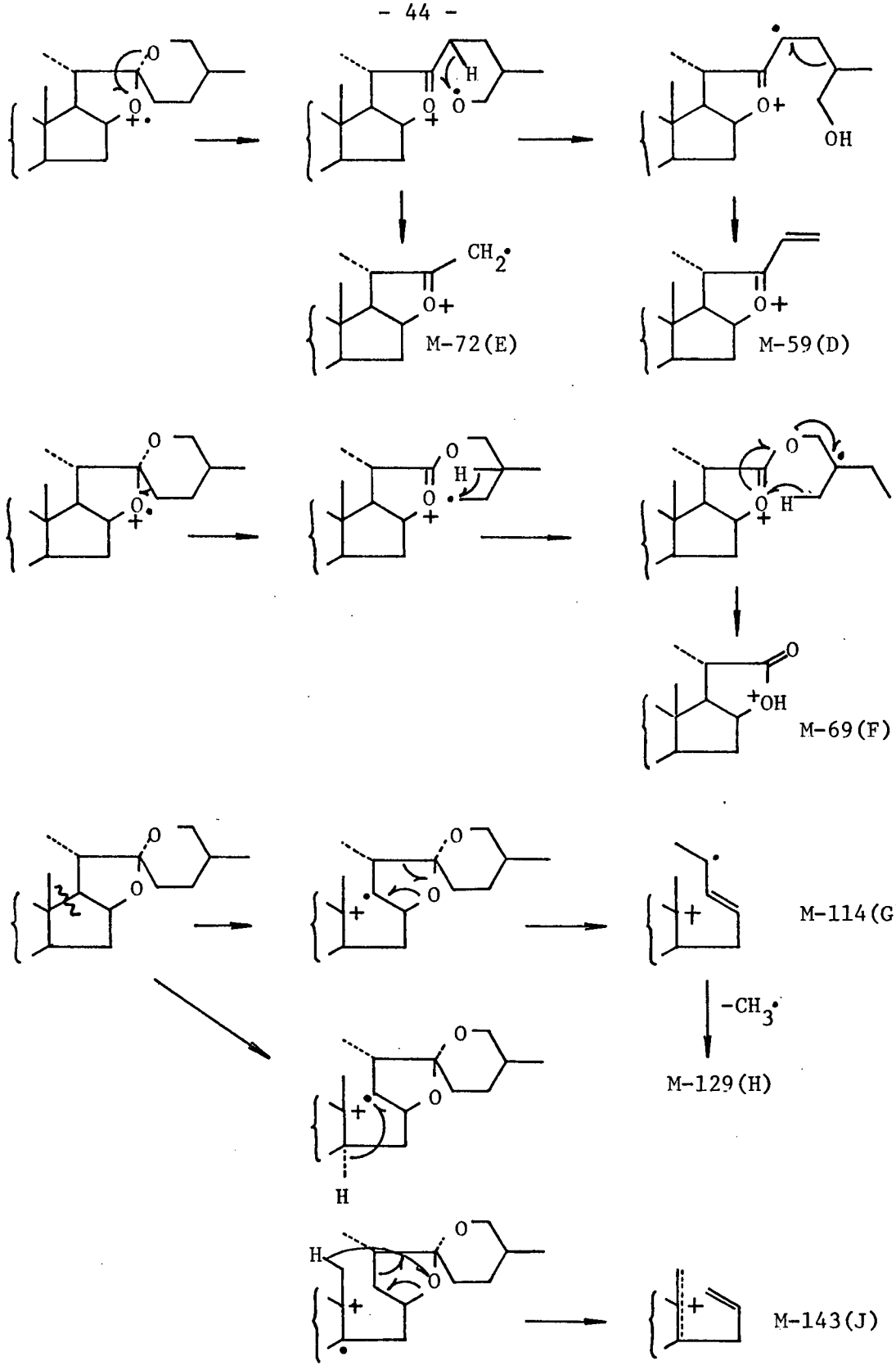


Figure 11 (continued): Mass spectrometric fragmentation of the spiroketal system.

Examination of the literature revealed that there were three types of reactions documented for the exocyclic olefin (119a). The first example is catalytic hydrogenation of (119a) with palladium-charcoal in acetic acid.[148] The structure with the 13β-methyl group (122) was inferred from inspection of the molecular models. The stereochemistry at C-13 was further corroborated by examination of the O.R.D. curves of the two epimeric ketones (125,126) which had been derived from the α,β-unsaturated ketone (123) via the Beckmann rearrangement followed by acid hydrolysis.[167] The minor, thermodynamically less stable epimer (125) was correlated with the α,β-unsaturated ketone (123) through the 3,17-diketone (129),[174] so that they have the same configuration at C-13. The major ketone (126) showed an unusually strong positive Cotton effect (a = +216°). Using as models the enantiomeric B-nor-coprostan-3-one (130) and (+)-cis-8-methyl-hydrindan-5-one, which exhibit negative Cotton effects, it was concluded that the major ketone (126) contains a cis C/D ring juncture. Further support for this assignment was provided by the demonstration that the 12β-etiojervane derivative (132) had the expected negative Cotton effect (a = -92°).[175] The D-ring of (125) and (126) can exist theoretically in four distinct conformations as outlined in Figure 13. Consideration of octant projections and the principle of conformational analysis which states that for 2-methylcyclohexanones the equatorial isomer is more favorable than the axial one,[176-178] two conformers (K and L) for (126) remain as possibilities.

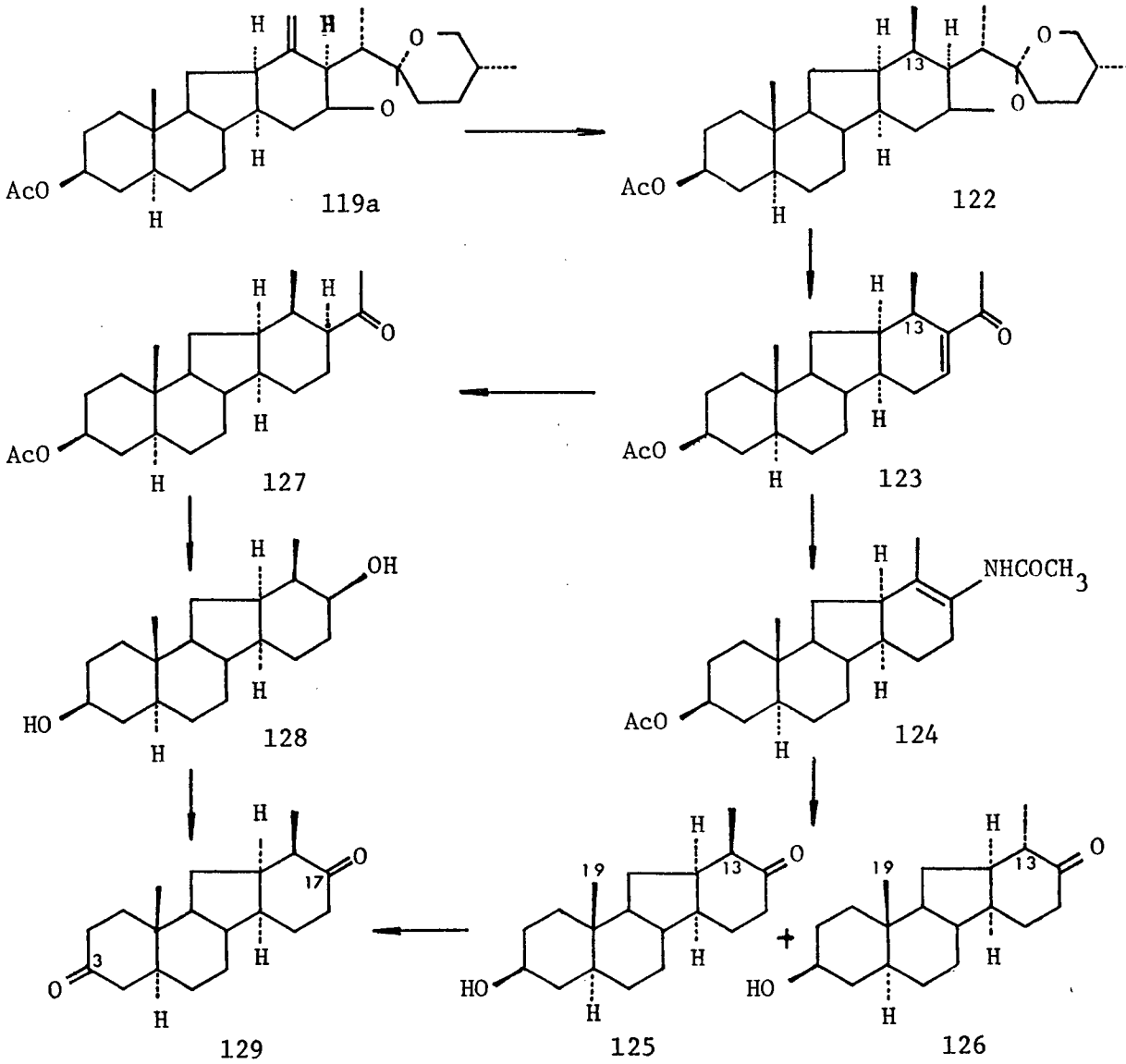
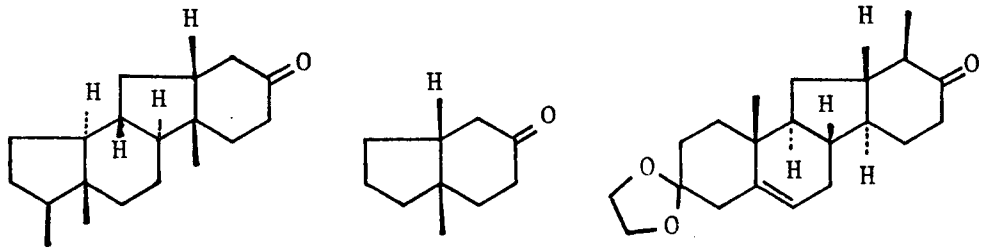


Figure 12: Elucidation of stereochemistry of hydrogenation product of (119a).



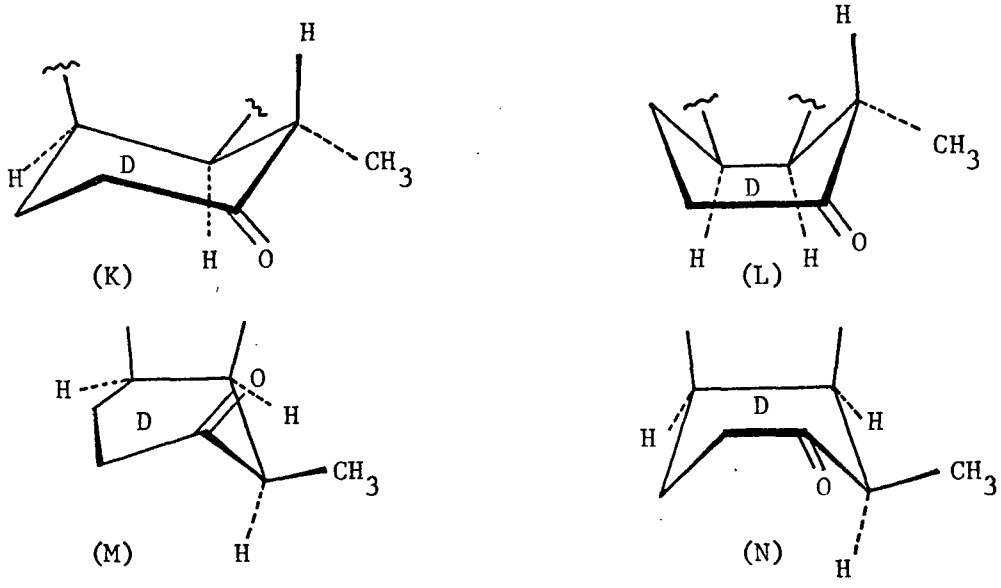


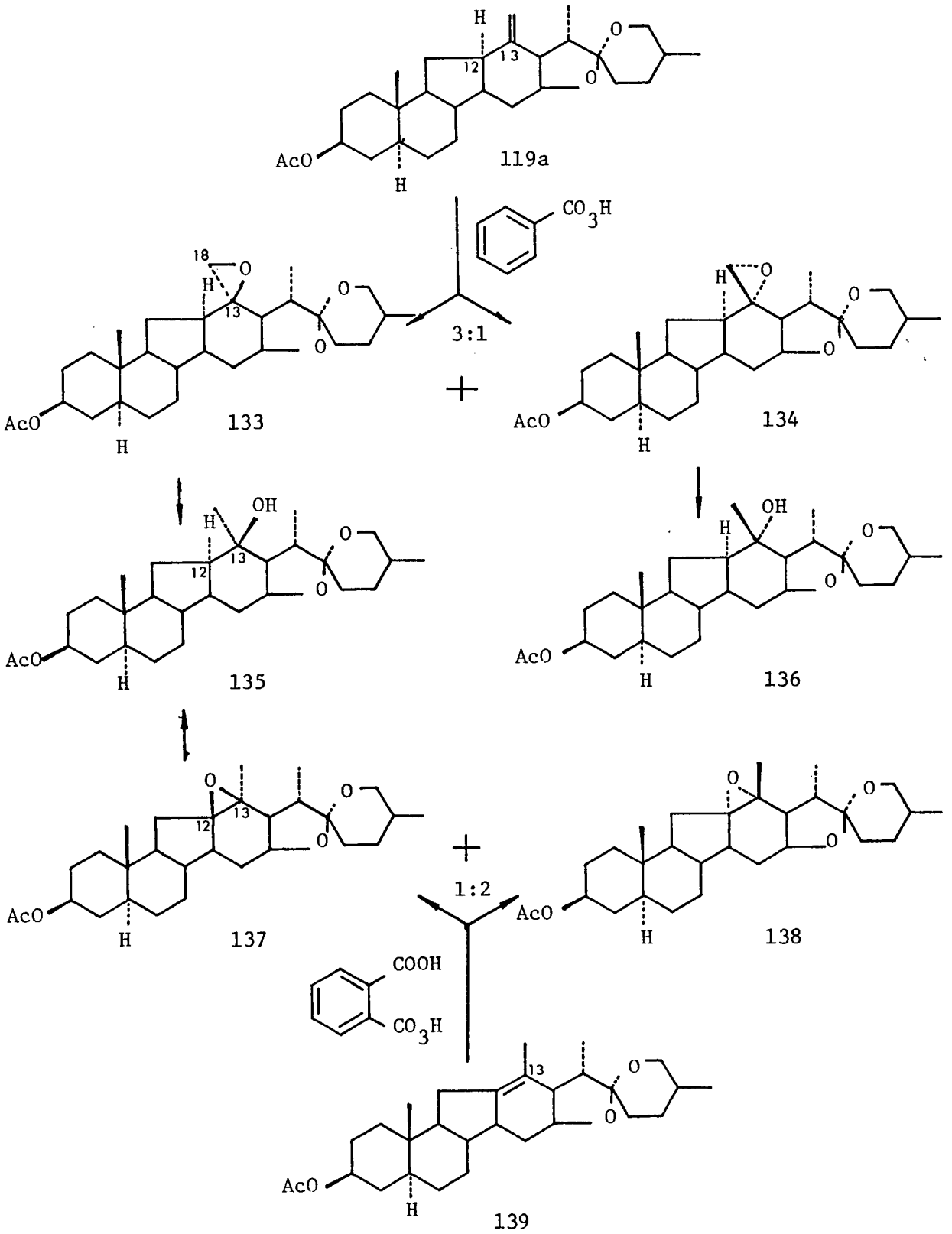
Figure 13: Four possible conformations for the D-ring of (125) and (126)

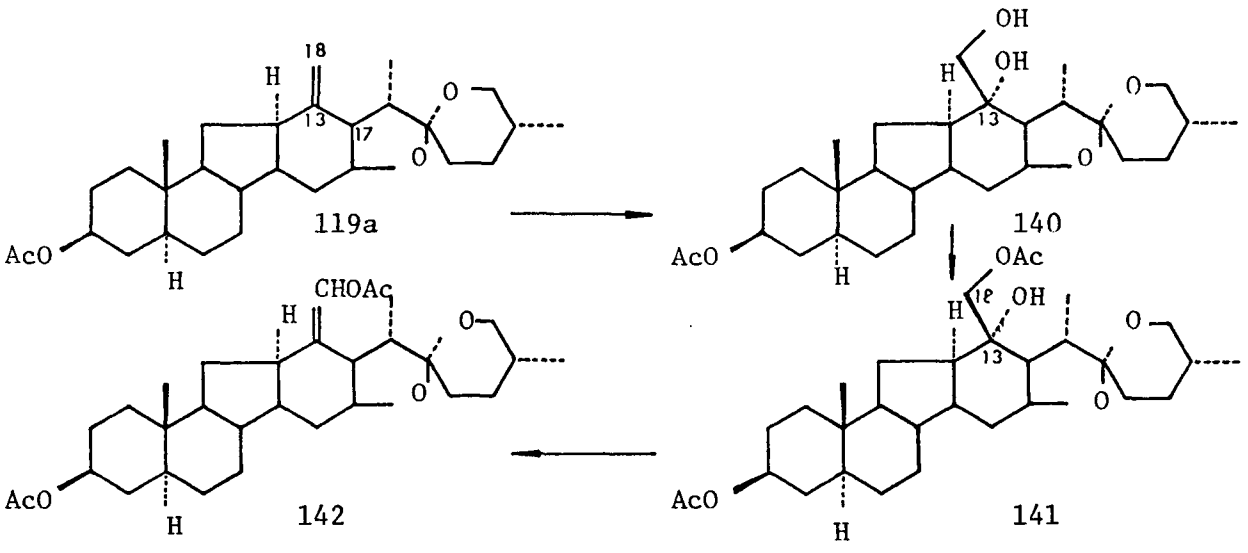
The O.R.D.curve of the epimeric ketone (125) displayed a negative Cotton effect (a = -94°).[167] This change in sign is explained by the conformational changes of the D-ring (see conformations M and N) so that a C-13 substituent occupies the equatorial position as illustrated in Figure 13. The octant rule predicts that the forms (M) and (N) would have a negative Cotton effect. Since the C-17 carbonyl group in (M) and (N) lies above the C-19 methyl group, the upfield shift[179] (0.10 p.p.m.) of the C-19 methyl signal revealed in the n.m.r. spectrum of (125), when compared to that of (126), also agrees with the conformational changes in the D-ring.

Catalytic hydrogenation of the olefin (119a) has therefore taken place with hydrogen attacking from the α face of the molecule to give the C-13β methyl compound (122).

The second example[109,112] of reaction involving the exocyclic olefin (119a) was epoxidation with perbenzoic acid. It was found that two epimeric epoxides, β (133) and α (134), were formed in the ratio of 3:1. The structure of the β-epoxide (133) has been established through the finding that the lithium aluminum hydride reduction product (135) was identical with the reduction product of one of the epoxides derived from the endocyclic olefin (139).[111] Since the alcohol (135) was obtained from both (119a) and (139), the structure of the alcohol (135) must contain a hydroxyl group at C-13. However, the exocyclic olefin (119a) and the derived epoxides (133,134) possess the 12α-H configuration.[110,112,167] The alcohol (135), therefore, has the same 12α-H configuration, so that the identical alcohol, which was derived by trans attack of hydride ion on the epoxide (137), must have a 13β-hydroxyl group. Consequently the epoxides (133) and (137) must have the 13β,18- and 12β,13β-epoxy structures respectively. Thus in the case of the exocyclic olefin (119a) the attack by perbenzoic acid has occurred predominantly from the β face of the molecule. Since the peracid usually attacks an olefin from its less hindered side to produce the less hindered epoxide as the major product, the above results indicate the β face to be less hindered and are notably in disagreement with the results of hydrogenation.

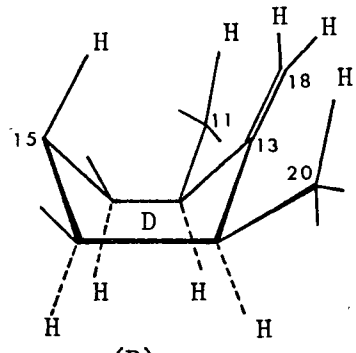
The third example is the oxidation of the olefin (119a) with osmium tetroxide.[110,113] Acetylation of the derived diol (140) followed by treatment with thionyl chloride-pyridine was demonstrated to give a mixture containing the enol acetate (142).[113] The formation of the


119a
133
134
3:1
135
136
137
138
139
1:2
AcO
OH
COOH
CO3H



exocyclic rather than the endocyclic enol acetate from the diol 18-acetate (141) was regarded as establishing the 13α configuration of the tertiary hydroxyl group on the basis that the epimeric alcohols (135) and (136) in the foregoing discussion gave, under the same reaction conditions, the endocyclic olefin (139) and the exocyclic olefin (119a) respectively. In this third example a quite bulky reagent, namely osmium tetroxide, appears to have approached the exocyclic double bond from the α face, although the structure of the resulting diol (140) has not been conclusively proved.

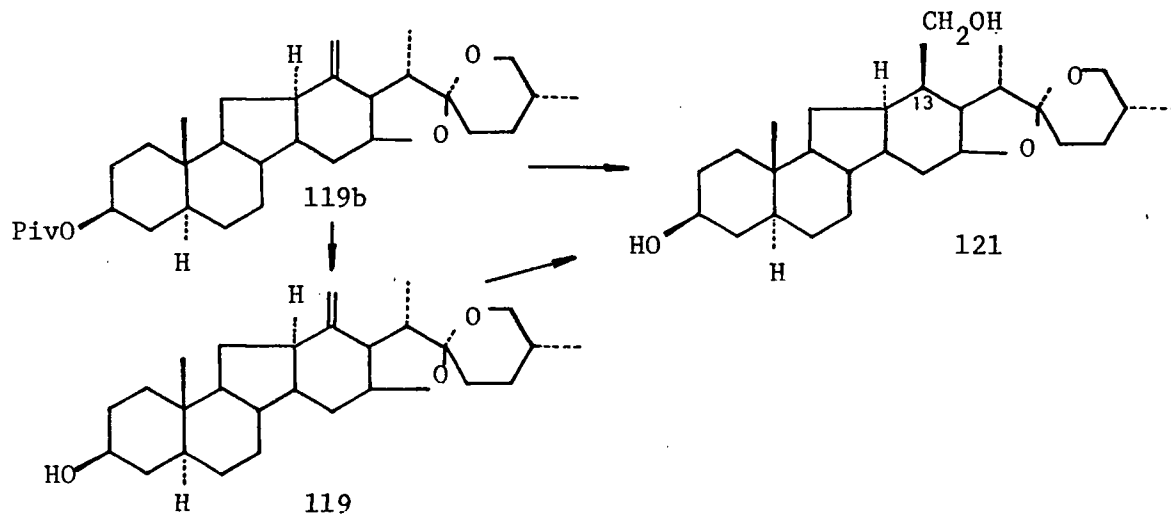
Examination of the molecular models indicates that the D-ring in the exocyclic olefin (119a) takes a modified boat conformation (P) which is imposed by the two adjacent cis-fused five-membered rings, and that the entire molecule is nearly planar in shape. With regard to the $\Delta^{13(18)}$ double bond the α face appears to be more accessible than the β face since the latter is shielded by the 11β,15β and 20β hydrogen atoms.



(P)

The addition of diborane has been found to occur in a cis manner from the less hindered face of the double bond.[181]

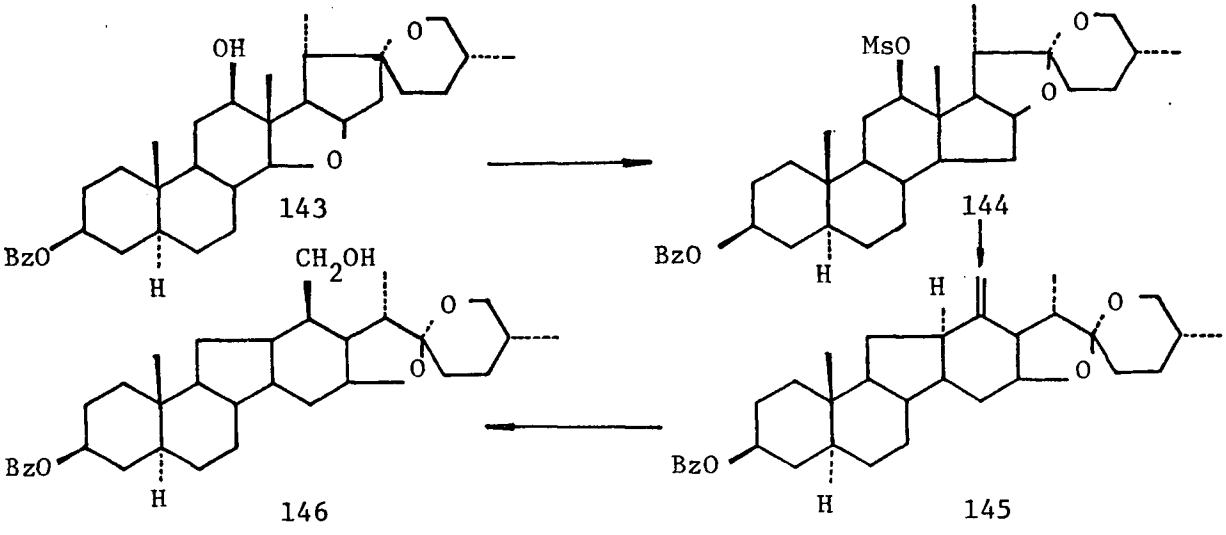
On the basis of the data presented above it was speculated that the hydroboration reaction occurred from the α face and the resulting primary alcohol was tentatively regarded as having the 13β-hydroxylmethyl group as shown in structure (121).



When the exocyclic olefin 3-pivalate (119b) was submitted to the same hydroboration conditions as (119a), a complex mixture of products was obtained and after base catalyzed hydrolysis of the product mixture the desirable diol (121) could be isolated in only about 10%

yield. It was then decided that the pivalate group should be removed by the action of lithium aluminum hydride. The reaction proceeded smoothly in ether and gave the free alcohol (119) in almost quantitative yield. The n.m.r. and the mass spectra of the alcohol (119) are shown in Figures 14 and 15 respectively. The spiroketal system in (119b) was evidently untouched by lithium aluminum hydride, although lithium aluminum hydride with aluminum chloride[138,183-185] or with hydrogen halides[182] has been shown to cleave the spiroketal side chain.

Hydroboration of the exocyclic olefin alcohol (119) proceeded normally giving rise to the diol (121) in better than 70% yield. The exocyclic olefin 3-benzoate (145), which was derived from rockogenin (116) in an analogous manner, was also submitted to the hydroboration conditions and in this case the primary alcohol (146) (see Figure 16)



was obtained in 54% yield. It is noteworthy that the success of hydroboration of the $\Delta^{13(18)}$ double bond on ring D is remarkably dependent on the functionality at C-3 on ring A. This type of phenomenon may presumably be accounted for in terms of "conformational transmission."[180]

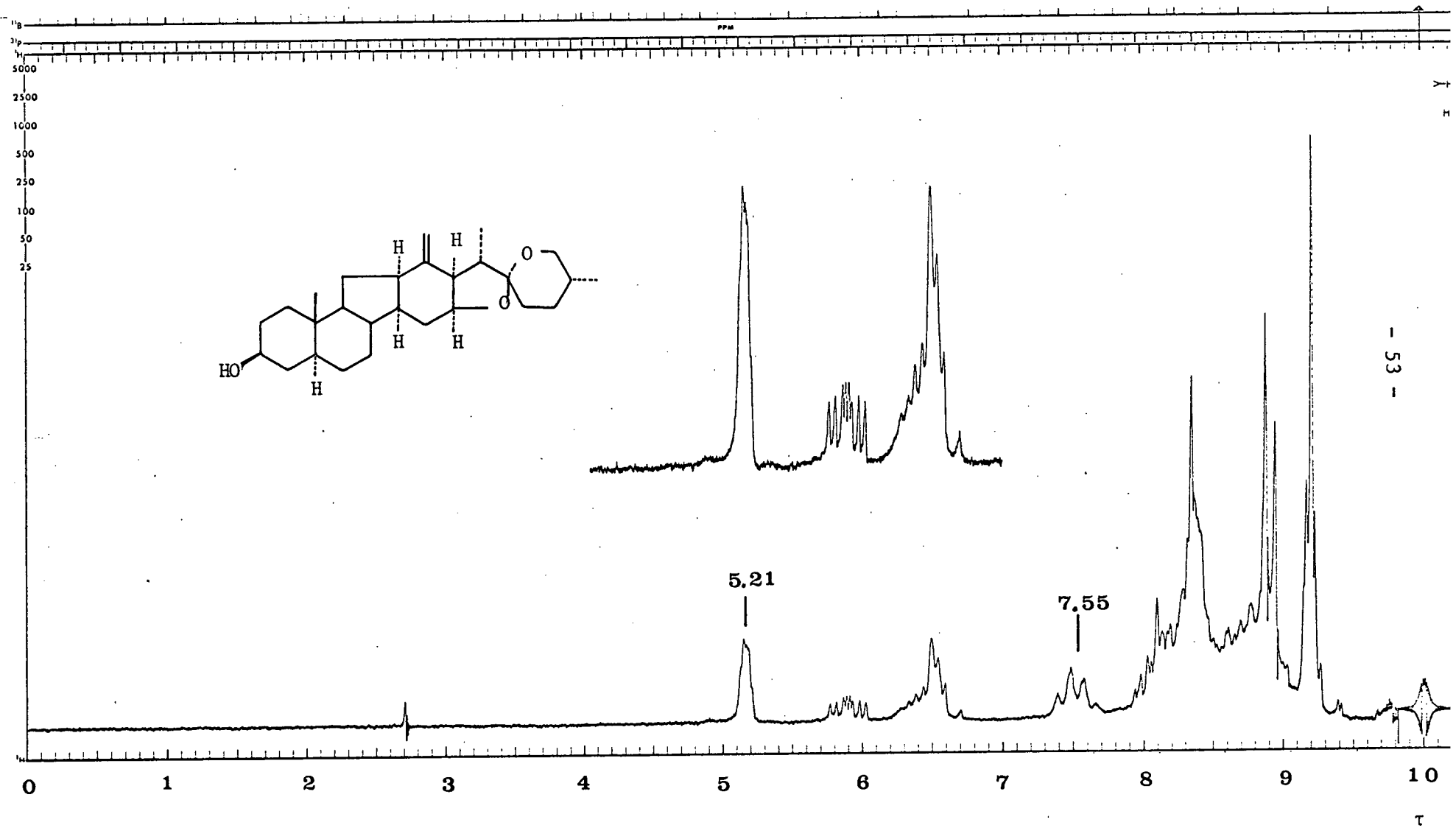


Figure 14: N.m.r. spectrum of compound (119).

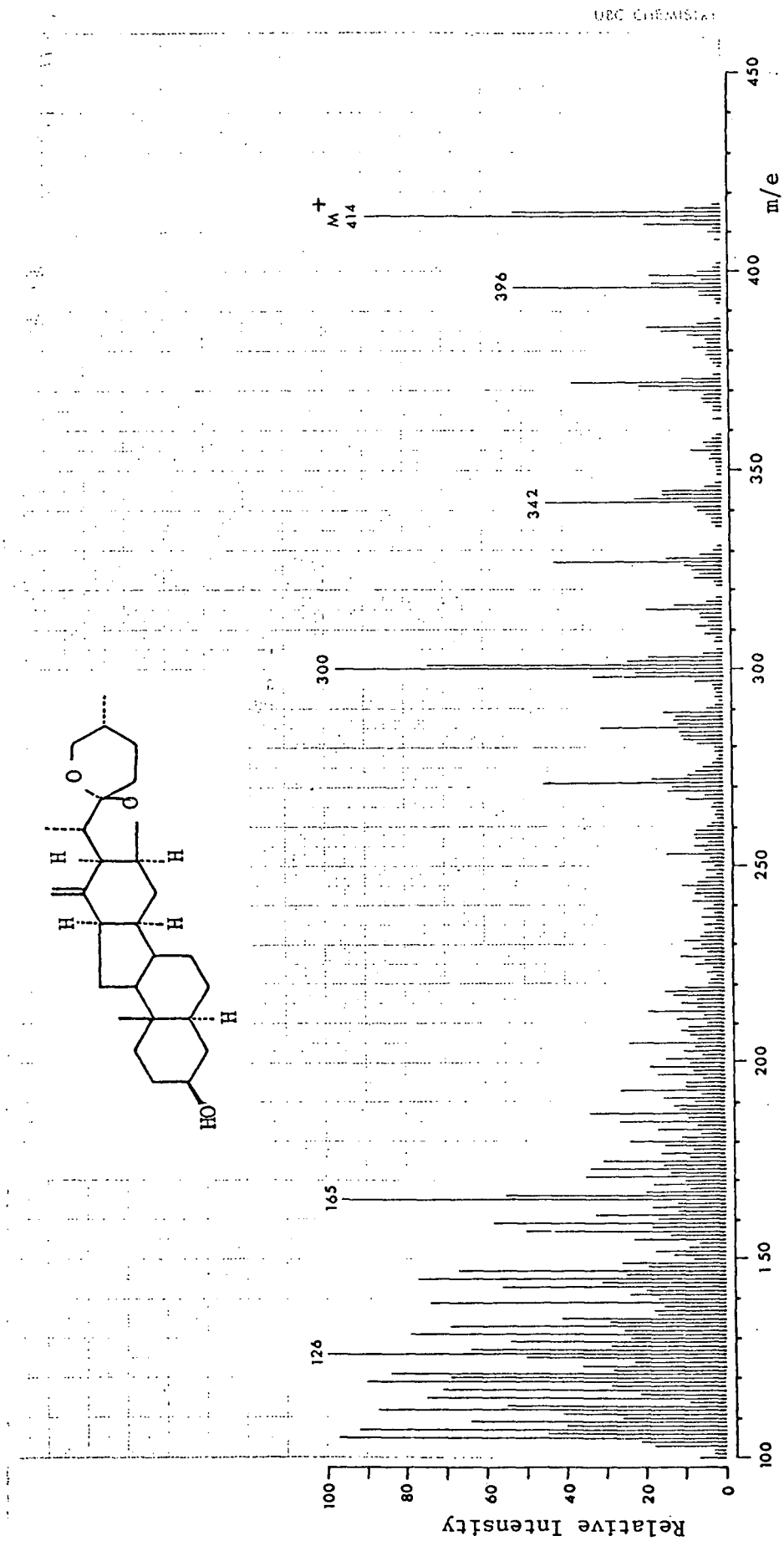


Figure 15: Mass spectrum of compound (119).

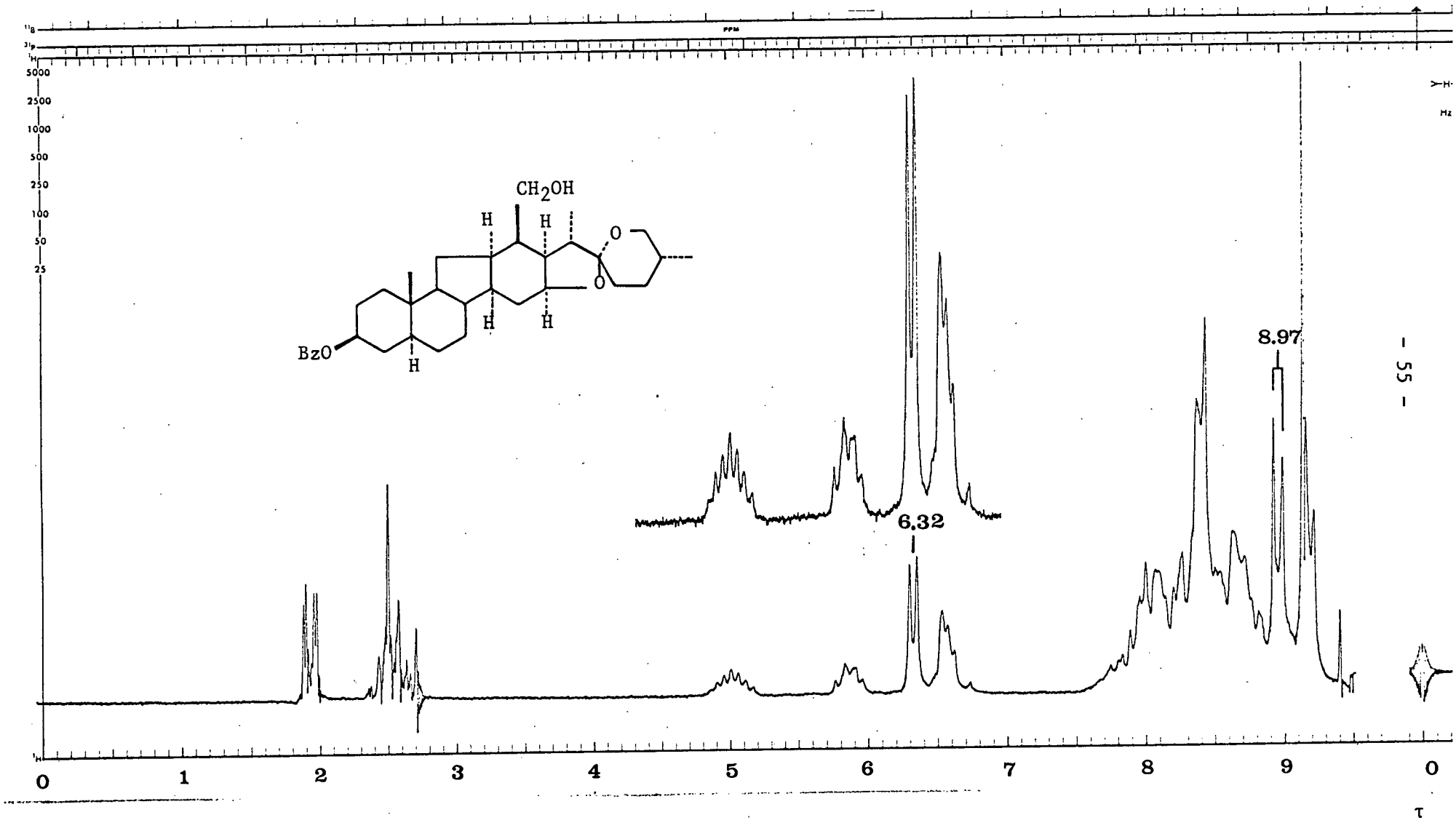
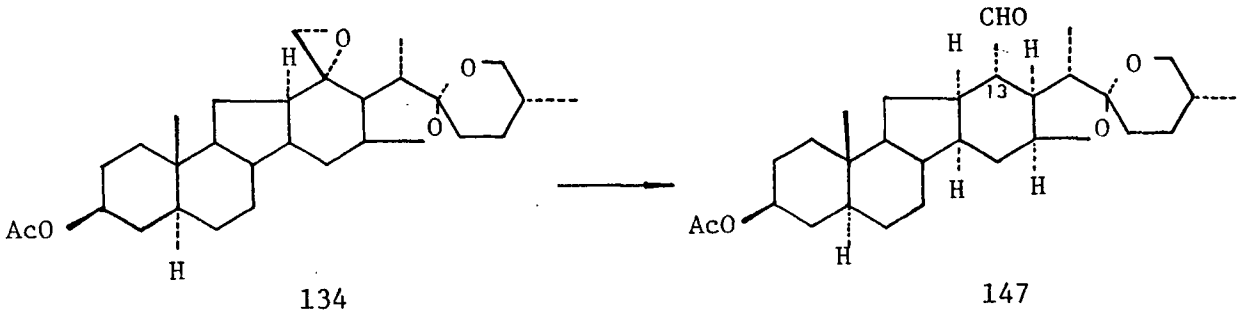
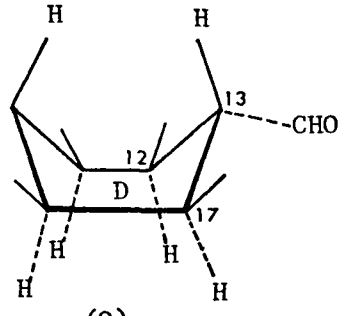


Figure 16: N.m.r. spectrum of compound (146).



134 147

Coxon et al.[113] described a conversion of the α-epoxide (134) by either boron trifluoride etherate or perchloric acid into an aldehyde to which they assigned the 13α-formyl structure (147) on the basis of i.r. and n.m.r. spectra. The compound (147), they reported, was not epimerized by base, confirming the more stable α-configuration of the formyl group. As the partial structure (Q) shows, a α-substituent at C-13 occupies the pseudo-equatorial position in the boat-like ring D.



(Q)

In order to shed some light upon the configurational assignment of the hydroboration product (121) it was decided to prepare an aldehyde derivative for the purpose of comparisons with the previous data.[113] When the diol (121) was subjected to Moffatt oxidation,[162-164] a compound was isolated by rapid chromatography on silica gel. The n.m.r. spectrum of this compound (see Figure 17) showed an aldehydic proton as a doublet (J = 5.5 Hz) at τ 0.22. Similarly the 3-benzoate

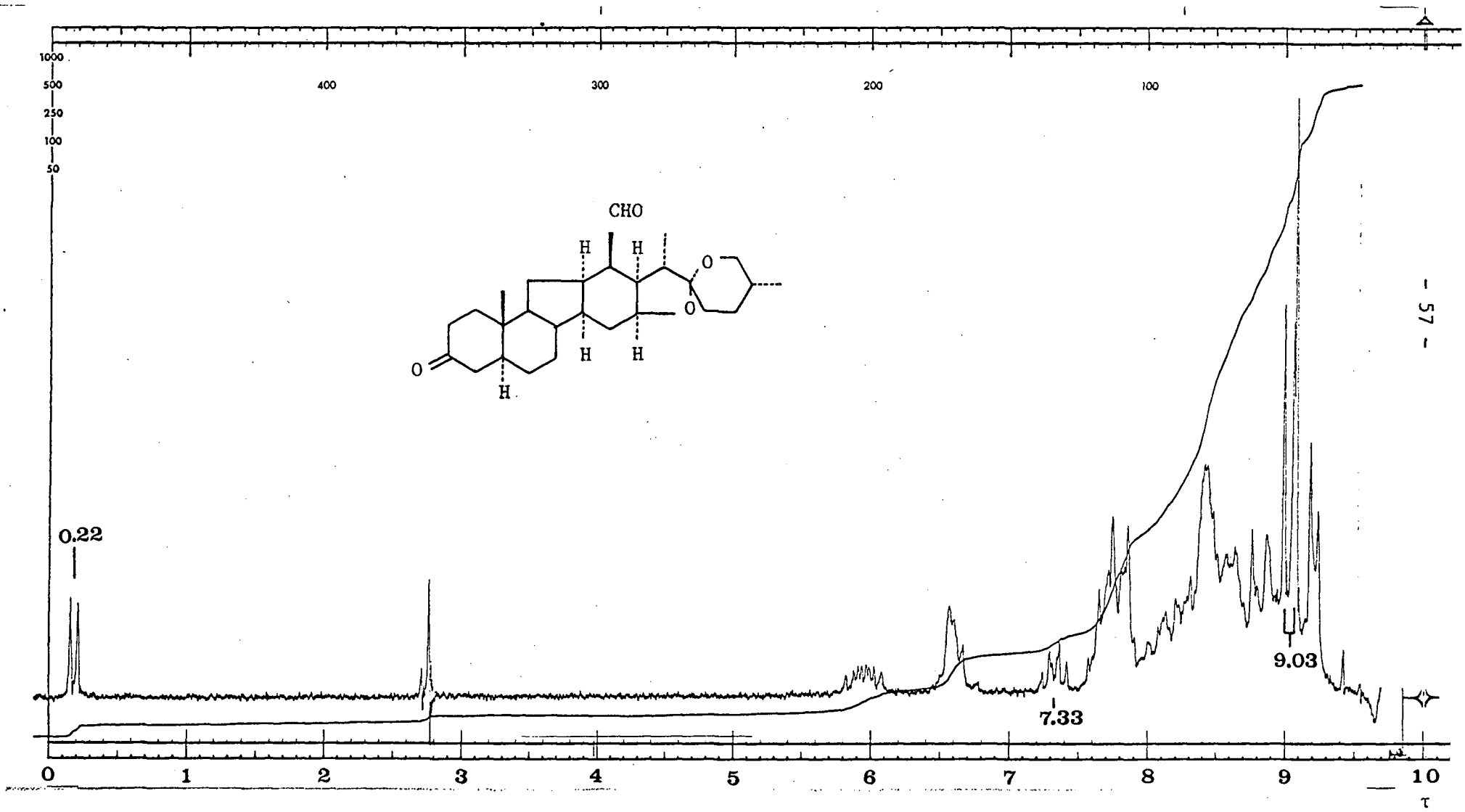


Figure 17: N.m.r. spectrum of compound (148).

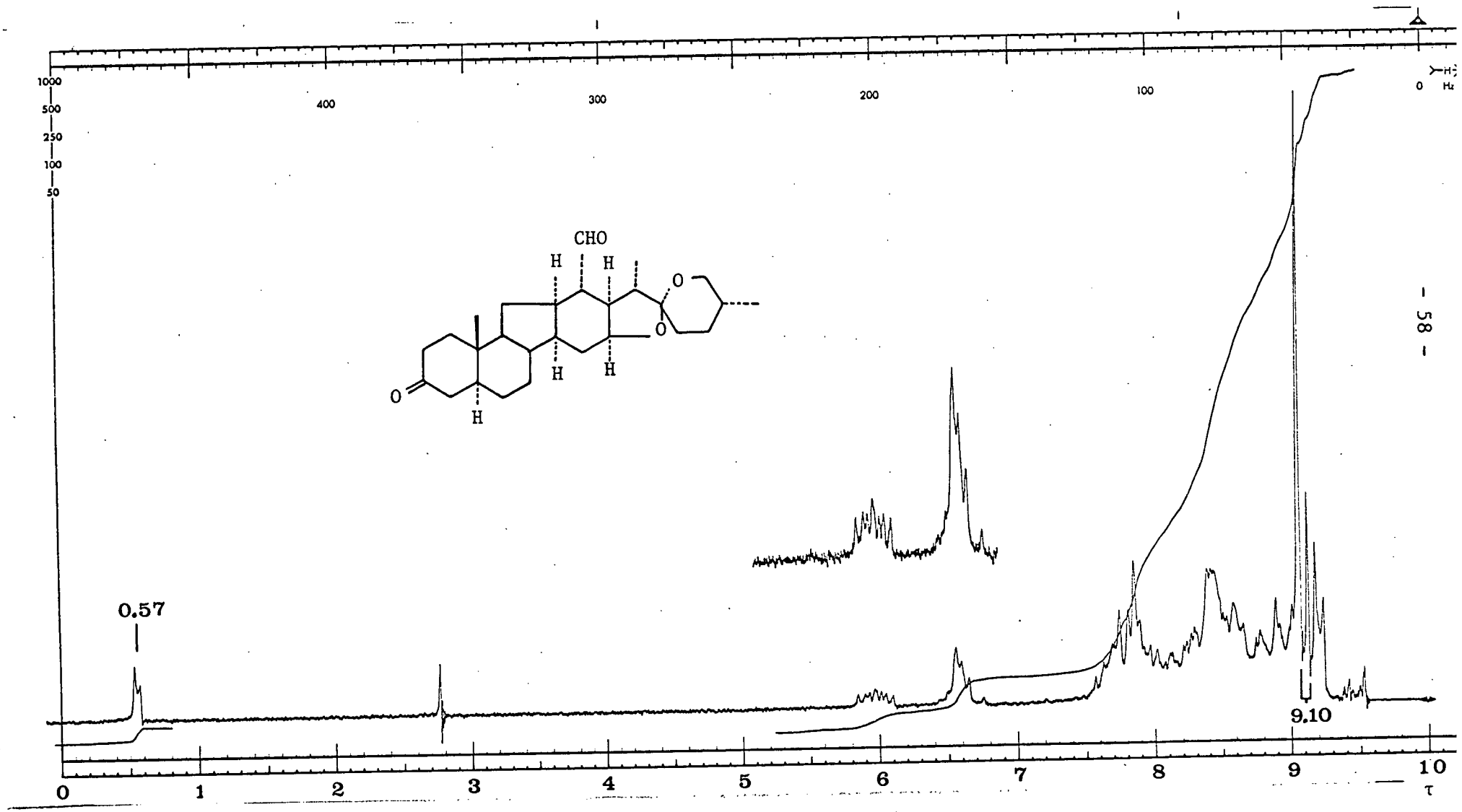
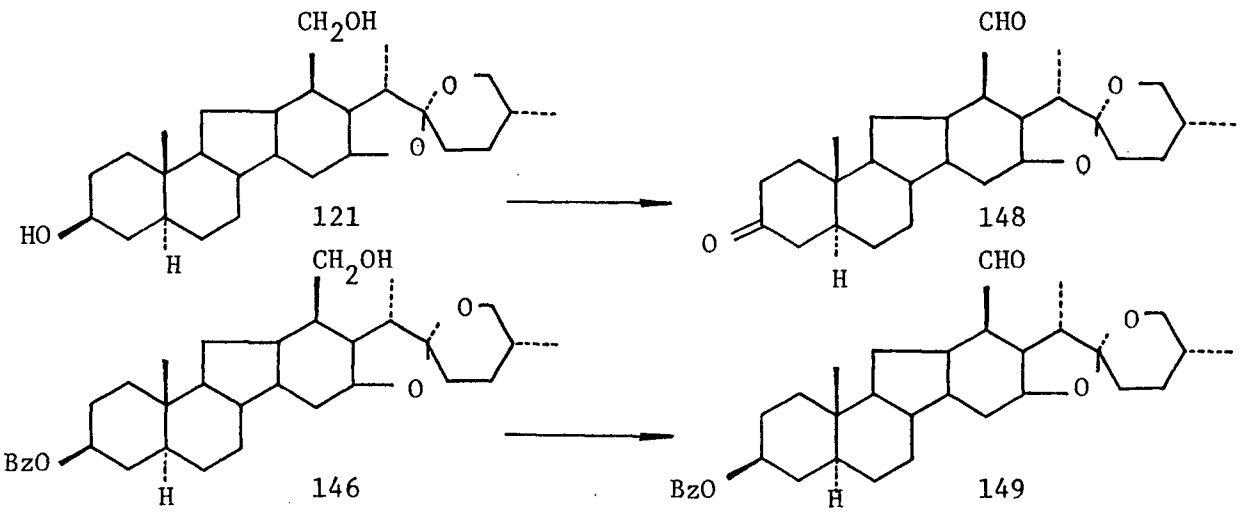


Figure 18: N.m.r. spectrum of compound (150).



derivative (146) was converted to an aldehyde, which also showed a doublet (J = 5.5 Hz) at τ 0.12 (see Figure 19) attributable to the aldehydic proton. The n.m.r. signals (at 60 MHz) reported by Coxon et al.[113] for the 13α-formyl compound (147) were a doublet (J = 7 Hz) at τ 0.21 for the aldehydic proton and a doublet (J = 7 Hz) for the C-13β proton at τ 7.32. As for the latter doublet, a doublet of triplets with coupling constants of 5.5 and 7.0 Hz were found in the same region of the n.m.r. spectra of both aldehydes (see Figure 17 and 19) obtained from (121) and (146).

It was not clear at this point which epimer of the aldehyde had been obtained, since on the one hand no information was available for the 13β-formyl compound and on the other hand the possibility of epimerization during the reaction or the isolation process could not be ruled out. Only if our assignment of configuration for the hydroboration product (121) was correct and no epimerization took place, then the aldehydic product obtained by Moffatt oxidation would have a 13β-formyl

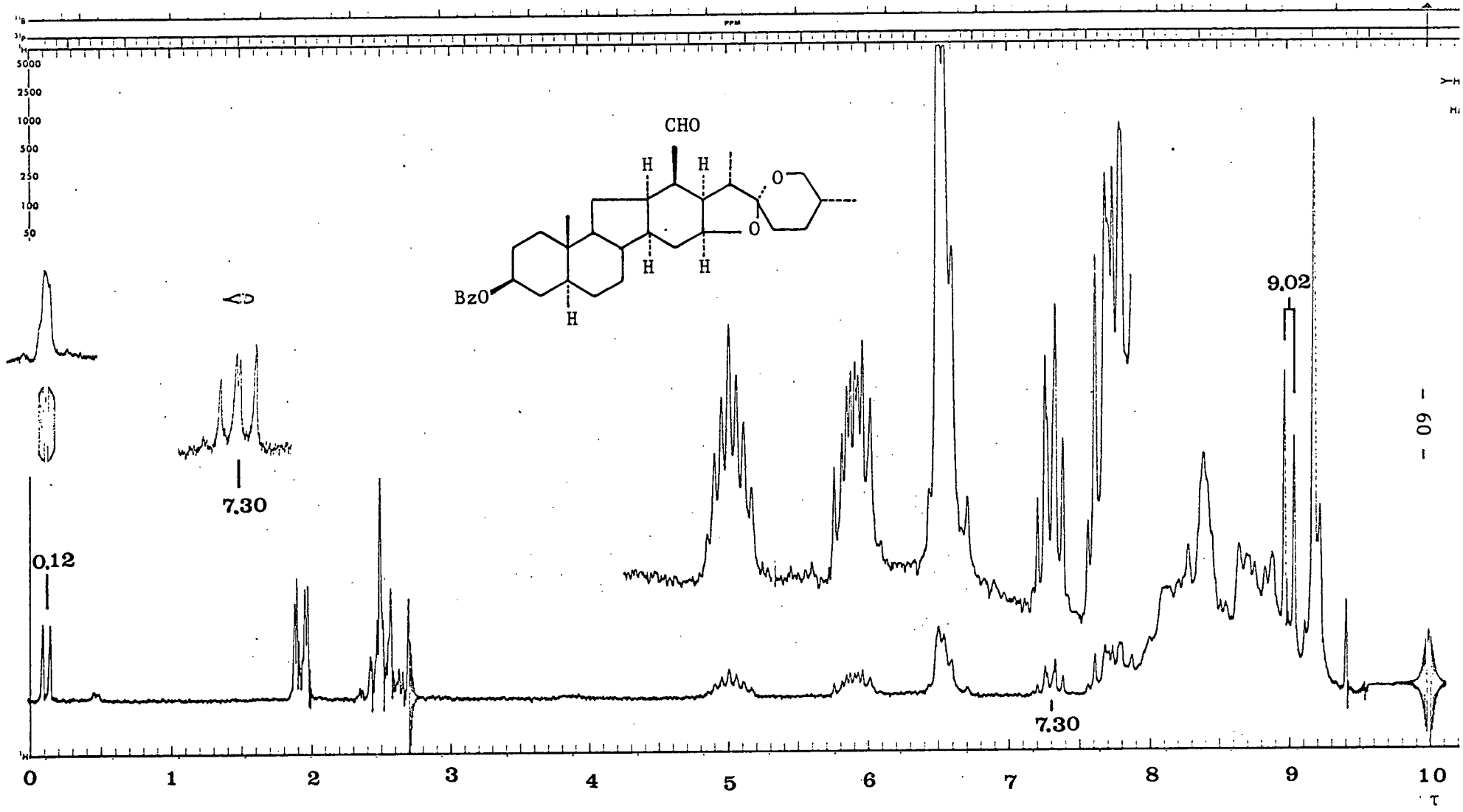


Figure 19: N.m.r. spectrum of compound (149).

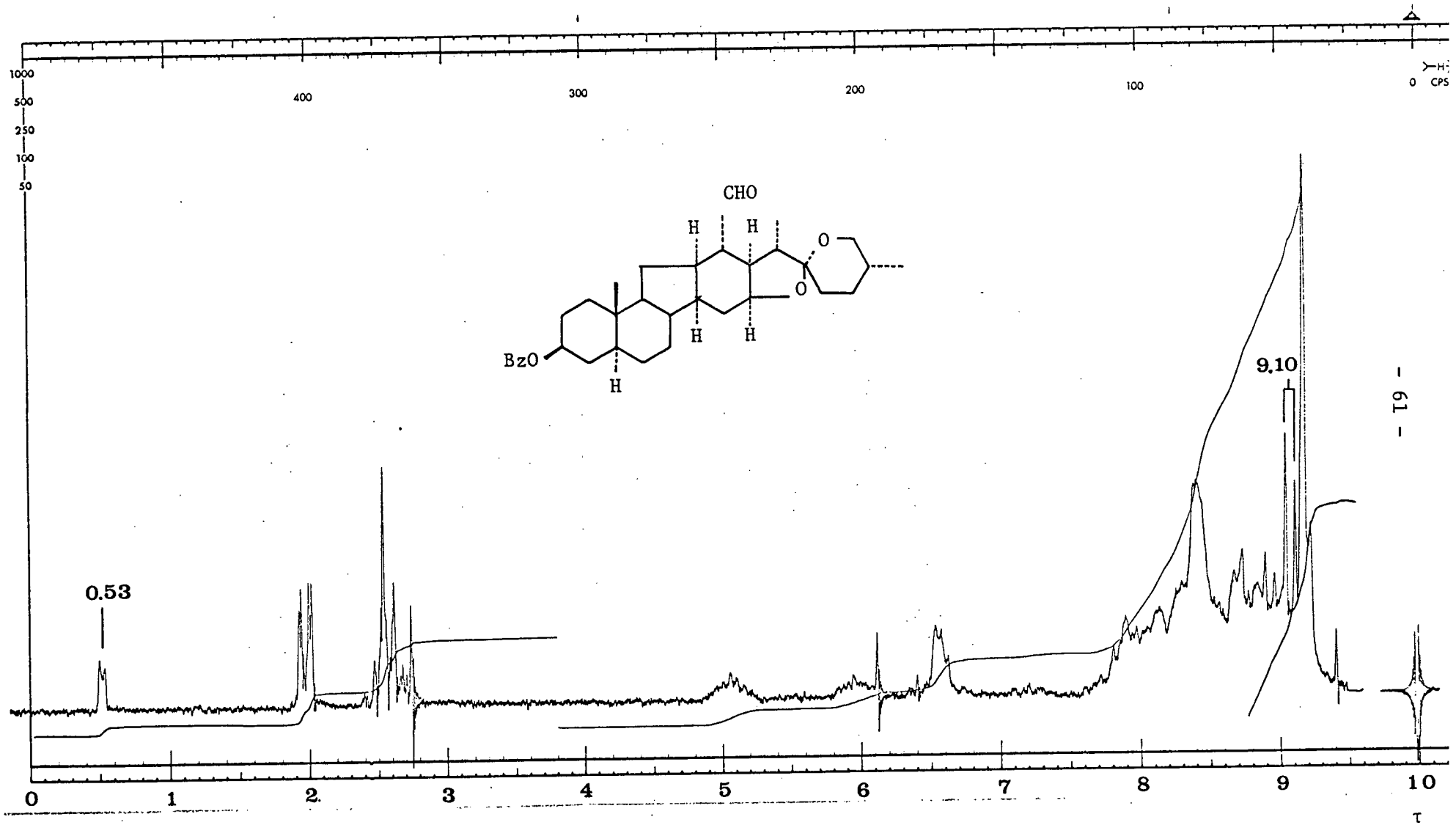
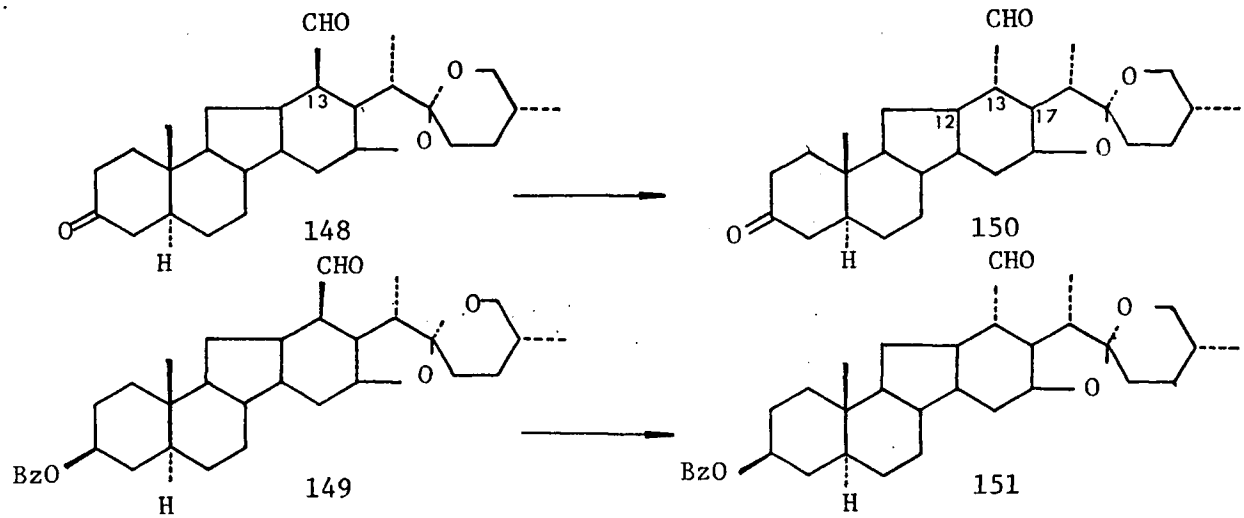


Figure 20: N.m.r. spectrum of compound (151).

group (see 148) and would be the less stable epimer of the two possible products. In fact, it was subsequently found that treatment with potassium carbonate in methanol at room temperature smoothly and completely converted the initial Moffatt oxidation product into the epimeric aldehyde (see Figure 18 for n.m.r. spectrum). It was thus concluded that on Moffatt oxidation of the diol (121) the thermodynamically less stable epimer is formed and that this epimer is completely epimerized on contact with weak base. Furthermore, providing that the D-ring (Q) is in a boat-like conformation and that a pseudoequatorial (α) substituent is more favorable than a pseudoaxial (β) one, it is possible to assign the structures (148) and (150) to the less stable and the more stable epimers respectively. An analogous assignment can also be made for the pair (149) and (151). In the n.m.r. spectra of both (150) (Figure 18) and (151) (Figure 20) the aldehydic proton appears as a poorly resolved doublet and the resonance for the C-13β proton is not visible in contrast with the epimeric aldehydes (148,149).



It is now evident that the structural assignment proposed by Coxon et al.[113] does not agree with ours, and that additional, more direct evidence is necessary to clarify this point. Thus the β-aldehyde 3-benzoate (149) was subjected to a spin decoupling experiment and the results are schematically shown in Figure 21. Attention was focused on two sets of signals at τ 0.12 and τ 7.30 (Figure 19) corresponding to the aldehydic and the C-13α protons. The latter set is comprised of six transition lines and can be analyzed as shown in Figure 21. On irradiation of the higher field sextet the doublet collapsed into a singlet confirming the assignment of the sextet to the C-13 proton. Irradiation in turn of the lower field doublet reduced the sextet to a quartet in which coupling constants of 7.0 Hz and 5.5 Hz were recognized. These results enable us to conclude that the coupling constants between C-13α H and C-12α H and between C-13α H and C-17α H are 7.0 Hz and 5.5 Hz, though a further distinction is not possible.

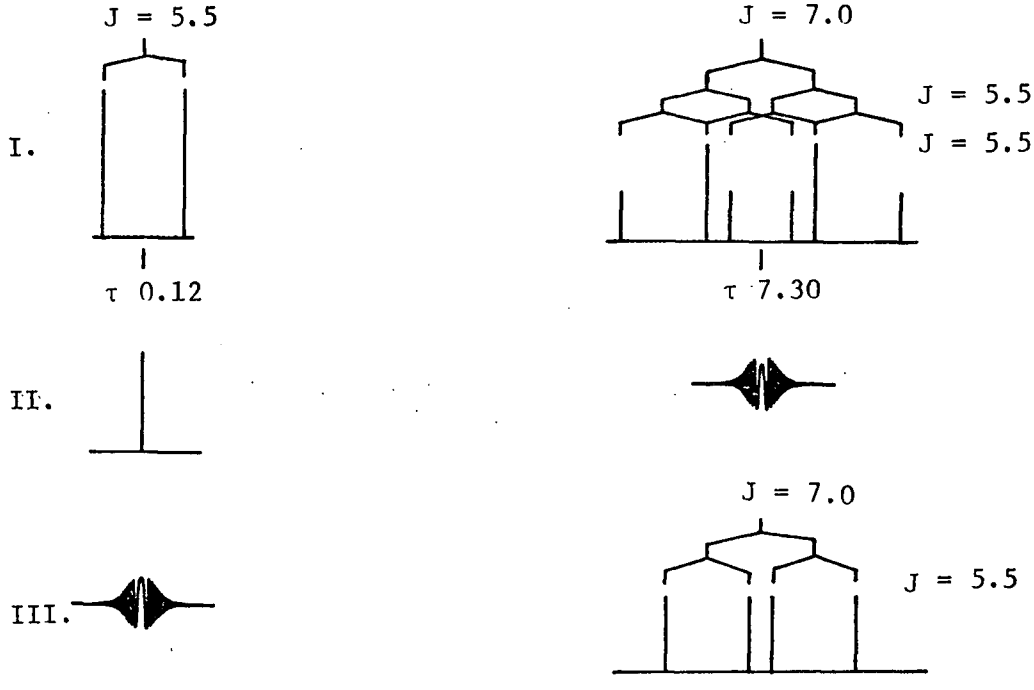


Figure 21: Results of spin decoupling experiment with (149).

It is well known that the magnitude of a vicinal proton-proton coupling constant is related to the dihedral angle separating the two C-H bonds.[165,166] By using the graphic method the spin coupling constants observed above were related to the dihedral angles as symmarized in Table 1. If the 13-formyl group were oriented β as in

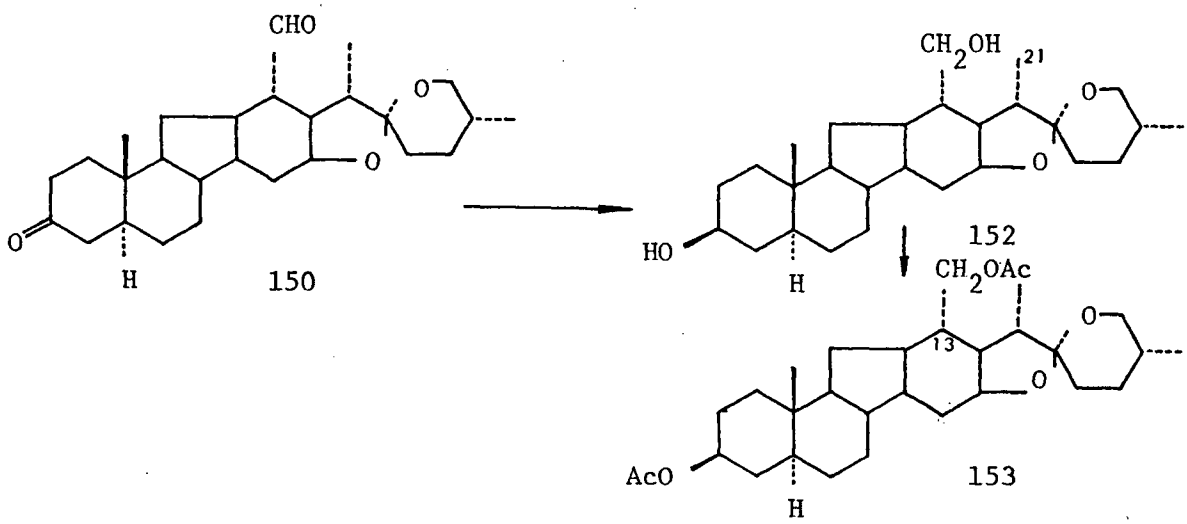
Table 1. Dihedral angles

	Karplus[165]		Williamson and Johnson[166]	
J_{vic} 5.5	35°	140°	40°	125°
	(-25°)	(-40°)	(-20°)	(-55°)
J_{vic} 7.0	22°	150°	23°	130°
	(-38°)	(-30°)	(-37°)	(-50°)

structure (149), it would not be unreasonable to assume that C-13 and C-15 would shift away from each other to give a flattened boat conformation for ring D. If this is so, then the dihedral angles between C-12αH and C-13α H as well as between C-17α H and C-13α H would become less than 60°. A similar deformation, however, would not be as preferable for the 13α-formyl compound (151), since the α substituent at C-13 is already in the pseudo-equatorial position. Considering the deviations of the angles (60—180°) shown in Table 1 which are expected in the ideal boat conformation, it seems more

plausible to assign the α configuration to the hydrogen at C-13, in agreement with the previous discussions.

The diacetate (153) represents the key synthetic intermediate which possesses not only the C-nor-D-homo ring system but also the functionalized C-18 methyl group with the desired stereochemistry at C-13. The aldehyde (150) was treated with sodium borohydride in methanol and the resulting diol (152) was obtained pure by crystallization from ethyl acetate-petroleum ether. The n.m.r. and the mass spectra of the diol (152) are shown in Figures 22 and 23, respectively. The mass



spectra of the isomeric diols (121 and 152) showed quite similar fragmentation patterns (Figure 10 and Figure 23). Comparison of the n.m.r. spectra of the isomeric diols (Figure 9 and Figure 22) shows that the C-19 methyl resonance in (152) is shifted upfield (+0.03 p.p.m.) compared to that in (121), whereas the doublet due to the C-21 methyl group is shifted downfield as much as -0.08 p.p.m. On the basis of the empirical rules summarized by Zurcher,[186,187] these

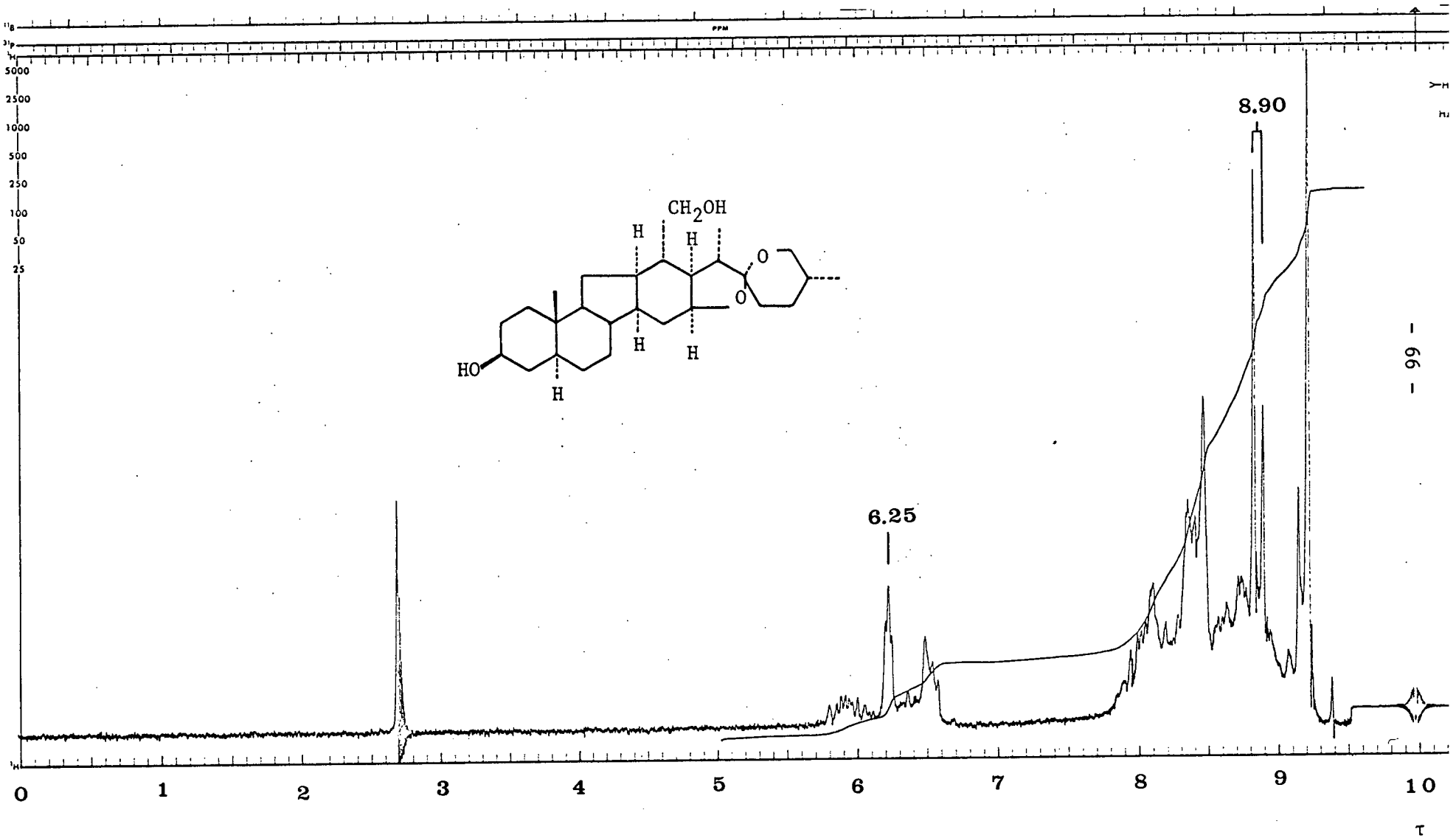


Figure 22: N.m.r. spectrum of compound (152).

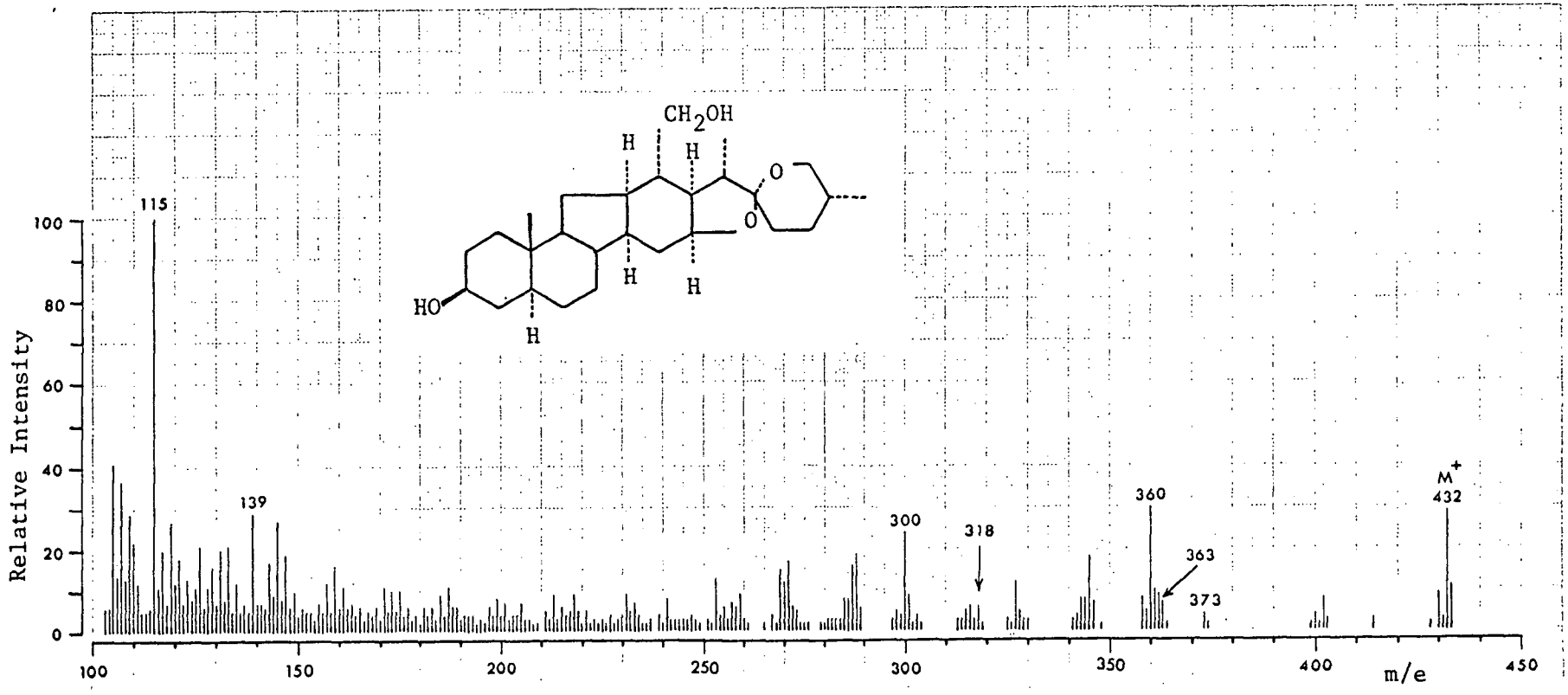


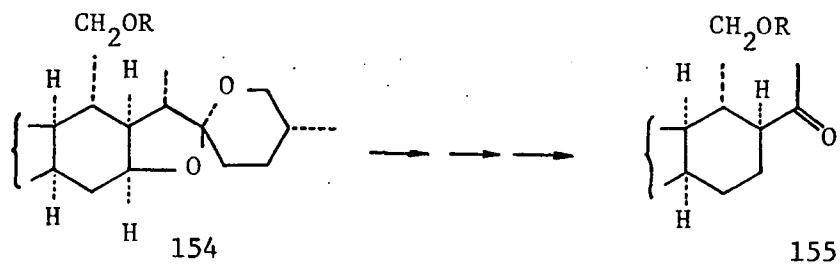
Figure 23: Mass spectrum of compound (152).

observed shifts are qualitatively consistent with the configurational change of the 13-hydroxymethyl group from the β (121) to the α (152) position. The diol (152) was subsequently acetylated by the usual method to yield the corresponding diacetate (153) which was characterized by its n.m.r. (Figure 24) and mass spectra.

In the i.r. spectra of C-nor-D-homo-spirostan derivatives, such as (119), (119a), (119b), (121), (145), (146) , (149) , (152) and (153), the four characteristic bands associated with the spiroketal side chain were observed at approximately their predicted positions, namely 982, 922, 900 and 866 cm^{-1}. However, the above compounds exhibited the 922 cm^{-1} band with reduced intensity, this phenomenon being characteristic of (22R)-C-nor-D-homo-spirostans. In general, the order of intensity of the aforementioned bands in (22R)-C-nor-D-homo-spirostans is as follows,

$$\varepsilon_{982\ cm^{-1}} > \varepsilon_{900} > \varepsilon_{866} > \varepsilon_{922}$$

With the characterization of 3β-acetoxy-13α-acetoxymethyl-C-nor-D-homo-18-nor-5α,12α-spirostan (153) the present synthesis entered the third phase in which the spiroketal system (154) has to be modified to give the suitable C_{21} "pregnajervane" derivative (155).



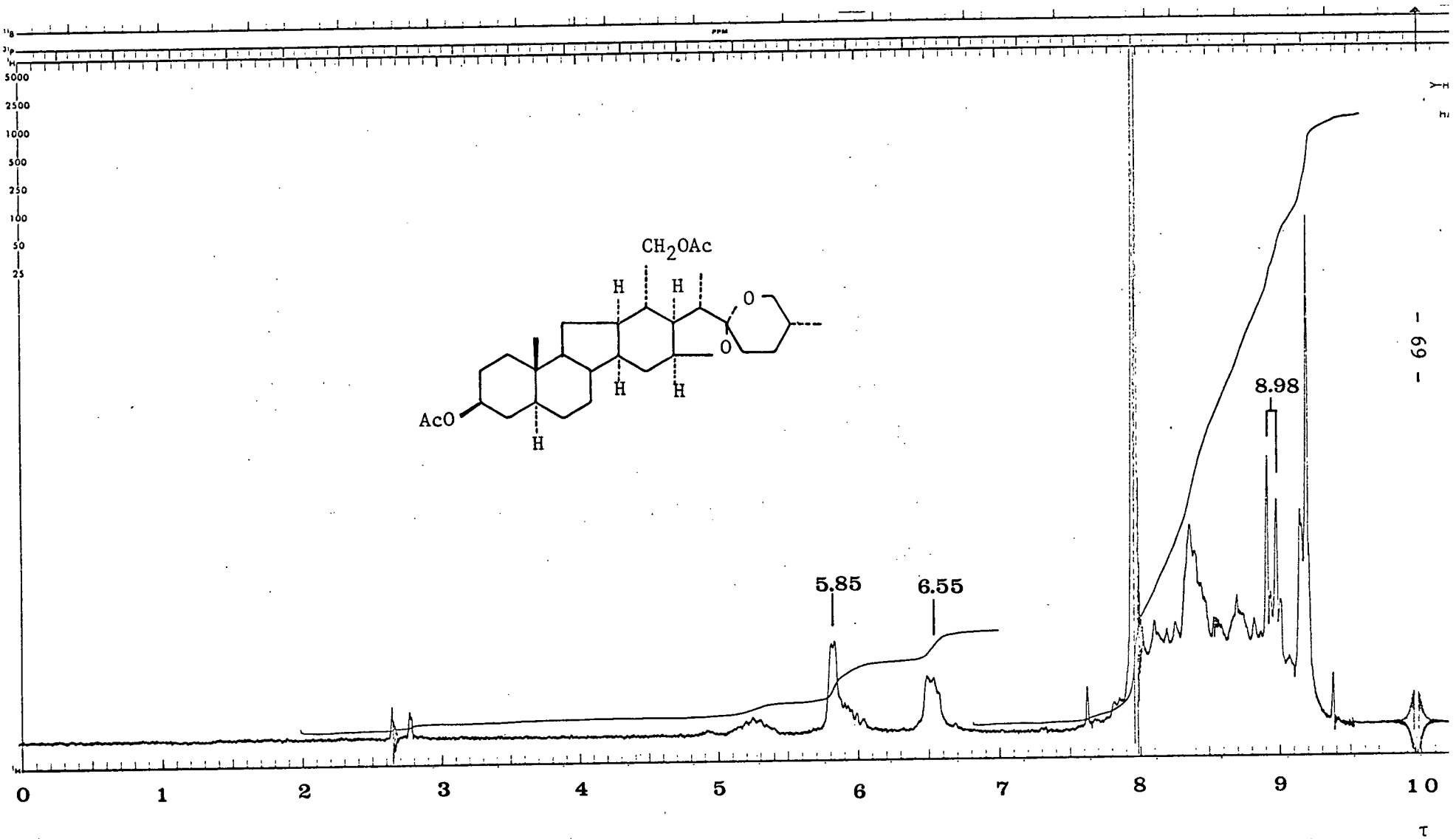
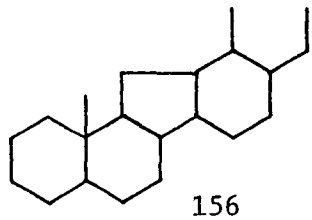


Figure 24: N.m.r. spectrum of compound (153).

Since there are presently no officially accepted rules of nomenclature for C_{21} C-nor-D-homo steroids such as those described in the following part of the thesis , it is appropriate here to make some comments about this matter. The IUPAC nomenclature for the C_{21} tetracyclic hydrocarbon (156) is cumbersome, and adaptation of



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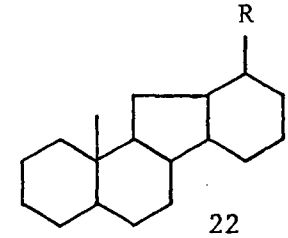
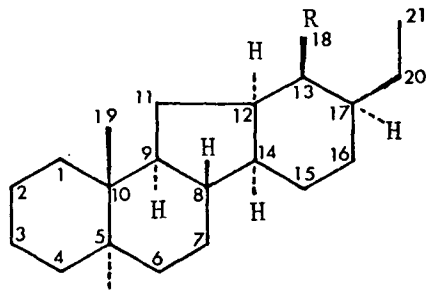


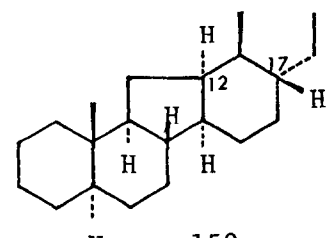
22

"etiojervane" (22; R = CH_3)[58] as parent compound leads to other complications, particularly with respect to the numbering of the two-carbon side chain and designation of the stereochemistry of substituents attached to it. Chang and Ebersole[122] proposed that the term "pregnajervane" be adopted for the C_{21} hydrocarbon (156) as a substitute for 17-ethyletiojervane.[175,188,189] The numbering system for the "pregnajervane" skeleton is illustrated for 12α-pregnajervane (157).[174,190] It is noteworthy here that the term "pregnajervane" implies the configurations at all the asymmetric centers except C-12. On this basis, the tetracyclic hydrocarbon which lacks the methyl



157 R = CH_3

157a R = H



158

group at C-13 must be named 18-nor-12α-pregnajervane (157a, R = H). The C-17 epimer of 12α-pregnajervane (157) is, according to W.F. Johns,[174] denoted as 12α,17α-pregnajervane (158).

Two methods have been reported for degradation of the spiroketal system of sapogenins to a Δ^{16}-20-one[130-136,139] or a 16,20-diol[141-145] derivative. The first method, as already outlined in Figure 6, involves the classical pseudomerization followed by chromic acid oxidation. This method, however, has been reported[129] to be unsuccessful in the 18-substituted C-nor-D-homo sapogenins. Hence, in the present synthesis this particular method was not investigated to any extent. The second method for the degradation, originally developed by Marker et al.,[141,142] involves treatment of sapogenins under Baeyer-Villiger oxidation conditions followed by base-catalyzed hydrolysis of the resultant mixed esters. Some examples of this method are illustrated in Figure 25.

W.F. Johns[129] has developed quite an efficient method to convert the spiroketal system to its Δ^{16}-20-one derivative, namely the conversion of 3β-acetoxy-C-nor-D-homo-(25R)-5α,12α-spirostan (122) to 3β-hydroxy-12α-pregnajerv-16-en-20-one (167). The mixed ester (164), which had been obtained directly from the performic acid oxidation of the sapogenin (122), was hydrolyzed by contact with alumina and the resulting diol (165) was oxidized to the keto-acid (166). The keto-acid (166), after base treatment, afforded the α,β-unsaturated ketone (167) in 50% over-all yield from the C-nor-D-homo sapogenin (122). This conversion compared favorably with that from the classical pseudomerization procedure (45%).[148] The Baeyer-Villiger oxidation

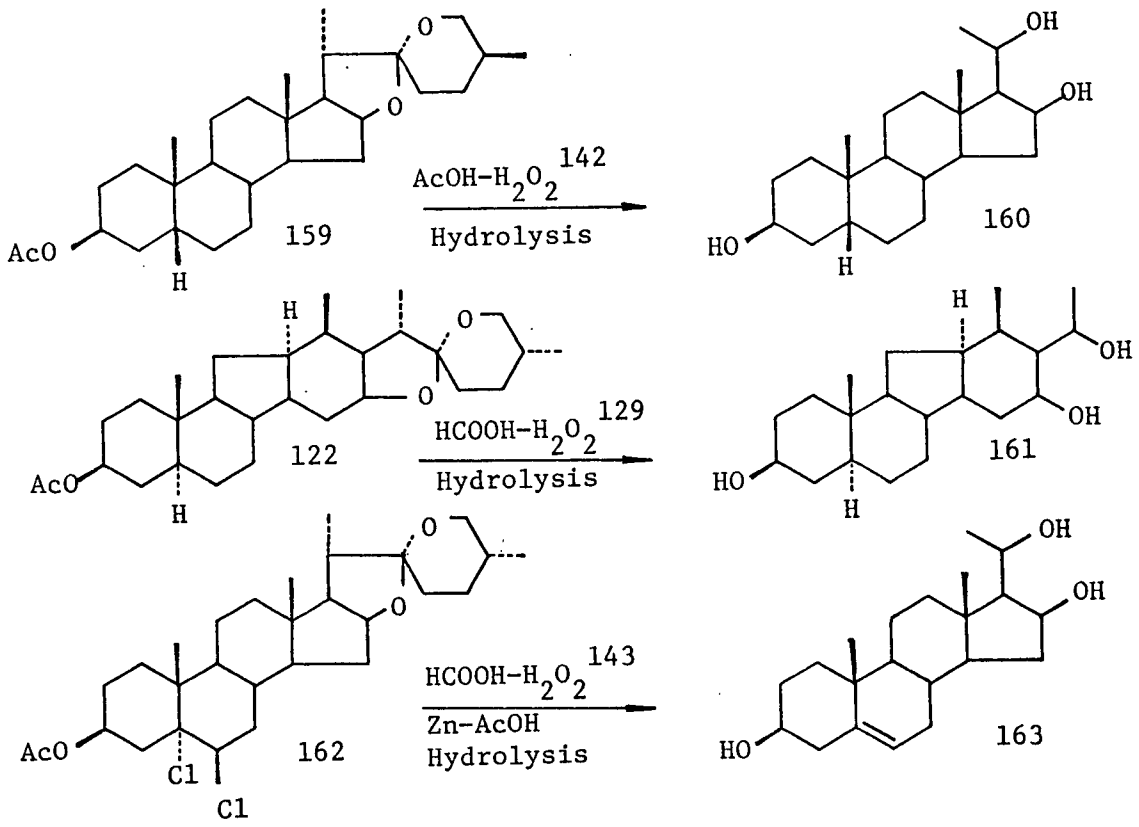
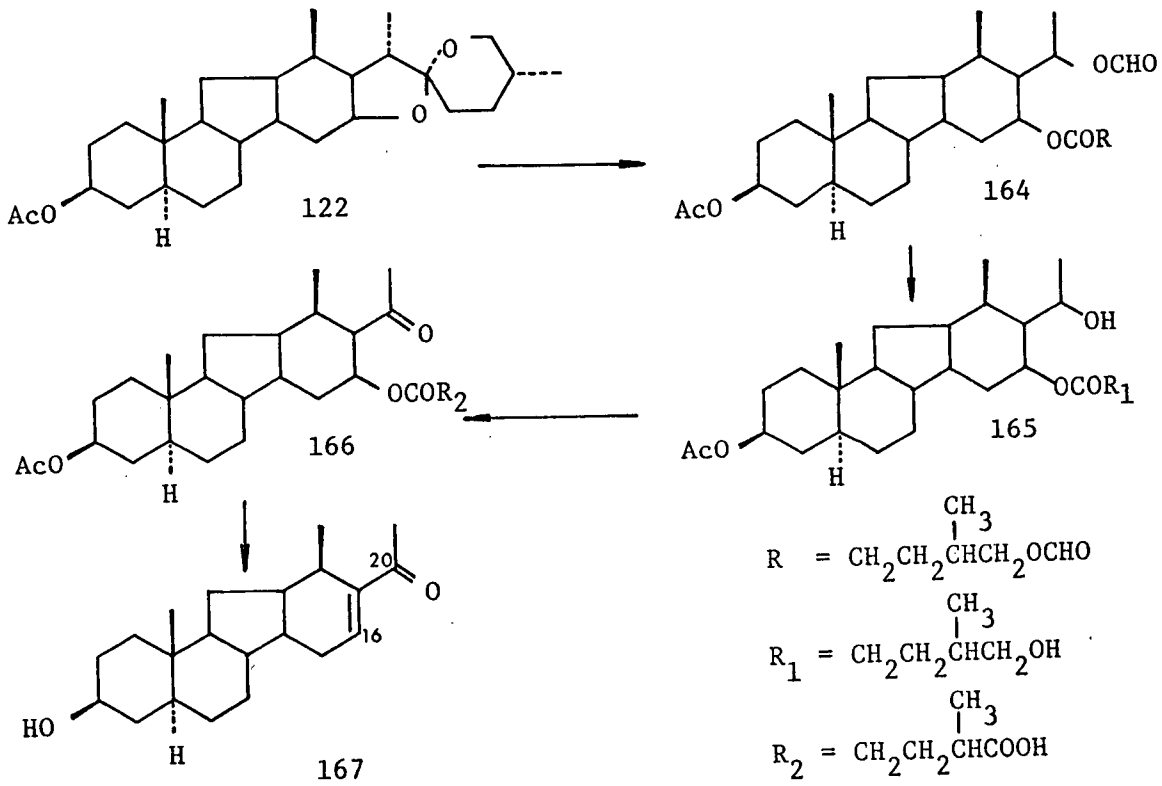


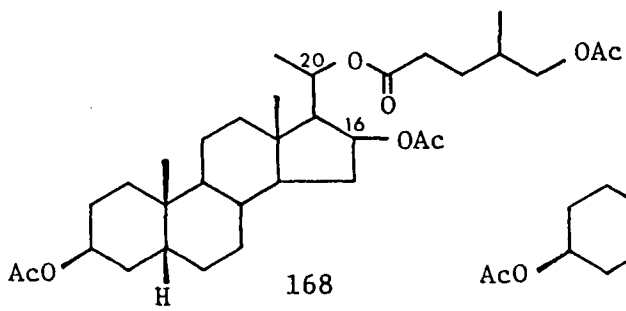
Figure 25: Some examples of peracid degradation of the spiroketal system.



procedure was therefore attempted with the intermediate diacetate (153).

When (153) was treated with performic acid generated *in situ* at 41-44° for 4 hr, a gummy material was obtained which exhibited two major spots by t.l.c. along with more polar, minor products but no starting material could be detected. A similar result was obtained when the oxidation was carried out at room temperature for 36 hours. When this oxidation mixture was chromatographed on Florisil, the combined amount of the two major spots was found to be about 85% of the total mixture. Since it was impossible to isolate any single compound from this mixture, no further insight into the selectivity of the oxidation could be obtained at this stage.

The structure of the reaction intermediate postulated for the conversion of sarsasapogenin 3-acetate (159) to 5β-pregnane-3,16,20-triol (160)[142] was the mixed-ester (168) resulting from the Baeyer-Villiger oxidation of the potential ketonic group at C-22 in the spiroketal system. However, for the performic acid oxidation of the C-nor-D-homo sapogenin (122)[129] it was demonstrated that one of the formate groups incorporated during the oxidation is located at C-20. This result suggested that the actual structure (169) of the Baeyer-



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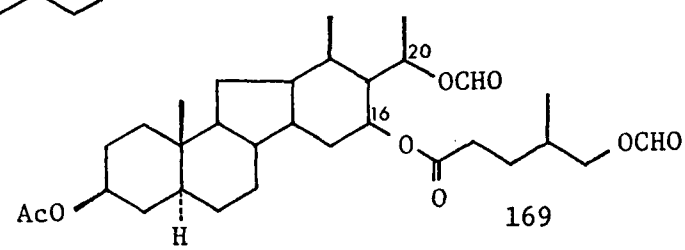


169

Villiger product, or at least that of the major product, was the reverse of that expected.

The mechanistic pathway, initially suggested by W.F. Johns,[129] for the performic acid oxidation of the spiroketal system, may be elaborated for the diacetate (153) as given in Figure 26. Protonation of one of the ether oxygens preferentially opens the six-membered ring F. The same mode of preferential cleavage of ring F of sapogenins has been observed in such examples as the classical pseudomerization[130,131] and the reductive cleavage with lithium aluminum hydride-aluminum chloride[138] or by catalytic hydrogenation in acetic acid.[148] Attack of peracid at C-22 and subsequent bond migration would result in the dioxolane intermediate (172) analogous to Winstein's postulated intermediate in solvolysis with neighboring ester group participation.[191] The nucleophilic attack by formate anion at either C-16 or C-20 produces two isomeric mixed-ester intermediates (173,174). The C-26 hydroxyl group may be formylated separately.[192]

Although the structures of the products obtained from performic acid oxidation of the diacetate (153) were uncertain, selective hydrolysis of the formate esters was attempted. A survey of the literature revealed that ester hydrolysis is a common occurrence on active alumina, and that a modified chromatographic technique affords a synthetically useful tool especially for formate hydrolysis[193-195] as well as for selective hydrolysis of acetates of primary alcohols.[196] Adsorption of the crude oxidation products on alumina of various pH's however, resulted in quite complex mixtures.[197] When the same material was treated with potassium carbonate in methanol for a very brief

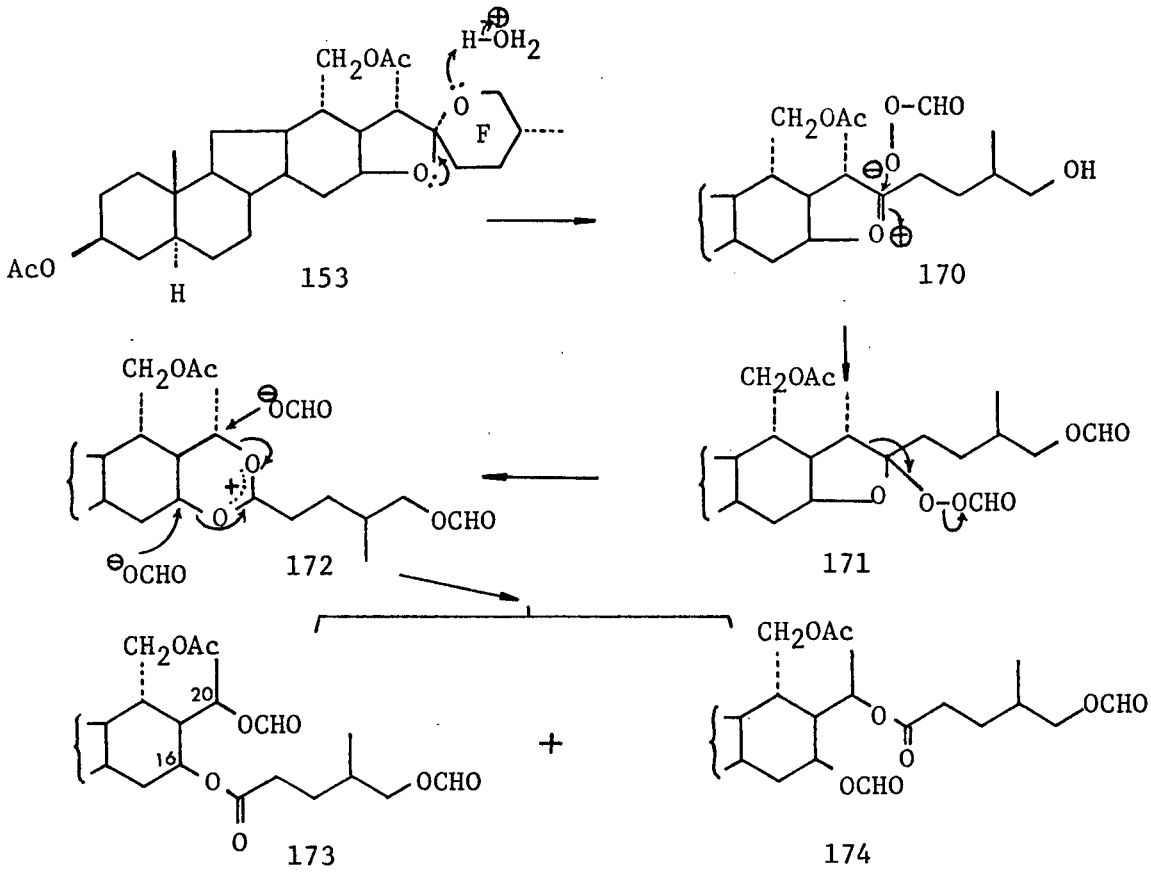
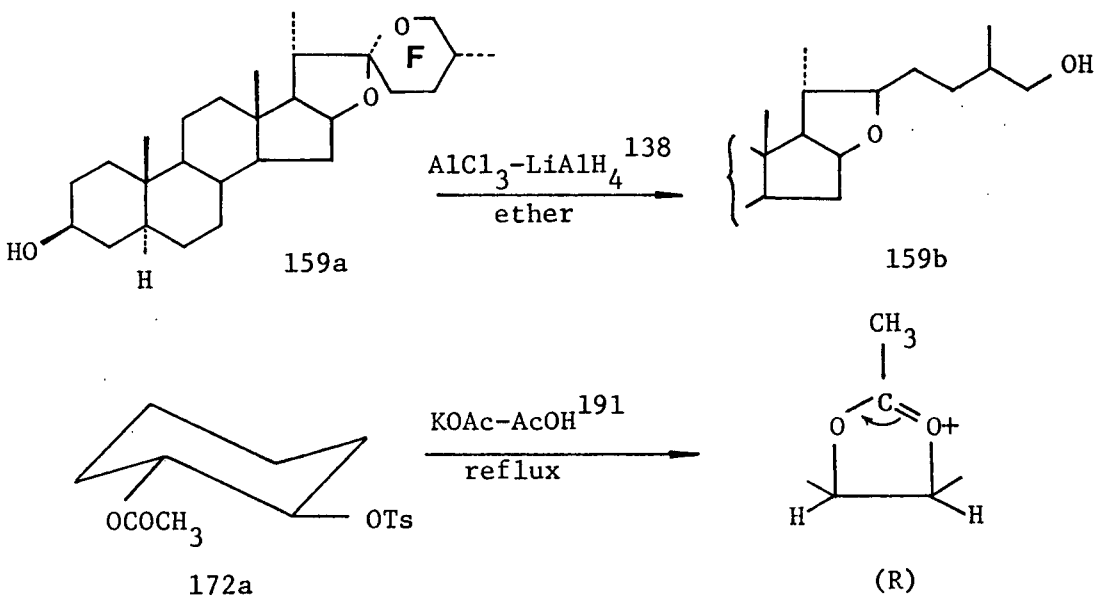
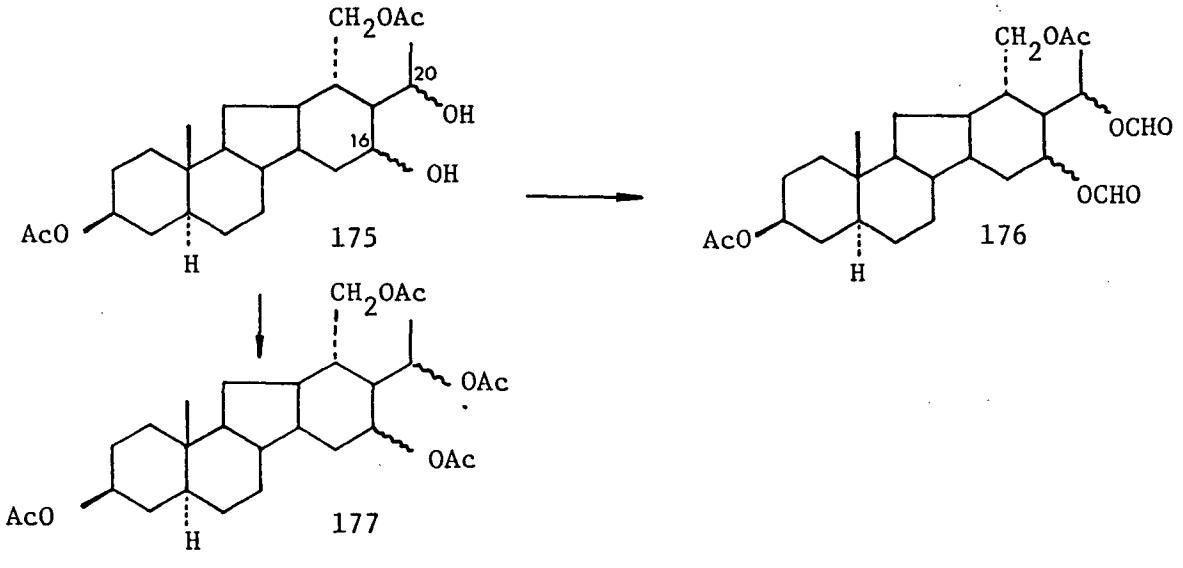


Figure 26. Postulated mechanism of the performic acid oxidation of Diacetate (153).



period, a multitude of products, as evidence by t.l.c., were observed. Chromatography on deactivated alumina led to the isolation of the major product of hydrolysis, in 21% yield, from the diacetate (153). The structure of this material was determined as 3β-acetoxy-13α-acetoxymethyl-18-nor-pregnajervan-16ξ,20ξ-diol (175) on the basis of n.m.r. (see Figure 27), i.r. and mass spectral data as well as the results of elemental analysis. The configurations at C-16 and C-20 are not known.

That the diol (175), in which the ring-opened side chain had been lost, was formed in substantial quantity by potassium carbonate treatment suggests either that the conditions are too drastic or that the diformate ester of the tetracyclic structure (176) was already present in the Baeyer-Villiger oxidation products. Thus the diol (175) was treated with acetic-formic anhydride[198] in pyridine and the diformate derivative (176) (Figure 28) was obtained in good yield. Comparisons by t.l.c. showed that the diformate (176) had not been



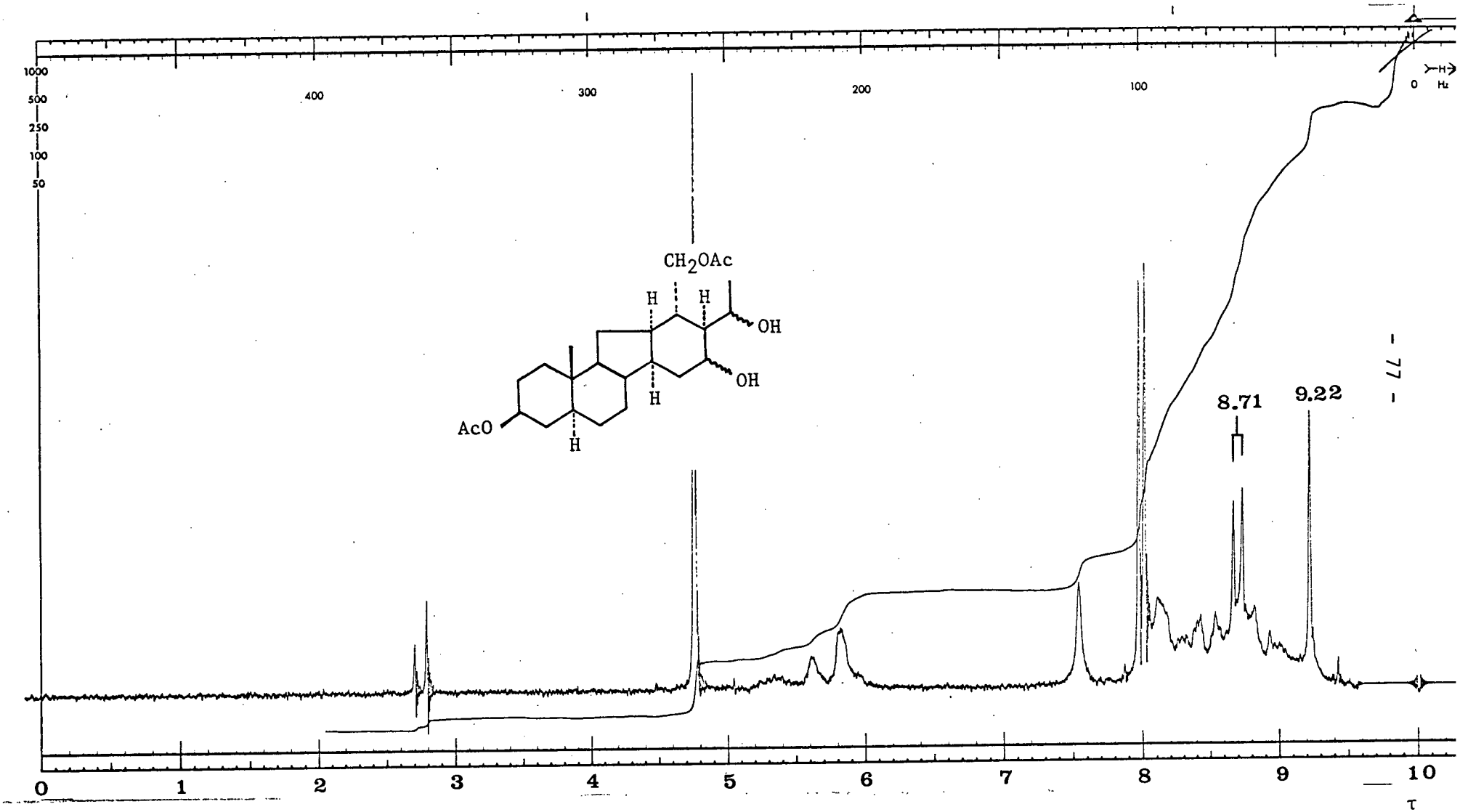


Figure 27: N.m.r. spectrum of compound (175).

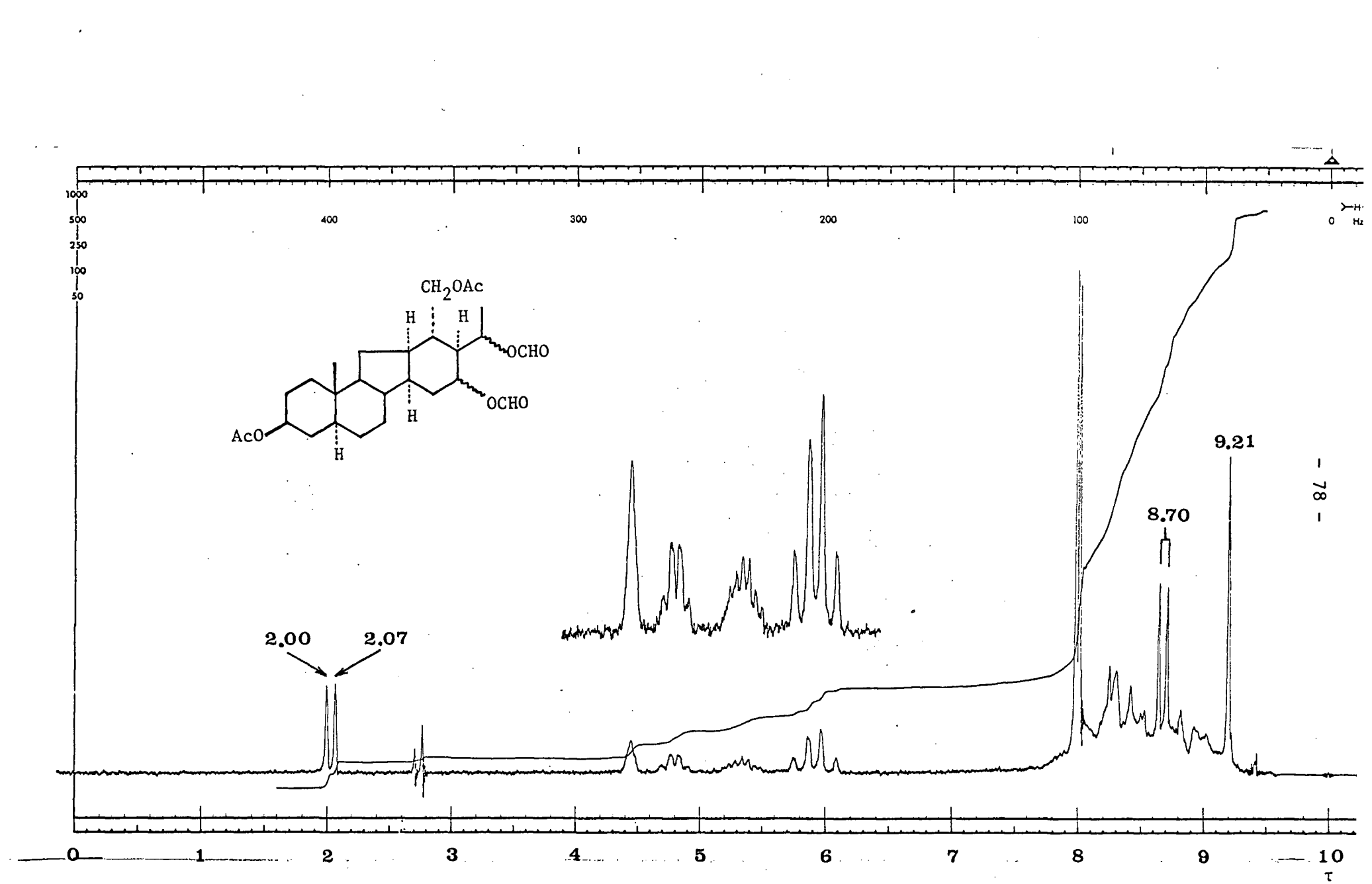


Figure 28: N.m.r. spectrum of compound (176).

formed by the Baeyer-Villiger oxidation of the diacetate (153). It was concluded that potassium carbonate in methanol is too drastic for selective removal of formate ester groups. However, chromic acid oxidation of the hydrolysis mixture followed by base treatment and acetylation gave, among many other products, the tetraacetate (177) which was apparently derived from the incompletely hydrolyzed products such as (178) and (179). The structure of the tetraacetate (177) was established on the basis of n.m.r. (see Figure 29), i.r., and mass spectral data as well as the results of elemental analysis. The configurations at C-16 and C-20 were not assigned. It was also found that acetylation of the diol (176) gave a compound which was identical in every respect with the tetraacetate (177). Summarizing these results it is clear that hydrolysis of the Baeyer-Villiger product with potassium carbonate in methanol results in indiscriminate removal of the ester groups. The extent of hydrolysis is probably dependent mainly on the period of reaction.

The pH of the system used for the hydrolysis described above was found to be over 11. It was speculated that better selectivity in the hydrolysis of formates, which are normally expected to be more readily hydrolyzed than their higher homologues, may be attained if a less basic system is employed. Thus the crude Baeyer-Villiger oxidation mixture was treated with a methanolic buffer system adjusted to pH 8.06 with sodium barbital-hydrochloric acid.[199] Hydrolysis was carried out at 42-45° for 8 days readjusting the pH to 8.06 every 24 hours until most of the starting material was consumed. T.l.c. studies of the crude hydrolysis mixture showed that more than ten

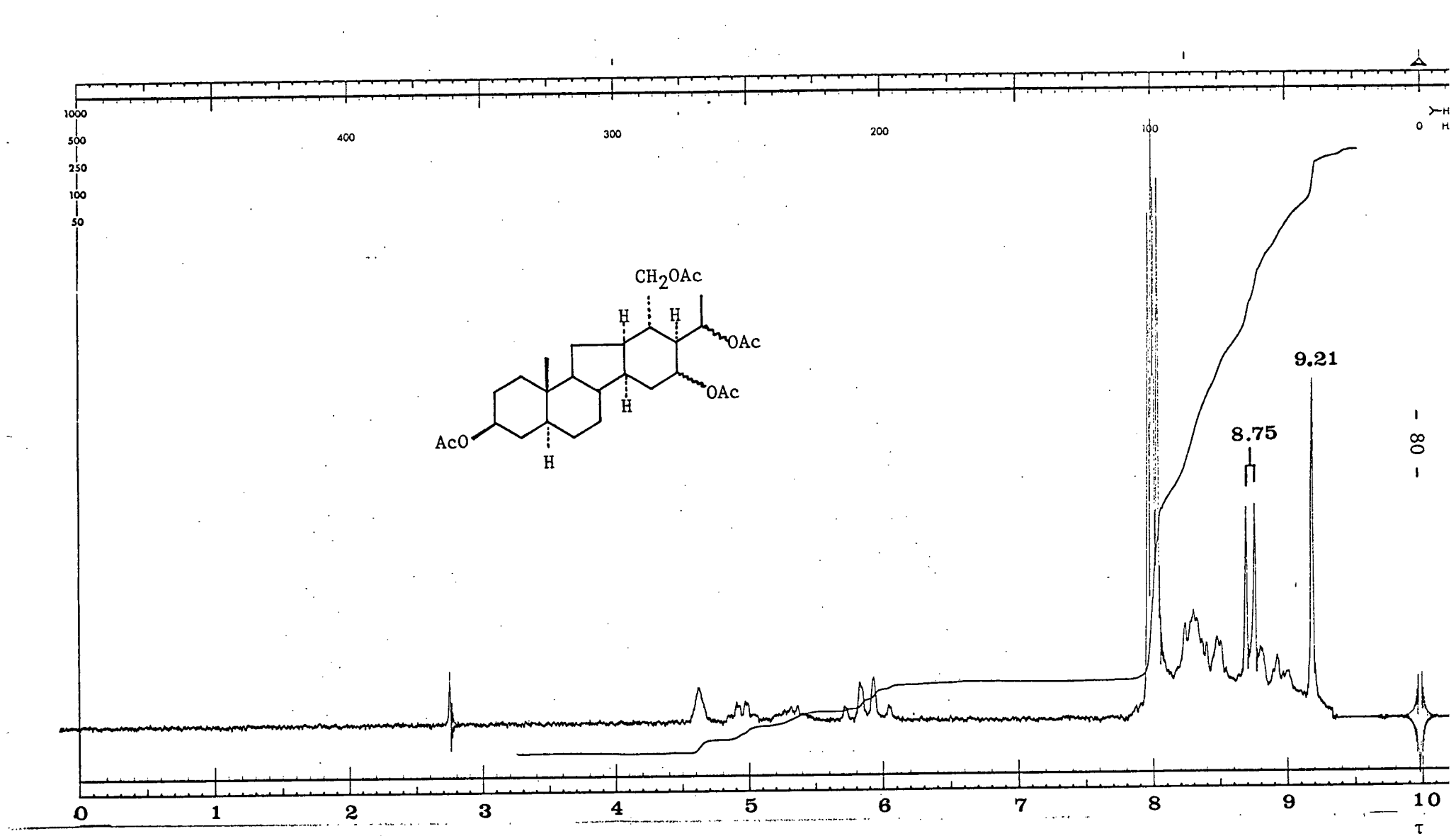


Figure 29: N.m.r. spectrum of compound (177).

products, with one of them predominant, were visible (spraying with sulfuric acid and subsequent heating of the chromatoplate) and that the diol (175) was present as one of the minor products. The formation of the diol (175) here may be explained in terms of an assisted hydrolysis[200] which is presumably operative in the intermediate diols (180 and 181) behaving as a mono-esters of 1,3-diols. The predominant product was then considered to correspond to either of the intermediate diols, (180) and (181), or a mixture of both. However, subsequent investigation showed that this assignment was incorrect.

For structural studies of chemical compounds various physical methods are now available. In the course of this synthesis extensive use was made of spectroscopic techniques, particularly those of i.r., n.m.r. and mass spectroscopy. However, when it comes to investigating a mixture of compounds, these techniques are usually inadequate and isolation of each component in pure forms becomes essential. For solving the structural problems associated with the Baeyer-Villiger oxidation and the subsequent hydrolysis, i.r. spectroscopy provided no useful information except to show the presence or absence of hydroxyl groups. Mass spectroscopy was also useless due to pyrolysis prior to electron bombardment, a phenomenon which has been frequently observed with high molecular weight polyesters and polyalcohols. N.m.r. spectroscopy, however, offered a little help in following the possible structural change in the reaction sequence shown in Figure 30.

In the n.m.r. spectrum of the Baeyer-Villiger oxidation mixture

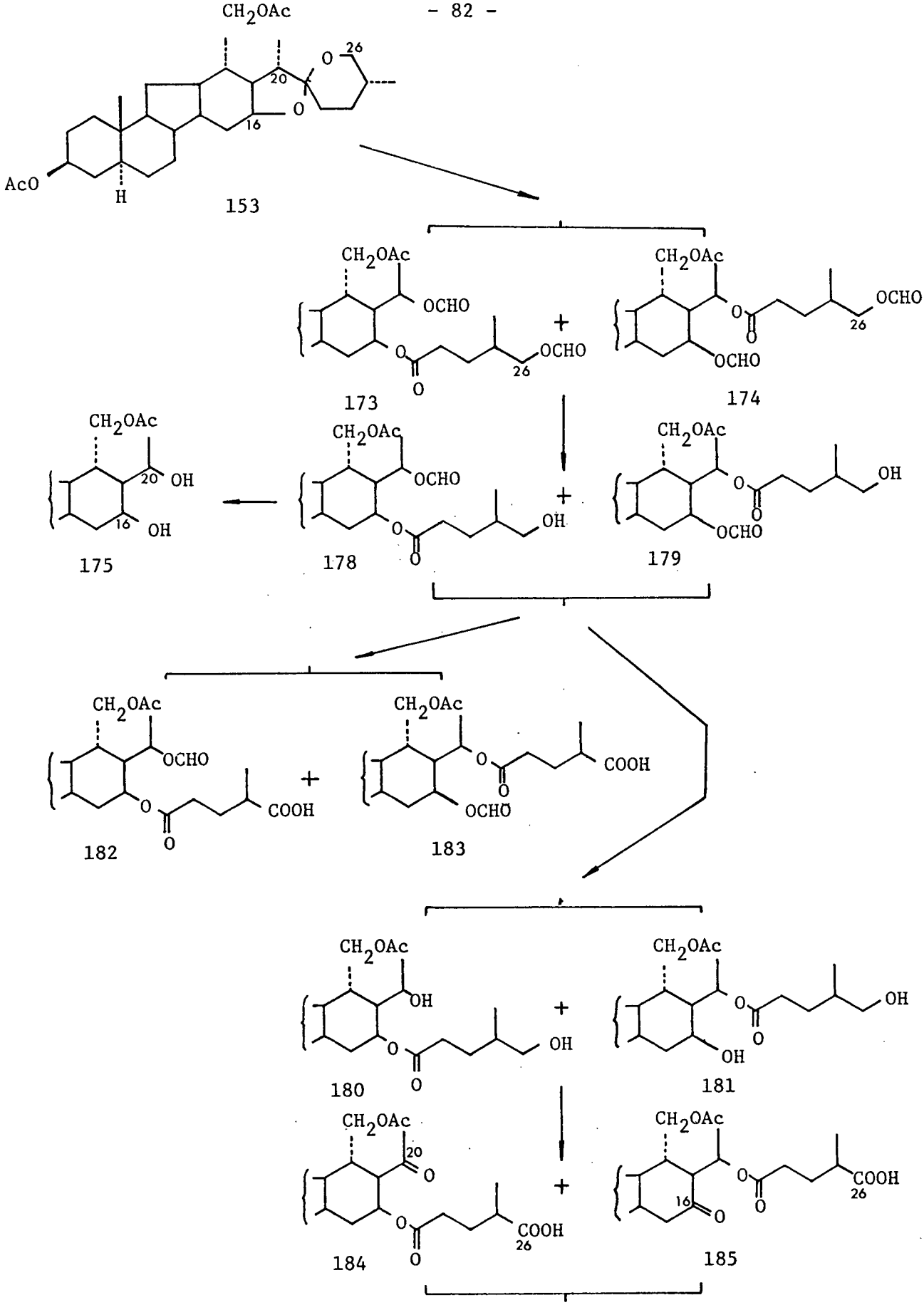


Figure 30: Performic acid degradation of the spiroketal system of (153).

there were two groups of signals at τ 2.00 and τ 1.92 with an area ratio of 2:1. This suggested the presence of at least two different formate groups in the products. In addition a doublet appeared at τ 5.97 (J = 6.5 Hz) with corresponding disappearance of the multiplet at τ 6.55 found in the diacetate (153) and assigned to the C-26 methylene protons. These results indicated that ring F of the diacetate (153) had been opened and that C-26 now carries the formyloxy group (see Figure 30). Upon treatment with either the potassium carbonate-methanol system or the buffered medium the n.m.r. spectra underwent similar changes. The formate signals diminished in intensity while at the same time their relative area ratio was reversed. Another noticeable change was the upfield shift (+ 0.58 p.p.m.) of the C-26 methylene doublet from τ 5.97 to τ 6.55. This shift can be explained by the loss of the formyl group at C-26 resulting in the formation of the primary hydroxyl group (178-182). Incidentally, the magnitude of the shift (+ 0.58 p.p.m.) was identical with that expected from Shoolery's additive constants.[201]

A significant insight into the structural problems was obtained when the Baeyer-Villiger oxidation mixture was treated with potassium carbonate in methanol-water with added sodium formate. It was found (by t.l.c.) that under the above conditions one of the two major components in the product mixture was consumed within 10 min at 0° whereas the other remained apparently unchanged. Comparison studies by t.l.c. revealed that the major product obtained by this method had an R_f value identical with the major component observed in the

barbital buffer treatment. Since the potassium carbonate-sodium formate method yielded a fairly simple mixture, chromatographic separation using a Florisil column was attempted. The series of fractions collected were subsequently combined to give three fractions. The least polar fraction (30%) contained the product which had survived the hydrolysis. While the n.m.r. spectrum of this fraction suggested the possibility of a mixture of more than one compound, it was quite noteworthy that there were no signals attributable to the C-26 methylene and C-27 methyl protons. Furthermore this fraction also showed the two formate signals of comparable intensities at their original positions, namely at τ 2.00 and τ 1.92. The second fraction (33%) contained, as a major component, the substance which had appeared previously in the hydrolysis studies. The n.m.r. spectrum shown in Figure 31 has all the required features for both (178) and (179). While it is possible that epimerization at C-16 and C-20 is also involved, the C-26 formate group has evidently been removed. The most polar fraction (35%) consisted mainly of three products, detectable by t.l.c., and its n.m.r. spectrum showed the presence of relatively less intense formate signals. The retention of the ring-opened C_6 side chain was evidenced by the doublets at τ 6.55 (J = 5.5 Hz, C-26 CH_2) and τ 9.09 (J = 6.0 Hz, C-27, CH_3).

Summarizing the results of the potassium carbonate-sodium formate hydrolysis of the Baeyer-Villiger oxidation mixture, it is firstly clear that the oxidation gives in about 30% yield the side product(s) which is not either (173) or (174), secondly the formate group at C-26 is completely eliminated under the above conditions, and thirdly the

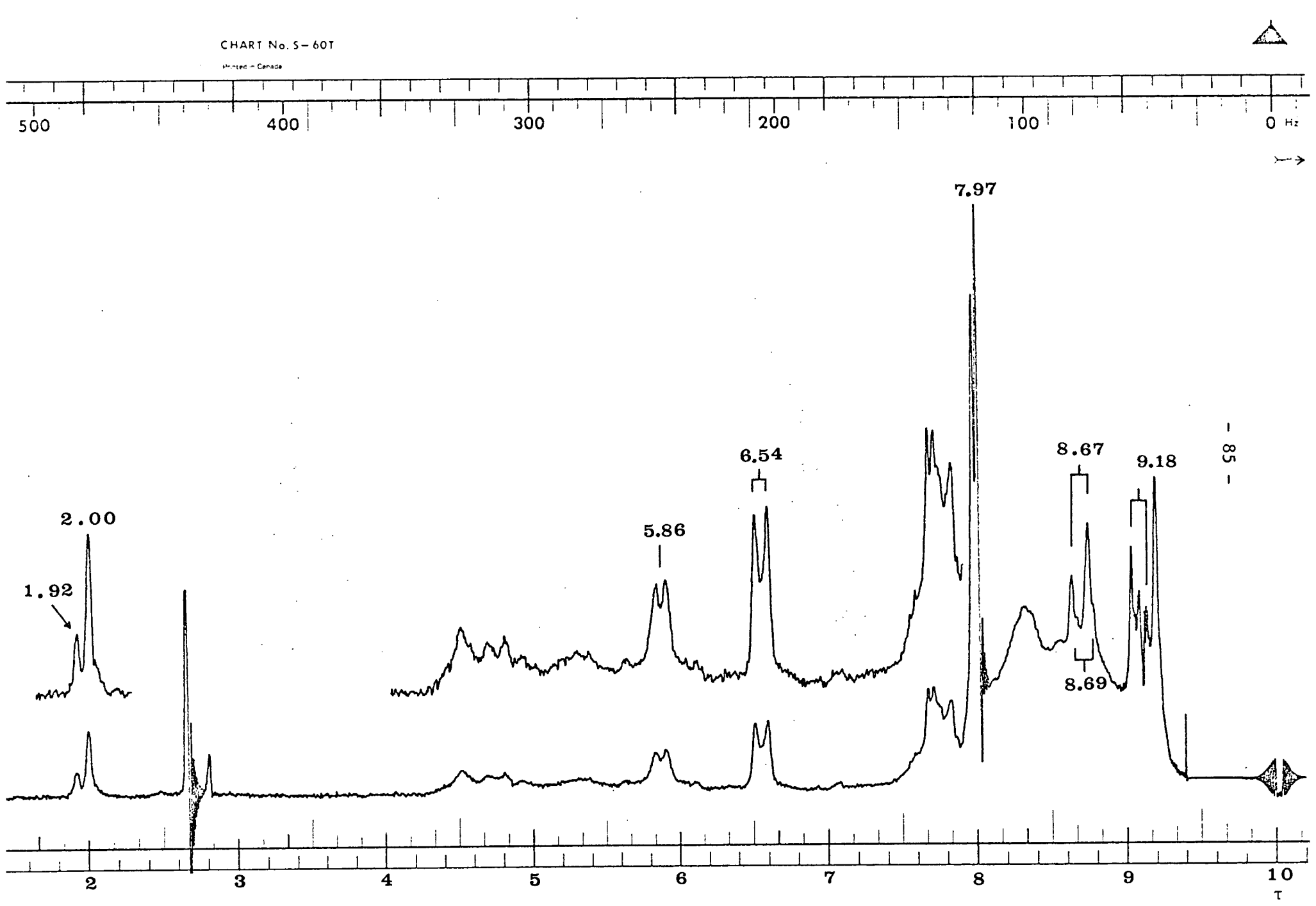


Figure 31: N.m.r. spectrum of the second fraction (p. 84).

the ring-opened C_6 side chain is not lost to an appreciable degree.

The problem still remained as to how to eliminate the formate groups in the intermediates (178 and 179) which were possibly present as the major products of hydrolysis. When the crude mixture from the barbital buffer treatment was titrated with Jones reagent (chromium trioxide-acetone) at 12-14° for a 1 hr period, a weak singlet appeared at τ 7.83 in the n.m.r. spectrum of the mixture, thereby indicating the formation of the methyl ketone group as in (184). The doublet at τ 6.55 (J = 5.5 Hz) had almost completely disappeared suggesting the transformation of the primary hydroxyl group at C-26 to the carboxylic acid functionality. As the titration was repeated the intensity of the singlet at τ 7.83 kept increasing. This could only be explained by assuming hydrolysis of the formate groups during the chromic acid oxidation, since the oxidation of alcohols by chromic acid is normally a quite rapid process.

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Corey et al.[202] reported that the formate ester (186) was directly converted to the ketone (187) by oxidation with chromic acid-acetic acid-water in excellent yield. As a possible mechanism Corey suggests that the chromate ester formed by carbonyl addition may be involved instead of ordinary acid hydrolysis prior to oxidation. This suggestion

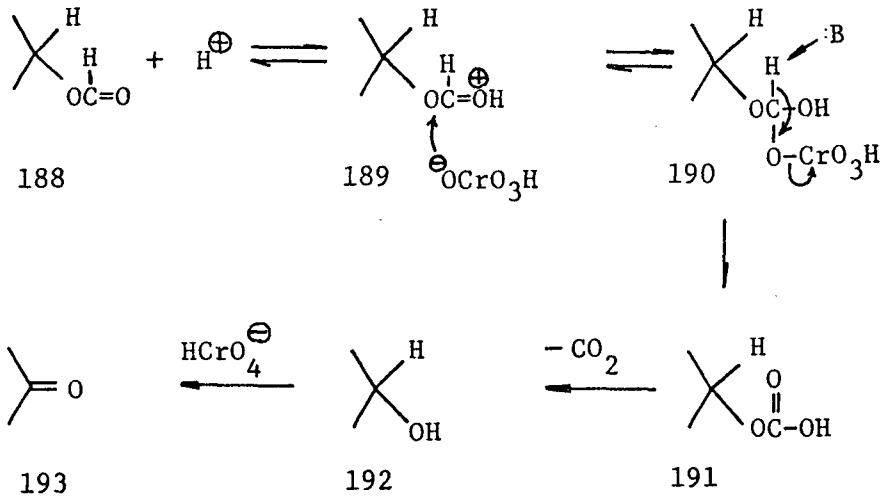
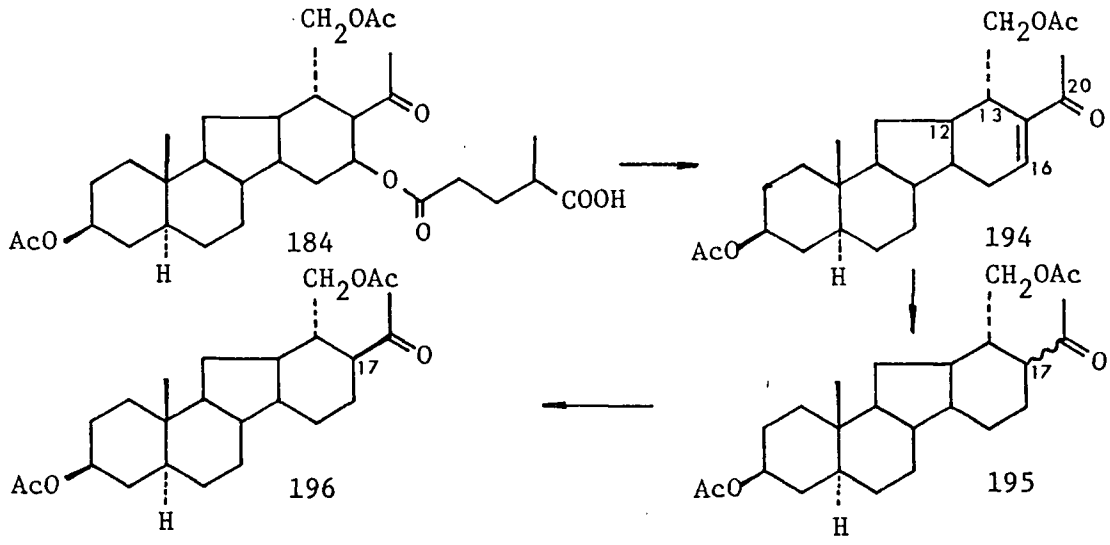


Figure 32: Postulated mechanism of chromic acid oxidation of formate esters.

may be visualized as shown in Figure 32 in analogy to the oxidation mechanism for aldehydes. According to this mechanism the formates may be selectively oxidized in the presence of the homologous esters. Thus the possibility was tested by treating the Baeyer-Villiger oxidation mixture with excess Jones reagent at room temperature for 20 hr. The n.m.r. spectrum of the acidic fraction extracted from the resultant mixture showed a remarkable similarity with that of the oxidation products obtained previously. However, subsequent base treatment and acetylation yielded the α,β-unsaturated ketone (194) only as a very minor product. The direct oxidation method therefore was not pursued in detail.

The hydrolysis mixtures obtained by the barbital buffer and the potassium carbonate-sodium formate hydrolyses were oxidized with the Jones reagent until the intensity of the methyl ketone signal at τ 7.83 reached its maximum. Base treatment of the resulting products followed



by acetylation yielded a complex mixture of products in both series. Chromatography on alumina led to isolation of 3β-acetoxy-13α-acetoxymethyl-18-nor-12α-pregnajerv-16-en-20-one (194). The n.m.r. spectrum (Figure 33) and the mass spectrum (Figure 34) and other spectral data were all in accord with the proposed structure (194).

Hydrogenation of the α,β-unsaturated ketone (194) proceeded smoothly and gave the saturated ketone (195) as a mixture of two C-17 epimers. In the n.m.r. spectrum the major ketone showed two singlets at τ 9.20 and τ 7.87 attributed to C-19 and C-21 methyl groups respectively, while the minor ketone showed corresponding singlets at τ 9.22 and τ 7.91. Upon treatment with boron trifluoride etherate only the minor ketone was found to be epimerized. On the basis of these observations coupled with conformational considerations the major, more stable ketone was assigned as having structure (196). The n.m.r. and mass spectra of (196) are given in Figures 35 and 36 respectively.

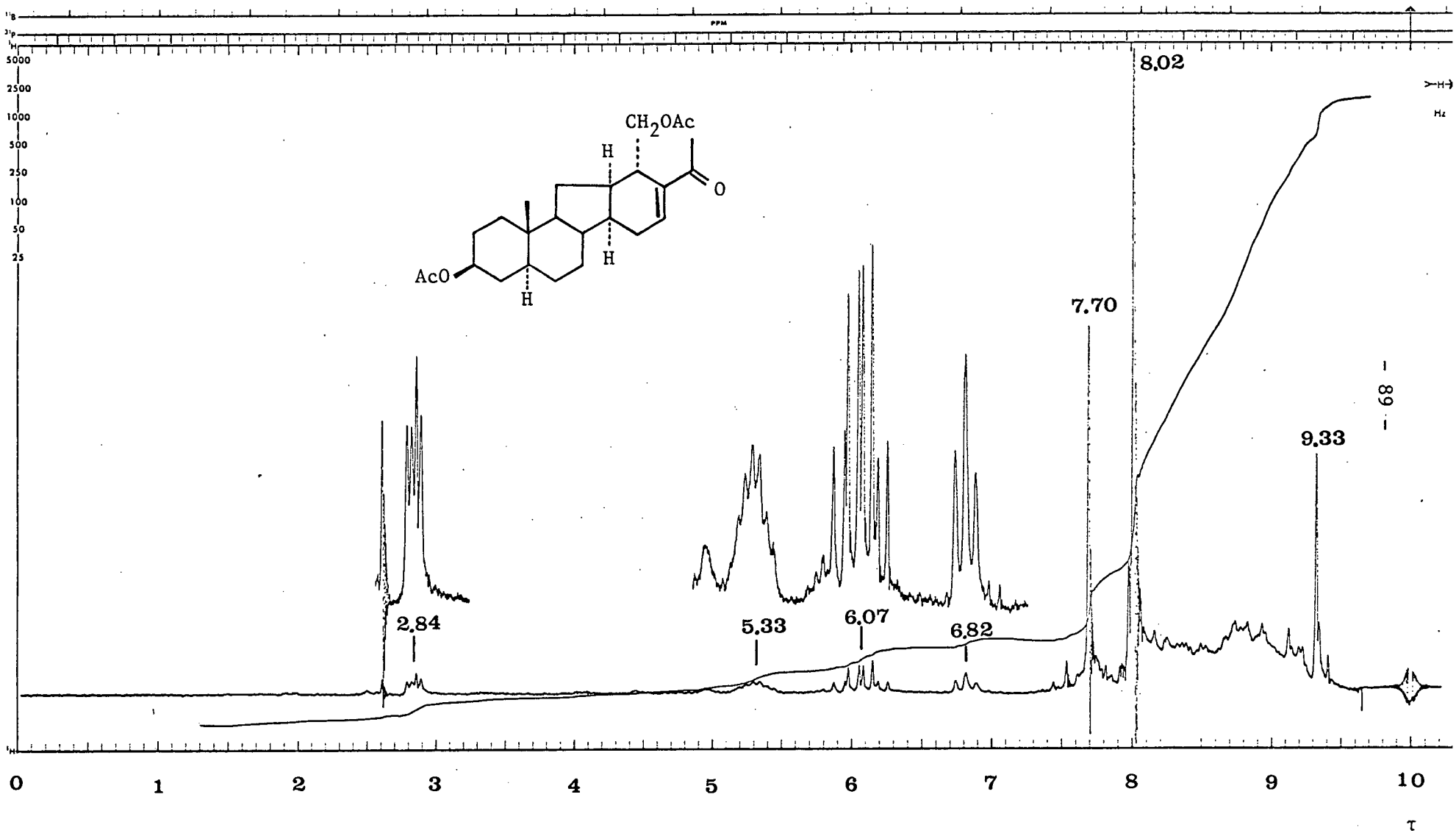


Figure 33: N.m.r. spectrum of compound (194).

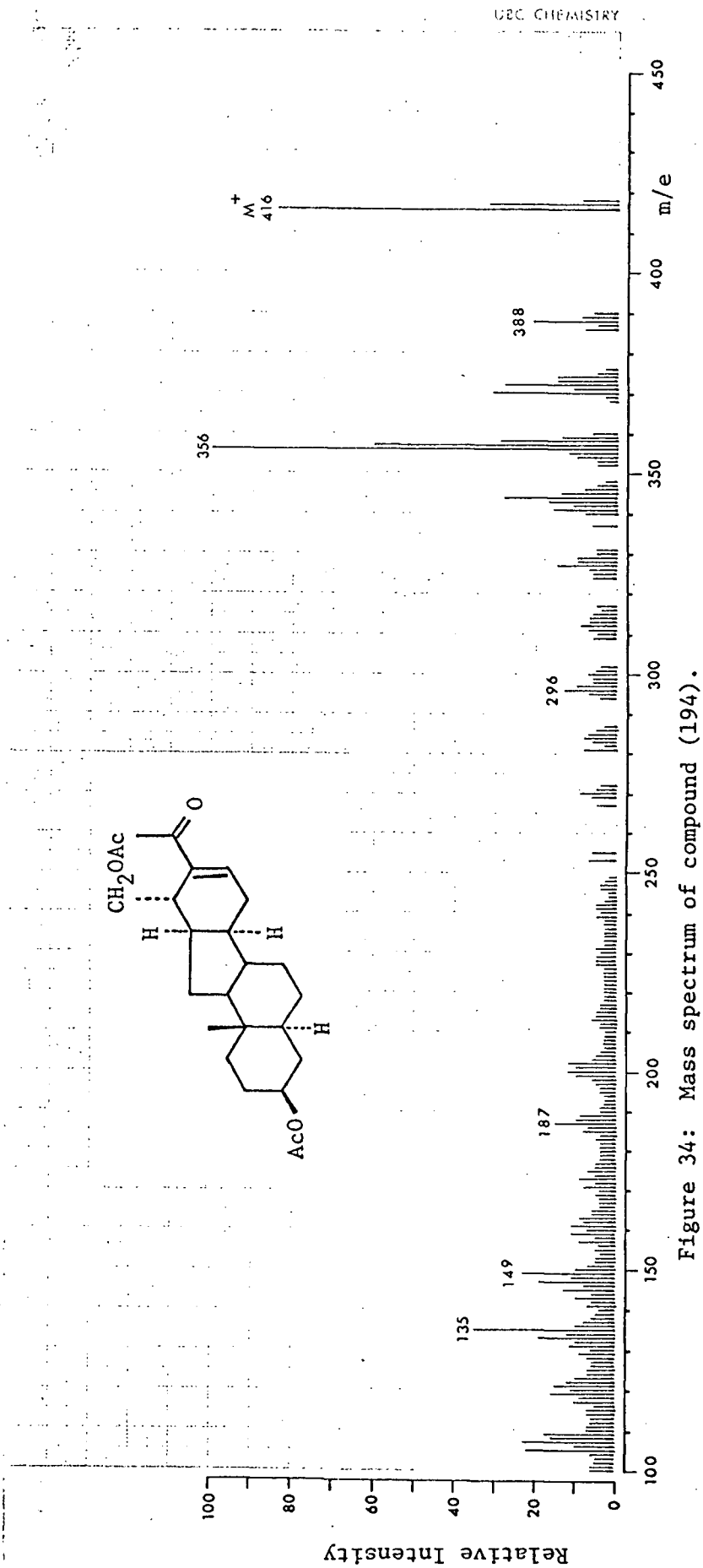


Figure 34: Mass spectrum of compound (194).

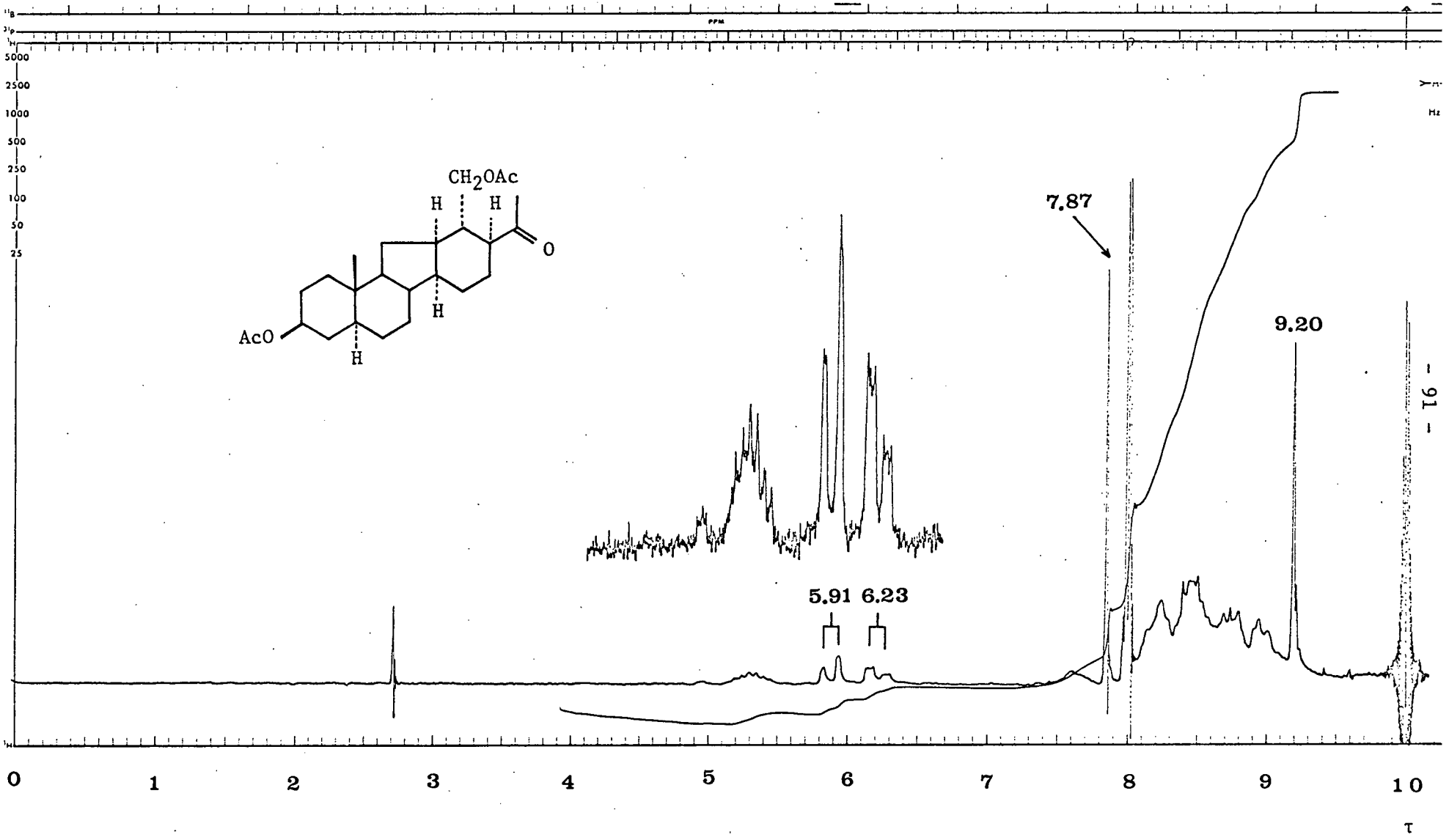


Figure 35: N.m.r. spectrum of compound (196).

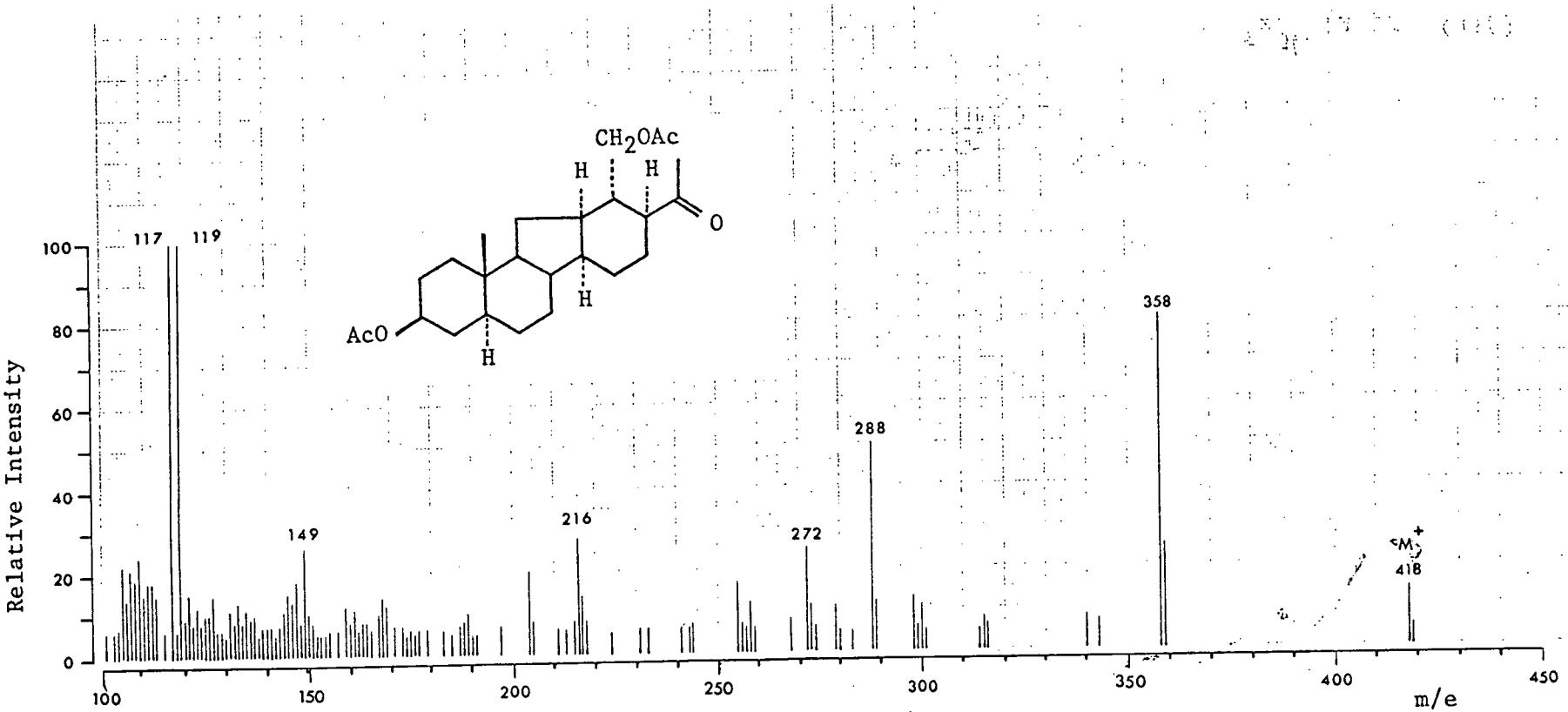


Figure 36: Mass spectrum of compound (196).

The overall yield of 3β-acetoxy-13α-acetoxymethyl-18-nor-12α-pregnajervan-20-one (196) from 3β-acetoxy-13α-acetoxymethyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan (153) was found to be 4 to 5% in both the series described above.

As the preparation of the pregnajervane ketone (196) had been accomplished, the present synthesis entered the fourth phase in which attachment of a suitably functionalized pyridine to the pregnajervane portion (196) was to be performed.

A survey of the literature revealed that 2-lithio-5-methylpyridine reacted with 3β-acetoxypregn-5-en-20-one (197) to produce the two epimeric condensation products (198) in fairly good yield.[203-205] It was then decided to first apply this reaction to a model compound. The α,β-unsaturated ketone (199) was chosen as the model compound, since it was available from previous studies[169,170] in our laboratory. 2-Bromo-5-methylpyridine was prepared *via* a known procedure from 2-amino-5-methylpyridine.[206-208] As for the base to generate 2-lithio-5-methylpyridine, phenyllithium and n-butyllithium were tried, but the latter was eventually employed on account of its better accessibility. The coupling reaction of (199) was performed under helium at -60 to -50°. Extraction by acid and subsequent chromatography on basic alumina provided a mixture of two compounds in about 55% yield. The n.m.r. and the mass spectra of this mixture are given in Figures 37 and 38. These data are in agreement with the proposed structure (200). The u.v. spectrum, i.r. spectrum and elemental analysis further confirmed the structure (200).

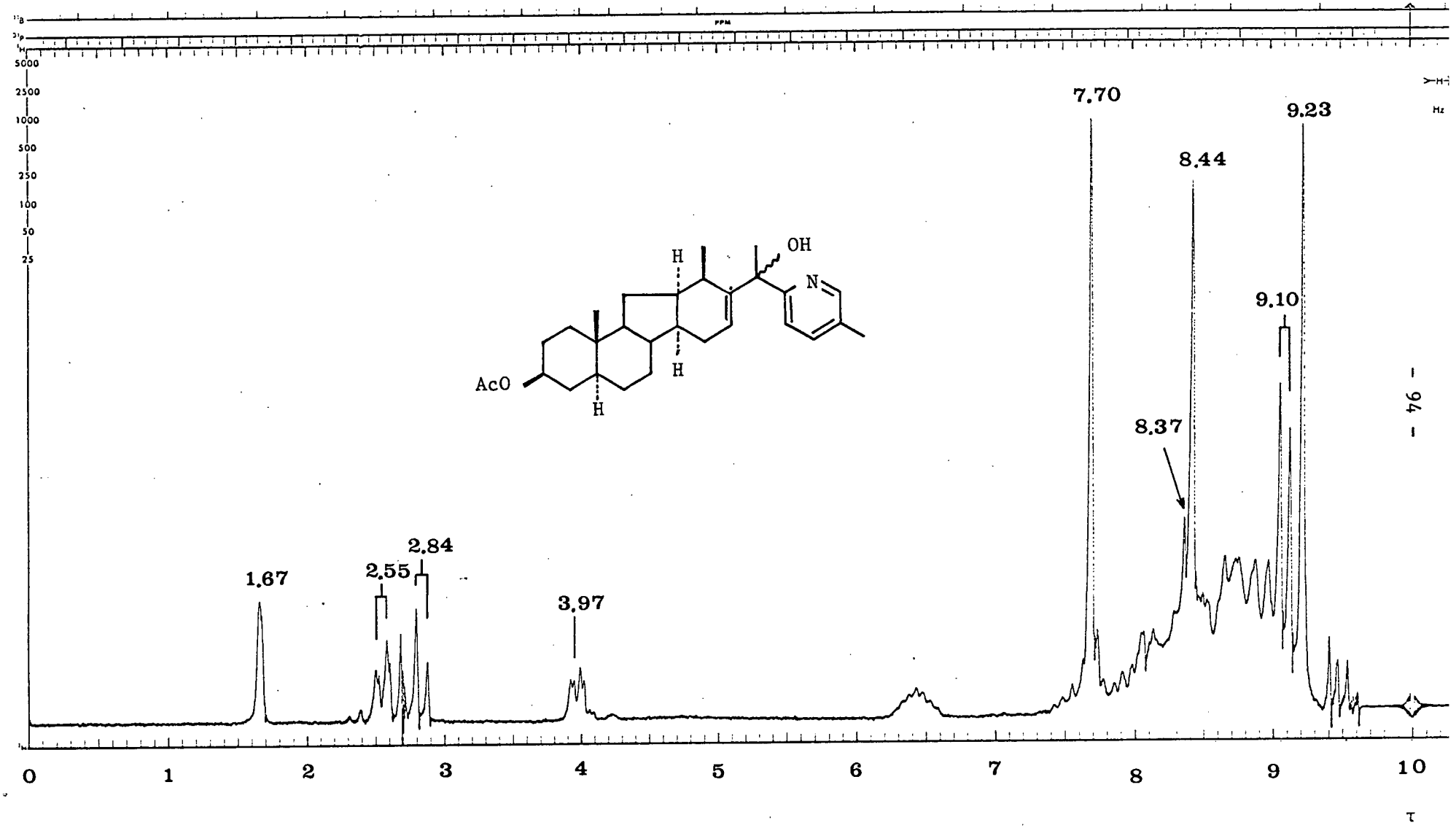


Figure 37: N.m.r. spectrum of compound (200).

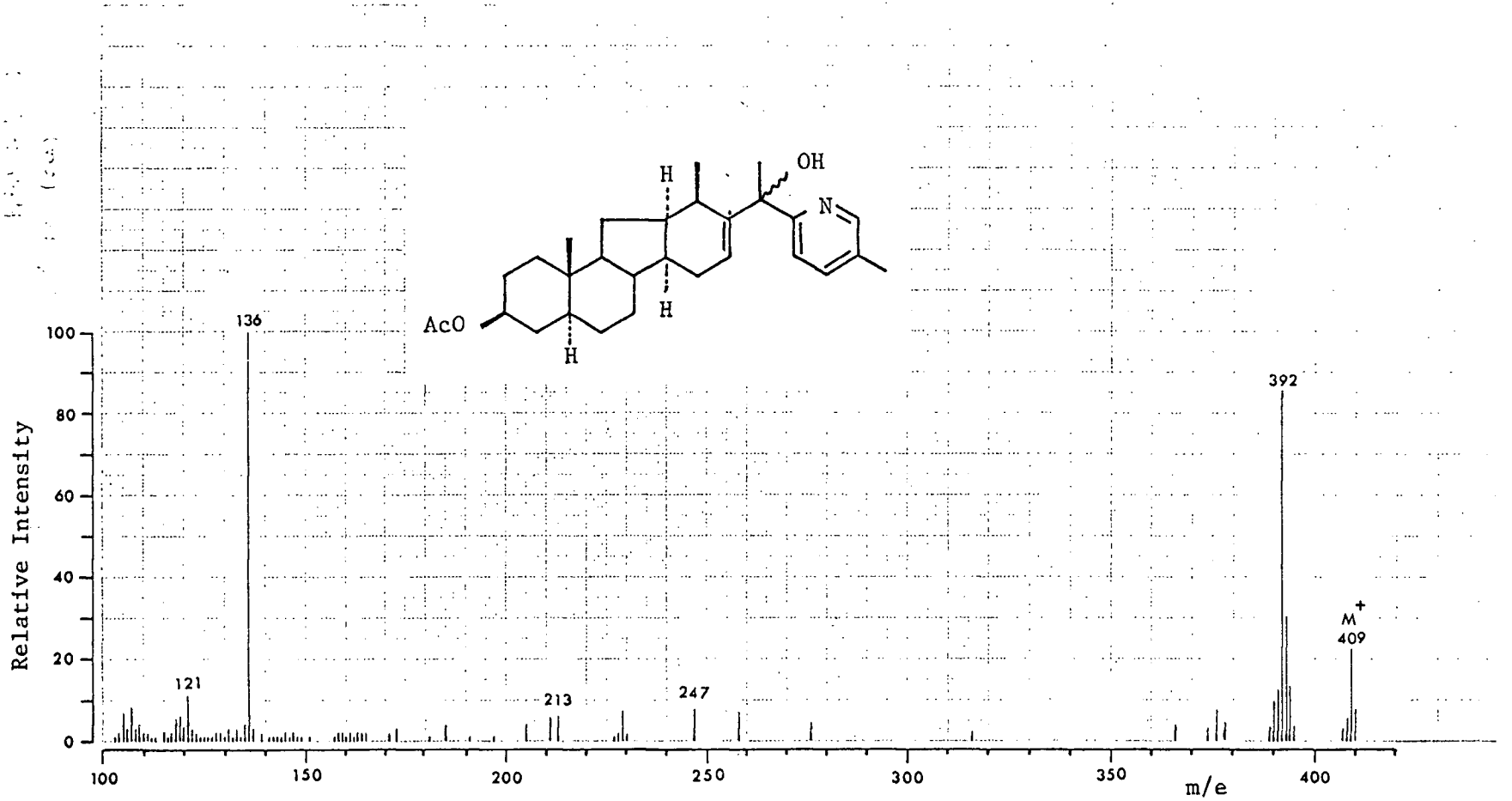
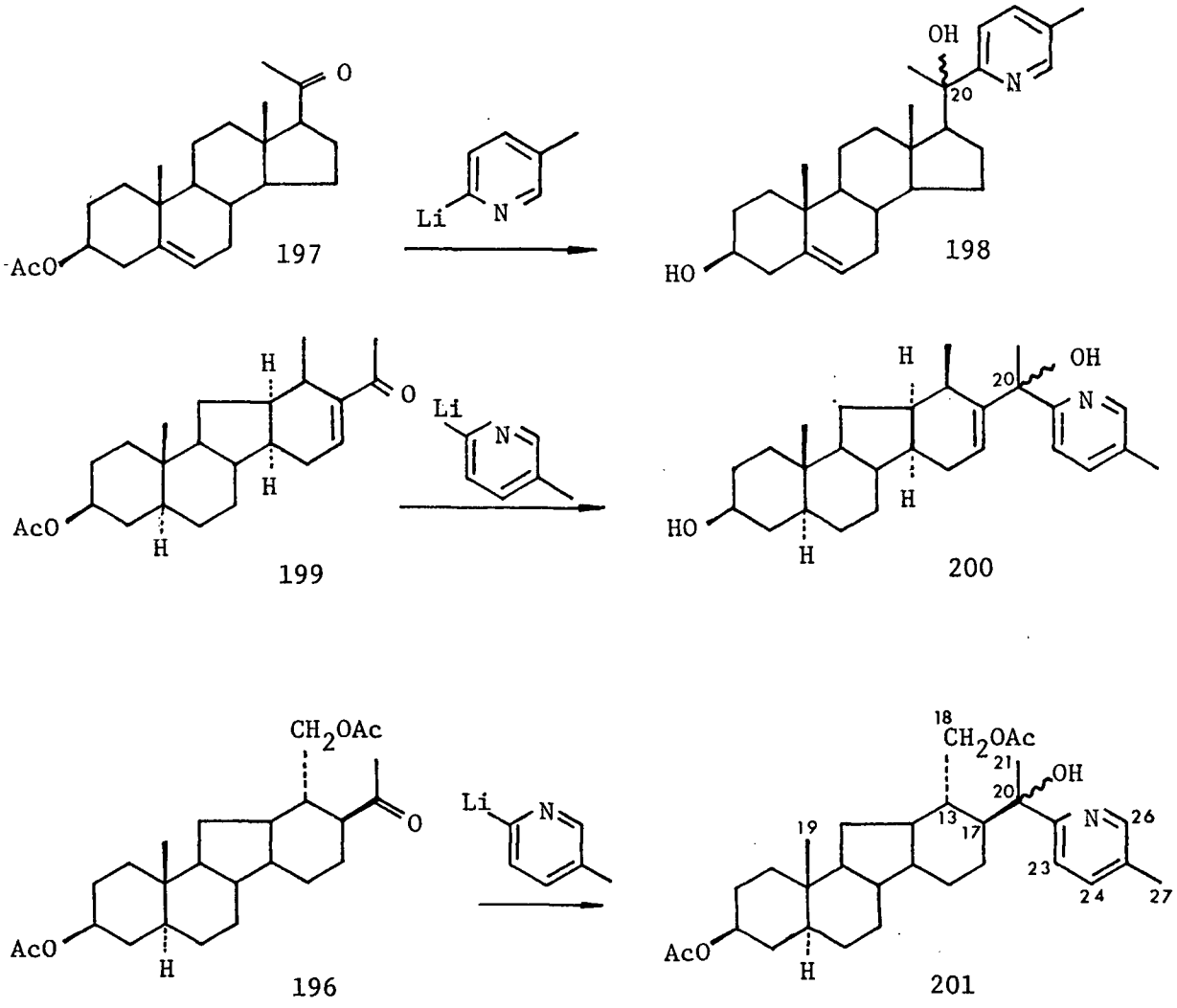


Figure 38: Mass spectrum of compound (200).



In a similar way the pregnajervane ketone (196) (1 mole) was reacted with 2-lithio-5-methylpyridine prepared in advance from 2-bromo-5-methylpyridine (15 moles) and n-butyllithium (13 moles). The crude products were extracted with dilute hydrochloric acid and the basic material was acetylated with acetic anhydride-pyridine prior to chromatographic separation on basic alumina. A chromatographically pure material was isolated in 28.5% yield. The mass spectrum (Figure 39)

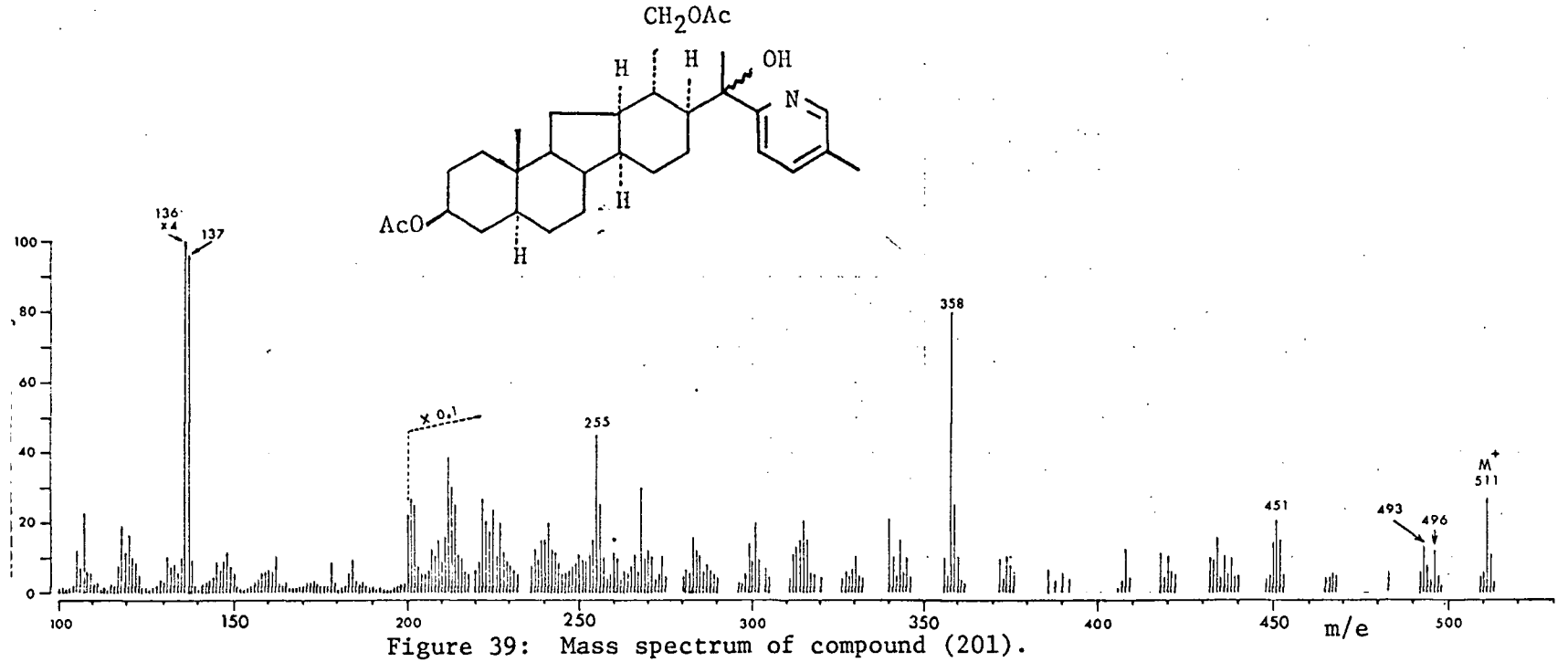


Figure 39: Mass spectrum of compound (201).

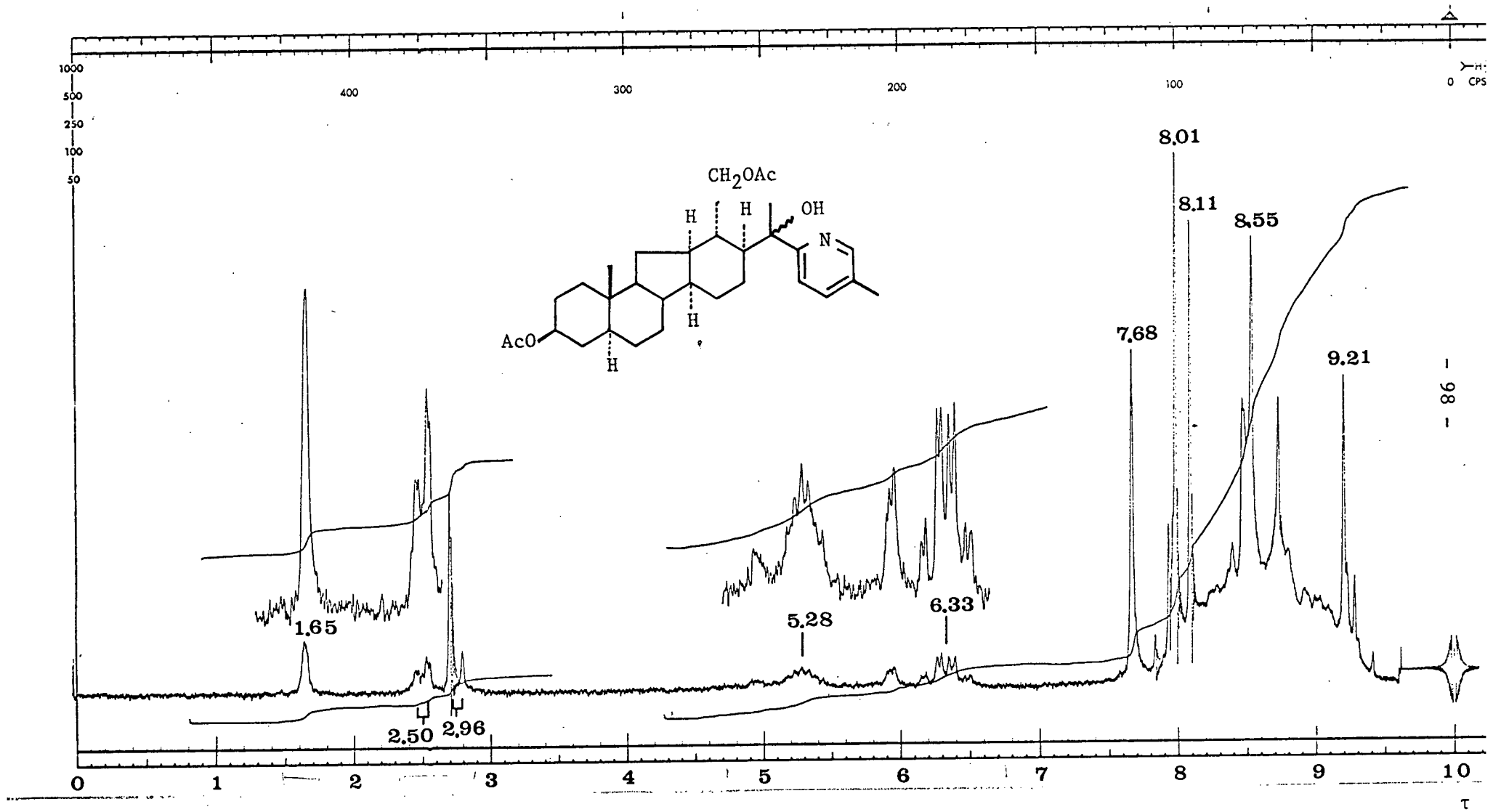
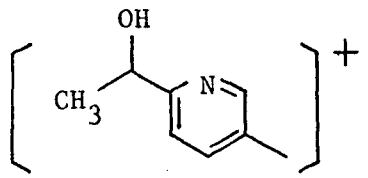


Figure 40: N.m.r. spectrum of compound (201).



(S)

of this material showed the base peak at m/e 136 corresponding to the expected fragment (S). A significant peak at m/e 511 was attributed to the molecular ion of the coupling product (201) on the basis that the fragments with m/e 496 ($M-CH_3$), m/e 451 (M-AcOH) and m/e 358 (M-AcOH-picoline) were also present. The n.m.r. spectrum (Figure 40) showed methyl signals with the chemical shifts expected for the structure (201). Other features of the spectrum were also in good agreement with the desired coupling product (201). Although this material was obviously a mixture of two epimers at C-20, it was impossible to determine the stereochemistry of the major epimer.

Now that all the carbon atoms necessary for building up the fundamental skeleton of verticine (205) and related alkaloids, have been incorporated in one molecule, the next phase of the present synthesis will be concerned with intramolecular cyclization leading to a hexacyclic intermediate such as (203). Reduction of the pyridine ring will give the key intermediate 6-deoxyverticine (204) which would allow stereochemical correlation with a sample from the natural source. Finally, introduction of a 6α-hydroxyl will complete the total synthesis of verticine (205).

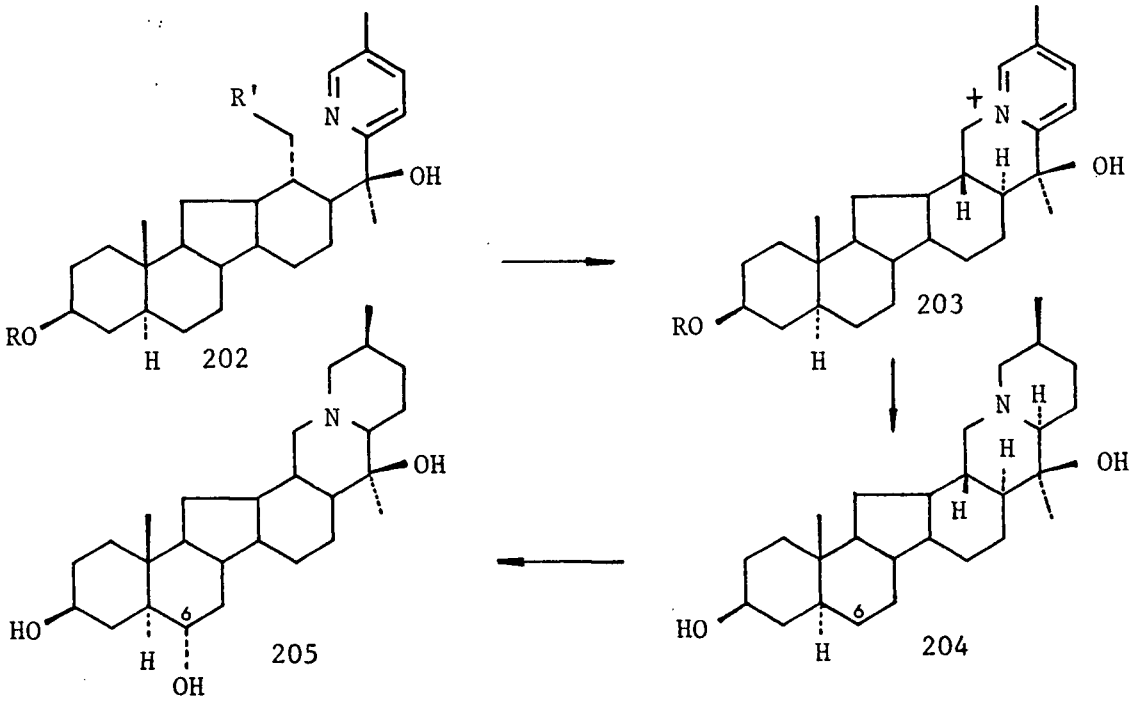


Figure 41: Suggested conclusion of the synthesis of Verticine (205).

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (u.v.) spectra were recorded in methanol on a Cary 15 recording spectrophotometer. Infrared (i.r.) spectra were recorded on a Perkin Elmer model 457 spectrophotometer as potassium bromide pellets unless otherwise specified. Nuclear magnetic resonance (n.m.r.) spectra were determined at 60 MHz on a Varian T-60 spectrometer and at 100 MHz on either a Varian HA-100 spectrometer or a Varian XL-100 spectrometer using deuteriochloroform with tetramethyl silane as an internal standard. The chemical shifts are recorded in the Tiers τ scale and the types of protons, integrated areas, multiplicities, spin coupling constants J (in Hz) are indicated in parentheses. Mass spectra were determined on an Atlas CH-4 spectrometer or an Associated Electrical Industries MS-902 spectrometer, high resolution measurements being determined on the latter instrument. Elemental analyses were performed by Mr. P. Borda of the Microanalytical Laboratory, University of British Columbia. For column chromatography Shawinigan alumina or Woelm neutral or anionotropic alumina were used.

Reduction of hecogenin (3β-hydroxy-(25R)-5α-spirostan-12-one) (115)

A sample of hecogenin (67.67 g) was weighed in a 2-liter 3-necked round-bottomed flask and to this were added dry 2-propanol (250 ml) and dry tetrahydrofuran (400 ml). The flask was equipped with a thermometer, a condenser, a stirrer and a nitrogen gas inlet. The system was kept under a static pressure of nitrogen and heated up with a heating mantle to 65°. A clear, light brown solution resulted after a short period. Then the heating mantle was disconnected and potassium (40 g), which had been cut and kept under petroleum ether (b.p. 30-60°), was introduced in a 30 min period. After the addition of potassium was completed the heating mantle was switched on again and the reaction mixture was gently refluxed for 4 hr. At the end of this period a portion of ethyl acetate was added to destroy the remaining potassium. Then the reaction mixture was cooled to room temperature and poured with vigorous stirring onto crushed ice (2,000 g) containing acetic acid (80 g). A white precipitate formed immediately. Further addition of crushed ice (2,000 g) was made and the mixture was left overnight at room temperature. The precipitate was collected by suction filtration and transferred into a 1-liter beaker to be digested thoroughly with water. The resultant suspension was again filtered, washed with water, suction dried, and finally dried in a vacuum oven at 80-100° overnight to give crude rockogenin (116) (64.12 g, 94.3%). A portion of the product was crystallized from ethanol/dioxane to give rockogenin ((25R)-5α-spirostan-3β,12β-diol) (116), m.p. 217-218° (lit. m.p. 216-219°,[111] 218.5-220.5,[109] 210-213° [132]). N.m.r. signals: 9.25 (singlet, 3H, C-18 CH_3), 9.18

(singlet, 3H, C-19 CH_3), 9.22 (doublet, J = 6, 3H, C-27 CH_3), 8.97 (doublet, J = 6.5, 3H, C-21 CH_3), ca. 6.6 (broad multiplet, 4H, C-3 CH + C-12 CH + C-26 CH_2), 5.59 (quartet, J = 7.5, 1H, C-16 CH). I.r.: 3360, 1455, 1245, 1181, 1056, 981, 958, 921, 898, 864 cm^{-1}. Mass spectrum: M.W. 432; base peak at m/e 139; main peaks at m/e 432, 417, 402, 373, 363, 360, 318, 303, 300, 289, 248, 139, 115. Elemental analysis, found: C 74.72, H 10.08; calc. for $C_{27}H_{44}O_4$: C 74.95, H 10.25.

No attempt was made at this stage to eliminate epirockogenin from the product mixture.

Reduction of hecogenin acetate (3β-acetoxy-(25R)-5α-spirostan-12-one) (115a)

Hecogenin acetate was reduced by the method used in the reduction of hecogenin (115). Thus, hecogenin acetate (54.0 g, m.p. 240-3°) in 2-propanol (150 ml) and tetrahydrofuran (350 ml) was treated with potassium (42.5 g) for 2.5 hr. Precipitation of the products with crushed ice containing acetic acid (100 g) gave, after drying in a vacuum oven overnight, a crude rockogenin mixture (49.20 g).

Rockgenin 3-pivalate (3β-pivaloyloxy-(25R)-5α-spirostan-12β-ol) (117)

Freshly distilled pivaloyl chloride (28.8 g, 0.239 mole) was added at 0° to a stirred solution of crude rockogenin (116) (85.94 g, 0.199 mole), dry pyridine (250 ml) and benzene (500 ml) in a 1-liter round-bottomed flask. The flask was left at room temperature for 10 hr, then a small portion of water was added to destroy the excess of

pivaloyl chloride and the precipitate (consisted mainly of pyridine hydrochloride and epirockogenin 3-pivalate) was collected by suction filtration. The filtrate was evaporated and the residue was dissolved in benzene (1,000 ml), washed with half saturated aqueous sodium chloride containing hydrochloric acid, and subsequently with 2% potassium hydroxide in half saturated aqueous sodium chloride. Concentration of the organic layer followed by the usual crystallization procedure gradually replacing benzene with ethyl acetate gave rockogenin 3-pivalate (117) (73.30 g, 71.3%) as colourless needles. An analytical sample was obtained by recrystallization from acetone, m.p. 250-252° (lit.[148] m.p. 255-257°). N.m.r. signals: 9.25 (singlet, 3H, C-18 CH_3), 9.21 (doublet, J = 5.5, 3H, C-27 CH_3), 9.14 (singlet, 3H, C-19 CH_3), 8.97 (doublet, J = 6.5, 3H, C-21 CH_3), 8.85 (singlet, 9H, C-3 pivalate protons), 6.5-6.7 (3H, C-26 CH_2 + C-12 CH), 5.59 (quartet, J = 7, 1H, C-16 CH), 5.35 (multiplet, 1H, C-3 CH). I.r.: 3505, 1710, 1292, 1246, 1182, 1057, 984, 959, 923, 903, 866 cm^{-1}. Mass spectrum: M.W. 516; base peak at m/e 139; main peaks at m/e 516, 498, 457, 447, 444, 429, 402, 384, 373, 332, 139, 115 and 109. Elemental analysis, found: C 74.22, H 10.03; calc. for $C_{32}H_{52}O_5$: C 74.37, H 10.14.

The precipitate formed during the reaction was washed with water and the residue was dried <u>in vacuo</u> to yield epirockogenin 3-pivalate (10.85 g, 14.1%), m.p. 286-287°. An analytical sample was obtained by recrystallization from methylene chloride, m.p. 299-299.5° (lit.[148] m.p. 297-299°). I.r.: 3540, 1707, 1293, 1242, 1183, 1054, 983, 956, 921, 902, 865 cm^{-1}. Mass spectrum: M.W. 516; base peak at m/e 139;

main peaks at m/e 516, 498, 457, 447, 444, 429, 402, 384, 373, 327, 139, 115, 109. Elemental analysis, found: C 74.44, H 10.28; calc. for $C_{32}H_{52}O_5$: C 74.37, H 10.14.

Rockogenin 12-methanesulfonate 3-pivalate (12β-mesyloxy-3β-pivaloyloxy-(25R)-5α-spirostan) (118)

Crystalline rockogenin 3-pivalate (117) (66.90 g) was dissolved in dry pyridine (500 ml) in a 2-liter 3-necked round-bottomed flask equipped with a stirrer, a dropping funnel and a Drierite tube. The solution was chilled below 5° and freshly distilled mesyl chloride (40 ml) was added dropwise. The reaction mixture was allowed to stand at room temperature for 20 hr, then the flask was immersed in an ice-salt bath and 10% aqueous sodium bicarbonate (350 ml) was added slowly so that the temperature did not exceed 5°. During this operation evolution of a gas was observed and an oily layer, which first appeared on the wall of the flask, turned to a granular solid. Ice water (700 ml) was added and the mixture was stirred for 1 hr below 5°. The solid was filtered off, washed with water, digested with ice water, filtered again and suction dried over 3 hr, then dried in a vacuum oven overnight with temperature slowly rising to 50°. The grayish granular crude product (74.68 g, 97.0%) obtained appeared homogeneous by t.l.c. and was immediately used for the next reaction without further purification. N.m.r. signals: 8.83 (singlet, 9H, C-3 pivalate protons), 6.99 (singlet, 3H, $-OSO_2CH_3$). I.r.: 1715, 1358, 1283, 1242, 1170, 1058, 982, 930, 908, 888, 838 cm^{-1}.

3β-Acetoxy-C-nor-D-homo-(25R)-5α,12α-spirost-13(18)-en (119a)

Potassium metal (14.5 g) was dissolved in warm tert-butanol (900 ml) in a 1-liter 3-necked round-bottomed flask equipped with a nitrogen inlet and a condenser. Dry rockogenin 12-methanesulfonate 3-pivalate (118) (39.71 g) was added to the solution and the mixture was refluxed for 4 hr. The resultant suspension was allowed to cool, and water (100 ml) was added to give a clear, yellow solution. Most of the solvent alcohol was removed in vacuo with occasional addition of water. After concentration to about 200 ml the mixture was poured onto crushed ice (2,000 g). Addition of sodium chloride to the resultant creamy suspension caused a solid to separate out. The solid was collected by filtration, washed with water, and dried in a vacuum oven at 80° to give a mixture of products (30.67 g). This mixture was acetylated by refluxing with acetic anhydride (200 ml) for 30 min. On cooling to room temperature the desired product, 3β-acetoxy-C-nor-D-homo-(25R)-5α,12α-spirost-13(18)-en (119a),crystallized out as white leaflets. The crystals were collected, washed with acetic acid and dried in a vacuum oven to yield the exocyclic olefin acetate (119a) (8.03 g, 26.3% from the methanesulfonate), m.p. 212-218° (lit. 221-225.5°,[110] 215°[111]). N.m.r. signals: 9.21 (doublet, J =5.5, C-27 CH_3), 9.20 (singlet, 3H, C-19 CH_3), 8.92 (doublet, J = 6, C-21 CH_3), 8.00 (singlet, 3H, $-OCOCH_3$), 7.53 (multiplet, 2H, C-12 CH + C-17 CH), 6.54 (multiplet, 2H, C-26 CH_2), 5.90 (octet, 1H, C-16 CH), 5.30 (multiplet, 1H, C-3 CH), 5.17 (broad singlet, 2H, C-18 $=CH_2$). I.r.: 1735, 1636, 1250, 1242, 1055, 980, 917, 901, 882, 864 cm^{-1}. Mass spectrum: M.W. 456; base peak at m/e 126; main peaks at m/e 456, 438, 414, 384, 342, 313,

165, 139, 126, 115, 112. High resolution mass spectrum, found: 456.3320; calc. for $C_{29}H_{44}O_4$: 456.3239. Elemental analysis, found: C 76.43, H 9.90; calc. for $C_{29}H_{44}O_4$: C 76.27, H 9.71.

3β-Pivaloyloxy-C-nor-D-homo(25R)-5α,12α-spirost-13(18)-en (119b)

The methanesulfonate derivative (118) (43.6 g) was dissolved in dry pyridine (350 ml) in a 1-liter round-bottomed flask equipped with a condenser and a nitrogen inlet. The solution was refluxed for 14 hr, and pyridine was removed in vacuo. Benzene (600 ml) and ethyl acetate (600 ml) were added and the resultant solution was washed successively with water, dilute hydrochloric acid, 5% aqueous sodium bicarbonate and then with water. The organic layer was dried over sodium sulfate and the solvents were removed in vacuo to yield a solid (37.0 g). Crystallization from petroleum ether (b.p. 65-73°) afforded exocyclic olefin pivalate (119b) (30.2 g, 82.5%), m.p. 193.5-194.5°. N.m.r. signals: 9.21 (doublet, J = 6, 3H, C-27 CH_3), 9.20 (singlet, 3H, C-19 CH_3), 8.92 (doublet, J = 6.5, 3H, C-21 CH_3), 8.85 (singlet, 9H, C-3 pivalate protons), ca. 7.50 (multiplet, 2H, C-12 CH + C-17 CH), ca. 6.54 (multiplet, 2H, C-26 CH_2), 5.90 (octet, 1H, C-16 CH), 5.31 (multiplet, 1H, C-3 CH), 5.17 (broad singlet, 2H, C-18 $=CH_2$). I.r.: 1728, 1719, 1644, 1285, 1247, 1171, 1061, 983, 902, 885, 868 cm^{-1}. Mass spectrum: M.W. 498; base peak at m/e 165; main peaks at m/e 498, 480, 456, 439, 429, 426, 411, 397, 384, 355, 253, 165, 139, 126, 115, 105. Elemental analysis, found: C 76.92, H 10.08; calc. for $C_{32}H_{50}O_4$: C 77.06, H 10.11 . (see Figure 7 and Figure 8).

C-Nor-D-homo-(25R)-5α,12α-spirost-13(18)-en-3β-ol (119)

The exocyclicolefin pivalate (119b) (13.76 g) was dissolved in dry ether (300 ml) in a 1-liter round-bottomed flask, and lithium aluminum hydride (1.00 g) was added slowly. The reaction mixture was stirred for 2 hr at room temperature, and ethyl acetate (5 ml) in ether (400 ml) was added. Celite (10 g), water (3 ml) and anhydrous sodium sulfate (30 g) were introduced consecutively and the mixture was stirred for 30 min before the solid was filtered off. Removal of solvents from the filtrate followed by drying in a vacuum oven at 50° gave crude C-nor-D-homo-(25R)-5α,12α-spirost-13(18)-en-3β-ol (119) (11.63 g). An analytical sample was prepared by recrystallization from ethyl acetate, m.p. 184-186°. N.m.r. signals: 9.22 (singlet, 3H, C-19 CH_3), 9.21 (doublet, J = 6, 3H, C-27 CH_3), 8.93 (doublet, J = 6.5, 3H, C-21 CH_3), ca. 7.55 (multiplet, 2H, C-12 CH + C-17 CH), ca. 6.55 (multiplet, 3H, C-3 CH + C-26 CH_2), 5.93 (octet, 1H, C-16 CH), 5.21 (broad singlet, 2H, C-18 $=CH_2$). I.r.: 3475, 3360, 1619, 1247, 1053, 983, 903, 884, 866 cm^{-1}. Mass spectrum: M.W. 414; base peak at m/e 126; main peaks at m/e 414, 396, 372, 355, 345, 342, 327, 300, 285, 271, 165, 139, 126, 115, 105. Elemental analysis, found: C 78.30, H 10.11; calcd. for $C_{27}H_{42}O_3$: C 78.21, H 10.21. (see Figure 14 and Figure 15).

Hydroboration of 3β-acetoxy-C-nor-D-homo-(25R)-5α,12α-spirost-13(18)-en (119a)

The exocyclic olefin acetate (119a) (8.02 g, m.p. 212-218°) was dissolved in dry tetrahydrofuran (150 ml) in a 500 ml, 3-necked round-bottomed flask equipped with a stirrer, a condenser and a nitrogen inlet. Diborane in tetrahydrofuran (1 M, 40 ml) was added over a 30 min period, and the solution was stirred at room temperature for 3 hr. Aqueous sodium hydroxide (10%, 50 ml) and aqueous hydrogen peroxide (30%, 40 ml) were added and the mixture was stirred overnight. Removal of solvents in vacuo, neutralization with dilute hydrochloric acid followed by ether extraction gave crude 13β-hydroxymethyl —C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan-3β-ol (121) (7.83 g). This material was chromatographed on alumina (neutral, activity III, 150 g), and elution with chloroform-ethyl acetate (3:1) yielded the pure diol (121) (6.22 g, 82%). An analytical sample was obtained by crystallization from ethyl acetate-petroleum ether (b.p. 65-73°), m.p. 182-184.5°. N.m.r. signals (see Figure 9): 9.22 (singlet, 3H, C-19 CH_3), 9.20 (doublet, J = 6.0, 3H, C-27 CH_3), 8.98 (doublet, J = 6.0, 3H, C-21 CH_3), 8.26 (singlets, 2H, C-3 OH + $-CH_2OH$), 5.58 (multiplet, 2H, C-26 CH_2), 6.4 (multiplet, 1H, C-3 CH), 6.35 (doublets, J = 5.5, 2H, $-C\underline{H}_2OH$), and 5.87 (distorted quartet, 1H, C-16 CH). I.r.: 3385, 1241, 1029, 1017, 985, 907, 900, 863 cm^{-1}. Mass spectrum (see Figure 10): M.W. 432; base peak at m/e 115; main peaks at m/e 432, 402, 373, 363, 360, 345, 318, 300, 288, 145, 139, 126, 115, and 107. High resolution mass spectrum, found: 432.3200; calc. for $C_{27}H_{44}O_4$: 432.3239. Elemental analysis, found: C, 74.98, H, 10.26; calc. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25.

Hydroboration of C-nor-D-homo-(25R)-5α,12α-spirost-13(18)-en-3β-ol (119)

The crude exocyclic olefin alcohol (119) (7.69 g) was dissolved in dry tetrahydrofuran (100 ml) in a 250 ml, 3-necked, round-bottomed flask equipped with a stirrer, a condenser and a nitrogen inlet. Diborane in tetrahydrofuran (1 M, 50 ml) was added over a 30 min period and the solution was stirred at room temperature for 12 hr. Then aqueous sodium hydroxide (10%, 25 ml) and aqueous hydrogen peroxide (30%, 50 ml) were added and the mixture was stirred at 45-50° for 1 hr. Removal of the solvent in vacuo, neutralization with dilute hydrochloric acid followed by extraction with chloroform gave the crude product (8.48 g). This material was chromatographed on alumina (neutral, activity III, 150 g), and eluted with ethyl acetate to give the pure diol (121) (5.84 g). An analytical sample was obtained by crystallization from ethyl acetate-petroleum ether (b.p. 65-73°), m.p. 182-184°, and was shown to be identical with the pure diol (121) prepared by hydroboration of the exocyclic olefin acetate (119a).

Rockogenin 3-benzoate (3β-benzoyloxy-(25R)-5α-spirostan-12β-ol) (143)

Crude rockogenin (116) (6.79 g) in pyridine (50 ml) was treated with benzoyl chloride (2.55 g) at 0° for 1 hr. Addition of aqueous sodium bicarbonate (5%) followed by extraction with ether gave a crystalline solid (7.64 g), which was chromatographed on alumina (Shawinigan, 130 g). Elution with petroleum ether (b.p. 65-73°)-ethyl acetate and recrystallization from ethyl acetate-methylene chloride gave pure rockogenin 3-benzoate (143), m.p. 226-228°. N.m.r. signals: 9.23 (singlet, 3H, C-18 CH_3), 9.23 (doublet, J = 5.5, 3H, C-27 CH_3),

9.11 (singlet, 3H, C-19 CH_3), 8.96 (doublet, J = 6.0, 3H, C-21 CH_3). I.r.: 3555, 1703, 1600, 1585, 1455, 1275, 980, 923, 899, 864, 710 cm^{-1}. Mass spectrum: M.W. 536; base peak at m/e 139; main peaks at m/e 536, 477, 467, 464, 423, 404, 393, 352, 139, 126, 115 , 105. Elemental analysis, found: C 75.65, H 9.03; calc. for $C_{34}H_{48}O_5$: C 76.08, H 9.01.

Rockogenin 3-benzoate 12-methanesulfonate (3β-benzoyloxy-12β-mesyloxy-(25R)-5α-spirostan (144)

Rockogenin 3-benzoate (143) (5.00 g) was dissolved in pyridine (50 ml) and was treated with freshly distilled mesyl chloride (3.5 ml) at 0° for 30 hr. The reaction mixture was then diluted with ethyl acetate and washed consecutively with water, dilute hydrochloric acid (1 N), aqueous sodium bicarbonate (5%), and water. Evaporation of the solvent yielded crude rockogenin 3-benzoate 12-methanesulfonate (144) (5.54 g). Part of the crude mixture was crystallized from ethyl acetate to give grayish needles, m.p. 128-131°. N.m.r. signals: 9.23 (doublet, J = 5.5, 3H, C-27 CH_3), 9.15 (singlet, 3H, C-18 CH_3), 9.10 (singlets, 3H, C-19 CH_3), 8.96 (doublet, J = 6.5, 3H, C-21 CH_3), 7.04 (singlet, 3H, $-OSO_2CH_3$). I.r.: 1720, 1340, 1280, 1177, 1170, 983, 926, 906, 830, 713 cm^{-1}. Elemental analysis, found: C 68.28, H 8.25; calc. for $C_{35}H_{50}O_7S$: C 68.37, H 8.20.

3β-Benzoyloxy-C-nor-D-homo-(25R)-5α,12α-spirost-13(18)-en (145)

A solution of the methanesulfonate derivative (144) (4.74 g) in dry pyridine (100 ml) was refluxed under nitrogen for 25 hr. Removal

of pyridine and extraction with ethyl acetate gave the crude exocyclic olefin benzoate (145) (4.08 g). Crystallization from ethyl acetate-petroleum ether (b.p. 65-73°) afforded an analytical sample of (145) as needles, m.p. 197-199°. N.m.r. signals: 9.20 (doublet, J = 6.0, 3H, C-27 CH_3), 9.15 (singlets, 3H, C-19 CH_3), 8.92 (doublets, J = 7.0, 3H, C-21 CH_3), 5.15 (broad singlets, 2H, C-18 $=CH_2$). I.r.: 1716, 1642, 1452, 1282, 1118, 1074, 983, 903, 887, 863, 710 cm^{-1}. Mass spectrum: M.W. 518; base peak at m/e 105; main peaks at m/e 518, 490, 476, 446, 431, 404, 375, 282, 165, 139, 126, 115, 105. High resolution mass spectrum, found: 518.3354; calc. for $C_{34}H_{46}O_4$: 518.3393. Elemental analysis, found: C 78.58, H 8.84; calc. for $C_{34}H_{46}O_4$: C 78.72, H 8.94.

3β-Benzoyloxy-13β-hydroxymethyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan (146)

The exocyclic olefin benzoate (145) (3.53 g, m.p. 193-196°) was dissolved in dry tetrahydrofuran (150 ml) and treated under nitrogen with diborane in tetrahydrofuran (1 M, 17.5 ml). After stirring at room temperature for 2 hr aqueous hydrogen peroxide (30%, 15 ml) and aqueous potassium hydroxide (10%, 1 ml) were added, and the mixture was stirred for 3 hr. The solvent was removed and the products were extracted with ethyl acetate. The crude product mixture (3.79 g) was chromatographed on alumina (Shawinigan, 130 g), and elution with petroleum ether (b.p. 65-73°)-methylene chloride (1:1) gave the primary alcohol derivative (146) (1.96 g, 54%) as a crystalline solid. An analytical sample was obtained by recrystallization from methanol-methylene chloride as leaflets, m.p. 175-176°. N.m.r. signals (see

Figure 16): 9.20 (doublet, J = 5.5, 3H, C-27 CH_3), 9.16 (singlet, 3H, C-19 CH_3), 8.97 (doublet, J = 6.0, 3H, C-21 CH_3), 6.32 (doublets, J = 5.5, 2H, $\underline{CH_2}OH$). I.r.: 3440, 1716, 1601, 1583, 1453, 1279, 1113, 1025, 979, 897, 865, 710. Mass spectrum: M.W. 536; base peak at m/e 105; main peaks at m/e 536, 464, 404, 362, 270 , 241, 139, 126, 115, 105. Elemental analysis, found: C 76.00, H 8.93; calc. for $C_{34}H_{48}O_5$: C 76.08, H 9.01.

13β-Formyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan-3-one (148)

The diol (121) (8.00 g, m.p. 182-184.5°) was dissolved in benzene (150 ml) and dimethyl sulfoxide (100 ml) in a 1-liter round-bottomed flask. Dicyclohexylcarbodiimide (30.5 g), pyridine (4 ml) and trifluoroacetic acid (2 ml) were added and the solution was stirred for 40 hr at room temperature under nitrogen. The reaction mixture was diluted with ether (500 ml), and oxalic acid (12.0 g) in methanol (50 ml) was introduced slowly. The mixture was stirred for 1 hr and the white crystalline dicyclohexylurea precipitate was filtered off, washed with ether (300 ml), and the combined organic layer was washed consecutively with water, saturated aqueous sodium bicarbonate, and water. The ether layer was dried over sodium sulfate, and the solvent was evaporated in vacuo to yield the aldehyde (148) as an oil (10.15 g). An analytical sample was obtained as an amorphous solid by chromatography on silica gel. N.m.r. signals (see Figure 17): 9.21 (doublet, J = 6.0, C-27 CH_3), 9.08 (singlet, 3H, C-19 CH_3), 9.03 (doublet, J = 6.0, 3H, C-21 CH_3), 7.33 (doublet of triplets, J = 5.5 and 7.0, 1H, C-13 CH), 6.60 (multiplet, 2H, C-26 CH_2), 5.95 (octet, 1H, C-16 CH), 0.22 (doublet, J = 5.5, 1H,-CHO).

Mass spectrum: M.W. 428; base peak at m/e 149; main peaks at m/e 428, 369, 359, 356, 341, 314, 285, 256, 206, 149, 135, 126, 115. High resolution mass spectrum, found: 428.2953; calc. for $C_{27}H_{40}O_4$: 428.2926.

3β-Benzoyloxy-13β-formyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan (149)

A solution of the primary alcohol derivative (146) (563.5 mg, m.p. 175-176°) in benzene (10 ml) and dimethylsulfoxide (5 ml) was treated with dicyclohexylcarbodiimide (862 mg), pyridine (0.2 ml) and trifluoroacetic acid (0.3 ml). The mixture was stirred at room temperature for 15 hr. A methanolic solution (5 ml) of oxalic acid (205 mg) was added and after 1 hr of stirring the products were extracted with petroleum ether (b.p. 30-60°). The crude product mixture (1.29 g) was chromatographed on Florisil eluting with carbon tetrachloride to give the aldehyde (149) (557.4 mg) as an amorphous solid. N.m.r. signals (see Figure 19): 9.21 (doublet, J = 6.0, 3H, C- 27 CH_3), 9.20 (singlet, 3H, C-19 CH_3), 9.02 (doublet, J = 6.5, 3H, C-21 CH_3), 7.30 (doublet of triplets, J = 5.5 and 7.0, 1H, C-13 CH), 0.12 (doublet, J = 5.5, 1H, -CHO). I.r.: 2713, 1711, 1601, 1583, 1453, 1279, 1115, 980, 900, 867, 711 cm^{-1}. Mass spectrum: M.W. 534: base peak at m/e 239; main peaks at m/e 534, 475, 465, 462, 420, 406, 362, 325, 270, 239, 197, 157, 152, 139, 126, 119, 117. High resolution mass spectrum, found: 534.3320; calc. for $C_{34}H_{48}O_5$: 534.3345.

13α-Formyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan-3-one (150)

The crude aldehyde (148) (13.26 g) in methanol (400 ml) was treated with anhydrous potassium carbonate (3.00 g) for 1.5 hr at room temperature. Most of the solvent was removed in vacuo and water was added to the residue. Extraction with methylene chloride gave, after drying over sodium sulfate, the crude epimeric aldehyde (150) (12.69 g). Part of the crude mixture was chromatographed on silica gel to give an analytical sample. N.m.r. signals (see Figure 18): 9.22 (doublet, J = 6.0, 3H, C-27 CH_3), 9.10 (doublet, J = 5.0, 3H, C-21 CH_3), 9.08 (singlet, 3H, C-19 CH_3), 6.58 (multiplet, 2H, C-26 CH_2), 5.98 (octet, 1H, C-16 CH), 0.57 (distorted doublet, J = 4, 1H,-CHO). Mass spectrum: M.W. 428; base peak at m/e 115; main peaks at m/e 428, 369, 359, 356, 341, 314, 285, 257, 206, 149, 135, 126, 115. High resolution mass spectrum, found: 428.2933; calc. for $C_{27}H_{40}O_4$: 428.2926.

13α-Hydroxymethyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan-3β-ol (152)

The crude aldehyde (150) (12.00 g) was dissolved in methanol (300 ml) and the solution was treated with sodium borohydride (2.00 g) at 0°. After 3 hr of stirring most of the solvent was removed in vacuo, and the products were extracted from the acidified aqueous layer with chloroform. Drying the organic layer over sodium sulfate followed by evaporation of the solvent gave the crude diol (152) (12.36 g). An analytical sample was obtained by crystallization from ethyl acetate-petroleum ether (b.p. 65-73°), m.p. 243.5-245°. N.m.r. signals (see Figure 22): 9.25 (singlet, 3H, C-19 CH_3), 9.21 (doublet, J = 6.0, 3H, C-27 CH_3), 8.90 (doublet, J = 6.5, 3H, C-21 CH_3), 6.55 (multiplet, 2H, C-26 CH_2),

6.4 (multiplet, 1H, C-3 CH), 6.25 (doublet, J = 4.5 , $C\underline{H}_2OH$), 5.95 (octet, 1H, C-16 CH). I.r.: 3400, 1247, 1058, 1027, 981, 922, 900, 867 cm^{-1}. Mass spectrum (see Figure 23): M.W. 432; base peak at m/e 115; main peaks at m/e 432, 402, 373, 363, 360, 345, 318, 300, 288, 145, 139, 126, 115, 105. Elemental analysis, found: C 74.79, H 10.38; calc. for $C_{27}H_{44}O_4$: C 74.95, H 10.25.

3β-Acetoxy-13α-acetoxymethyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan (153)

The diol (152) (5.00 g, m.p. 243.5-245°) was treated with pyridine (30 ml) and acetic anhydride (30 ml) overnight at room temperature. The reaction mixture was then chilled in an ice bath, and water (200 ml) was added. The mixture was stirred for 1 hr, extracted with ether and worked up to give the crude diacetate (153) (6.09 g) which was chromatographed on alumina (neutral, activity I). Elution with petroleum ether (b.p. 65-73°) gave the pure diacetate (5.63 g) as an amorphous solid. This material resisted crystallization. N.m.r. signals (see Figure 24): 9.23 (singlet, 3H, C-19 CH_3), 9.21 (doublet, J = 6.0, 3H, C-27 CH_3), 8.98 (doublet, J = 6.0, 3H, C-21 CH_3), 8.01 (singlet, 3H, $-OCOCH_3$), 7.99 (singlet, 3H, $-OCOCH_3$), 6.55 (multiplet, 2H, C-26 CH_2), 5.95 (multiplet, 1H, C-16 CH), 5.85 (doublet, J = 2.0, $-C\underline{H}_2OAc$), 5.28 (multiplet, 1H, C-3 CH). I.r.: 1735 , 1385, 1366, 1243, 1057 1028, 981, 920, 902, 864 cm^{-1}. Mass spectrum: M.W. 516; base peak at m/e 105; main peaks at m/e 516, 486, 457, 447, 444, 402, 387, 384, 342, 327, 264, 253, 145, 129, 126, 115, 105. High resolution mass spectrum, found: 516.3486; calc. for $C_{31}H_{48}O_6$: 516.3451.

3β-Benzoyloxy-13α-formyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan (151)

A reasonably pure sample of the β-aldehyde benzoate (149) was dissolved in methanol (50 ml) and benzene (20 ml) and the solution was treated with potassium carbonate (0.2 g) at room temperature for 45 min. The solvents were evaporated and the crude product was taken up with petroleum ether (65-73°). Concentration of the organic layer gave a clear glass (510 mg) which was chromatographed on alumina (neutral, activity II, 15 g). Elution with ether-petroleum ether (65-73°) (1:1) yielded the title compound (151) (476 mg). N.m.r. signals (see Figure 20): 9.22 (doublet, J = 6.5, 3H, C-27 CH_3), 9.20 (singlet, 3H, C-19 CH_3), 9.10 (doublet, J = 6.5, 3H, C-21 CH_3), 0.53 (distorted doublet, J = 4.0, 1H, -CHO).

Baeyer-Villiger oxidation of 3β-acetoxy-13α-acetoxymethyl-C-nor-D-homo-18-nor-(25R)-5α,12α-spirostan (153).

(Method BV-I)

The diacetate (153) (4.90 g) was dissolved in formic acid (98-100%, 100 ml) containing water (5 ml). The solution was heated to 40° and aqueous hydrogen peroxide (30%, 20 ml) was added dropwise in 20 min, while the temperature was maintained at 41-44°. Three additional portions of aqueous hydrogen peroxide (30%, 5 ml) were introduced at hourly intervals. The reaction mixture was then diluted with water (1,000 ml) and extracted with methylene chloride. The organic layer was washed with aqueous sodium bicarbonate and with water, then dried and concentrated to give the Baeyer-Villiger oxidation products as a clear glass (5.13 g).

(Method BV-II)

Aqueous hydrogen peroxide (17%, 35 ml) was added dropwise to a solution of the diacetate (153) (10.62 g) in chilled formic acid (98-100%, 200 ml) at ice bath temperature. The ice bath was removed and the mixture was stirred at room temperature for 24 hr. Additional aqueous hydrogen peroxide (30%, 5 ml) was then introduced and stirring was continued for a 12 hr period. The reaction mixture was worked up as in Method I to give a clear glass (10.82 g). N.m.r. signals: 9.18 (singlet, C-19 CH_3), 7.98 and 7.97 (two singlets, two $-OCOCH_3$ groups), 5.97 (doublet, J = 6.5, C-26 CH_2), 2.00 and 1.92 (two groups of signals, -OCHO).

Hydrolysis of the Baeyer-Villiger oxidation products

(Method I)

The crude performic acid oxidation mixture (5.13 g) was dissolved in methanol (100 ml) and placed in a 100 ml separatory funnel. Saturated aqueous potassium carbonate (5 ml) was added to the solution and the mixture was shaken for 1.5 min. The reaction mixture was promptly transferred to a 2-liter separatory funnel containing dilute hydrochloric acid (0.1 N, 500 ml), saturated aqueous sodium chloride (500 ml) and methylene chloride (400 ml). The organic layer was washed with aqueous sodium bicarbonate and with water, then dried over sodium sulfate. Evaporation of the solvent in vacuo gave the hydrolysis products as a clear glass (4.83 g). This material was chromatographed on alumina (neutral, activity III, 150 g). Elution with benzene-ethyl acetate (1:2) gave the diol (175) (875 mg, 21% based on the diacetate

(153)) as a crystalline solid. An analytical sample was obtained by recrystallization from benzene-methylene chloride, m.p. 211-212°. N.m.r. signals (see Figure 27): 9.22 (singlet, 3H, C-19 CH_3), 8.71 (doublet, J = 6.5, 3H, C-21 CH_3), 8.03 (singlet, 3H, $-OCOCH_3$), 7.99 (singlet, 3H, $-OCOCH_3$), 7.55 (singlet, 2H, two-OH groups; disappeared with D_2O), 5.90 (multiplet, 1H, C-20 CH), 5.82 (broad singlet, 2H, C-18 CH_2), 5.52 (broad singlet, 1H, C-16 CH), 5.35 (multiplet, 1H, C-3 CH). I.r.: 3240, 1733, 1378, 1365, 1245, 1025 cm^{-1}. Mass spectrum: M.W. 436; base peak at m/e 107; main peaks at m/e 400, 358, 340, 314, 273, 260, 254, 187, 147, 145, 107, 105. Elemental analysis, found: C 68.61, H 9.19; calc. for $C_{25}H_{40}O_6$: C 68.77, H 9.24. The rest of the material (2.78 g) from the column consisted of about six compounds.

(Method II)

The crude performic acid oxidation mixture (2.18 g), obtained by the room temperature reaction, was dissolved in methanol (100 ml). A barbital buffer solution (0.05 M, pH 8.24, 15 ml)[199] was added while adjusting the pH of the solution to 8.06 at 42°. The solution was stirred at 42-45° for a period of 8 days readjusting the pH to 8.06 $\pm$ 0.02 every 24 hr. The solvent was then removed in vacuo and the products were taken up with methylene chloride. The organic layer was washed with dilute aqueous sodium bicarbonate and with water, then dried over sodium sulfate and concentrated in vacuo to give an amorphous solid (2.29 g). N.m.r. signals: 9.18 (singlet C-19 CH_3), 7.98 and 7.95 (two singlets, two $-OCOCH_3$ groups), 6.55 (doublet, J = 5.5, C-26 CH_2), 2.00 and 1.92 (two singlets, -OCHO).

(Method III)

The crude performic acid oxidation mixture (10.82 g), obtained by the room temperature reaction, was chromatographed on Florisil (25 g). Elution with petroleum ether (b.p. 65-73°)-methylene chloride mixture gave the major product (9.33 g) shown to be two compounds by t.l.c. Further elution with methanol gave a mixture of minor products (1.23 g) which yielded no α,β-unsaturated ketone (194) on subsequent treatment.

The above mixture of the major products (5.80 g) was dissolved in methanol (200 ml) and chilled to 0° in an ice bath. Then aqueous sodium formate (2.0 g in 4.0 ml water) and aqueous potassium carbonate (1.0 g in 4.0 ml water) were added and the mixture was stirred for 10 min. The reaction mixture was diluted with water and the products were taken up with methylene chloride. The organic layer was washed with aqueous sodium carbonate and with water, then dried over sodium sulfate and concentrated in vacuo to give an amorphous solid (5.15 g). Part of this material (2.74 g) was chromatographed on Florisil (30 g). Elution with petroleum ether (b.p. 65-73°)-chloroform (4:1) gave a clear glass (827 mg) which had an R_f value by t.l.c. identical with one of the Baeyer-Villiger oxidation products. N.m.r. signals: 9.19 (singlet, C-19 CH_3), 8.01 and 7.98 (two singlets, two $-OCOCH_3$ groups), 5.30 (multiplet, C-3 CH), 2.00 and 1.92 (two singlets, -OCHO). Further elution with petroleum ether (b.p. 65-73°)-chloroform (1:1) gave a clear glass (916 mg) which appeared homogeneous by t.l.c. This material was shown to have an R_f value identical with the major product obtained by Method II. N.m.r. signals (see Figure 31): 9.18 (singlet, 3H, C-19

CH_3), 8.69 and 8.67 (two doublets, J = 6.5, 3H, C-21 CH_3), 7.97 and 7.94 (two singlets, 6H, two $-OCOCH_3$ groups), 6.54 (doublet, J = 5.5, 3H, C-26 CH_2), 5.86 (doublet (probably inner signals of an AB quartet), J = 4.5, 2H, $-C\underline{H}_2OAc$), 2.00 and 1.92 (two singlets, 1H, -OCHO (two types)). Elution with chloroform and methanol gave a mixture of products (967 mg) as an amorphous solid. N.m.r. signals: 9.09 (doublet, J = 6.0, C-27 CH_3), 6.55 (doublet, J = 5.5, C-26 CH_2), 2.00 and 1.92 (two singlets, -OCHO).

Formylation of the diol (175)

The diol (175) (108 mg) was treated with acetic-formic anhydride (6 ml) and pyridine (4 ml) at 50° for 3 hr. Extraction with benzene gave the crude product as a crystalline solid (121 mg). An analytical sample of the diformate derivative (176) was obtained by recrystallization from heptane-benzene, m.p. 167-169°. N.m.r. signals (see Figure 28): 9.21 (singlet, 3H, C-19 CH_3), 8.70 (doublet, J = 6.5, 3H, C-21 CH_3), 8.02 and 8.00 (two singlets, 6H, two $-OCOCH_3$ groups), 6.01 and 5.82 (AB quartet, J = 12, 2H, C-18 CH_2), 5.35 (multiplet, 1H, C-3 CH), 4.80 (quartet, J = 6, 1H, C-20 CH), 4.44 (broad singlet, 1H, C-16 CH), 2.07 and 2.00 (two singlets, 2H, two -OCHO groups). I.r.: 1736, 1726, 1720, 1711, 1248, 1208-1176 cm^{-1} (four bands). Mass spectrum: M.W. 492 (M^+ not visible); base peak at m/e 340; main peaks at m/e 432, 386, 340, 326, 280, 265, 187, 147, 145, 143, 131, 119, 107, 105. Elemental analysis, found: C 65.76, H 8.30; calc. for $C_{27}H_{40}O_8$: C 65.83, H 8.19.

13α-Acetoxymethyl-3β,16ξ,20ξ-triacetoxy-18-nor-12α-pregnajervane (177)

The combined fractions (2.33 g) obtained from column chromatography of the crude hydrolysis mixture (Method I) was dissolved in acetone (spectro grade, 60 ml) and treated with Jones' reagent (4.0 ml) at 5° for 15 min. Isolation of the products with methylene chloride gave a greenish amorphous solid (2.48 g). A solution of this material in t-butanol (150 ml) was then refluxed with aqueous sodium hydroxide (5%, 30 ml) for 1 hr. The bulk of the solvent was evaporated in vacuo and the residue was neutralized with dilute hydrochloric acid. Extraction with methylene chloride gave a gummy material which was immediately acetylated with acetic anhydride-pyridine at room temperature overnight. The product was taken up with benzene, then the organic layer was washed successively with dilute hydrochloric acid, saturated aqueous sodium bicarbonate and water. The crude product (874 mg) was chromatographed on alumina (neutral, activity II, 75 g) and elution with ethyl acetate gave a crystalline material (511 mg) which was recrystallized from methanol to give the tetraacetate (177) as fine needles, m.p. 149-150°. The identical compound was obtained by acetylation of the diol (175) as follows.

The diol (175) (135 mg) was treated with acetic anhydride (3 ml) and pyridine (3 ml) at 60° for 5 hr. Extraction with benzene gave the crude tetraacetate (177) (152 mg). An analytical sample was obtained by crystallization from methanol, m.p. 149-150°. N.m.r. signals (see Figure 29): 9.21 (singlet, 3H, C-19 CH_3), 8.75 (doublet, J = 6, 3H, C-21 CH_3), 8.06, 8.04, 8.02, 8.00 (four singlets, 12H, four $-OCOCH_3$ groups), 5.81 and 5.99 (AB quartet, J = 12, 2H, C-18 CH_2),

5.35 (multiplet, 1H, C-3 CH), 4.96 (doublets of quartets, J = 6 and 2, 1H, C-20 CH), 4.64 (broad singlet, 1H, C-16 CH). I.r.: 1727, 1380, 1250, 1068, 1021. Mass spectrum: M.W. 520 (M^+ not visible); base peak at m/e 340; main peaks at m/e 460, 400, 358, 340, 280, 273, 265, 254, 187, 147, 145, 107, 105. Elemental analysis, found: C 67.10, H 8.48; calc. for $C_{29}H_{44}O_8$: C 66.90, H 8.52.

3β-Acetoxy-13α-acetoxymethyl-18-nor-12α-pregnajervan-20-one (196)

(Series I)

The crude hydrolysis product (2.29 g) obtained from the barbital buffer treatment (Method II) was dissolved in acetone (spectro grade, 100 ml) and treated with Jones reagent (2.5 ml) at 12-14° for 1 hr. The reaction was quenched with methanol (5 ml), acetone was removed in vacuo, and the products were isolated by chloroform extraction. This material was subjected to further oxidation with Jones reagent (2.0 ml) under the same conditions as above. The acidic products (1.24 g) were extracted with aqueous potassium hydroxide (3%, 10 ml x 3) from the ethereal solution of the oxidation products. The acidic material was further subjected to the oxidation conditions (Jones reagent, 2.0 ml, 1 hr; 1.0 ml, 1.5 hr) to give the crude keto-acid (184) (1.21 g) as an amorphous solid after chloroform extraction followed by evaporation of the solvent. N.m.r. signals: 9.19 (singlet, C-19 CH_3), 7.97 (singlet, with a shoulder, $-OCOCH_3$), 7.83 (singlet, $-COCH_3$). Aqueous potassium hydroxide (10%, 6 ml) was added to the warm solution of the crude keto-acid (184) (566 mg) in t-butanol (70 ml), and the mixture was refluxed under nitrogen for 1 hr. The solvent was removed

in vacuo and water was added to the residue. Methylene chloride extraction of the slightly basic aqueous layer yielded a clear glass (318 mg) which was immediately treated with acetic anhydride-pyridine (1:1) overnight. The crude α,β-unsaturated ketone (194) (389 mg, λ_{max} 234.5 nm) was obtained as a mixture by methylene chloride extraction. This mixture was hydrogenated with Adams catalyst (40 mg) in ethanol (20 ml) and benzene (5 ml) for 1 hr. The catalyst was filtered off, the solvent was evaporated, and the residual material dissolved in methylene chloride was passed through an alumina column (neutral, activity I, 2.0 g) to give the crude saturated ketones (195) (346 mg). This mixture was dissolved in dry benzene (15 ml) and boron trifluoride etherate (0.2 ml) was added. The mixture was stirred at room temperature for 24 hr and boron trifluoride etherate (0.15 ml) was added. Stirring was continued for 16 hr and the reaction mixture was diluted with ether. The organic layer was washed consecutively with water, aqueous potassium bicarbonate (5%) and water. The crude saturated ketone (196) was obtained as a clear glass (336 mg). Crude ketone (196) (206 mg) was also obtained from the crude keto-acid (184) (535 mg) via the same series of reactions. The combined, crude saturated ketone (542 mg) was chromatographed on alumina (neutral, activity I, 54 g) and elution with ethyl acetate-petroleum ether (65-73°) (1:19) gave the pure, crystalline saturated ketone (196) (62 mg, 4.5% from the diacetate (153)). N.m.r. signals (see Figure 35): 9.20 (singlet, 3H, C-19 CH_3), 8.04 and 8.02 (two singlets, 6H, two $-OCOCH_3$ groups), 7.87 (singlet, 3H, C-21 CH_3), 6.23 and 5.91 (distorted AB quartet, J = 11, $-C\underline{H}_2OAc$), 5.33 (multiplet, 1H,

C-3 CH). I.r. spectrum: 1733, 1705, 1447, 1368, 1240, 1023 cm^{-1}. Mass spectrum (see Figure 36): M.W. 418; base peak m/e 119 and 117; main peaks at m/e 418, 359, 358, 298, 288, 272, 216, 204, 149, 119, 117. High resolution mass spectrum, found: 418.2702; calc. for $C_{25}H_{38}O_5$: 418.2718. Elemental analysis, found: C 71.59, H 9.00; calc. for $C_{25}H_{38}O_5$: C 71.74, H 9.15. Further elution with ethyl acetate-petroleum ether (65-73°)(1:19) yielded the tetraacetate (177) (238 mg) as a crystalline solid.

(Series II)

The crude hydrolysis mixture (5.54 g), obtained from the potassium carbonate-sodium formate procedure, was dissolved in acetone (200 ml) and treated with Jones reagent (13 ml) at 0° for 1 hr. Methanol was added to quench the reaction and the crude product (5.47 g) was taken up with ether after removal of acetone. This material was dissolved in ether (400 ml) and shaken with dilute aqueous sodium hydroxide (0.5%, 50 ml x 3) to isolate the acidic compounds. Neutralization of the aqueous layer with hydrochloric acid and back-extraction with methylene chloride yielded a slightly yellow amorphous solid (3.04 g). This was again treated with Jones reagent (5 ml) in acetone (200 ml) at room temperature for 18 hr, then with Jones reagent (20 ml) in acetone (200 ml) at room temperature for 7 hr to yield the crude keto-acid (184) (2.41 g). This material in t-butanol (150 ml) was treated with aqueous potassium hydroxide (10%, 25 ml) as in Series I. Subsequent acetylation gave the crude α,β-unsaturated ketone (194) as a clear glass (1.80 g). This material

was chromatographed on alumina (neutral, activity II, 125 g) and elution with ethyl acetate-petroleum ether (65-73°) (1:6) yielded the α,β-unsaturated ketone (194) (265 mg) as a clear glass. N.m.r. signals (see Figure 33): 9.33 (singlet, 3H, C-19 CH_3), 8.02 (singlet, 6H, two $-OCOCH_3$ groups), 7.70 (singlet, 3H, C-21 CH_3), 6.82 (distorted triplet, J = 7.5, 1H, C-13 CH), 6.07 (octet, 2H, $-C\underline{H}_2OAc$), 5.33 (multiplet, 1H, C-3 CH), 2.84 (quartet, J = 3 and 7, 1H, C-16 CH). U.v. spectrum: λ_{max} 233 nm. Mass spectrum (see Figure 34): M.W. 416; base peak at m/e 356; main peaks at m/e 416, 388, 372, 370, 356, 343, 327, 296, 202, 187, 149, 135, 107, 105. High resolution mass spectrum, found: 416.2569; calc. for $C_{25}H_{36}O_5$: 416.2563.

The α,β-unsaturated ketone (194) (133 mg) was hydrogenated with Adams catalyst (20 mg) in ethanol (30 ml) for 1 hr. The catalyst was filtered off and the solvent was removed <u>in vacuo</u> to yield a mixture of the saturated ketones (195) (132 mg). N.m.r. signals: the major ketone (196), 9.20 and 7.87; the minor ketone, 9.22 and 7.91. T.l.c. of this mixture on silica gel (ethyl acetate-petroleum ether (65-73°) (1:3)) revealed two spots in about 3:1 ratio. This material was dissolved in dry benzene (30 ml) and treated with boron trifluoride etherate (0.25 ml) for 24 hr to give the saturated ketone (196) (112 mg) as a solid. This substance was shown by t.l.c. and n.m.r. to be identical with the saturated ketone obtained in Series I.

22,26-Imino-13β-jerva-16,22,24,26-tetraene-3β,20-diol or 3β,20-dihydroxy-16,17,22,23,24,25,26-N-octadehydro-5α-veratranine (200).[59]

Dry ether (10 ml) was placed in a 100 ml 3-necked round bottomed flask equipped with a stirrer, a thermometer, and a condenser topped with a helium inlet. The flask was cooled to -45° with an acetone-dry ice bath. Addition of a hexane solution of n-butyllithium (2.35 M, 2.1 ml) followed by 2-bromo-5-methylpyridine (3.60 g) in ether (40 ml) resulted in the immediate development of a deep red colour in the solution. The temperature was maintained at -45° to -40° for 45 min, the 3β-acetoxy-12α-pregnajerv-16-en-20-one (199) (377 mg)[148,169,170] in tetrahydrofuran (5 ml) and ether (20 ml) was introduced over 15 min at -60° to -50°. The acetone-dry ice bath was removed and the reaction mixture was stirred for 15 min. Aqueous hydrochloric acid (2 N, 30 ml) was added to quench the reaction and the aqueous layer was shaken with ether. The aqueous layer was neutralized with ammonium hydroxide (28-30%) and the basic reaction products were extracted with methylene chloride. The organic layer was dried over sodium sulfate, and evaporation of the solvent gave the reaction products (877 mg) as a yellow amorphous solid. This material was chromatographed on alumina (anionotropic, activity III, 38 g) and elution with methanol gave an amorphous solid (300 mg). The solid was dissolved in methylene chloride (50 ml), and was shaken with hydrochloric acid (1 N, 20 ml x 4). The organic layer was dried over sodium sulfate and concentrated to give 3β,20-dihydroxy-16,17,22,23,24,25,26-N-octadehydro-5α-veratranine (200) (279.7 mg, 57%). N.m.r. signals (see Figure 37): 9.23 (singlet, 3H, C-19 CH_3), 9.10 (doublet, J = 7.0, 3H, C-18 CH_3), 8.44 and 8.37 (two singlets, C-21 CH_3), 7.70 (singlet, 3H, C-27 CH_3), 6.43 (multiplet,

1H, C-3 CH), 3.97 (doublet of doublets, J = 3 and 7, 1H, C-16 =CH), 2.84 (doublet, J = 8.0, 1H, C-23 CH), 2.55 (doublet of doublets, J = 8.0 and 2.0, 1H, C-24 CH), 1.67 (doublet, J = 2.0, 1H, C-26 CH). I.r.: 3400, 1602, 1571, 1485, 1450, 1382, 1043, 1031, 836 cm^{-1}. U.v.: λ_{max} 267.5 nm (ε = 4440). Mass spectrum (see Figure 38): M.W. 409; base peak at m/e 136; main peaks at m/e 409, 392, 247, 229, 213, 211, 136, 121. Elemental analysis, found: C 78.97, H 9.71, N 3.40; calc. for $C_{27}H_{43}O_2N$: C 79.17, H 9.60, N 3.42.

3β,18-Diacetoxy-20-hydroxy-22,23,24,25,26-N-hexadehydro-5α,13β(H),17α(H)-veratranine (201)[59]

Dry ether (5 ml) was placed in a flame-dried 25 ml 3-necked round bottomed flask equipped with a stirrer, a thermometer, and a condenser topped with a helium inlet. The flask was cooled to -60° with an acetone-dry ice bath. Addition of 2-bromo-5-methylpyridine (161 mg, 15 equivalents) in ether (1 ml) and tetrahydrofuran (2 ml) followed by n-butyllithium (1.8 M in hexane, 0.45 ml, 13 equivalents) resulted in a deep red solution when the temperature was allowed to rise to -40° in 45 min. 3β-Acetoxy-13α-acetoxymethyl-18-nor-12α-pregnajervan-20-one (196) (25.9 mg, 1 equivalent) in tetrahydrofuran (1 ml) was then added in 2 min and the mixture was stirred at -40° for 3 min before dilute hydrochloric acid (2 N, 20 ml) was added to quench the reaction. The aqueous layer was shaken with petroleum ether (65-73°)-methylene chloride (4:1, 50 ml) and the organic layer was washed with dilute hydrochloric acid (2 N, 5 ml). The combined aqueous layers were neutralized with aqueous sodium hydroxide and extracted

with methylene chloride to give the basic reaction products (60.6 mg) which were acetylated with acetic anhydride-pyridine at room temperature overnight. The reaction mixture was worked up by extracting with products with methylene chloride to yield a yellow glass (62.9 mg). This material was chromatographed on alumina (anionotropic, activity II, 10.5 g) and the desired coupling product (201) (15 mg) was eluted with ethyl acetate-petroleum ether (65-73°) (1:1). 3β,18-Diacetoxy-20-hydroxy-22,23,24,25,26-N-hexadehydro-5α,13β(H),17α(H)-veratranine (201) (9.0 mg, 28.5%) was isolated by further purification by preparative t.l.c. (silica gel, 0.5 m/m, petroleum ether (65-73°)-ethyl acetate (2:1)). N.m.r. signals (see Figure 40): 9.21 (singlet, 3H, C-19 CH_3), 8.55 (singlet, 3H, C-21 CH_3), 8.11 and 8.01 (two singlets, 6H, two $-OCOCH_3$ groups), 7.68 (singlet, 3H, C-27 CH_3), 6.33 (octet, 2H, C-18 CH_2), 5.28 (multiplet, 1H, C-3 CH), 2.96 (doublet, J = 8.0, 1H, C-23 CH), 2.50 (doublet of doublets, J = 8.0 and 2.5, 1H, C-24 CH), 1.65 (broad singlet, 1H, C-26 CH). U.v.: λ_{max} 267 nm (ε = 3700). I.r.: 3400, 1725, 1603, 1581, 1381, 1367, 1260, 1026, 831 cm^{-1}. Mass spectrum (see Figure 39): M.W. 511; base peak at m/e 136; main peaks at m/e 511, 496, 493, 451, 358, 255, 137, 136, 107. High resolution mass spectrum, found: 511.3294; calc. for $C_{31}H_{45}NO_5$: 511.3298.

BIBLIOGRAPHY

1. P.J. Pelletier, and J.B. Caventou, Ann. Chim. Phys., 14, 69 (1820).
2. E. Simon, Pogg. Ann., 41, 569 (1937).
3. J. Fried, O. Wintersteiner, M. Moore, B.M. Iselin, and A. Klingsberg, J. Amer. Chem. Soc., 73, 2970 (1951).
4. O. Wintersteiner, and M. Moore, J. Amer. Chem. Soc., 78, 6193 (1956).
5. J. Sicher, and M. Tichy, Tetrahedron Letters, 6 (1959).
6. S. Okuda, H. Kataoka, and K. Tsuda, Chem. Ind. (London, 512 (1961).
7. H. Mitsuhashi, and Y. Shimizu, Tetrahedron, 19, 1027 (1963).
8. D.H. Bailey, D.P.G. Hamon, and W.S. Johnson, Tetrahedron Letters, 555 (1963).
9. H. Mitsuhashi, and K. Shibata, Tetrahedron Letters, 2281 (1964).
10. T. Masamune, M. Takasugi, and Y. Mori, Tetrahedron Letters, 489 (1965).
11. R.L. Augustine, Chem. Ind. (London), 1448 (1961).
12. T. Masamune, I. Yamazaki, and M. Takasugi, Bull. Chem. Soc. Japan, 39, 1090 (1966).
13. J.W. Scott, L.J. Durham, H.A.P. de Jongh, U. Burkhardt, and W.S. Johnson, Tetrahedron Letters, 2381 (1967).
14. G.N. Reeke, Jr., R.L. Vincent, and W.N. Lipscomb, J. Amer. Chem. Soc., 90, 1663 (1968).
15. S.M. Kupchan, and M.I. Suffness, J. Amer. Chem. Soc., 90, 2730 (1968).
16. W.A. Jacobs, and S.W. Pelletier, J. Org. Chem., 18, 765 (1953).
17. D.H.R. Barton, O. Jeger, V. Prelog, and R.B. Woodward, Experientia, 10, 81 (1954).
18. S.M. Kupchan. W.S. Johnson, and S. Rajagopalan, Tetrahedron, 7, 46 (1959).

19. W.T. Eeles, Tetrahedron Letters, No. 7, 24 (1960).

20. L.F. Fieser and M. Fieser, in "Steroids", p. 876, Reinhold Publ. Corp., New York (1959).

21. S.M. Kupchan, and A.W. By, in "The Alkaloids" (R.H.F. Manske, ed.), Vol. X, pp. 193-280 (1968).

22. J. Tomko, and A. Vassova, Pharmazie, 20, 385 (1965).

23. J. Tomko, Z. Voticky, G. Spiteller, and M. Spiteller-Friedmann, Arch. Pharm., 299, 347 (1966).

24. E. Hohne, K. Schreiber, H. Ripperger, and H.H. Worch, Tetrahedron 22, 673 (1966).

25. J. Tomko, I. Bendik, S. Baner, and I. Mokry, Pharm. Zentralhalle, 99, 313 (1960).

26. J. Tomko and I. Bendik, Coll. Czech. Chem. Commun., 27, 1404 (1962).

27. J. Tomko, A. Vassova, G. Adam, K. Schreiber, and E. Hohne, Tetrahedron Letters, 3907 (1967).

28. E. Hohne, G. Adam, K. Schreiber, and J. Tomko, Tetrahedron, 24, 4875 (1968).

29. J. Tomko, and A. Vassova, Chem. Zvesti, 18, 266 (1964).

30. J. Tomko, A. Vassova, G. Adam, and K. Schreiber, Tetrahedron 24, 6839 (1968).

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

32. J. Tomko, Z. Voticky, A. Vassova, G. Adam, and K. Schreiber, Coll. Czech. Chem. Commun., 33, 4054 (1968).

33. G. Adam, K. Schreiber, J. Tomko, and A. Vassova, Tetrahedron, 23, 167 (1967).

34. J. Tomko, V. Brazdova and Z. Voticky, Tetrahedron Letters, 3041 (1971).

35. Y. Sato, H. Kaneko, E. Bianchi, and H. Kataoka, J. Org. Chem., 34, 1557 (1969).

36. J. McKenna, Quart. Rev., 7, 231-254 (1953).

37. V. Prelog, and O. Jeger, in "The Alkaloids" (R.H.F. Manske, H.L. Holmes, Ed.) Vol. III, p. 270, Academic Press, New York (1953).

38. A. Stoll, Gazzetta Chim. Ital., 84, 1190 (1954).

39. K.J. Morgan, and J.A. Barltrop, Quart. Rev. (London), 12, 34 (1958).

40. Reference 37, Vol. VII, pp. 363-417 (1960).

41. H.B. Boit, in "Ergebnisse der Alkaloid-Chemie bis 1960", pp. 798-832, Akademie-Verlag, Berlin (1961).

42. C.R. Narayanan, in "Progress in the Chemistry of Organic Natural Products" (L. Zechmeister, Ed.), Vol. XX, pp. 298-371, Springer-Verlag, Wien (1962).

43. K. Schreiber, Pure and Applied Chemistry, 21, 131 (1970).

44. K.S. Brown, Jr., in "Chemistry of Alkaloids" (S.W. Pelletier, Ed.), p. 631 Van Nostrand Reinhold Co., New York (1970).

45. D.G. Crosby, in "Naturally Occurring Insecticides" (M. Jacobsen

a and D.G. Crosby, Eds.) pp. 186-198, Marcel Dekker, New York (1971)1

46. E. Merck, Justus Liebig's Ann., 95, 200 (1855).

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

48. W. Poethke, Arch. Pharm., 275, 357, 571 (1937).

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

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

51. W. Fahrig, Pharmazie, 8, 83 (1953).

52. O. Krayer in "Pharmacology in Medicine" 2nd Ed., pp. 515-524, McGraw-Hill, New York (1958).

53. L.S. Goodman, and A. Gilman, "The Pharmacological Basis of Therapeutics" 3rd. ed., MacMillan, New York (1965).

54. S.M. Kupchan, and W.E. Flacke, in "Antihypertensive Agents" (E. Schlitter, Ed.), Academic Press, New York (1967).

55. J.M. Kingsbury, "Poisonous Plants of the United States and Canada", Prentice-Hall, New Jersey (1964).

56. W. Binns. J.L. Shupe, R.F. Keeler, and L.F. James, J. Amer. Vet. Med. Assoc., 147, 839 (1965).

57. R.F. Keeler and W. Binns, Can. J. Biochem., 44, 819, 829 (1966).

58. J. Fried and A Klingsberg, J. Amer. Chem. Soc., 75, 4929 (1953).

59. "Revised Tentative Rules for Nomenclature of Steroids", Steroids, 13, 277 (1969).

60. T. Masamune, M. Takasugi, A. Murai and K. Kobayashi, J. Amer. Chem. Soc., 89, 4521 (1967).

61. T. Masamune, M. Takasugi, and A. Murai, Tetrahedron, 27, 3369 (1971).

62. W.S. Johnson, H.A.P. de Jongh, C.E. Coverdale, J.W. Scott and Urs Bruckhardt, J. Amer. Chem. Soc., 89, 4523 (1967).

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

64. W.F. Johns and I. Laos, J. Org. Chem., 30, 4220 (1965).

65. W.S. Johnson, J.M. Cox, D.W. Graham and H.W. Whitlock, Jr., J. Amer. Chem. Soc., 89, 4524 (1967).

66. T. Masamune, K. Orito, and A. Murai, Bull. Chem. Soc., Japan, 39, 2503 (1966).

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

68. H. Mazur, N. Danieli, and F. Sondheimer, J. Amer. Chem. Soc., 82, 5889 (1960).

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

70. 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. Chem. Soc., 4356 (1956).

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

72. J.P. Kutney, John Cable, W.A.F. Gladstone, H.W. Hanssen, E.J. Torupka and W.D.C. Warnock, J. Amer. Chem. Soc., 90, 5332 (1968).

73. J.P. Kutney, John Cable, G.V. Nair, W.D.C. Warnock, Intra-Science Chemistry Reports, 4, 265 (1970).

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

75. M. Fukuda, Nippon Kagaku Zasshi, 50, 74 (1929); CA, 23, 3920 (1929).

76. M. Fukuda, Sci. Repts. Tohoku Univ., Ser. A, 18, 323 (1929); Chem. Zentr, 101, 988 (1930).

77. T.-Q. Chou and K.-K. Chen, Chinese J. Physiol., 6, 265 (1932); CA, 26, 5703 (1932).

78. T.-Q. Chou and K.-K. Chen, Chinese J. Physiol., 7, 41 (1933); CA, 27, 3033 (1933).

79. T.-T. Chu and T.-Q. Chou, J. Amer. Chem. Soc., 69, 1257 (1947).

80. T.-T. Chu, W.-K. Hwang, and J.-Y. Loh, Acta Chim. Sinicia, 21, 232 (1955); CA, 51, 444 (1957).

81. Y.-F. Chi, Y.-S. Kao, and K.-J. Chang, J. Amer. Chem. Soc., 58, 1306 (1936).

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

83. S. Ito, M. Kato, K. Shibata and T. Nozoe, Chem. Pharm. Bull. (Tokyo), 11, 1337 (1963).

84. M.E. Gross and H.P. Lankelma, J. Amer. Chem. Soc., 73, 3439 (1951).

85. T.-C. Chu, J.-Y. Lo, W.-K. Huang, and F.-C. Ho, K'o Hsueh T'ung Pao, 12, 371 (1957); CA, 55, 27384 (1961).

86. T.-C. Chu, J.-Y. Lu, W.-K. Huang, F.-C. Ho, and C.-C. Liu, Hua Hsueh Hsueh Pao, 24, 377 (1958); CA, 53, 20117 (1959).

87. T.-T. Chu and J.-Y. Loh, Acta Chim. Sinicia, 21, 227 (1955); CA, 51, 444 (1957).

88. L.C. Craig and W.A. Jacobs, J. Biol. Chem., 129, 79 (1939).

89. L.C. Craig and W.A. Jacobs, J. Biol. Chem., 139, 263 (1941).

90. T.-Q. Chou and T.-T. Chu, J. Amer. Chem. Soc., 63, 2936 (1941).

91. F.-C. Ho, J.-Y. Lo, C.-C. Liu, and T.-C. Chu, K'o Hsueh T'ung Pao, 12, 372 (1957); CA, 55, 27384 (1961).

92. M. Fukuda, Nippon Kagaku Zasshi, 69, 165 (1948); CA, 46, 4555 (1952).

93. H. Morimoto and S. Kimata, Chem. Pharm. Bull. (Tokyo), 8, 302 (1960).

94. H. Morimoto and S. Kimata, Chem. Pharm. Bull. (Tokyo), 8, 871 (1960).

95. W. Zimmermann, Z. Physiol., 300, 141 (1955).

96. S. Ito, J.B. Stothers and S.M. Kupchan, Tetrahedron, 20, 913 (1964).

97. S. Ito, Y. Fukazawa, T. Okuda, and Y. Iitaka, Tetrahedron Letters, 5373 (1968).

98. Y.-F. Chi, Y.-S. Kao, and K.-J. Chang, J. Amer. Chem. Soc., 58, 1306 (1936).

99. Y.H. Wu, J. Amer. Chem. Soc., 66, 1778 (1944).

100. K. Fragner, Chem. Ber., 21, 3284 (1888).

101. T.-T. Chu and J.-Y. Loh, Acta Chim. Sinicia, 21, 241 (1955); CA, 51, 445 (1957).

102. T.-T. Chu, J.-Y. Loh, and W.K. Hwang, Acta Chim. Sinicia, 21, 401 (1955); CA, 51, 445 (1957).

103. T.-T. Chu, J.-Y. Loh, and W.-K. Hwang, Acta Chim. Sinicia, 22, 205 (1956); CA, 51, 445 (1957).

104. T.-T. Chu and J.-Y. Loh, Acta Chim. Sinicia, 22, 210 (1956); CA, 51, 445 (1957).

105. H. Boit, Chem. Ber., 87, 472 (1954).

106. H.G. Boit and L. Paul, Chem. Ber., 90, 723 (1957).

107. H. Suginome, N. Sato and T. Masamune, Tetrahedron Letters, 2671 (1969).

108. H. Suginome, T. Kojima, K. Orito and T. Masamune, Tetrahedron, 27, 291 (1971).

109. R. Hirschmann, C.S. Snoddy, Jr., and N.L. Wendler, J. Amer. Chem. Soc., 74, 2693 (1952).

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

111. J. Elks, G.H. Phillipps, D.A.H. Taylor, and L.Y. Wyman, J. Chem. Soc., 1739 (1954).

112. J.M. Coxon, M.P. Hartshorn and D.N. Kirk, Aust. J. Chem., 18, 759 (1965).

113. J.M. Coxon, M.P. Hartshorn and D.N. Kirk, Tetrahedron, 21, 2489 (1965).

114. R.E. Marker and E. Rohrmann, J. Amer. Chem. Soc., 61, 3592 (1939).

115. R.E. Marker and E. Rohrmann, J. Amer. Chem. Soc., 62, 518 (1940).

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

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

118. H. Mitsuhashi, and N. Kawahara, Tetrahedron, 21, 1215 (1965).

119. H. Mitsuhashi, Y. Shimizu and N. Kawahara, Tetrahedron, 24, 2789 (1968).

120. Y. Shimizu and H. Mitsuhashi, Tetrahedron, 24, 4207 (1968).

121. F.C. Chang and R.C. Ebersole, Tetrahedron Letters, 1985 (1968).

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

123. J.M. Coxon, M.P. Hartshorn, D.N. Kirk, and M.A. Wilson, Tetrahedron, 25, 3107 (1969).

124. G. Lukacs, P. Longevialle, and X. Lusinchi, Tetrahedron, 26, 583 (1970).

125. G. Lukacs, P. Longevialle, and X. Lusinchi, Tetrahedron, 27, 1891 (1971).

126. G. Lukacs, L. Cloarec, L. Lacombe, and X. Lusinchi, Bull. Soc. Chim. Fr., 180 (1972).

127. J.W. Huffman and R.R. Sobti, Steroids, 16, 755 (1970).

128. G. Van de Woude and L. van Hove, Tetrahedron Letters, 1305 (1972).

129. W.F. Johns, J. Org. Chem., 32, 4086 (1967).

130. R.E. Marker and E. Rohrmann, J. Amer. Chem. Soc., 61, 3592 (1939).

131. R.E. Marker and E. Rohrmann, J. Amer. Chem. Soc., 62, 518 (1940).

132. G.P. Mueller, R.E. Stobaugh and R.S. Winniford, J. Amer. Chem. Soc., 75, 4888 (1953).

133. A.F.B. Cameron, R.M. Evans, K.C. Hamlet, J.S. Hunt, P.G. Jones, and A.G. Long, J. Chem. Soc., 2807 (1955).

134. D.H. Gould, H. Staeudle, and E.B. Hershberg, J. Amer. Chem. Soc., 74, 3685 (1952).

135. W.G. Dauben and G.J. Fonken, J. Amer. Chem. Soc., 74, 4618 (1954).

136. M.E. Wall, H.E. Kenney, and E.S. Rothman, J. Amer. Chem. Soc., 77, 5665 (1955).

137. C. Djerassi, O. Halpern, G.R. Pettit, and G.H. Thomas, J. Org. Chem., 24, 1 (1959).

138. G.R. Pettit and W.J. Bowyer, J. Org. Chem., 25, 84 (1960).

139. H.I. Fakih and Y.K. Hamied, Indian J. Chem., 2, 508 (1964).

140. A.G. Gonzalez, R. Freire, M.G. Garcia-Estrada, J.A. Salazar, and E. Suarez, Am. Quim., 67, 903 (1971); CA, 76, 34480 (1972).

141. R.E. Marker, E. Rohrmann, H.M. Crooks, E.L. Wittle, E.M. Jones and D.L. Turner, J. Amer. Chem. Soc., 62, 525 (1940).

142. R.E. Marker, E.M. Jones and J. Krueger, J. Amer. Chem. Soc., 62, 2532 (1940).

143. K. Morita, S. Noguchi, H. Kono and T. Miki, Chem. Pharm. Bull. (Tokyo), 11, 90 (1963) and following papers.

144. H. Mitsuhashi, K. Shibata, T. Sato and Y. Shimizu, Chem. Pharm. Bull. (Tokyo), 12, 1 (1964).

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

146. F.C. Uhle, J. Org. Chem., 27, 656 (1962).

147. F.C. Uhle, J. Org. Chem., 30, 3915 (1965).

148. W.F. Johns, J. Org. Chem., 29, 2545 (1964).

149. J.W. Huffmann, D.M. Alabran and A.C. Ruggles, J. Org. Chem., 33, 1060 (1968).

150. R. Anliker, O. Rohr and H. Heusser, Helv. Chim. Acta, 38, 1171 (1955).

151. R.B. Turner and R.H. Garner, J. Amer. Chem. Soc., 80, 1424 (1958).

152. Y. Pocker, "Molecular rearrangements", Chapter 1, Interscience Publishers (1963).

153. M. Nussim, Y. Mazur and F. Sondheimer, J. Org. Chem., 29, 1120 (1964).

154. E.S. Rothmann, M.E. Wall and C.R. Eddy, J. Amer. Chem. Soc., 74, 4013 (1952).

155. M.E. Wall, C.R. Eddy, M.L. McClennan and M. Kumpp, Anal. Chem. 24, 1337 (1952).

156. C.R. Eddy, M.E. Wall and M. Klumpp Scott, Anal. Chem., 25, 266 (1953).

157. R.N. Jones, E. Katzenellenbogen and K. Dobriner, J. Amer. Chem. Soc., 75, 158 (1953).

158. J.E. Page, Chem. & Ind., 58 (1957).

159. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. 2, Holden-Day, Inc., San Francisco (1964).

160. C. Djerassi, Pure Appl. Chem., 21, 205 (1970).

161. W.H. Faul and C. Djerassi, Org. Mass Spectrom., 3, 1187 (1970).

162. K.E. Pfitzner and J.G. Moffatt, J. Amer. Chem. Soc., 87, 5561 (1965).

163. K.E. Pfitzner and J.G. Moffatt, J. Amer. Chem. Soc., 87, 5670 (1965).

164. A.H. Fenslau and J.G. Moffatt, J. Amer. Chem. Soc., 88, 1762 (1966).

165. M. Karplus, J. Amer. Chem. Soc., 85, 2870 (1963).

166. K.L. Williamson and W.S. Johnson, J. Amer. Chem. Soc., 83, 4623 (1961).

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

168. F. Johnson, Chem. Rev., 68, 375 (1968).

169. Ph.D. Thesis, John Cable, University of British Columbia (1968).

170. Ph.D. Thesis, G.V. Nair, University of British Columbia (1969).

171. J.M. Coxon, M.P. Hartshorn and D.N. Kirk, Tetrahedron Letters, 119 (1965).

172. R. Hirschmann, C.S. Snoddy, Jr., and N.L. Wendler, J. Amer. Chem. Soc., 74, 2693 (1952).

173. C.F. Hiskey, R. Hirschmann and N.L. Wendler, J. Amer. Chem. Soc., 75, 5135 (1953).

174. W.F. Johns, J. Org. Chem., 35, 3524 (1970).

175. S.M. Kupchan and S.D. Levine, J. Amer. Chem. Soc., 86, 701 (1964).

176. N.L. Allinger and H.M. Blatter, J. Amer. Chem. Soc., 83, 994 (1961).

177. B. Rickborn, J. Amer. Chem. Soc., 84, 2414 (1962).

178. Reference 20, cf. p. 213.

179. O.L. Chapman, H.G. Smith, and R.W. King, J. Amer. Chem. Soc., 85, 806 (1963).

180. D.H.R. Barton, A.J. Head and P.J. May, J. Chem. Soc., 935 (1957).

181. H.C. Brown and G. Zweifel, J. Amer. Chem. Soc., 86, 393 (1964).

182. H.M. Doukas and T.D. Fontaine, J. Amer. Chem. Soc., 75, 5355 (1953).

183. E.L. Eliel, V.G. Badding and M.N. Rerick, J. Amer. Chem. Soc., 84, 2371 (1962).

184. B.E. Leggetter and R.K. Brown, Can. J. Chem., 43, 1030 (1965).

185. G.R. Pettit, A.H. Albert and P. Brown, J. Amer. Chem. Soc., 94, 8095 (1972).

186. R.F. Zurcher, Helv. Chim. Acta, 44, 1380 (1961).

187. R.F. Zurcher, Helv. Chim. Acta, 46, 2054 (1963).

188. S.M. Kupchan, T. Masamune and G.W.A. Milne, J. Org. Chem., 29, 755 (1964).

189. S.M. Kupchan and M.J. Abu El-Haj, J. Org. Chem., 33, 647 (1968).

190. W.F. Johns, J. Org. Chem., 36, 711 (1971).

191. S. Winstein and R.E. Buckles, J. Amer. Chem. Soc., 65, 613 (1943).

192. L.F. Fieser and S. Rajagopalan, J. Amer. Chem. Soc., 71, 3938 (1949).

193. R.H. Levine, A.V. McIntosh, Jr., G.B. Spero, D.E. Raymen and E.M. Meinzer, J. Amer. Chem. Soc., 70, 511 (1948).

194. T. Reichstein and C.W. Shoppee, Discussions Faraday Soc., 7, 305 (1949).

195. J.C. Chang and R.T. Blickenstoffe, J. Amer. Chem. Soc., 80, 2906 (1958).

196. W.F. Johns and D.M. Jerina, J. Org. Chem., 28, 2922 (1963).

197. M.Sc. Thesis, R.W. Brooks, University of British Columbia (1969).

198. L.F. Fieser and M. Fieser, "Reagents for Organic Synthesis" p. 4, John Wiley and Sons, Inc. (1967).

199. L. Michaelis, J. Biol. Chem., 87, 33 (1930).

200. H.B. Henbest and B.J. Lovell, J. Chem. Soc., 1965 (1957).

201. R.H. Bible, "Guide to the NMR Empirical Method", Plenum Press, New York (1967), p. 275.

202. E.J. Corey, M. Ohno, S.W. Chou and R.A. Scherrer, J. Amer. Chem. Soc., 81, 6305 (1959).

203. F.C. Uhle, J. Amer. Chem. Soc., 73, 883 (1951).

204. F.C. Uhle, J. Amer. Chem. Soc., 83, 1464 (1961).

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

206. L.C. Craig, J. Amer. Chem. Soc., 56, 232 (1934).

207. F.H. Case, J. Amer. Chem. Soc., 68, 2576 (1946).

208. E. Shaw, J. Bernstein,K. Losee and W.A. Lott, J. Amer. Chem. Soc., 72, 4763 (1950).