STUDIES RELATED TO:

BARK EXTRACTIVES OF SOME FIR AND SPRUCE SPECIES; AND

SYNTHESIS AND BIOSYNTHESIS OF INDOLE ALKALOIDS

by

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Chemistry

We accept this thesis as conforming to the

required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September, 1970

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ABSTRACT

Part I of the thesis describes four investigations of some of the neutral components of bark extractives.

The petroleum ether extract of grand fir [Abies grandis (Dougl.) Lindl.] was found to contain two triterpene lactones. The first compound, cyclograndisolide, was shown by chemical and spectroscopic considerations and confirmed by X-ray analysis to be (23R)-3α-methoxy-9,19-cyclo-9β-lanost-24-ene-26,23-lactone (38). The second component, epi-cyclograndisolide, was isomeric with the first and was assigned as (23S)-3α-methoxy-9,19-cyclo-9β-lanost-24-ene-26,23-lactone (43).

In the second investigation, three triterpenes of the chloroform extract of Pacific silver fir [A. amabilis (Dougl.) Forbes] were examined. The main component, abieslactone, was known and had been assigned as (23R)-3α-methoxylanosta-9(11),24-diene-26,23-lactone (30). Chemical and spectroscopic evidence is considered which indicates that assignment to be incorrect and abieslactone is tentatively re-assigned as (23R)-3α-methoxy-9β-lanosta-7,24-diene-26,23-lactone (81). A minor component, AA_3 was assigned on the basis of methylation studies as 3-desmethylabieslactone or (23R)-3α-hydroxy-9β-lanosta-7,24-diene-26,23-lactone (83). Oxidation of AA_3 gave a ketone identical to the second minor component, AA_2, which is then (23R)-3-oxo-9β-lanosta-7,24-diene-26,23-lactone (82).

The third investigation concerns the structure of W_4, a triterpene ketone from the petroleum ether extract of Western white spruce [Picea glauca (Moench) Voss. var. albertiana (S. Brown) Sarg.]. The structure tentatively assigned on the basis of spectroscopic evidence is 3β-methoxy-8α-serrat-13-en-21-one (91).

The fourth investigation was a chemosystematic study of the petroleum ether extract of Engelmann spruce [P. engelmannii Parry]. The presence of methoxyserratene derivatives known to be present in other members of the same genus were not detected in the present investigation.

Part II of the thesis describes synthetic endeavors leading to possible bio-intermediates of indole alkaloids and the biosynthetic evaluation of one synthetic compound.

Condensation of 3-ethylpyridine with 2-carboethoxy-3(β-chloroethyl)indole (60) followed by reduction gave N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (64). The benzoxymethyl derivative 65 of compound 64 was treated with potassium cyanide to give the cyanomethyl derivative 66 which could be hydrolyzed to N-[β{3(2-carbomethoxymethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (67). Alkylation of the compound with methyl formate followed by reduction of the resulating enol, gave 16,17-dihydrosecodin-17-ol (69). This compound was shown to be not, or very slightly, incorporated into the alkaloids of Vinca rosea L. plants. Attempts to oxidize the tetrahydropyridine 64 with mercuric acetate under various conditions failed to give detectable amounts of the corresponding pyridinium salt.

In another synthetic sequence, condensation of the tryptophyl derivative 60 with 3-acetylpyridine ethylene ketal followed by the same sequence of reduction and homologation as employed before gave N-[β{3(2-carbomethoxymethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine (82). Attempts to oxidize 82 with mercurous acetate followed by hydrogenation failed to give the desired N-[β{3(2-carbomethoxymethylindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine (83).

In a second attempt to synthesize 83, the pyridinium chloride salt

84 from the condensation of 3-acetylpyridine with the tryptophyl derivative 60, was hydrogenated to N-[β{3(2-carboethoxyindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine (85). Reduction of 85 under a variety of conditions gave major amounts of N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-acetylpiperidine (86) with only trace amounts of N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine (87) containing the necessary vinylogous amide chromophore.

In a third approach to the synthesis of 83, methyl indole-2-carboxylate (88) was reduced and homologated as before to give methyl indole-2-acetate (92). Treatment of 92 with ethylene oxide and stannic chloride gave methyl 3(β-hydroxyethyl)indole-2-acetate (93). Treatment of the tryptophyl bromide derivative 94, produced by the action of phosphorous tribromide on 92, with 3-acetylpyridine gave the pyridinium bromide 95 which could be hydrogenated to the vinylogous amide 83. More conveniently, treatment of 93 in 3-acetylpyridine with phosphorous tribromide and immediate hydrogenation gave 83 in better yield.

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PART I

STUDIES RELATED TO BARK EXTRACTIVES OF SOME FIR AND SPRUCE SPECIES

Introduction

The mystery of man's natural world can be found in the earliest of records. It is probable that the utilization of substances now known as natural products started before the time of recorded history. Extracts of plants gave the ancient world indigo and alizarin used for dyeing. Aromatic plants afforded perfumes. Other naturally occurring materials were, and are still used for healing or killing.

Early investigations were deeply involved with the chemistry associated with these molecules. Many researchers were severely hampered by the volume of work needed to make the slightest progress. As chemical theory evolved two main areas of research proved to be stumbling blocks; purification of products and physical measurements on these products were inadequate. The past few decades have seen remarkable advances in both these areas.

The progress made in biosynthetic theory has also played a role in natural product investigation. Natural products are often classified in families, thus biogenetic considerations determined for one member are usually applicable to other members of the family. These considerations have led to predictions concerning the occurrence of new and, at the time, unknown structures and even to suggest that some structures already assigned should be re-investigated since they did not fit the existing biosynthetic patterns.

While much of the world is forested, surprisingly little is known about the chemical composition of the plant life. What is known is often

fragmented and incomplete making systematic studies nearly impossible. Despite the economic importance of the forestry industry the extractables found in the trees are poorly understood.

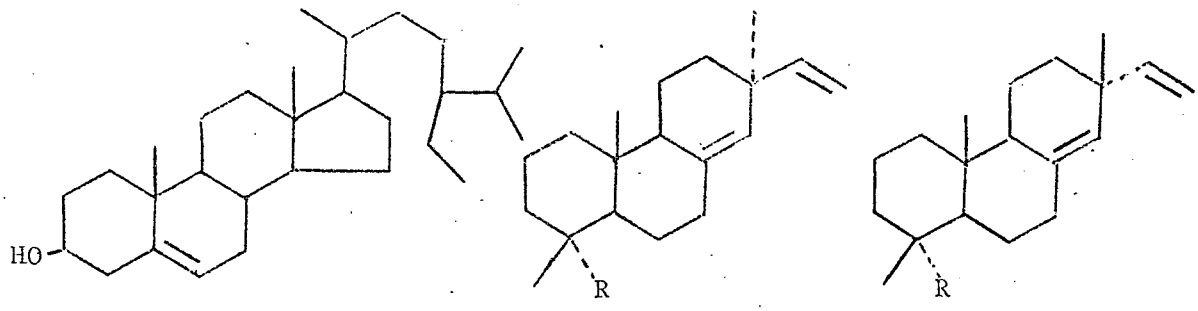
The pulp and paper industry has known of the existence of extractives but has often viewed them as a nuisance to be removed. Industrial ingenuity has accomplished their removal and in the kraft process two by-products, sulfate turpentine and tall oil, are of economic importance.

Sulfate turpentine consists of the volatile terpenes condensed from the relief gases. The production of synthetic pine oil which in turn is used for conversion to terpin hydrate and other chemicals, as well as a solvent and in ore flotation, uses a large portion of the turpentine. Paints, lacquers, synthetic resin, and the perfumery industry use smaller quantities.[1]

The composition of tall oil is variable depending on the kind of wood and the pulping and recovery processes. According to Browning[2] the range of composition may be from 35% to 55% resin acid, 35% to 60% fatty acid, and 10% to 20% unsaponifiable material. The unsaponifiable matter includes sterols, higher alcohols, hydrocarbons, and even some sterol esters of fatty acids which are difficult to saponify. Industrial uses of tall oil are in the manufacture of adhesives, binders, drying oils, soaps, printing ink, and varnishes. Esters of tall oil are used in drying oils, alkyd resins, plasticizers, and lubricant derivatives.

The resin ducts of certain trees when wounded secrete a viscous oil known as oleoresin. It consists essentially of a resin in a volatile oil. In the United States, oleoresin is collected from deliberately wounded

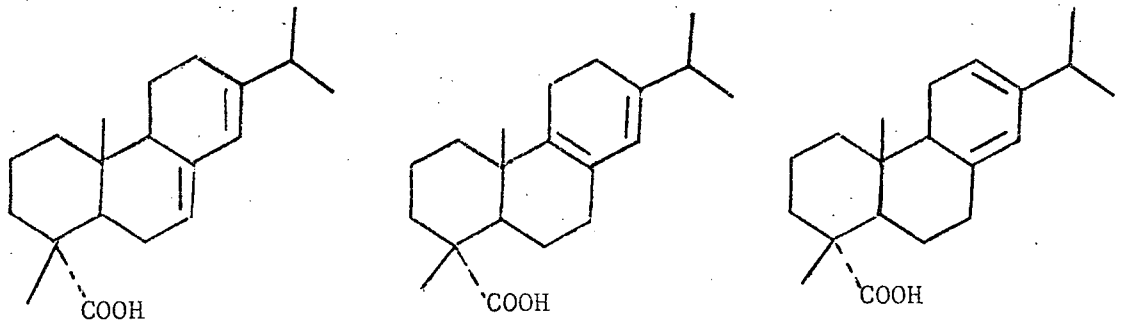
longleaf and slash pine. The volatile "wood turpentine" is removed and the remaining resin, consisting mainly of resin and fatty acid, is used much like tall oil. About 10% of the oleoresin is neutral or unsaponifiable material containing β-sitosterol (1) and other sterols; long chain fatty alcohols; two diterpene aldehydes, dextropimarinal (2) and isodextropimarinal (3); tricyclic diterpenes; diterpene alcohols; and 3,5 dimethoxystilbene.[3]



1 2, R = CHO 3, R = CHO

2b, R = COOH 3b, R = COOH

The resin acid fraction of tall oil or pine oleoresin consists mainly of diterpene acids with the abietane skeleton. Typical examples of the resin acids are dextropimaric (2b), isodextropimaric (3b), abietic (4), palustric (5), and levopimaric (6).



4 5 6

The volatile components, whether they be from wood, bark or leaves, are responsible for the characteristic odors associated with some trees. Some trained observers can distinguish certain woods on this basis. This approach when performed on a more scientific basis may be useful for differentiating or characterizing different species or variations of the same species.

The heartwood extracts of many conifers have been examined by Erdtman[4] and taxonomic correlations on the generic level in the families Pinaceae and Cupressaceae have been made. The heartwood of several Abies species has been studied and the presence of several diterpenoids has been noted.[5] The genus Pinus has been studied by Mirov[6] who investigated the monoterpenes found in the gum turpentine. The leaf oil of white spruce [Picea glauca (Moench) Voss] and black spruce [P. mariana (Mill) BSP.] were examined by von Rudloff.[7,8] He concluded that both species have a remarkably consistent and distinctive distribution pattern of leaf oil terpenes.

The significance of these studies is best illustrated by the chemosystematic studies by von Rudloff.[9] The Rosendahl spruce [P. glauca x mariana] is considered to be a hybrid of white and black spruce. Morphologically Rosendahl spruce is intermediate in some characteristics, like white or black spruce in others, and completely different from either in others. The analysis of the leaf oil showed a similar pattern thus reflecting the hybrid origin of the Rosendahl spruce.

A similar study of the cortical oleoresin from North American fir has been conducted by Zavarin.[10,11,12] Nine distinct Abies species are

recognized north of Mexico and seven of these may be grouped in three larger species complexes. A. magnifica A. Murr and A. procera Rehd. are in one complex; A. concolor (Gord. & Glend.) Lindl. and A. grandis (Dougl.) Lindl. in another complex; with A. lasiocarpa (Hook.) Nutt., A. balsamea (L.) Mill., and A. fraseri (Pursh) Poir. as the third complex. Both A. amabilis (Dougl.) Forbes and A. bracteata D. Don are separate and are not associated with any larger complex. The analysis of the monoterpenoids and the sesquiterpenoid hydrocarbons revealed chemical differences between many species and varieties.

Of the nine species, grand fir [A. grandis] and Pacific silver fir [A. amabilis] are of interest in connection with the present work. The major differences in chemical composition of the oleoresins may be seen in sections (a) and (b) of Table I.[10]

Compound	grandis	amabilis
(a) Sesquiterpenes		
β-Bisabolene	1%	30%
α-Cubebene	24	trace
α-Copaene	18	1
β-Copaene	trace	17
(b) Monoterpenes		
β-Phellandrene	15	46
Camphene	46	-
3-Carene	1	20

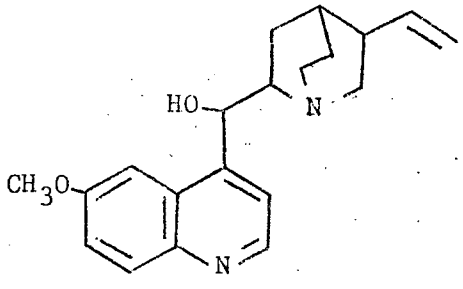
Table I. Hydrocarbons of Abies cortical oleoresins

As can be seen, there are major differences between these two species. The differences extend to the triterpenes isolated from the bark of these species and will be discussed later in this thesis.

At present, bark is a major waste product in the pulp and paper industry. Not only does it possess little commercial value but it also provides a difficult disposal problem. Bark represents 10% to 15% of the total weight of the wood treated and, in the long run, it is disadvantageous from the economic point of view to use it only as fuel.

The physical components of bark, cork, fiber, and bark powder, have been examined for possible uses.[13] Fibers may be used in fiberboard and as additions to fillings and insulation materials. Bark powder has found some use as a carrier for insecticides and in soil improvement. Fractions of cork have proved suitable for soil improvement and filling material.

Bark chemicals may be isolated either by pyrolysis or extraction. Alkaloids such as quinine (7) and strychnine (8) are isolated from bark. A flavanoid, quercitin (9), and some of its derivatives have been isolated from many barks. They have antioxidant properties which are of interest because of possible uses in food and are of some medicinal value in the treatment of capillary blood vessel disorders.[14]



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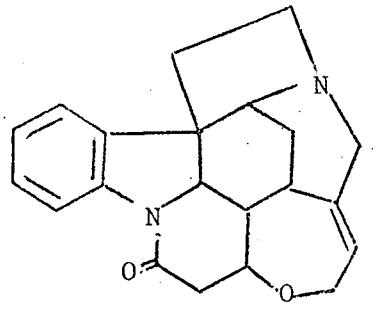

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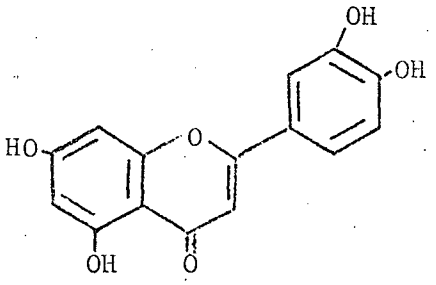

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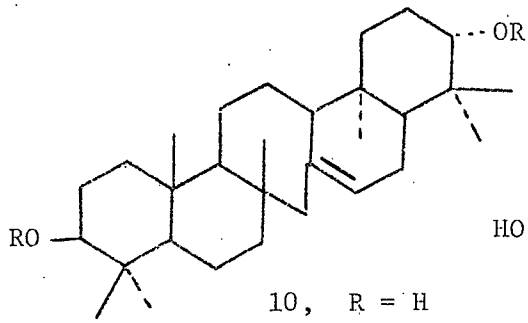
Extracts of western hemlock find use as soil stabilizers, cold-setting waterproof adhesives, and oil well drilling fluids.[15]

A study was made by Chang and Mitchell[16] on the amount of extractable material in the bark of some common North American pulpwood species. They successively extracted the bark with benzene, ethanol, hot water, and one per cent aqueous sodium hydroxide solution. The values obtained for Engelmann spruce [Picea engelmannii Parry] were 5.2% benzene and 25.9% ethanol soluble. The values for black spruce were 5.0% and 14.6% respectively. The only fir mentioned, balsam fir, had values of 13.2% and 3.3% respectively. Rowe[17] found that the benzene extract of pine bark varied from 28.7% for lodgepole pine [Pinus contorta Dougl.] to a low of 2.1% for sugar pine [P. lambertianna Dougl.]

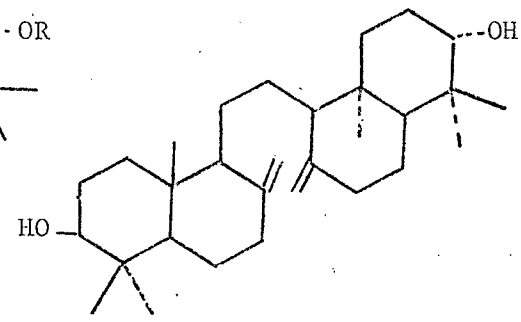
The composition of the extract obtained by Chang and Mitchell was not investigated fully. Rowe's study was more thorough and listed several sterols which he isolated. More interesting was the finding of a methoxy triterpenol in jack pine [P. banksiana Lamb.] and a triterpene diol, which he called pinusenediol, from loblolly pine [P. taeda L.] and jack pine.

The structure of pinusenediol was shown by Rowe[18] to be identical with that of serratenediol (10) isolated by Inubushi from a club moss, Lycopodium serratum Thumb. var. Thumbergie Makino.[19]



10, R = H

10b, R = OAc



11

Serratenediol was the first known triterpene containing a seven member ring C in its structure. Its biosynthesis was postulated[20] as occurring from α-onocerin (11a) with incorporation of one of the vinyl groups into ring C (Figure 1).

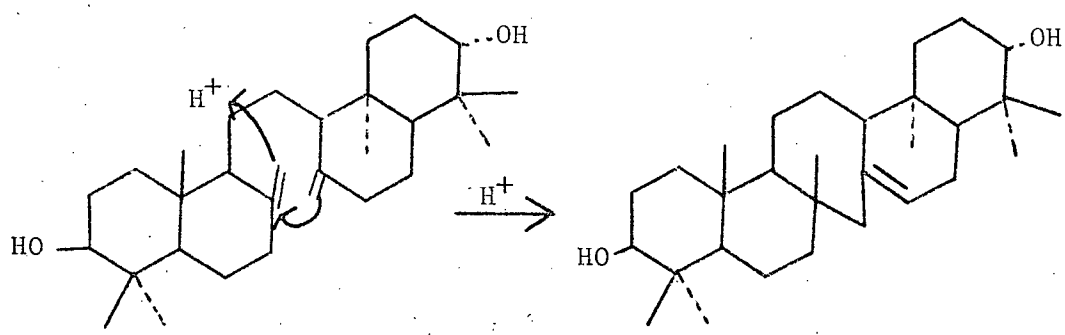
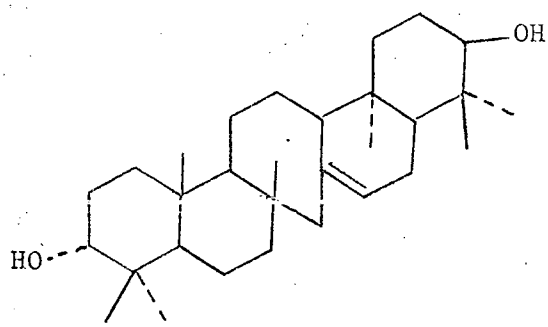


Figure 1. Postulated biosynthesis of serratenediol.

The result of the incorporation of one of the methylene groups into the ring system leaves seven angular methyl groups rather than the usual eight for other pentacyclic triterpenes.

Evidence for this biosynthesis is speculative since no labelling studies have been reported. Also neither α-onocerin nor its derivatives has been found to co-occur with the serratene derivatives in trees. A club moss, Lycopodium clavatum, was found to contain α-onocerin and also diepiserratenediol (12).[19]



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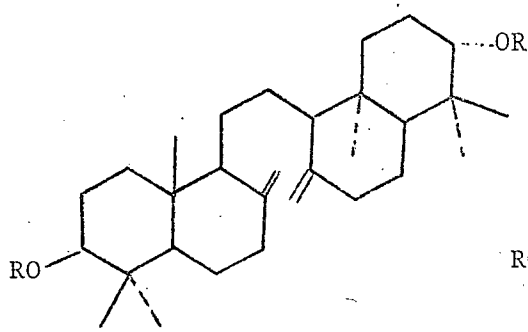
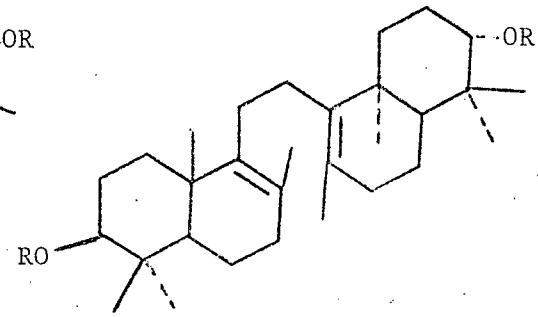
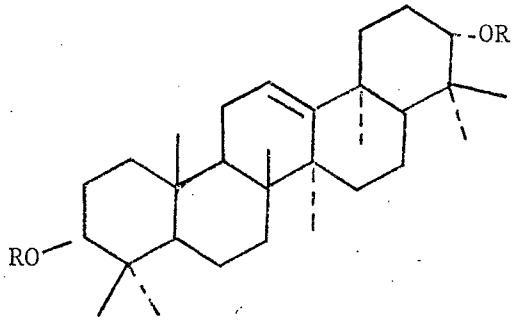
The isomerization of α-onocerin (11) by mineral acid into β- (13) and γ-onocerin (14) is known.[21] Inubushi[20] was able to confirm the earlier work by the isomerization of α-onocerin diacetate (11b) into β- and γ-onocerin diacetate (13b) and (14b) with the use of mineral acid. With a bulkier Lewis acid, boron trifluoride, Inubushi was able to isolate serratenediol diacetate (10b) and γ-onocerin diacetate. Similarly, starting with the corresponding diketone, serratenedione (15) was isolated. The conversion of α-onocerin into serratenediol represented a total synthesis of that compound since α-onocerin had been synthesized by Stork.[22] The position of the double bond in serratenediol and the position and configuration



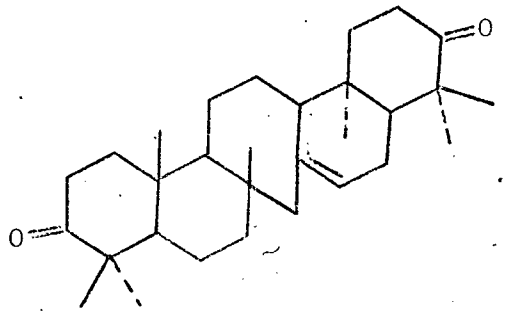
11, R = H
11b, R = Ac



13, R = H
13b, R = Ac

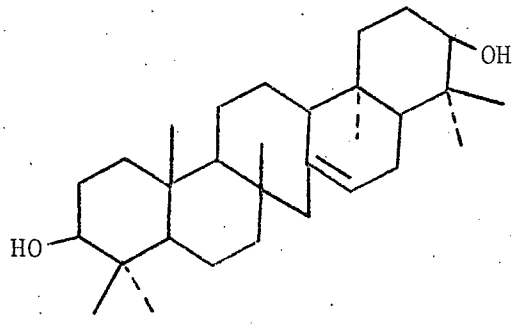


14, R = H
14b, R = Ac



15

of the methyl group arising from the vinyl group of α-onocerin were determined by degradation.



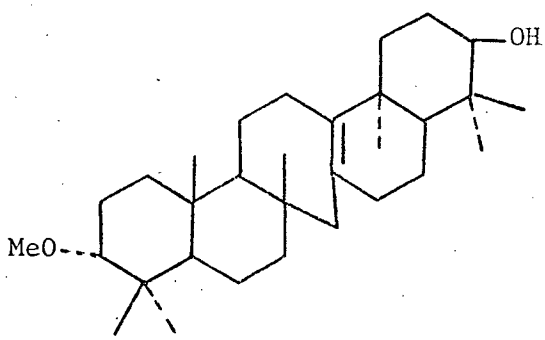
16

In a later paper Rowe[23] reported the isolation and structure of six serratenediol derivatives from pine bark. Of the six compounds only one, 21-episerratenediol (16), had been previously characterized. The remaining five were novel and three of them contained methoxy groups. The three

methoxy compounds were shown to be 3β,21α-dimethoxyserrat-14-ene (17); 3β-methoxyserrat-14-en-21α-ol (18); and 3β-methoxyserrat-14-en-21-one (19). The other two compounds were serrat-14-en-21-on-3β-ol (20) and serrat-14-en-3α,21β-diol (21). Further work on jack pine revealed 21β-methoxyserrat-14-en-3-one (22) and 21α-methoxyserrat-14-en-3β-ol (23).

The methylene chloride extract of the bark of Scots pine [P. sylvestus] contained 3β-methoxyserrat-14-en-21-one (19) and 3β,21α-dimethoxyserrat-14-ene (17).[24]

In a study of the bark of Sitka spruce [Picea sitchensis (Bong.) Carr.] conducted in our own laboratories and at the Forest Products Laboratory, Vancouver, several serratene derivatives were isolated.[25,26] The two in greatest abundance were 3β-methoxyserrat-14-en-21β-ol (24) and 3α-methoxyserrat-14-en-21β-ol (25). The first naturally occurring serratene compound with a 13-ene system, 3α-methoxyserrat-13-en-21β-ol (28), was also characterized. Among the minor components were the following serrat-14-enes: 3β,21β-diol (16); 3β,21α-diol (10a); 21-on-3β-ol (20); 3α-methoxy-21-one (26); 3β-methoxy-21-one (19); and 3α,21β-dimethoxy (27). The diterpene alcohol 13-epimanool (29) was characterized as its 3,5-dinitrobenzoate derivative.



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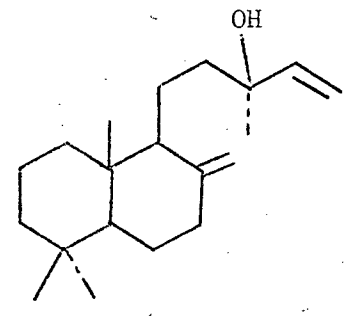

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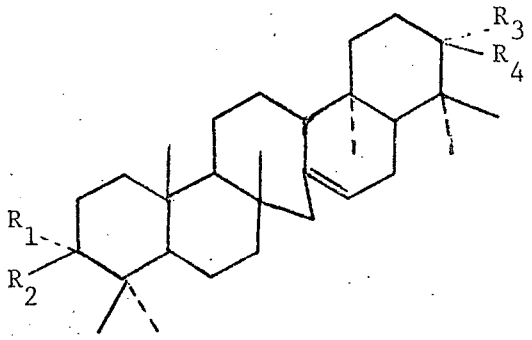


17, $R_1 = R_4 = H$, $R_2 = R_3 = OMe$

18, $R_1 = R_4 = H$, $R_2 = OMe$, $R_3 = OH$

19, $R_1 = R_3 = H$, $R_2 = OMe$, $R_4 = O$

20, $R_1 = H$, $R_2 = OH$, $R_3 = R_4 = O$

21, $R_2 = R_3 = H$, $R_1 = R_4 = OH$

22, $R_3 = H$, $R_4 = OMe$, $R_1 = R_2 = O$

23, $R_1 = R_4 = H$, $R_3 = OMe$, $R_2 = OH$

24, $R_1 = R_3 = H$, $R_2 = OMe$, $R_4 = OH$

25, $R_2 = R_3 = H$, $R_1 = OMe$, $R_4 = OH$

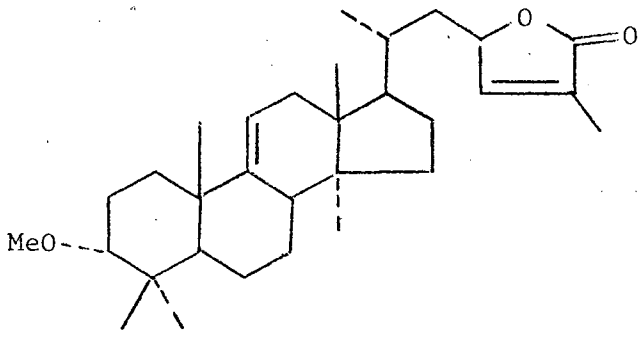
26, $R_2 = H$, $R_1 = OMe$, $R_3 = R_4 = O$

27, $R_2 = R_3 = H$, $R_1 = R_4 = OMe$

I. H. Rogers[27] has compiled a review on the wood and bark extractives of some spruces. Other than the above work on Sitka spruce most of the work on spruce has centered on the phenolics or resin acid and as such is not directly applicable to this thesis.

The occurrence of serratene derivatives in the family Pinaceae in the genus Pinus and later in the genus Picea is now well established. A methoxylated triterpene, abieslactone (30), has been reported from the genus Abies of the same family.[28] This compound, however, is not a serratene derivative but possesses rather a lanosterol skeleton. Abieslactone was initially isolated in 1938 from the bark and leaves of Abies mariessii Masters,[29] a fir tree of northern Japan. The same compound had been isolated by Hergert[30] from the North American Pacific silver fir [A. amabilis (Dougl.) Forbes] and Noble fir [A. procera Rehd.] and named by him as

methoxyabiesadienolide.



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On the basis of selenium dehydrogenation it was suggested in 1964 that abieslactone must contain the skeleton of trimethylsteroids.[31] The structure and absolute configuration suggested for abieslactone is 30. As will be seen later this compound occupies a rather central position in some of the discussion of the present work.

The above discussion provides a brief summary of investigations which relate to the first portion of this thesis concerning the isolation and characterization of compounds found in extracts of some spruce and fir trees.

Structural Studies on Triterpenes from Grand Fir

Discussion

As part of a long range study aimed at the eventual utilization of chemicals found in the bark of coniferous species, I.H. Rogers, Forest Products Laboratory, initiated an examination of the extractives of grand fir bark [*Abies grandis* (Dougl.) Lindl.].[32]

The bark for this study was obtained from a hundred year old tree growing on the University of British Columbia campus. The bark was air dried and ground to a coarse powder before being extracted in a large Soxhlet extractor using petroleum ether as solvent. Upon evaporation of the solvent the crude extract solidified to give a brown wax. Based on the weight of air dried bark, the crude extract represented a petroleum ether extractable content of 0.8%.

The crude extract was separated into numerous fractions using successive column chromatography on alumina. A typical separation emphasizing the fractions relevant to this discussion is shown in Figures 2 - 4. The initial fraction of interest in the present work is G (Figure 2) containing a mixture of fatty alcohols, lactones, and epimanool. Further purification provided three new fractions, M, N, and O.*

* The receipt of Fractions M, N, and O from I.H. Rogers[32] is gratefully acknowledged.

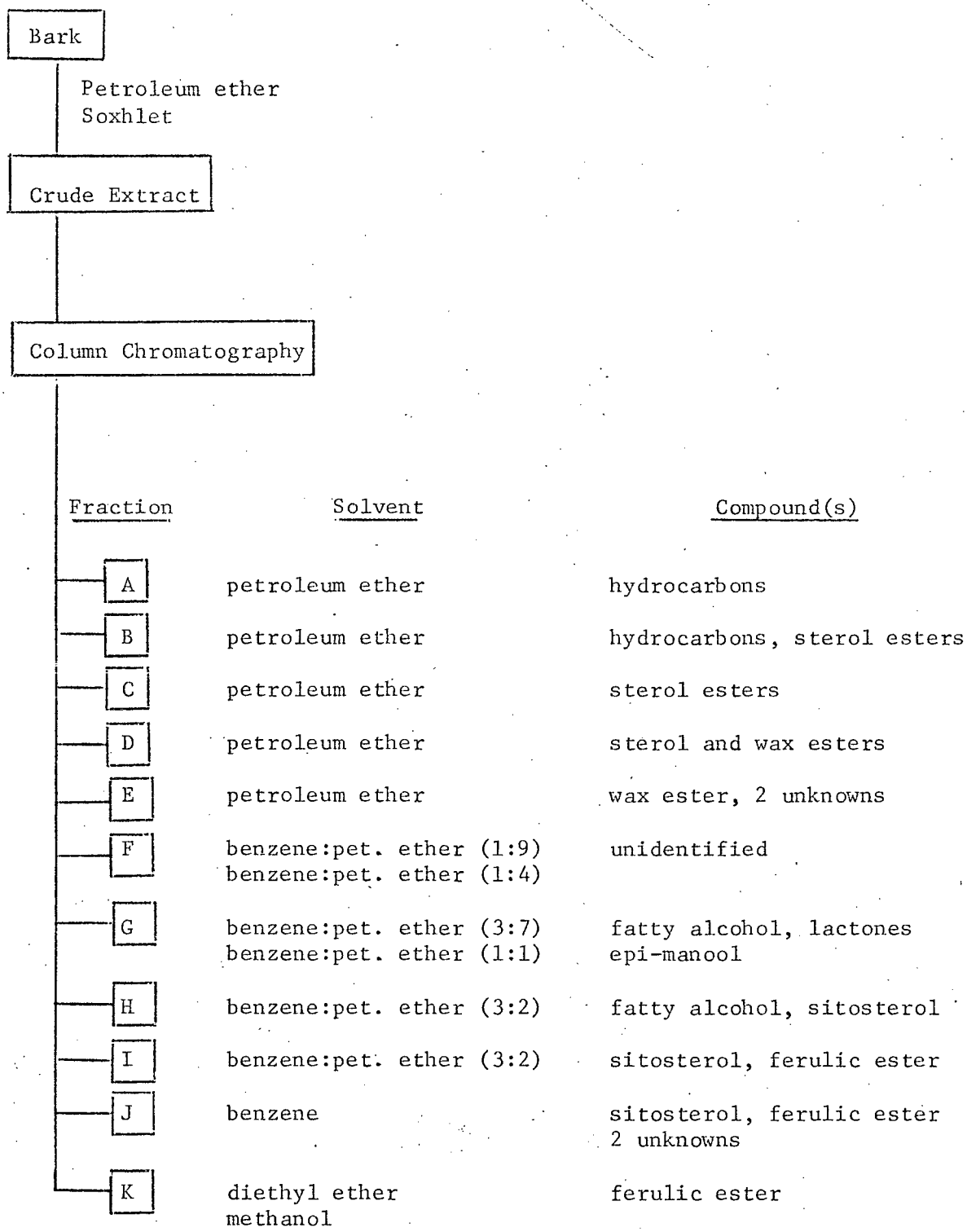


Figure 2. Typical purification sequence of components from grand fir bark.

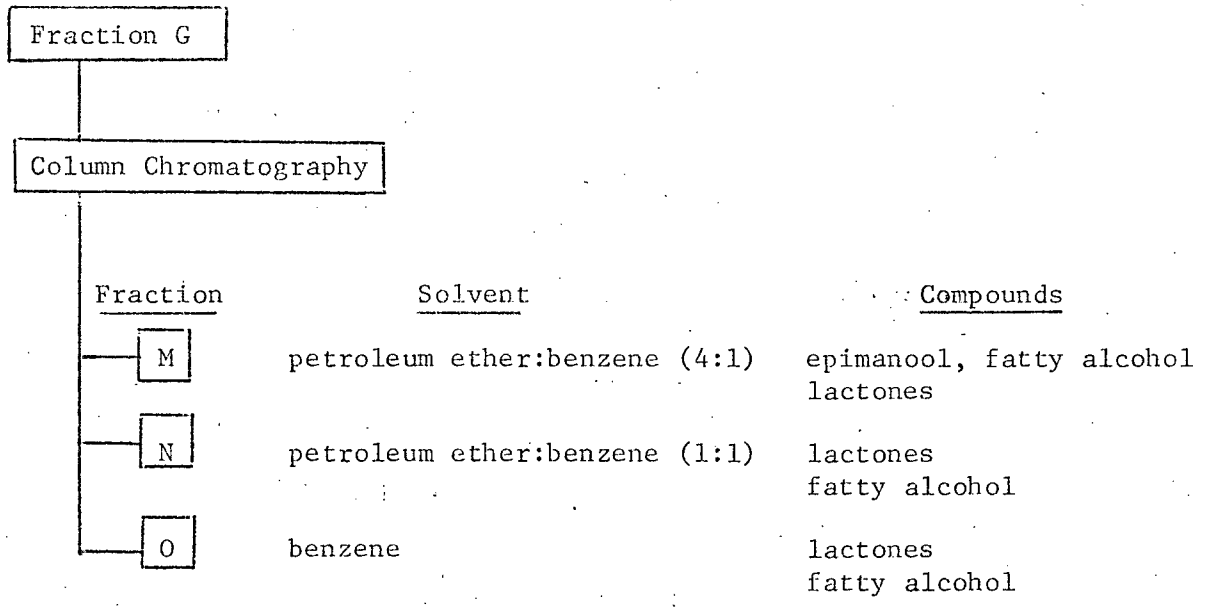
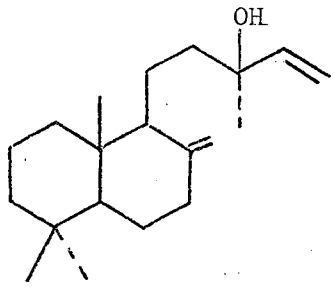


Figure 3. Purification of Fraction G.

Thin layer chromatography (TLC) of M, N, and O showed that all three fractions were mixtures. Fraction M contained at least four components: epimanool, rapidly recognized by comparison with an available authentic sample (see later); two components revealing lactonic absorption in the infrared; and fatty alcohols. Fractions N and O contained the same two lactones and fatty alcohol with the fatty alcohol being the main component.

On this basis it appeared that Fraction M would be the most profitable to examine initially. Chromatography of Fraction M on a column of alumina gave separation into two further fractions P and Q (Figure 4). Fraction P had TLC and nuclear magnetic resonance (NMR) properties which appeared consistent with manool (31) or epimanool (29). These two alcohols may be conveniently distinguished from each other as their 3,5-dinitrobenzoate derivative. The 3,5-dinitrobenzoate of Fraction P had a m.p. of 115 - 117°C

in agreement with the reported[33] value of 116 - 118°C for epimanool. On this basis the structure ascribed was that of epimanool (29).



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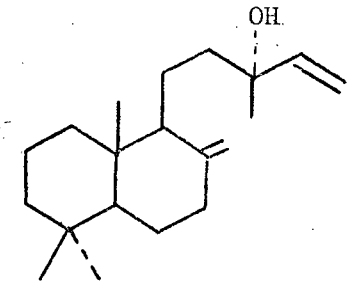

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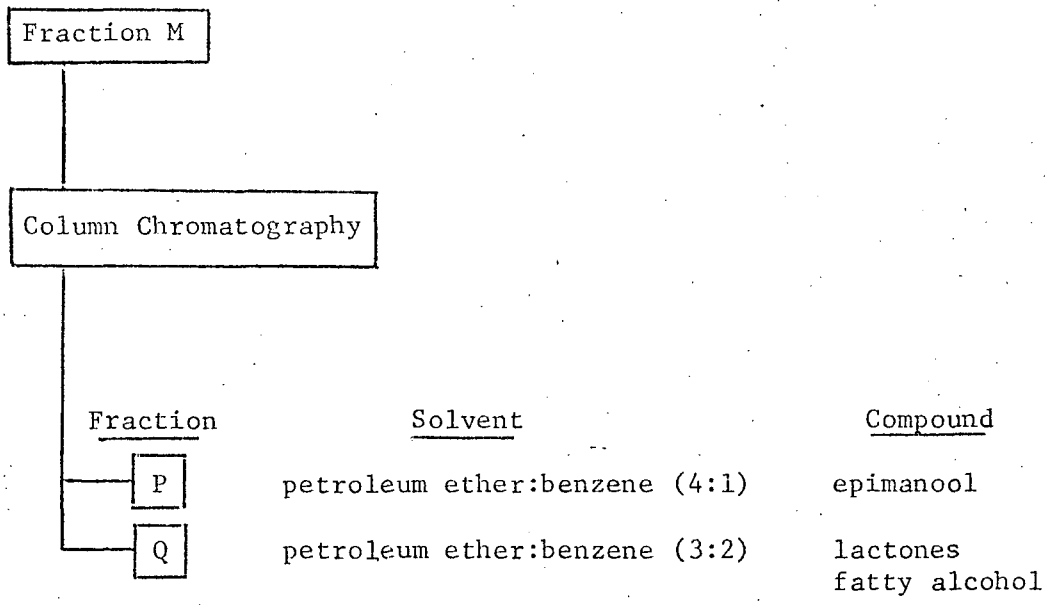
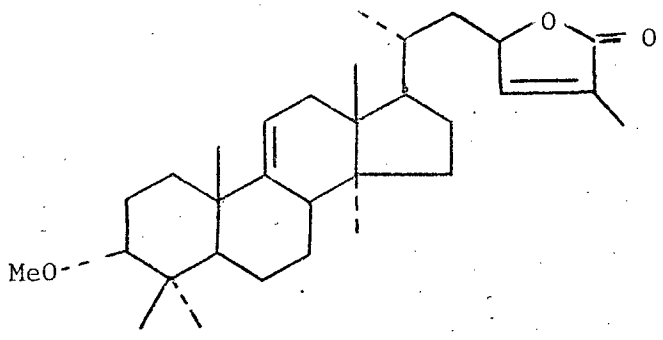


Figure 4. Purification of Fraction M.

Fraction Q contained at least two compounds. The NMR of this fraction had resonances at τ 3.0, 5.0, and 6.7 similar to those reported for abieslactone (30).[28] In addition, a vinylic methyl at τ 8.1 was distinguishable, a signal also found in abieslactone. A signal at τ 8.7 which was also present was assigned to the methylenes of the long chain fatty alcohol, one of the components in Fraction Q.



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The necessity to obtain material free of the fatty alcohol was obvious. Rowe in his work with pine bark extracts[33] had some success in removing this type of material with an urea channel complex. Consequently a portion of Fraction M was taken up in a warm alcoholic solution of urea and, after standing overnight, the crystalline complex containing the fatty alcohol component was removed by filtration. Thin layer chromatography of the mother liquors revealed a mixture of two compounds. These were conveniently separated on silica gel preparative layer plates impregnated with Rhodamine 6G.[34]

The top band was the greater of the two and could be eluted from the silica gel with chloroform. Evaporation of the chloroform left an

orange-red solid as some of the dye had also been eluted. A quick flush through a short column of deactivated alumina removed most of the color. The compound so obtained was initially coded as AG_1 and subsequently named cyclograndisolide. The second band was similarly treated to give a mixture of the second component, AG_2, and some AG_1. Additional quantities of this mixture were obtained from Fractions N and O in the manner described above.

For analytical purposes cyclograndisolide so obtained was crystallized from methanol to give a white solid, m.p. 191 - 193°C. The molecular formula by high resolution mass spectrometry and elemental analysis was found to be $C_{31}H_{48}O_3$. The infrared (IR) spectrum with a strong band at 1745 cm^{-1} and the ultraviolet (UV) spectrum with λ_{max} 209 mμ (log ε 4.33) suggested an α,β-unsaturated-γ-lactone, the same chromophore found in abieslactone.[28] The NMR spectrum at 60 MHz obtained in $CDCl_3$ revealed two high field doublets at τ 9.5 and 9.7, suggesting the presence of a cyclopropane ring; a C-methyl region (τ 8.95 - 9.15) corresponding to five C-methyls; a vinylic methyl at τ 8.1; an O-methyl at τ 6.7; a diffused triplet at τ 7.2; a one proton multiplet at τ 5.0; and an olefinic proton at τ 3.0. More accurate chemical shifts were obtained at 100 MHz but in this instance the spectrum had to be obtained in two portions. With $CDCl_3$ as solvent, tetramethylsilane (TMS) was used for the lock signal but this interfered with the high field doublets. Using $CHCl_3$ for the lock signal the high field doublets could be recorded but the low field olefinic proton at τ 3.0 was unobservable. Thus Figure 5 shows the region τ 4 - 10 in $CHCl_3$. A trace of TMS was added to mark τ 10.0. Figure 5a is the low

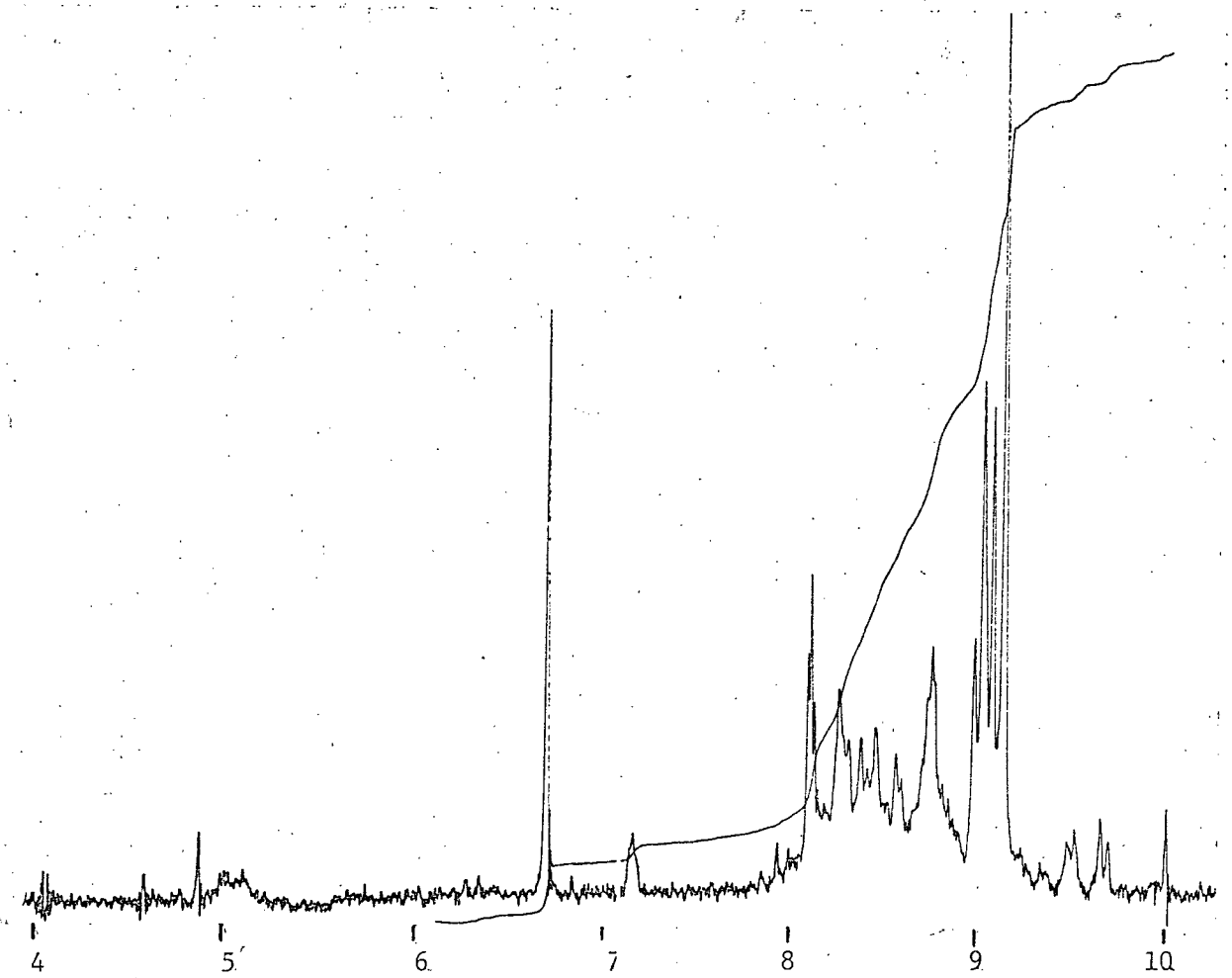


Figure 5. NMR spectrum of cyclograndisolide (τ 4 - 10 region).

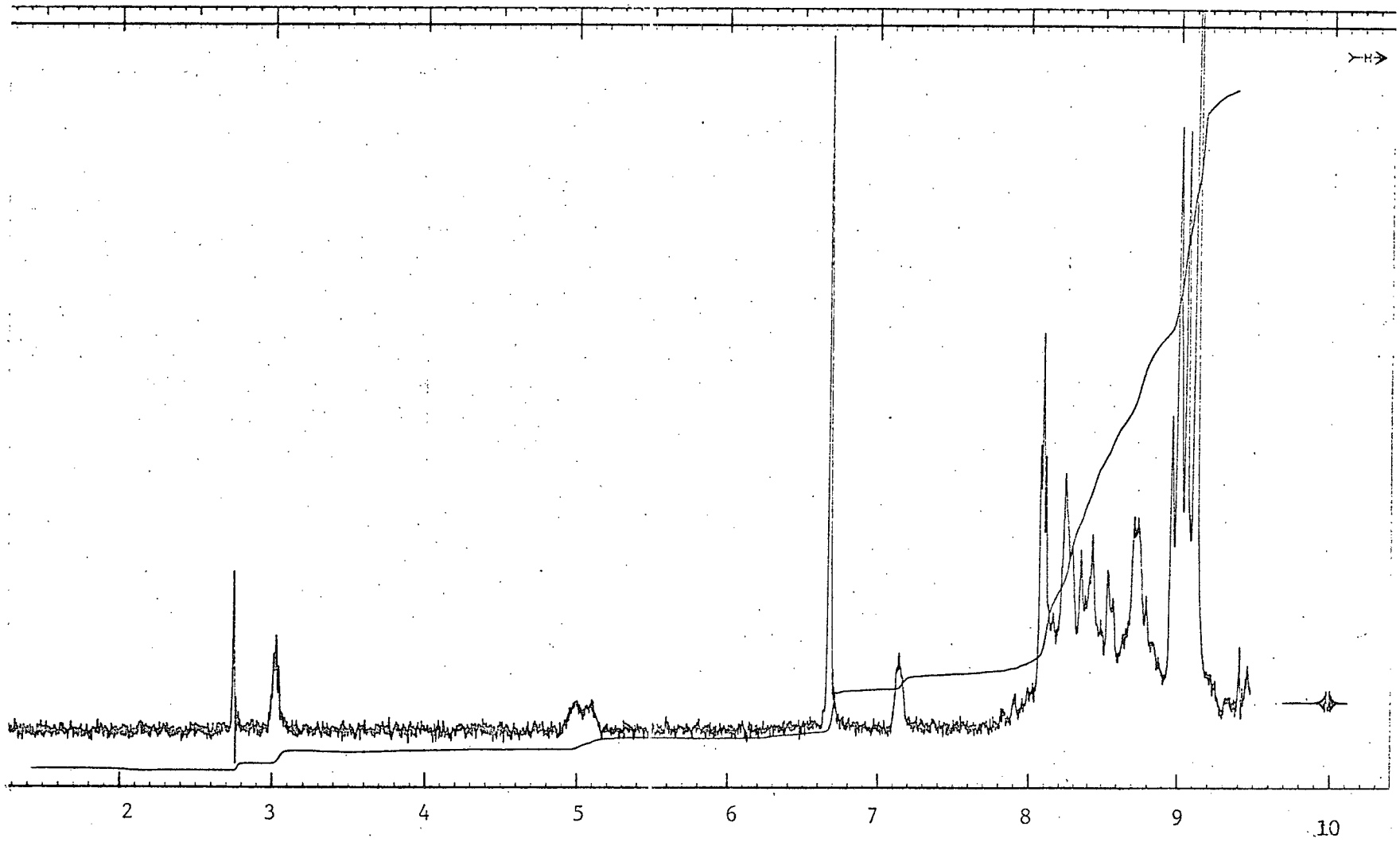


Figure 5a. NMR spectrum of cyclograndisolide (τ 2 - 9 region).

field region of cyclograndisolide in $CDCl_3$ relative to TMS. The cyclopropane methylene absorbs at τ 9.50 and 9.68 (J = 4 Hz); C-methyl groups absorb at τ 8.98 - 9.14; the vinylic methyl at τ 8.10 is an apparent triplet; the one proton triplet at τ 7.20 (J = 1.8 Hz) is assigned as an equatorial hydrogen geminal to the axial methyl ether at τ 6.72. The one proton multiplet at τ 5.05 was assigned to the proton which is geminal to the ring oxygen of the lactone and the apparent triplet at τ 3.02 was assigned to the olefinic proton on the lactone ring. The optical rotatory dispersion (ORD) curve (Figure 6) of cyclograndisolide (in dioxane) had a trough at 225 mμ ($[\phi]_{225}$ = -26,700°). The circular dichroism (CD) curve (Figure 7) had a weak positive peak, $[\theta]_{250}$ = +370°, and a strong negative value with no minimum observed above 215 mμ ($[\theta]_{215}$ = -44,000°). These measurements

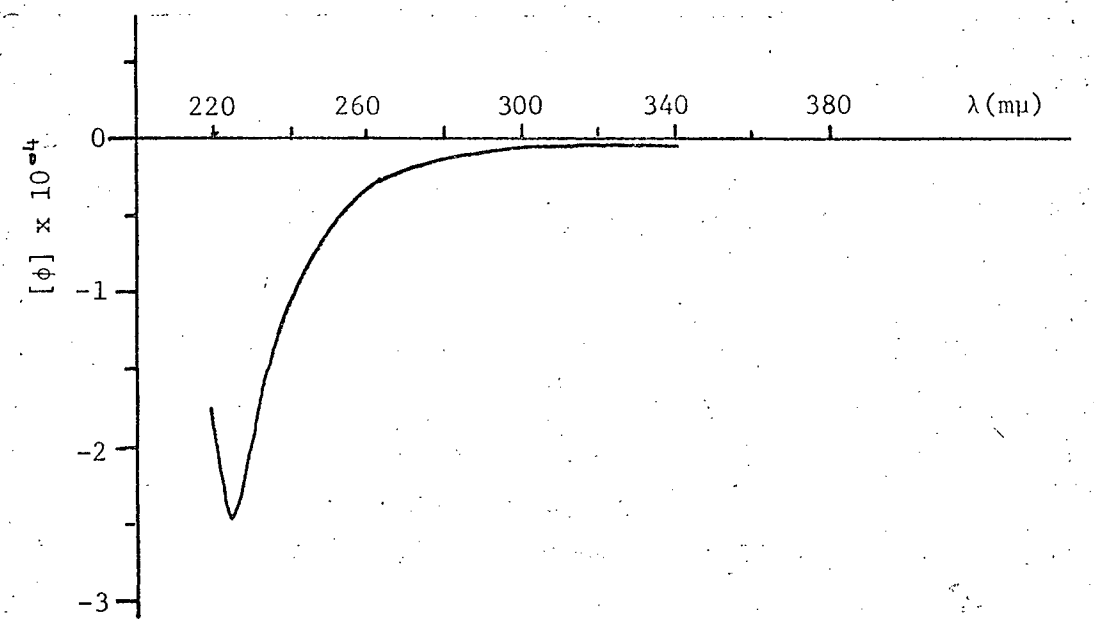


Figure 6. ORD curve of cyclograndisolide.

are of the same sign and of similar magnitude to those obtained for abieslactone[28] and would suggest the R configuration about the lactone.

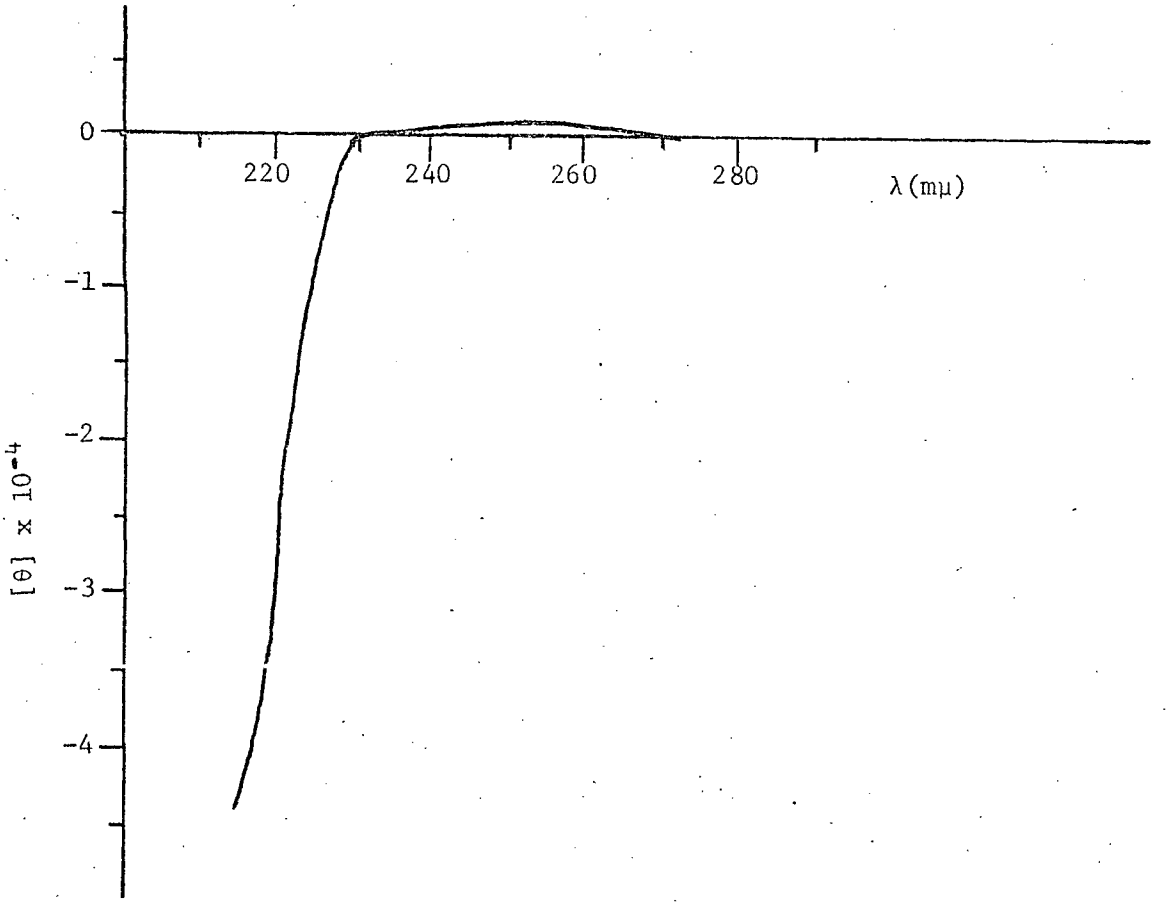
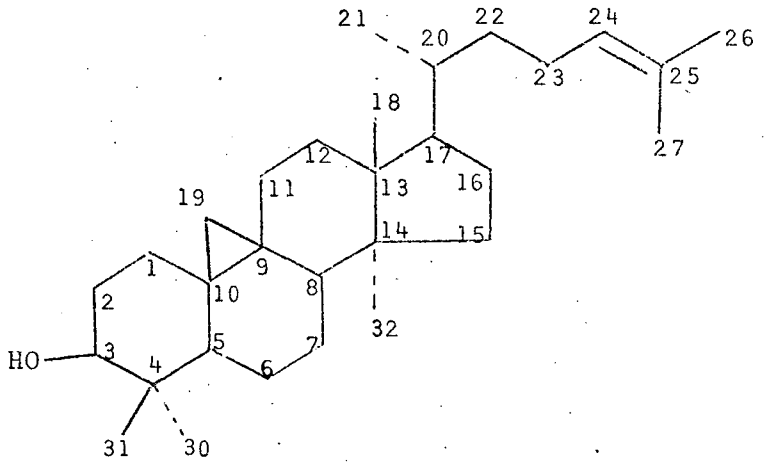


Figure 7. CD curve of cyclograndisolide.

The position of the cyclopropane ring in the above substance could not be determined from the spectral evidence but the known cyclopropanoid triterpenes[35] and the cyclopropanoid Buxus alkaloids[36] are all based on cycloartenol (32), providing some suggestions.

The chemistry of cycloartenol has been well studied.[37,38] It was known that treatment of cycloartanyl acetate with gaseous hydrogen chloride



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in chloroform resulted in a mixture of olefins. The major isomer was the 9(11)-ene with minor amounts of the 7-ene and 8-ene isomers. This reaction was extremely convenient for our purposes since opening of the cyclopropane in cyclograndisolide should give abieslactone (30) as the major isomer.

However, when gaseous HCl was bubbled into a chloroform solution of cyclograndisolide, the product isolated was not the expected abieslactone. In the NMR spectrum of abieslactone, the olefinic proton absorbs at τ 4.48 while the olefinic proton of the new reaction product, initially called iso-AG_1 and subsequently called grandisolide, absorbed at τ 4.80 (Figure 8). This latter value was in much better agreement with the reported[39] value of τ 4.75 for the C(11) olefinic proton of ester (33), a result which seemed at variance with that reported for abieslactone. In order to obtain further evidence on the expected chemical shift for the olefinic proton in a 9(11)-ene system, I examined dihydroparkeyl acetate (34) and observed a value of τ 4.81.

The other intriguing aspect of the NMR spectrum of grandisolide was the C-methyl group region which exhibited a series of signals in the

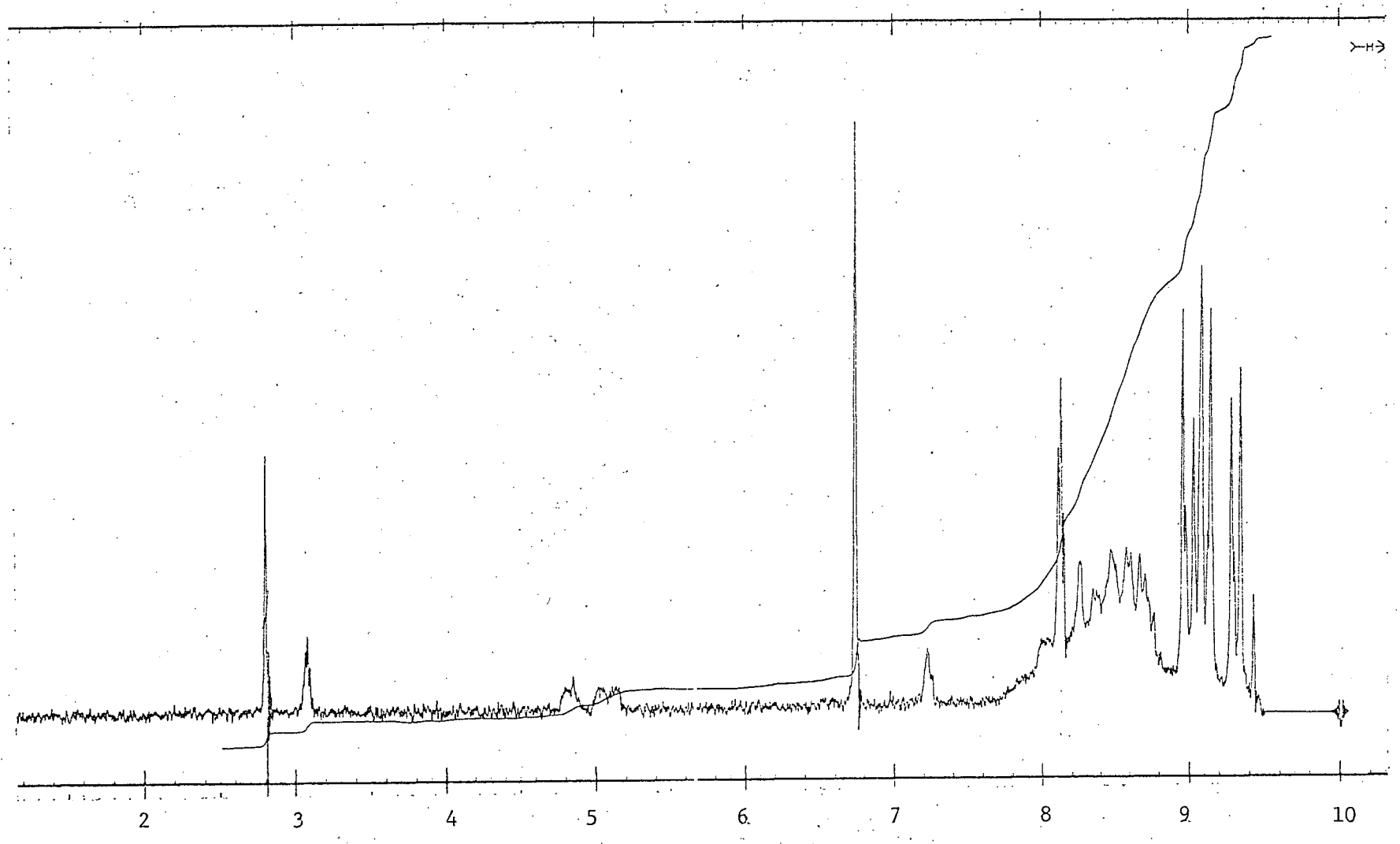
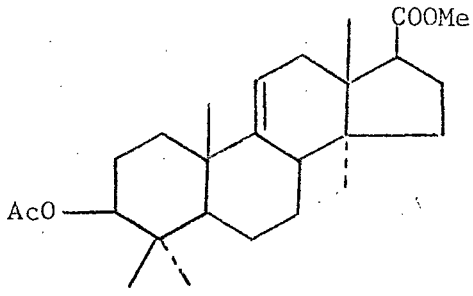


Figure 8. NMR spectrum of grandisolide.



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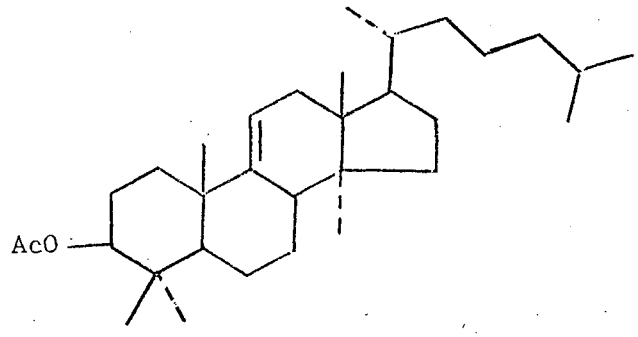

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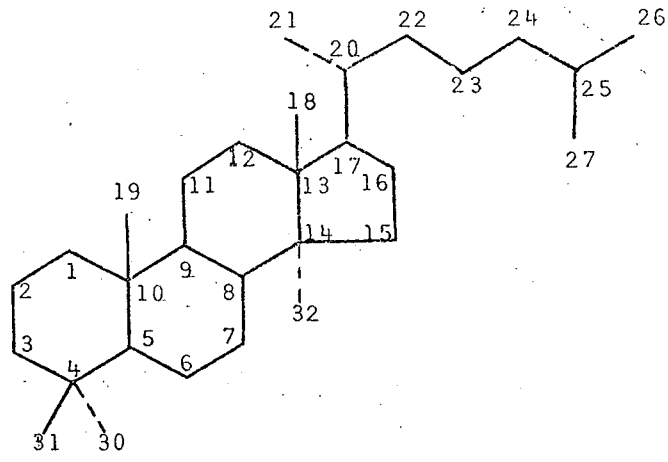
range τ 8.95 - 9.36. The C-methyl group region of abieslactone covered the range τ 8.90 - 9.08. This data provided a strong indication that although abieslactone may indeed be a member of the tetracyclic lanostane family, the position of the double bond and/or the methyl groups previously suggested in the structure 30 [28] may be open to question. For this reason I will discuss in some detail the chemistry of abieslactone in a later section of this thesis while at this time it is convenient to present the remaining data which provides the completion of the structure of cyclograndisolide.

Considerable systematic data[39,40,41,42] has been collected on the position of NMR signals due to methyl groups of triterpenes and complete assignments of these signals have become possible for certain triterpene families.

The facts available to this point suggested that cyclograndisolide was a member of the cycloartane family. Hence grandisolide would be a member of the lanostane family (35) for which a considerable body of NMR data was available. In particular the relative positions of the methyl



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groups had been extensively studied and the pertinent values for the lanostane family are found in Table II. Table III contains the changes in the chemical shifts ($\Delta\tau$) of the methyl group resonances produced by some functional groups attached to the skeleton.[39]

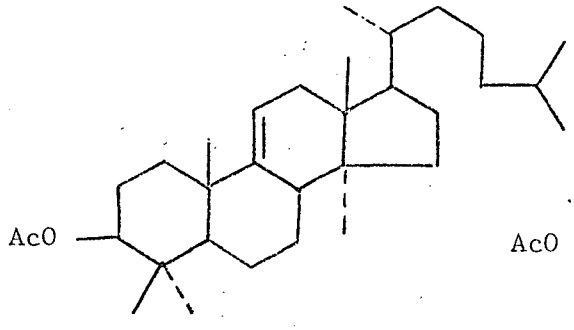
Compound	Methyl Group				
	30	31	19	18	32
lanostane	9.17	9.17	9.08	9.22	9.20
lanostan-3α-ol	9.13	9.07	9.07	9.23	9.19
lanostan-3α-acetate	9.09	9.17	9.09	9.23	9.17
lanostan-3β-ol	9.20	9.02	9.09	9.20	9.20
lanostan-3β-acetate	9.13	9.16	9.08	9.23	9.21
lanostan-3-one	8.94	8.94	8.94	9.22	9.22

Table II. Position of methyl groups in τ values (±0.03 τ).

Function	Methyl Group				
	30	31	19	18	32
8-ene	-0.01	-0.04	-0.13	+0.08	-0.07
9-ene	-0.02	-0.05	-0.19	+0.11	+0.05
8-ene-7,11-diketo	-0.08	-0.05	-0.44	-0.00	-0.34

Table III. Contribution of functional groups to the chemical shift change ($\Delta\tau$) of methyl groups.

Tables II and III in combination with each other may serve for the calculation of chemical shift values for other compounds in this series whether or not they are listed. As an example, the calculated values of



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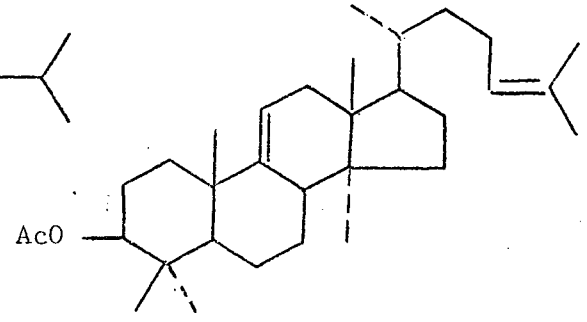

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parkeyl acetate (36) and dihydroparkeyl acetate (34) are given with the observed values in Table IV.

	Methyl Group				
	30	31	19	18	32
parkeyl acetate					
calculated	9.11	9.11	8.90	9.34	9.26
observed	9.14	9.14	8.94	9.38	9.28
dihydroparkeyl acetate					
calculated	9.11	9.11	8.90	9.34	9.26
observed	9.13	9.13	8.95	9.36	9.25

Table IV. Observed and calculated chemical shifts of methyl group resonances in typical lanost-9(11)-enes.

It is noted that the observed and calculated values are in good agreement. Attention is directed to the calculated and observed values for the 18, 19, and 32 methyl groups. The 19 methyl group is both observed and calculated to be in the region τ 8.90 to τ 8.95, an expected result since this methyl group is allylic to the 9(11)-ene system.

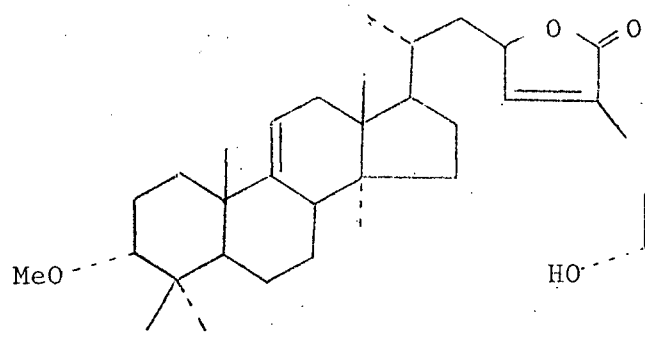
The 32 methyl group has a range τ 9.25 - 9.28, while the 18 methyl group is consistently at the highest field with a range of τ 9.34 to 9.36. Hence a typical lanost-9(11)-ene derivative should have the 19 methyl group resonating near τ 8.95 with the 18 and 32 methyl groups near τ 9.35 and τ 9.25 respectively.

If one makes the assumption that grandisolide is a member of the lanostane series, the most likely position for the methoxyl function would be C(3) while the α,β-unsaturated-γ-lactone would form part of the side chain. An examination of Table II shows the only functionality at C(3) which significantly affects the resonance frequencies of the 18, 19, and 32 methyl groups is the 3-ketone. Hence if the study is focussed on these three methyl groups in compounds with functionality at C(3) other than a ketone, the values presented in Table II should be applicable.

	Methyl Groups				
	30	31	19	18	32
lanost-9(11)-en-3α-ol calculated	9.11	9.02	8.89	9.34	9.24
grandisolide observed	9.12	9.02	8.95	9.36	9.29

Table V. Resonance frequencies of methyl groups of lanost-9(11)-enes

The effect of the lactone ring on the resonance frequencies of these methyl groups is unknown but it would be surprising if it was appreciable. A comparison of the calculated values for lanost-9(11)-en-3α-ol (37) with the observed data for grandisolide is made in Table V, and indeed the similarities are striking. This result provided further evidence in support of the general structural features present in grandisolide and,



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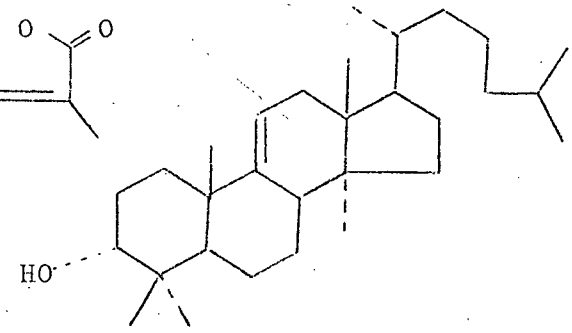

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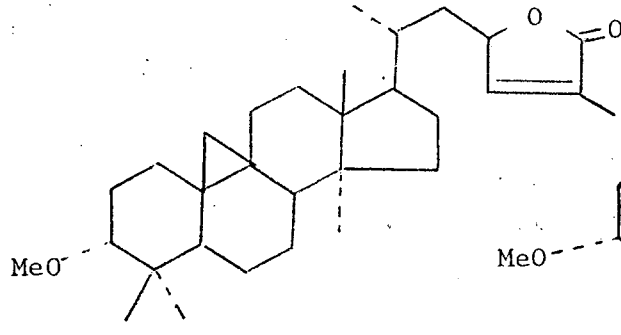
in turn, in cyclograndisolide.

At this stage it appeared desirable to establish the relationship, if any, between grandisolide and the only known tetracyclic triterpene which contained an unsaturated γ-lactone in the side chain, abieslactone (30). Indeed when abieslactone was treated with gaseous HCl in the manner already described for cyclograndisolide, a reaction product identical in all respects (m.p., mixed m.p., IR, and TLC) with grandisolide was obtained. This result established the suspected relationship between the two series and strengthened the argument that grandisolide possesses a tetracyclic system characteristic of the lanostane family. On this basis, the most likely structure for grandisolide would be 30. In turn, cyclograndisolide could be either 38 or 39 since both cyclopropane rings could lead to the 9(11)-ene shown in 30. The alternative 38 was somewhat more favored since the cycloartenol series discussed previously was already well established.



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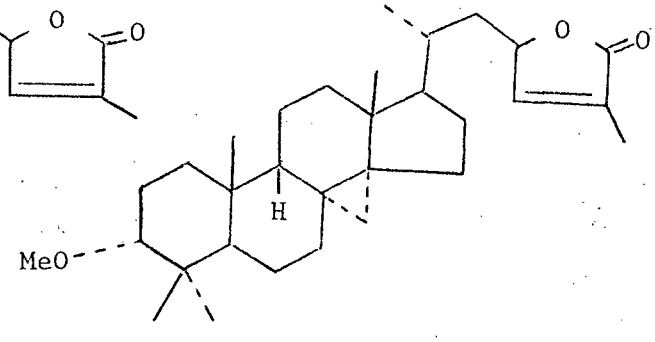

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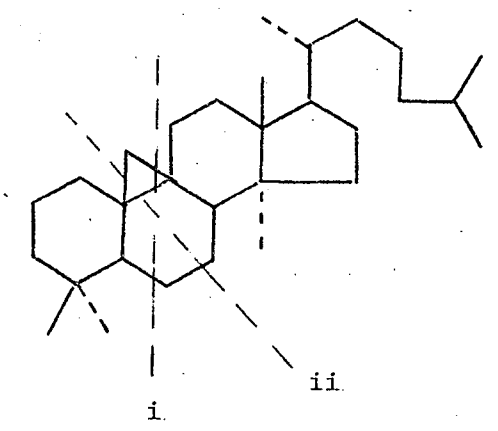
The quantities of cyclograndisolide were so small that further chemical investigations were not possible at this time. It was therefore decided that an extensive mass spectrometric study may reveal further evidence in support of 38 or 39. For this purpose the known cyclopropane system present in the cycloartenol family was first investigated. The mass spectrum of cycloartenol and some of its derivatives have been determined by several workers.[43,44] The presence of the 9,19 cyclopropane ring is manifested in the mass spectra by the appearance of an ion peak having an even mass number. The position of the peak is unaffected by the substitution pattern at C(4) or by the oxygen function at C(3). It is, however, shifted by varying the substitution in the side chain. Two proposals for the origin of this fragment have been advanced. One proposal envisages loss of ring A including the C(19) carbon (path i).[43] The other envisages loss of ring A with inclusion of C(6) but not with the C(19) carbon (path ii).[44] Without proper labelling studies these two paths cannot be distinguished. Regardless of the path followed, the resulting ion fragment for cycloartenol or cycloartenyl acetate has a value of m/e 286.



In order to compare our subsequent results under closely identical conditions, the spectrum of cycloartenyl acetate was run on our instrument and it is reproduced in Figure 9. The observed fragmentation pattern agrees well with the published data. A comparison of cycloartenyl acetate and cyclograndisolide (Figure 10) is now in order.

Cycloartenyl acetate (Figures 9 and 9a) exhibits a molecular ion at m/e 468. Loss of methyl from the molecular ion gives a M-15 ion at m/e 453 and a metastable at m/e 438.1. Acetic acid is also lost from the molecular ion to give ion a (m/e 408) and a metastable at m/e 356.2. Ion b at m/e 393 arises by loss of acetic acid from the M-15 ion and by loss of methyl from ion a as evidenced by metastables at m/e 341.1 and m/e 378.8.

Ion a loses $C_3H_7\cdot$ to give ion c at m/e 367 and a metastable at m/e 328.8 and also loses $C_5H_9\cdot$ to give ion d (m/e 339) and a metastable at m/e 281.8.

An ion e at m/e 286 corresponds to loss of ring A from the molecular ion. Although another group of workers reported the observation of a metastable ion from a similar fragmentation of cycloartenol, no metastable transition was observed in this instance. The ion e can also lose methyl to give ion f at m/e 271 with a metastable at m/e 256.9. A metastable ion at m/e 164.8 corresponds to loss of $C_5H_9\cdot$ from ion e to give an ion g at m/e 217. The entire side chain could cleave with a loss of 111 mass units from ion f to give ion h at m/e 175. Ion a could cleave through ring C to give ions h and i at m/e 203 and m/e 205; metastable ions at 101.0 and m/e 102.9 may arise by this process. The intense ion at m/e 69 likely arises, in part, from fragmentation of the side chain.

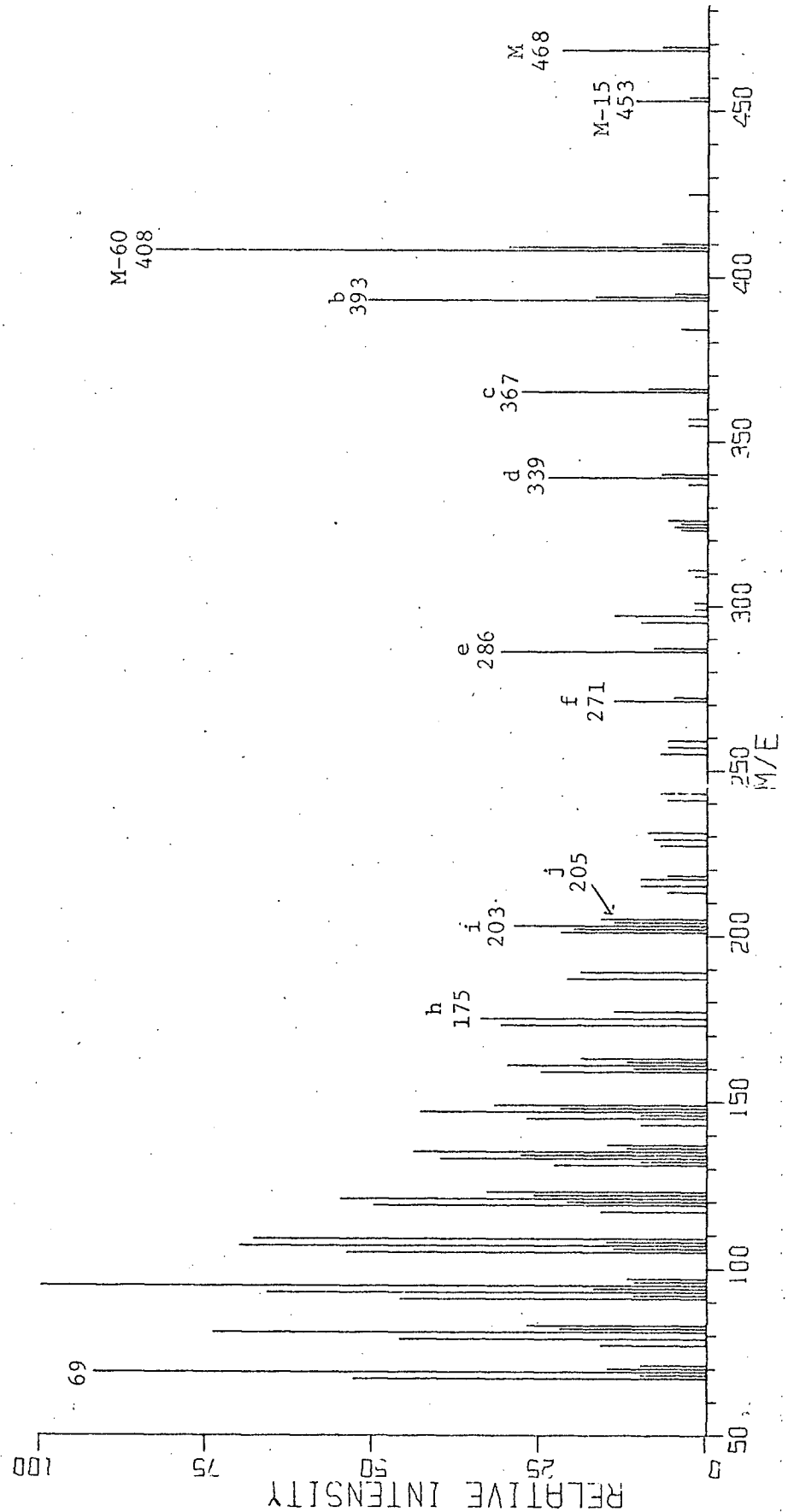


Figure 9. Mass spectrum of cycloartenyl acetate.

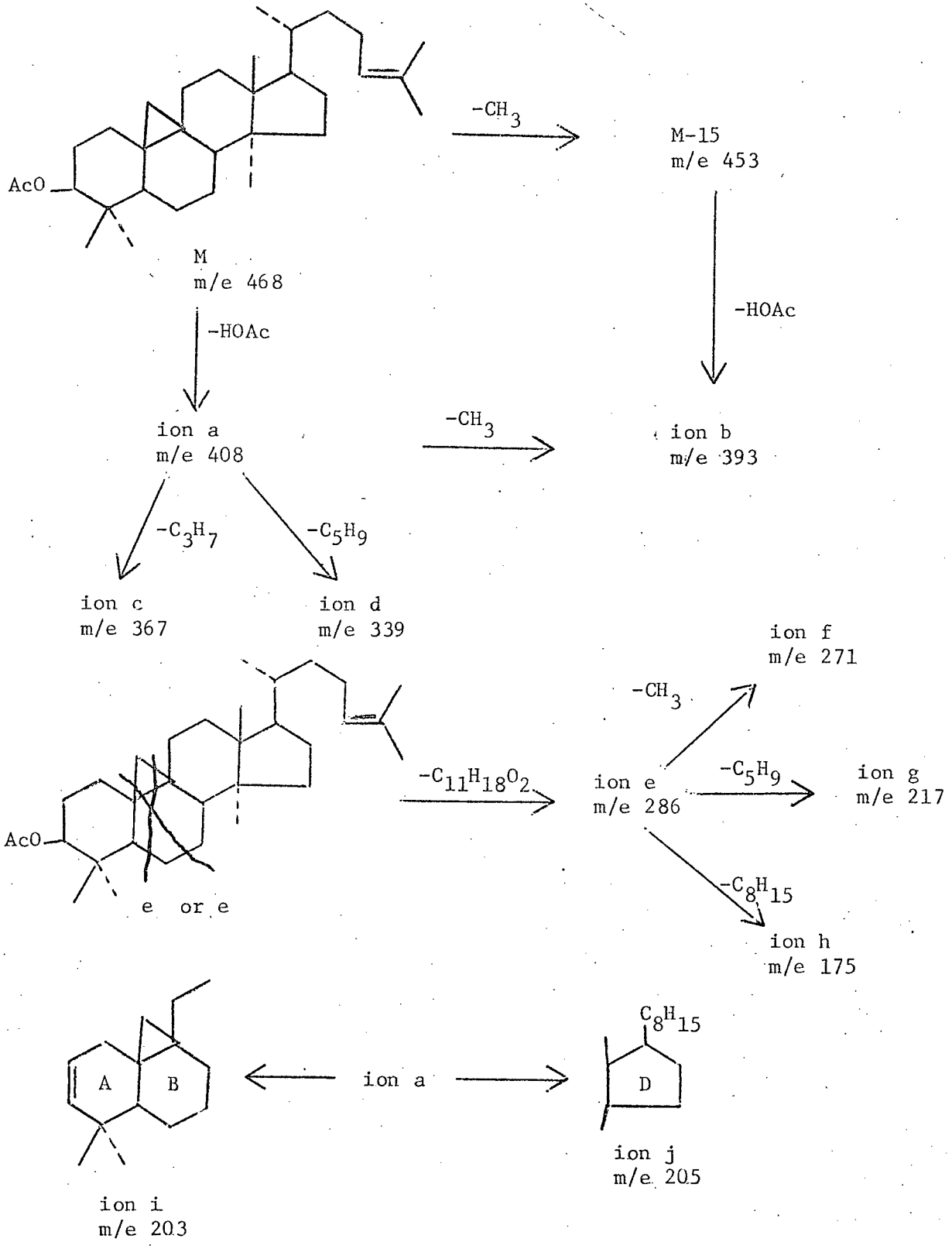


Figure 9a. Mass spectral fragmentation of cycloartenyl acetate.

Ion	Cycloartenyl acetate	Cyclograndisolide	Elemental Composition†
M	468	468	$C_{31}H_{44}O_3$
M-15	453*	453*	$C_{30}H_{45}O_3$
a	408*	436*	$C_{30}H_{44}O_2$
b	393*	421*	$C_{29}H_{41}O_2$
c	365*	393*	$C_{27}H_{37}O_2$
d	339*	367	$C_{25}H_{35}O_2$
e	286	314	$C_{31}H_{30}O_2$
f	271*	299	$C_{20}H_{27}O_2$
h	175	175	
j	205*	233	$C_{15}H_{21}O_2$

* metastable ion observed for process described in text
† obtained from high resolution mass spectrometry on cyclograndisolide

Table VI. Mass spectral comparison of cycloartenyl acetate and cyclograndisolide.

When the difference in functionality at C(3) and in the side chain is taken into account, the mass spectrum of cyclograndisolide (Figure 10) is remarkably similar to cycloartenyl acetate (Figure 9) as can be seen in Table VI. The molecular ion of cyclograndisolide is at m/e 468 ($C_{31}H_{48}O_3$). The M-15 ion for loss of methyl is at m/e 453 with a metastable at m/e 438.1. Loss of methanol gives ion a (m/e 436) of composition $C_{30}H_{44}O_2$. As was seen in cycloartenyl acetate, this ion may lose methyl to give ion b (m/e 421) or lose $C_3H_7\cdot$ to give ion c (m/e 393), both fragmentations being supported by metastable ions at m/e 406.4 and m/e 354.2 respectively. Ion d corresponding to loss of $C_5H_9\cdot$ from ion b is seen at m/e 367 ($C_{25}H_{35}O_2$).

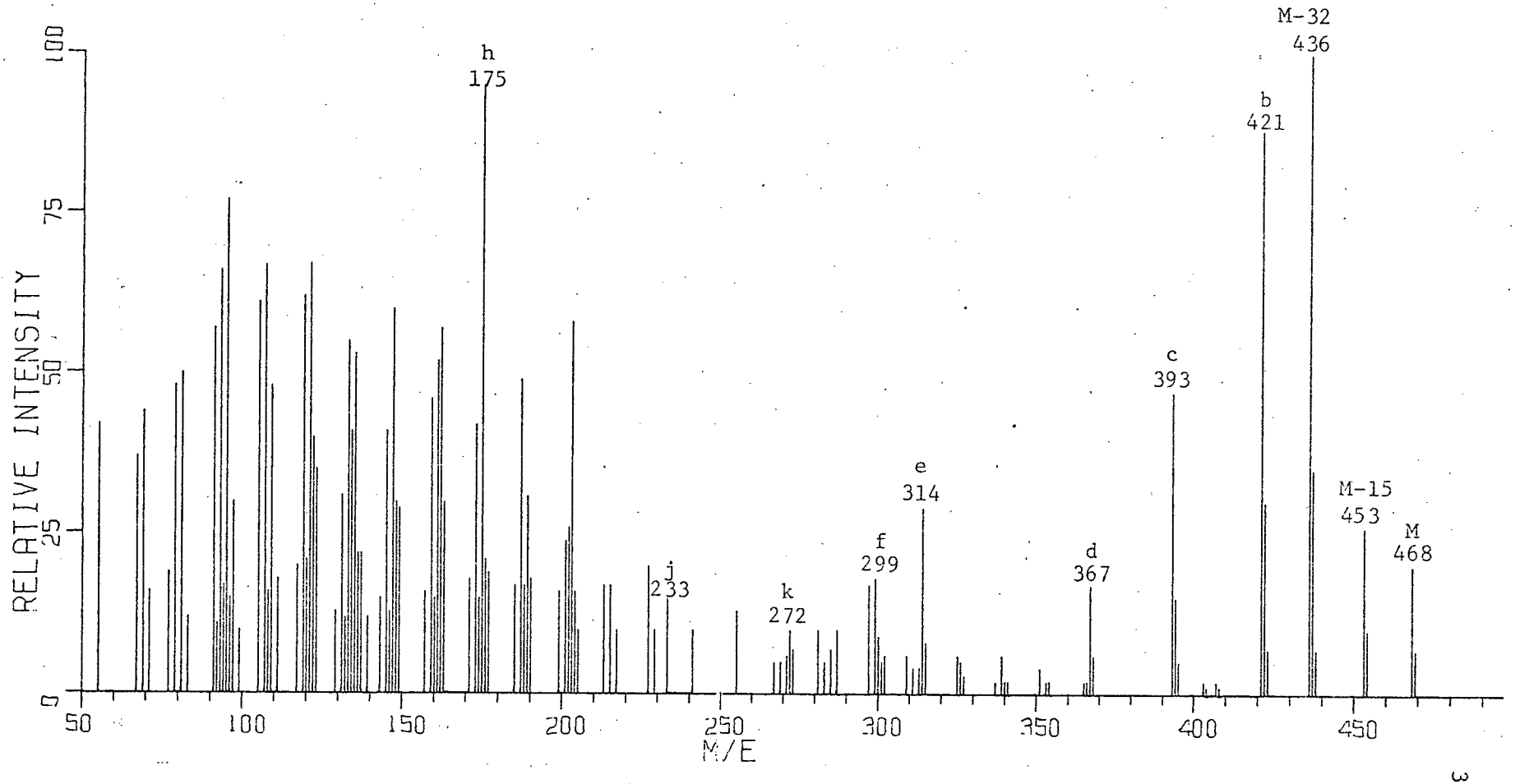
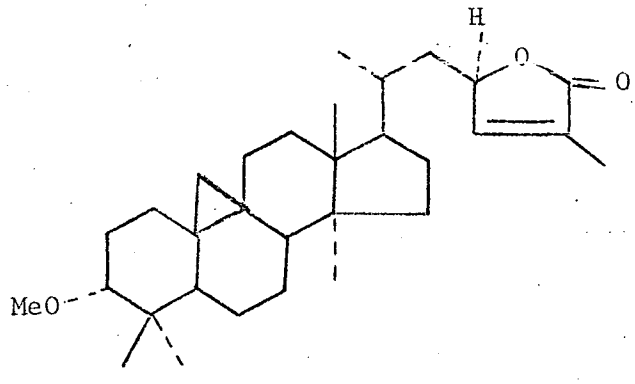


Figure 10. Mass spectrum of cyclograndisolide.

Ion e at m/e 314 ($C_{21}H_{30}O_2$) is observed along with the satellite ions f at m/e 299 ($C_{20}H_{27}O_2$) arising by loss of methyl and ion h at m/e 175 arising by loss of the entire side chain.

A fragment corresponding to ion j at m/e 233 is observed with composition $C_{15}H_{21}O_2$. An even mass ion, k, at m/e 272 with composition $C_{18}H_{24}O_2$ is of unknown origin.

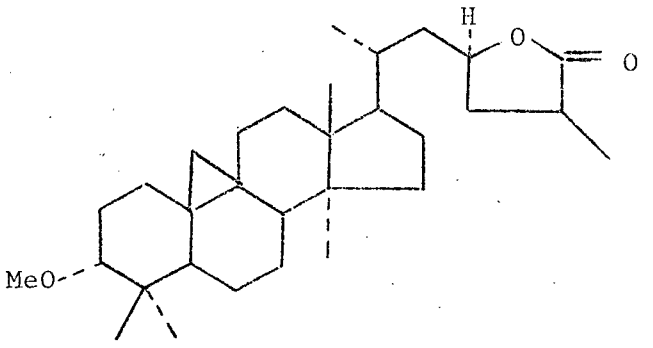
The striking similarities of the mass spectra of cycloartenyl acetate and cyclograndisolide coupled with the other chemical and spectral evidence presents a convincing argument for the presence of a 9,19 cyclopropane system. On the basis of all the evidence presented the structure 38 can be assigned for cyclograndisolide.



38

Since rather heavy reliance on spectral data was necessary in deducing this structure, it was decided to submit a derivative of this molecule for X-ray analysis.

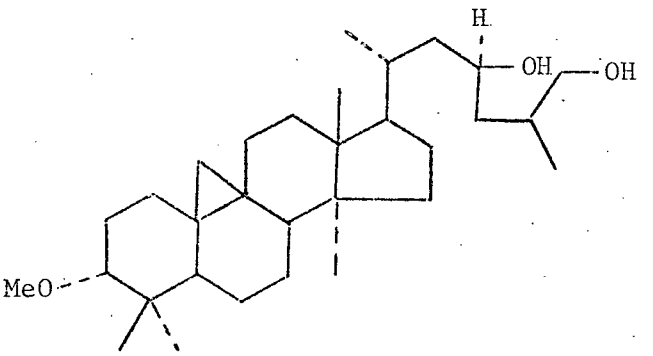
To this end cyclograndisolide was reduced under mild conditions to give dihydrocyclograndisolide (40) whose NMR spectrum still contained the



40

resonances for the cyclopropane protons. The mass spectral fragmentation of dihydrocyclograndisolide was like that of cyclograndisolide with ions containing the lactone ring now being observed at two mass units higher than in the cyclograndisolide.

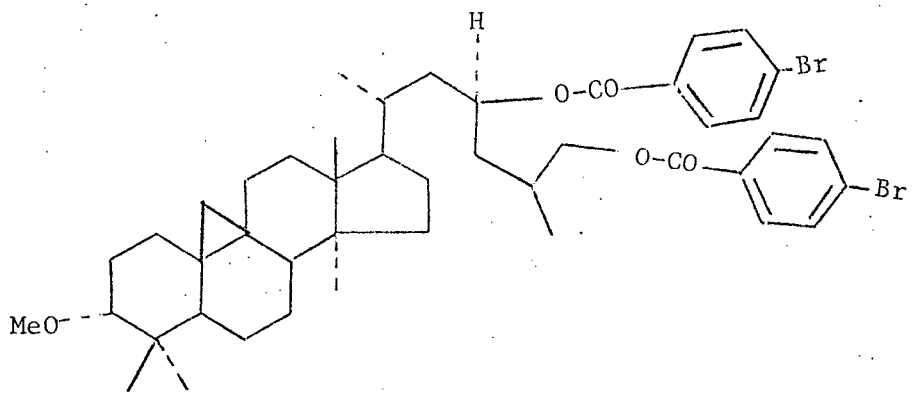
Dihydrocyclograndisolide could be reduced with lithium aluminum hydride to give a diol (41). The NMR spectrum of the latter revealed that



41

the cyclopropane ring was still intact and it was clear that the diol still contained all the asymmetric centers of the original natural product.

Treatment of the diol with p-bromobenzoyl chloride in pyridine gave the bis-p-bromobenzoate (42). The NMR spectrum of this derivative contained the resonances for the cyclopropane ring and for eight aromatic protons.

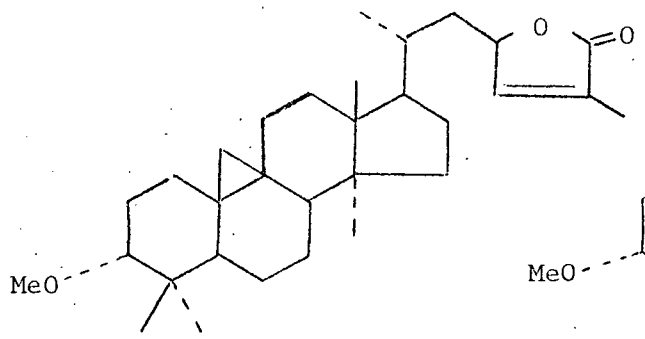


42

In addition, elemental analysis and molecular ions at m/e 838, 840, and 842 confirmed that the bis derivative had been obtained.

F. H. Allen of this department performed an X-ray analysis[45,75] on crystals of the bis-p-bromobenzoate derivative (42) which were orthorhombic, space group $P2_12_12_1$, with $\underline{a}$ = 6.635, $\underline{b}$ = 20.919, $\underline{c}$ = 30.530 Å, and four units of $C_{45}H_{60}O_5Br_2$ per unit cell. The intensities of the 2528 independent reflections with $2\theta \leq 100°$ were measured on a Datex-automated GE XRD6 diffractometer using Ni-filtered CuKα radiation. The structure was solved using Patterson and Fourier techniques and refined by block-diagonal least-squares methods to an $\underline{R}$-factor of 0.096. The absolute configuration was determined by the X-ray fluorescence technique.[76]

The analysis confirms the presence of the 9,19 cyclopropane ring and the configuration at C(23) as shown in structure 42. The absolute stereochemistry depicted for 42 is also correct. Hence, cyclograndisolide has the structure and absolute stereochemistry as shown in structure 38 and is (23R)-3α-methoxy-9,19-cyclo-9β-lanost-24-ene-26,23-lactone.



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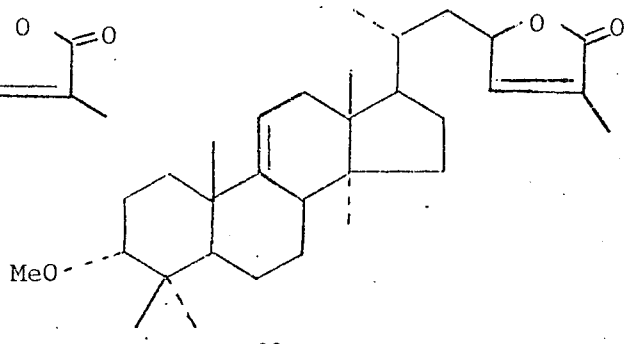


30

With the structure of cyclograndioslide established, grandisolide is (23R)-3α-methoxylanosta-9(11),24-diene-26,23-lactone (30) as suggested earlier.

As mentioned in the early portion of the present discussion, a second component containing a lactonic absorption had been isolated from Fraction M. This material, initially code named AG_2 and subsequently called epi-cyclograndisolide, was purified by preparative layer chromatography. Crystallization from methanol gave a white solid , m.p. 193 - 194°C. Elemental analysis and high resolution mass spectrometry gave a molecular formula of $C_{31}H_{48}O_3$. The IR and UV spectral properties were similar to those of cyclograndisolide and suggested an α,β-unsaturated-γ-lactone.

The NMR spectrum (100 MHz, Figures 11 and 11a) obtained in the manner used for cyclograndisolide exhibited, among other signals, cyclopropane protons at τ 9.50 and 9.68 (J = 4 Hz); the vinylic methyl at τ 8.12 as an apparent triplet (J = 1.7 Hz); the proton at τ 7.20 (J = 1.8 Hz) for an equatorial proton geminal to an O-methyl (τ 6.72); a one proton multiplet at τ 5.10 for a proton geminal to the lactone ring oxygen; and a one proton triplet at τ 2.98 for the olefinic proton of the lactone ring. The differences of note from cyclograndisolide are the downfield shift of the olefinic proton of the lactone, the upfield shift of the proton geminal to

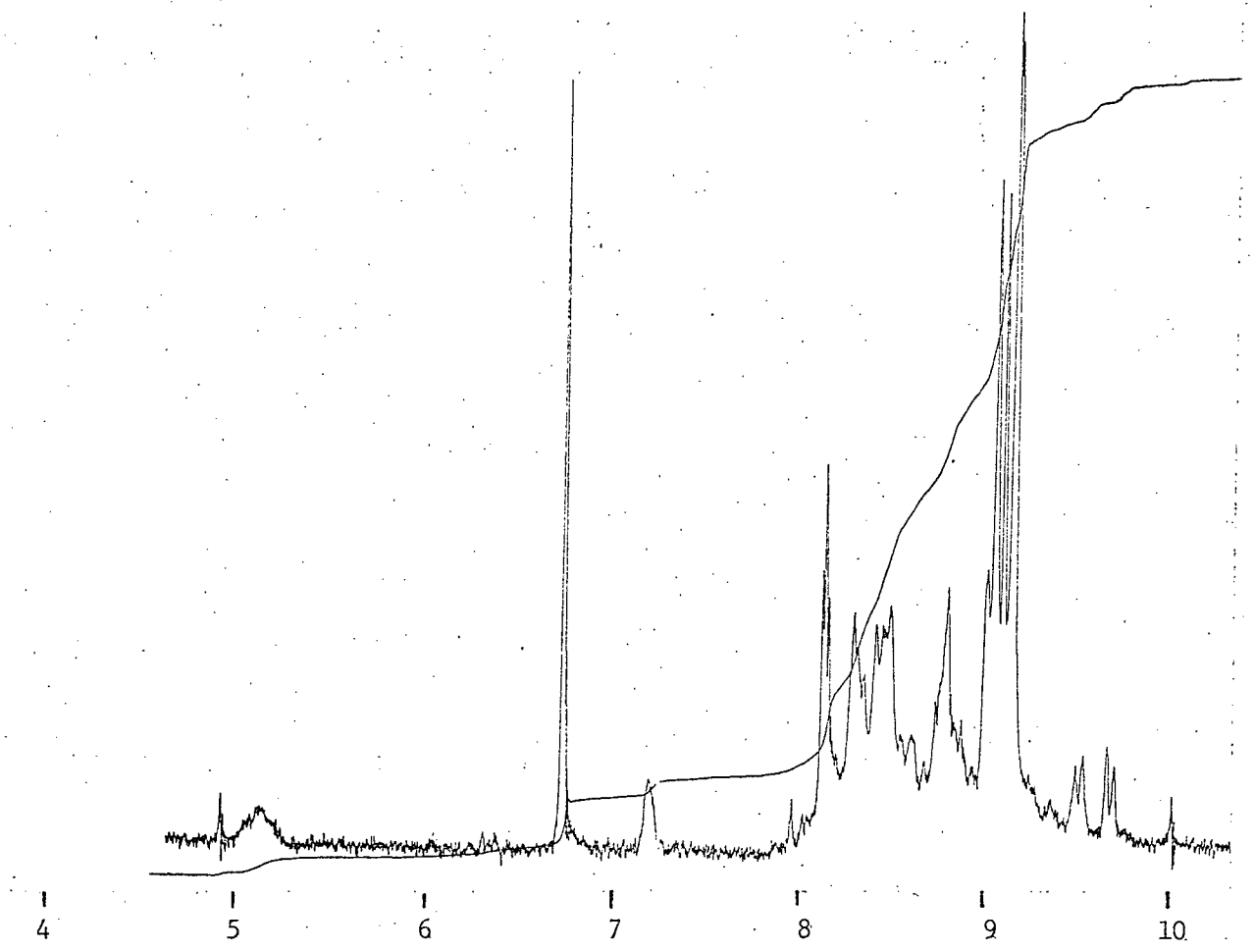


Figure 11. NMR spectrum of epi-cyclograndisolide (τ 4 - 10 region).

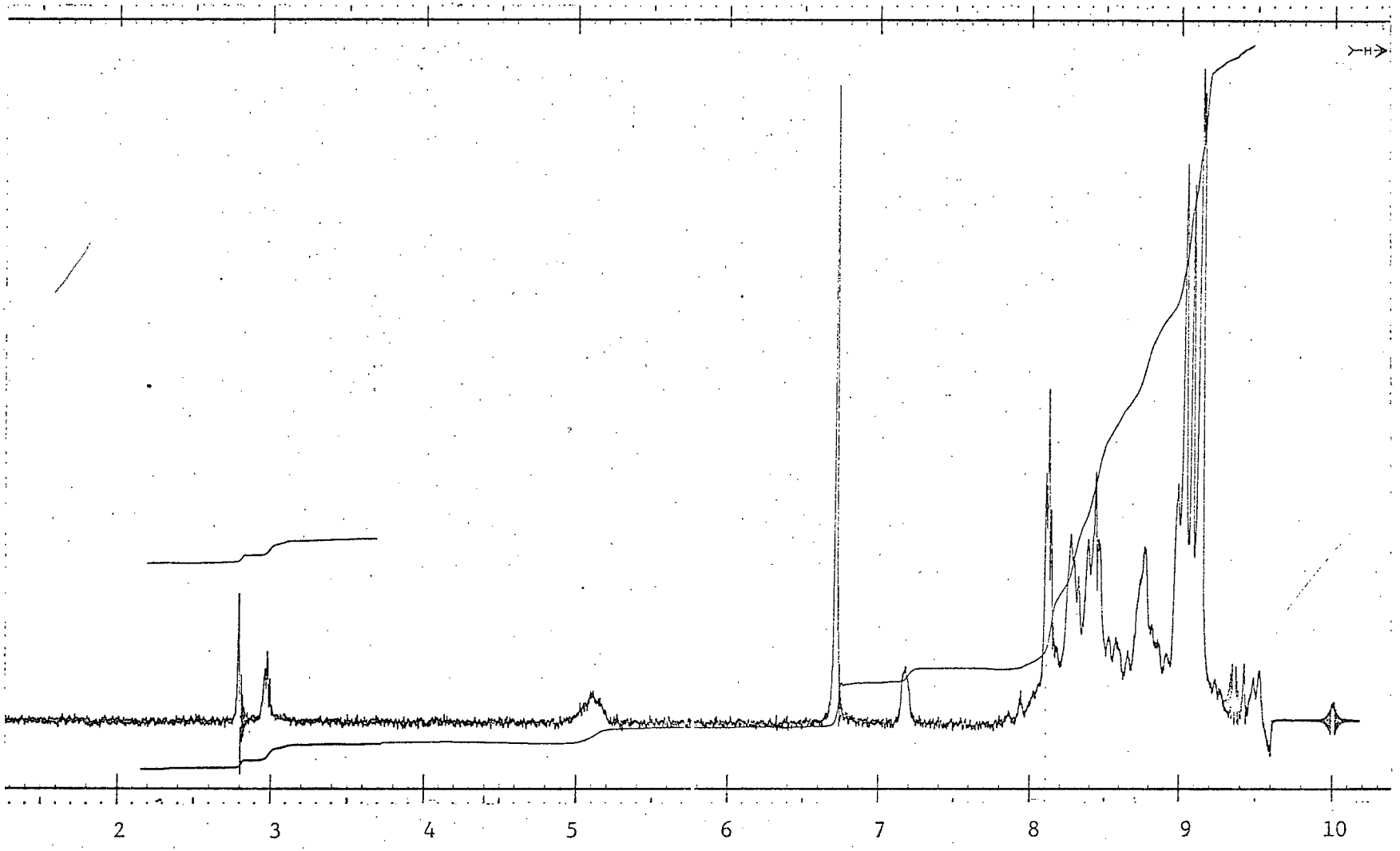


Figure 11a. NMR spectrum of epi-cyclograndisolide (τ 2 - 9 region).

the lactonic oxygen, and a slight upfield shift of the vinylic methyl group. The ORD curve (Figure 12) was of opposite sign to that of cyclograndisolide with a peak at $[\phi]_{225} = 22{,}640°$. The CD curve (Figure 13) had a shoulder ($[\theta]_{250} = 1708°$) with no maximum being observed above 220 mμ ($[\theta] = 39{,}140°$).

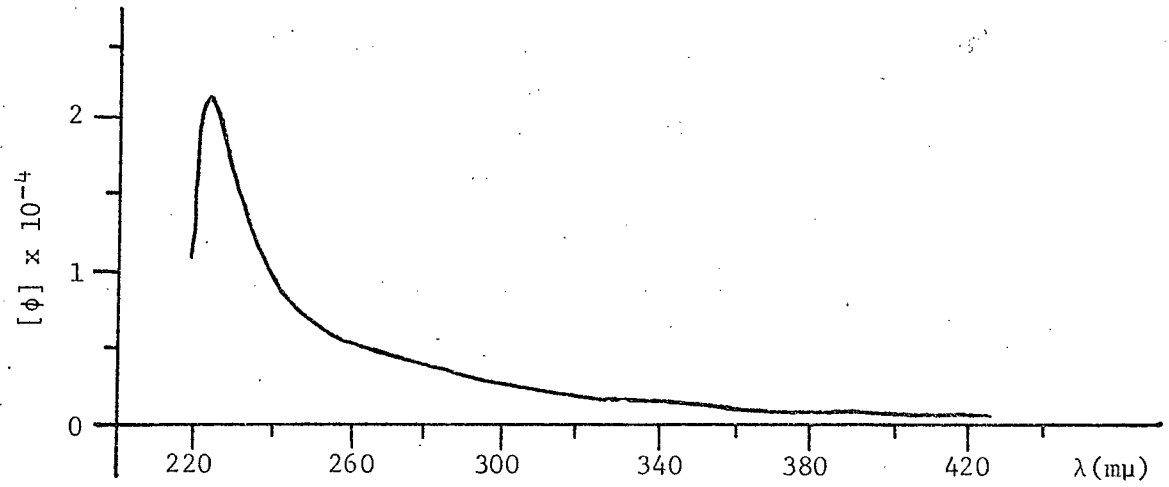


Figure 12. ORD curve of epi-cyclograndisolide.

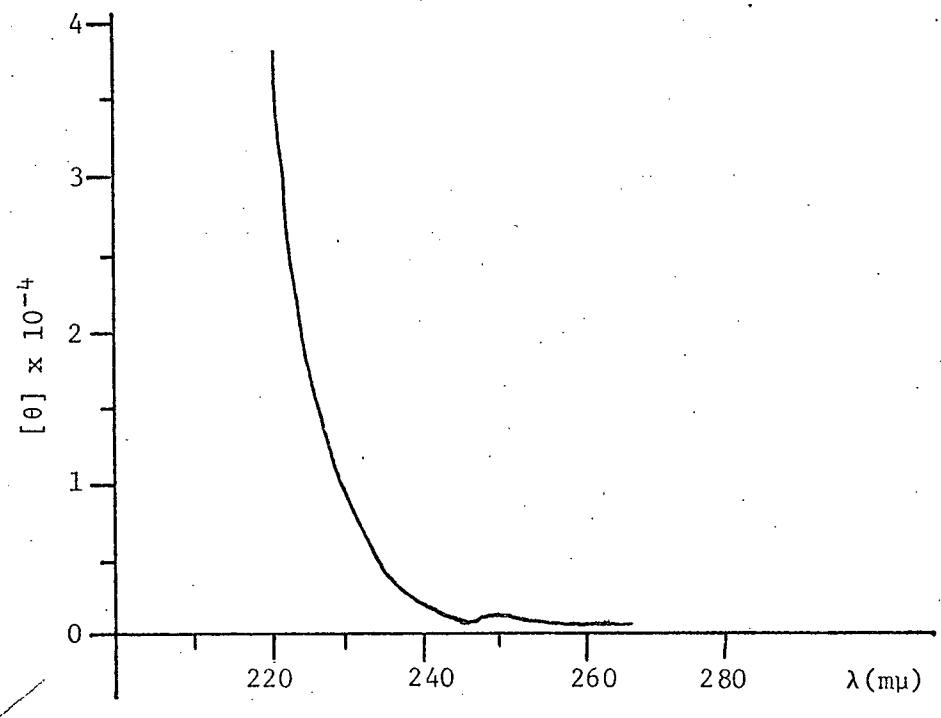
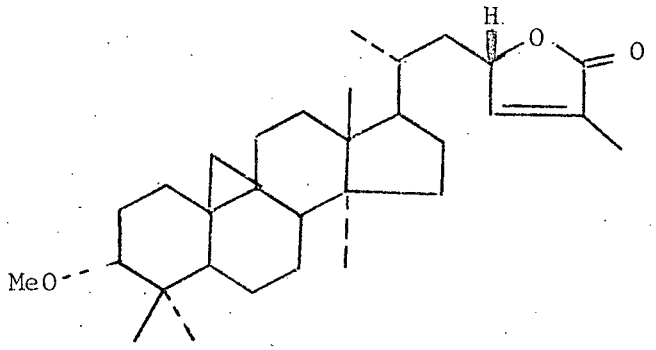


Figure 13. CD curve of epi-cyclograndisolide.

The positive nature of both these measurements suggests that the configuration about the lactone, namely C(23), is opposite to that of cyclograndisolide. In other words, the S configuration is tentatively assigned as shown in structure 43.



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The close similarity of this substance to cyclograndisolide was further illustrated when it was seen that the mass spectrum of epicyclograndisolide (Figure 14) was nearly identical to cyclograndisolide (Figure 10). In fact, the fragmentation pattern (Figure 14a) of both substances is the same. The molecular ion is seen at m/e 468. As in cyclograndisolide the M-15 ion for loss of methyl is at m/e 453 and ion a for loss of methanol is at m/e 436. Ion a may lose methyl to give ion b (m/e 421) or the M-15 ion may lose methanol to give the same ion. Ion a may lose $C_3H_7\cdot$ to give ion c (m/e 393) or $C_5H_9\cdot$ to give ion d (m/e 367). Ion e (m/e 314) corresponds to loss of ring A from the molecular ion. Loss of methyl from ion e results in ion f (m/e 299) and loss of the entire side chain gives ion h (m/e 175). A fragment corresponding to ion j is observed at m/e 233. As in cyclograndisolide ion k (m/e 272) is of unknown origin.

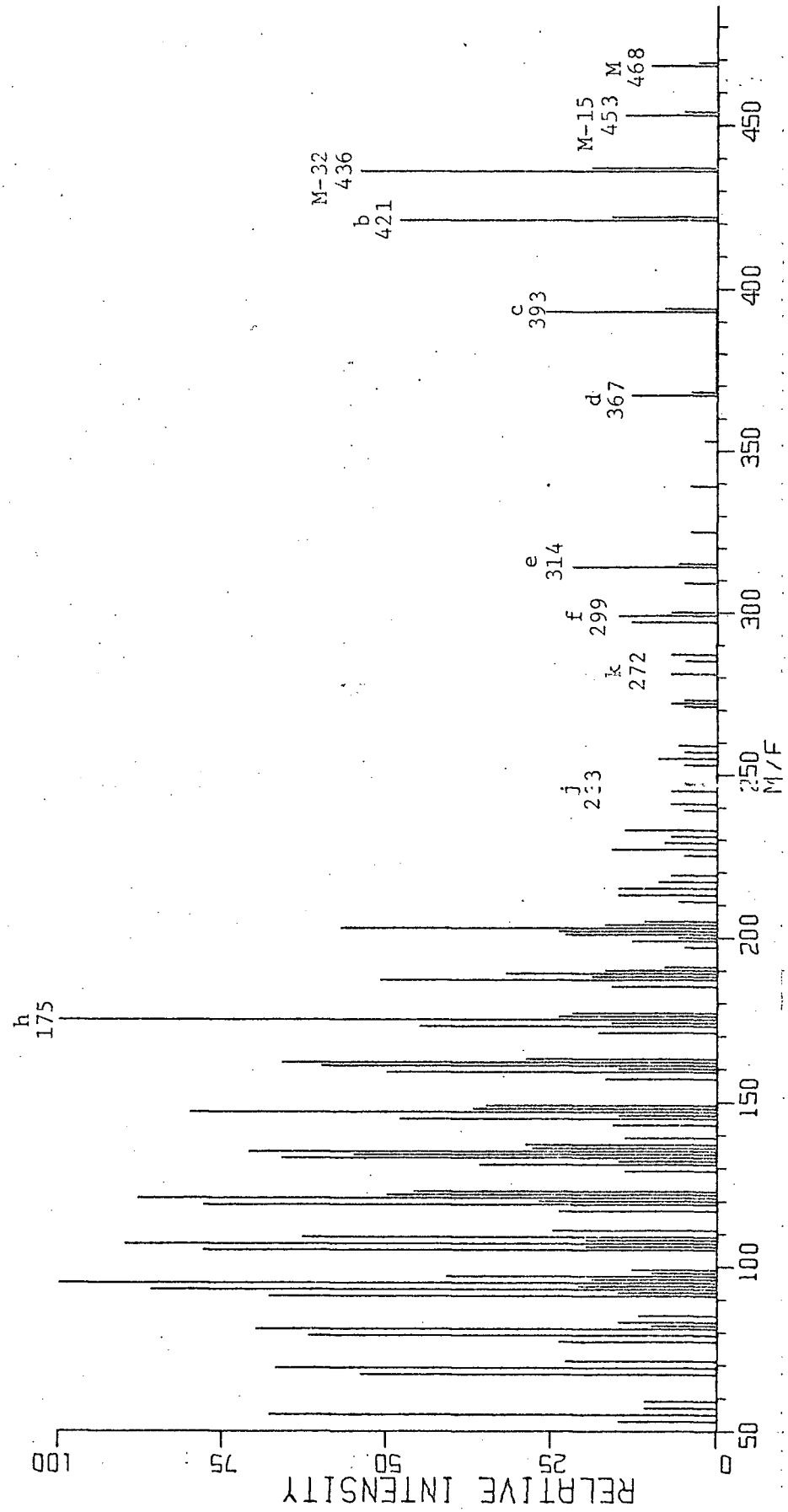


Figure 14. Mass spectrum of epi-cyclograndisolide.

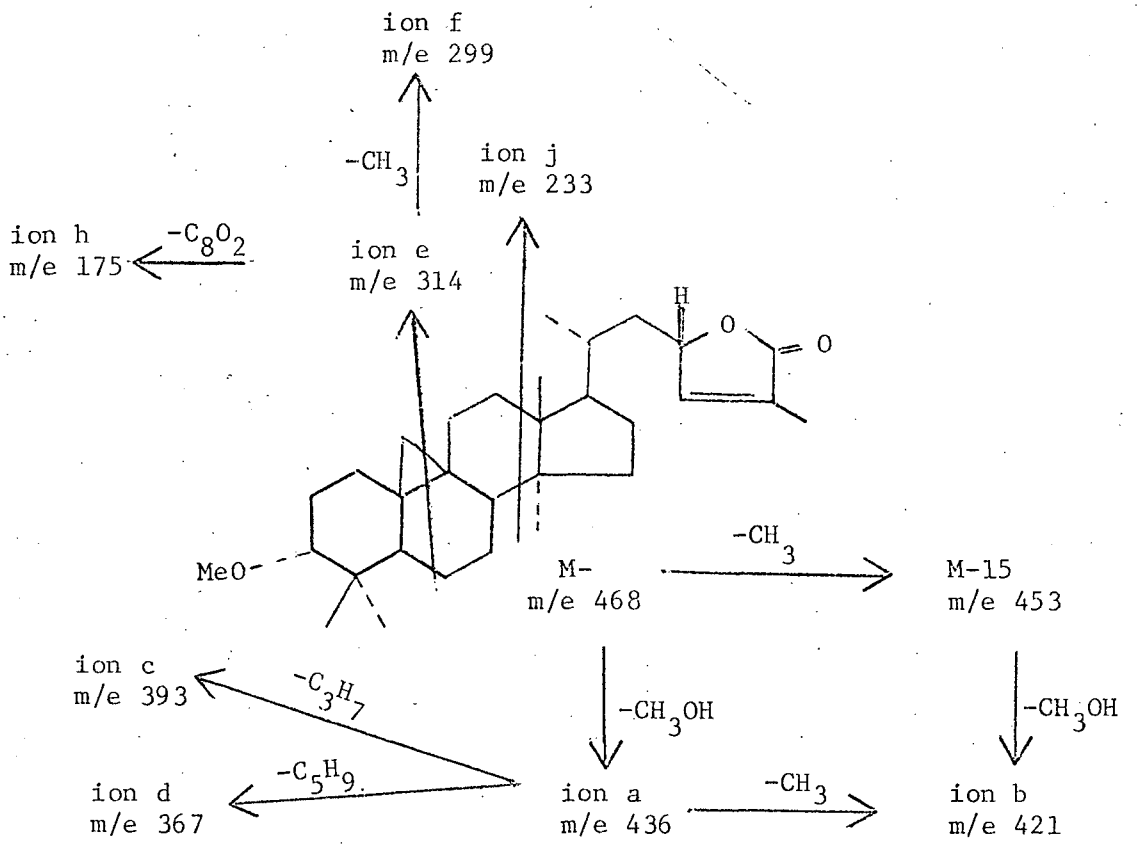
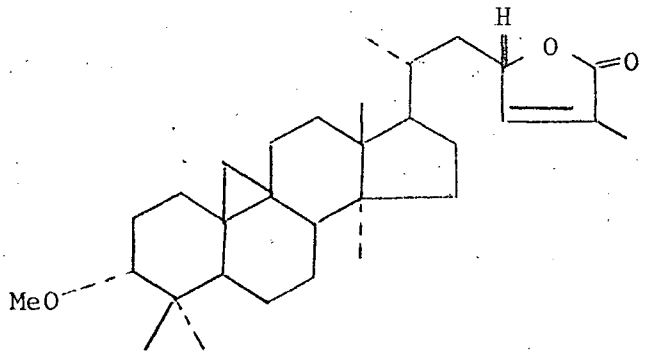


Figure 14a. Fragmentation pattern of epi-cyclograndisolide.

Because of the limited amount of epi-cyclograndisolide available, no chemical work was practical at this time. On the basis of the spectral evidence obtained for epi-cyclograndisolide, the structure suggested is (23S)-3α-methoxy-9,19-cyclo-9β-lanost-24-ene-26,23-lactone (43).



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Experimental

Throughout this work Merck silica gel G with added fluorescent indicator was used as adsorbent in thin layer chromatography (TLC). The chromatograms, 0.3 mm. in thickness, were air dried and activated in an oven at 100°C for three hours. The chromatograms were developed in chloroform and sprayed with antimony pentachloride in carbon tetrachloride (1:2) unless otherwise noted.

For preparative layer chromatography a thicker layer (0.5 mm.) of adsorbent was utilized, with 0.01% Rhodamine 6G added as indicator.[34] Spraying with antimony pentachloride was done only along one edge or not at all as detection of bands was possible with ultraviolet light in most instances.

Column chromatography was performed on either Woelm silica gel or neutral alumina. The preferred adsorbent was deactivated alumina (Activity III) prepared by the addition of water as directed by the manufacturers. Except in large scale work, the solvents were distilled before use.

The nuclear magnetic resonance (NMR) spectra were measured in chloroform or deuterochloroform at room temperature. The NMR spectra were obtained at either 60 MHz using a Jelco C-60, Varian A-60, or a Varian T-60 instrument or at 100 MHz using a Varian HA-100 instrument. The positions of all NMR resonances are given in the Tiers τ scale with tetramethylsilane as internal standard set at 10.0 units. For multiplets the τ values given

represent the center of the signal.

Mass spectra were measured on an Associated Electrical Industries MS 9 high resolution mass spectrometer or, where noted, on an Atlas CH 4 spectrometer. High resolution molecular weight determinations were determined on the MS 9 spectrometer.

Infrared (IR) spectra were measured on Perkin Elmer model 21, 137, or 457 instrument. The samples were usually measured as KBr pellets, however, some were measured in chloroform or neat. The positions of absorption maxima are quoted in wave numbers (cm^{-1}).

Ultraviolet (UV) absorptions were measured in methanol or ethanol on a Cary model 11 or model 15 spectrophotometer.

A Jasco model UV/ORD/CD 5 spectropolarimeter was used to measure the circular dichroism (CD) and optical rotatory dispersion (ORD) curves using methanol or dioxane as solvent.

Melting points were determined on a Kofler block and are uncorrected.

Elemental analyses were performed by Mr. P. Borda, University of British Columbia.

Extraction of grand fir bark[32]

Bark was obtained from a 100 year old grand fir tree growing on the campus of the University of British Columbia. The bark was air dried and ground in a Wiley mill to pass through a 3 mm. sieve. The ground bark was extracted for 24 hours in a large glass Soxhlet extractor. The extract was taken to dryness to provide a crude extract in a yield of 0.8% based on the air dried weight of bark extracted.

Chromatography of crude extract[32]

The crude extract (26.7 gms.) was chromatographed on alumina (450 gms.) into a number of fractions as shown below. The compounds subsequently isolated from each fraction are shown for clarity.

Fraction	Solvent (volume, mls.)	Weight, mgs.	Compounds
A	petroleum ether (200)	690	hydrocarbons
B	petroleum ether (100)	228	hydrocarbons, sterol ester
C	petroleum ether (200)	263	sterol ester
D	petroleum ether (200)	450	sterol and wax ester
E	petroleum ether (500)	994	wax ester, 2 unknowns
F	10% benzene in pet. ether (500) 20% benzene in pet. ether (400)	826	unidentified
G	30% benzene in pet. ether (400) 50% benzene in pet. ether (300)	1444	fatty alcohol, epimanool, lactones
H	60% benzene in pet. ether (400)	909	fatty alcohol, β-sitosterol
I	60% benzene in pet. ether (300)	1016	β-sitosterol, ferulic ester
J	benzene (400)	1925	β-sitosterol, ferulic ester, 2 unknowns
K	ether (500) methanol (500)	5004	ferulic ester

Total recovery 13.75 gms.*

* resin and fatty acids present in crude extract were irreversibly adsorbed on the alumina

Chromatography of Fraction G[32]

Fraction G (2.77 gms.) was chromatographed on alumina (200 gms.). Elution with 20% benzene in petroleum ether (400 mls.) gave Fraction M (770 mgs.) subsequently shown to contain epimanool, fatty alcohol, and lactones. Further elution with 50% benzene in petroleum ether (400 mls.) gave Fraction N (860 mgs.) subsequently shown to contain lactones and fatty alcohol. Finally elution with benzene (600 mls.) gave Fraction O (950 mgs.) containing fatty alcohol and lactones.

Chromatography of Fraction M

Fraction M (500 mgs.) was chromatographed on alumina (50 gms.). Elution with 20% benzene in petroleum ether (250 mls.) gave Fraction P (80 mgs.). Further elution with 40% benzene in petroleum ether (500 mls.) gave Fraction Q (400 mgs.).

Fraction P

Fraction P was a pale yellow oil. IR (neat) 3300 (OH), 3065 (vinyl), 1635 (C=C), 1410, 990, 915 (vinyl), 878 (terminal methylene), 1383 and 1365 (geminal dimethyl). NMR (60 MHz) 4.15 (1H, quartet, J = 17.5 Hz, J = 10.5 Hz), 4.88 (1H, quartet, J = 17.5 Hz, J = 1.5 Hz), 5.05 (1H, quartet, J = 10.5 Hz, J = 1.5 Hz), 5.26 and 5.55 (2H, multiplets, exocyclic methylene), 8.79 (CH_3-C-OH), and 9.17, 9.23, and 9.36 (angular methyl).

Fraction P (80 mgs.) was treated with freshly prepared 3,5-dinitrobenzoyl chloride (80 mgs.) in pyridine (2 mls.) for three days at room temperature. The pyridine was removed *in vacuo*. The residue was dissolved in methylene chloride, washed with water, dried over sodium sulfate, and evaporated. Crystallization of this residue from methylene chloride -

methanol gave needles m.p. 116 - 118°C, mixed m.p. with authentic epimanoyl-3,5-dinitrobenzoate 116 - 118°C.

Fraction Q

Fraction Q (400 mgs.) was obtained from Fraction M as a white low melting waxy solid. TLC showed the presence of at least three compounds (R_f 0.35, 0.25, 0.17). NMR (60 MHz) had resonances at 3.0, 5.0, 6.7, and 8.1 similar to abieslactone[28] in addition to broadened singlet at 8.7 assigned to methylene protons of a long chain fatty alcohol.

Removal of fatty alcohol from Fraction Q

A methanolic solution (4 mls.) of Fraction Q (200 mgs.) was heated to reflux and urea (2 gms.) was added followed by 1 ml. of benzene. The solution was allowed to cool slowly and was left standing for 2 days before filtering off the crystalline urea complex. The complex was washed twice with chloroform (2 mls.) and the combined filtrate was taken to dryness. The filtrate residue was partitioned between water and methylene chloride and the organic layer separated, washed with water, and dried over sodium sulfate. Evaporation of the solvent gave a white solid (85 mgs.) which contained by TLC two compounds, AG_1 and AG_2 (R_f 0.35 and 0.25).

The urea complex was dissolved in water and extraction with methylene chloride gave, upon drying and evaporation, the fatty alcohol as a low melting wax (R_f 0.17).

Purification of Fraction N

Fraction N (500 mgs.) was dissolved in hot methanol and left to cool for four hours after which time a white solid (120 mgs.) was removed by filtration. This solid was found to be fatty alcohol and not further

examined. The filtrate was concentrated to 4 mls. and urea (2 gms.) added and the solution was warmed to reflux to dissolve the urea; benzene (1 ml.) was added and the solution left to crystallize for 2 days. Removal of the urea complex by filtration and evaporation of the filtrate gave a residue which was partitioned between water and methylene chloride. The organic layer was washed with water, dried over sodium sulfate, and evaporated to give a residue of AG_1 and AG_2 (250 mgs.).

Purification of Fraction O

Fraction O (950 mgs.) was dissolved in hot methanol and left to cool for four hours after which time fatty alcohol (505 mgs.) was removed by filtration. The filtrate was concentrated to 4 mls. and urea (2 gms.) was added and dissolved at reflux, benzene (1 ml.) was added. After 2 days the urea complex was removed by filtration and the filtrate evaporated to give a residue which was partitioned between water and methylene chloride. The methylene chloride layer was washed with water, dried (sodium sulfate), and evaporated to give a residue of AG_1 and AG_2 (185 mgs.).

Preparative layer chromatography of AG_1 and AG_2

The mixture containing AG_1 and AG_2 (85 mgs.) was dissolved in chloroform (0.3 mls.) and applied to a preparative layer chromatogram (20x60 cm.). The plate was developed in chloroform and three bands were visible when the chromatogram was examined under UV light. The bands were scraped off the plate and extracted with chloroform. The top band (R_f 0.67) was of small amount (2 mgs.) and was not examined. The second band (R_f 0.40) was the major band (35 mgs.) and was one compound (AG_1) when examined by TLC. The third band (R_f 0.25) overlapped the second band and contained both

AG_1 and AG_2 (22 mgs.). Re-chromatography on preparative layer chromatograms as before gave pure AG_1 (3 mgs.) and pure AG_2 (15 mgs.).

AG_1 or cyclograndisolide (38)

The AG_1 from the preparative layer chromatography was flushed through a short column of alumina with benzene to remove most of the orange color which came from the Rhodamine 6G dye. Crystallization from methanol gave a white solid m.p. 191 - 193°C. ORD (c, 0.0368 in dioxane) $[\phi]_{300}$ - 890°, $[\phi]_{250}$ - 4,670°, $[\phi]_{225}$ - 26,700°, $[\phi]_{220}$ - 18,000°. CD (c, 0.0368 in dioxane) $[\theta]_{270}$ + 120°, $[\theta]_{250}$ + 370°, $[\theta]_{235}$ 0°, $[\theta]_{225}$ - 12,600°, $[\theta]_{215}$ - 44,000°. IR (KBr) 1745 (lactone carbonyl), 1665 (C=C). UV λ_{max}^{MeOH} 209 mμ (log ε 4.33). NMR (100 MHz) in $CDCl_3$, TMS lock 3.02 (1H, apparent triplet, J = 1.7 Hz, H-C=C-C=O), 5.05 (1H, multiplet, H-C-O), 6.72 (3H, singlet, OMe), 7.20 (1H, triplet, J = 1.8 Hz, equitorial H-C-OMe), 8.10 (3H, apparent triplet, J = 1.7 Hz, vinylic methyl), 8.98, 9.02, 9.07, 9.14 (5 C-methyls); in $CHCl_3$,$CHCl_3$ lock 3.02 unobservable, 5.05 - 9.14 region as before, 9.50 and 9.68 (2H, pair of doublets, J = 4 Hz, cyclopropane protons). Mass spectrum m/e 468 (M), 453 (M-15), 436 (M-32), 421 (M-47), and 314. (Found: C, 79.43; H, 10.17; $C_{31}H_{48}O_3$ requires C, 79.44; H, 10.32%); high resolution:

468.365 $C_{31}H_{48}O_3$ requires 468.360, 453.333 $C_{30}H_{45}O_3$ requires 453.336, 436.334 $C_{30}H_{44}O_2$ requires 436.334, 421.311 $C_{29}H_{41}O_2$ requires 421.311, 393.278 $C_{27}H_{37}O_2$ requires 393.279, 367.265 $C_{25}H_{35}O_2$ requires 367.264, 339.229 $C_{23}H_{31}O_2$ requires 339.232, 314.227 $C_{21}H_{30}O_2$ requires 314.224, 299.204 $C_{20}H_{27}O_2$ requires 299.201, 272.179 $C_{18}H_{24}O_2$ requires 272.178, 233.154 $C_{15}H_{21}O_2$ requires 233.154.

AG_2 or epi-cyclograndisolide (43)

The AG_2 from preparative layer chromatography was flushed through a short column of alumina with benzene to remove most of the orange color which came from the Rhodamine 6G dye. Crystallization from methanol gave a white solid m.p. 193 - 194°C. ORD (c, 0.0434 in dioxane) $[\phi]_{450}$ + 53°, $[\phi]_{300}$ + 2,370°, $[\phi]_{250}$ + 6,420°, $[\phi]_{225}$ + 22,640°, $[\phi]_{220}$ + 10,780°. CD (c, 0.0434 in dioxane) $[\theta]_{270}$ + 462°, $[\theta]_{260}$ + 1030°, $[\theta]_{255}$ + 1424°, $[\theta]_{250}$ + 1700°, $[\theta]_{245}$ + 1850°, $[\theta]_{215}$ + 39,140°. IR (KBr) 1740 (carbonyl), 1660 (C=C). UV λ_{max}^{MeOH} 210 mμ (log ε 4.15). NMR (100 MHz) in $CDCl_3$ TMS lock 2.98 (1H, apparent triplet, J = 1.7 Hz), 5.10 (1H, multiplet, H-C-O), 6.72 (3H, singlet, O-Me), 7.20 (1H, triplet, J = 1.7 Hz, equitorial, H-C-OMe), 8.12 (3H, triplet, J = 1.7 Hz, vinylic methyl), 8.98, 9.02, 9.07, 9.14 (5 C-methyls); in $CHCl_3$ with $CHCl_3$ lock 2.98 resonance unobservable, 5.10 - 9.14 region as before, 9.50 and 9.68 (2H, pair of doublets, J = 4 Hz, cyclopropane protons). Mass spectrum m/e 468 (M), 453 (M-15), 436 (M-32), 421 (M-47) and 314. (Found C, 79.32; H, 10.20; $C_{31}H_{48}O_3$ requires C, 79.44; H, 10.32%; high resolution 468.363 $C_{31}H_{45}O_3$ requires 468.360).

Grandisolide (30)

Cyclograndisolide (33 mgs.) was dissolved in dry chloroform (5 mls.) and hydrogen chloride, dried by passing throught concentrated sulphuric acid, was bubbled through for 2 hours. Evaporation of the chloroform gave grandisolide which was one spot on TLC. Crystallization from acetone gave a white solid m.p. 212 - 214°C. IR (KBr) 1740 (lactone carbonyl), 1665 (C=C). NMR ($CDCl_3$, 100 MHz) 3.02 (1H, apparent triplet,

J = 1.7 Hz, H-C=C-C=O), 4.80 (1H, multiplet, H-C=C), 5.05 (1H, multiplet, H-C-O), 6.72 (3H, singlet, OMe), 7.20 (1H, triplet, J = 1.7 Hz, equitorial, H-C-OMe), 8.10 (3H, triplet, J = 1.7 Hz, vinylic methyl), 8.95, 9.02, 9.12, 9.29, 9.36 (6 C-methyls). Mass spectrum (m/e) 468, 453, 436, 421. (Found C, 79.34; H, 10.36; $C_{31}H_{48}O_3$ requires C, 79.44; H, 10.32%).

Acid catalyzed isomerization of abieslactone

Abieslactone (35 mgs.) was dissolved in dry chloroform (5 mls.) and dry hydrogen chloride was bubbled through the solution for 2 hours. Evaporation of the chloroform gave a white solid which was one spot on TLC, m.p. 195 - 207°C. NMR (100 MHz) 3.02 (1H, apparent triplet, J = 1.7 Hz, H-C=C-C=O), 4.80 (non-integral, multiplet, H-C=C), 5.05 (1H, multiplet, H-C-O), 6.72 (3H, singlet, OMe), 7.20 (1H, triplet, J = 1.7 Hz), 8.10 (3H, triplet, J = 1.7 Hz, vinylic methyl), 8.94, 9.02, 9.07, 9.12, 9.14, 9.30, and 9.34 (18H, C-methyls). Repeated crystallizations from acetone gave m.p. 212 - 214°C, mixed m.p. with grandisolide 212 - 214°C. IR (KBr) 1740 (lactone carbonyl) 1665 (C=C); superimposable with IR of grandisolide.

Parkeyl acetate (36)

A sample of parkeyl acetate was received from Professors D.H.R. Barton (Imperial College, London) and G. Ourisson (C.N.R.S., Strasbourg). NMR (100 MHz) 4.80 - 5.00 (2H, multiplets, olefinic protons), 8.94, 9.14, 9.28, 9.38 (6 C-methyls).

Dihydroparkeyl acetate (34)

Parkeyl acetate (15 mgs.) was dissolved in tetrahydrofuran and hydrogenated for 2 hours over 10% palladium on charcoal (20 mgs.). The catalyst was removed by filtration and the filtrate evaporated and

crystallized from ethyl acetate m.p. 173 - 174°C (literature value 171 - 172°C[49]). NMR (100 MHz) 4.81 (1H, multiplet, H-C=C), 8.95, 9.13, 9.25, 9.36 (8 C-methyls). Mass spectrum (m/e) 470 (M), 455 (M-15), 410 (M-60), 395 (M-75). (Found C, 81.59; H, 11.53; $C_{32}H_{54}O_2$ requires C, 81.64; H, 11.56%).

Cycloartenyl acetate

An authentic sample of cycloartenyl acetate was received from Dr. Rowe (Forest Products Laboratory, Madison) m.p. 117 - 119°C (literature value 121 - 122°C[47]). NMR (100 MHz) in $CHCl_3$, $CHCl_3$ lock 4.97 (1H, multiplet, H-C=C), 5.51 (1H, quartet, J = 5 Hz and J = 10 Hz, H-C-OAc), 8.02 (3H, singlet, $OOCCH_3$), 9.05, 9.15, 9.19 (5 C-methyls), 9.47 and 9.70 (2H, pair of doublets, J = 4 Hz, cyclopropane protons). Mass spectrum (m/e) 468 (M), 453 (M-15), 408 (M-60), 393 (M-65) and 286.

Dihydrocyclograndisolide (40)

Cyclograndisolide (50 mgs.) in tetrahydrofuran (10 mls.) was hydrogenated over 10% palladium on charcoal (50 mgs.) at room temperature for 2 hours. The catalyst was removed by filtration and the filtrate evaporated and crystallized from ethyl acetate m.p. 198 - 199°C. IR (KBr) 1770 (lactone carbonyl). NMR (60 MHz) 5.50 (1H, multiplet, H-C-O), 9.50 and 9.68 (2H, pair of doublets, J = 4 Hz). Mass spectrum (m/e) 470 (M), 455 (M-15), 438 (M-32), 423 (M-47), and 316. (Found C, 79.00; H, 10.81; $C_{31}H_{50}O_3$ requires C, 79.10; H, 10.71%).

Dihydrocyclograndisolide diol (41)

Dihydrocyclograndisolide (30 mgs.) in tetrahydrofuran (10 mls.) was stirred with lithium aluminum hydride (10 mgs.) for 18 hours. A small amount of water was added and the solvent was evaporated. The residue

was carefully acidified with dilute hydrochloric acid and extracted with ether. The extract was washed with water, dried (sodium sulfate), and evaporated to give a white residue. Column chromatography on alumina (1 gm.) of the residue and elution with ether gave the diol, crystallization from petroleum ether - ether gave a white solid m.p. 133 - 134°C. IR (KBr) 3540, 3350 (OH). NMR (60 MHz) 6.2 - 6.6 (3H, overlapping multiplets, H-C-OH, CH_2-OH) 8.97, 9.03, 9.06, 9.10 (6 C-methyls) 9.50 and 9.68 (2H, pair of doublets, J = 4 Hz, cyclopropane protons). Mass spectrum (m/e) 474 (M), 459 (M-15), 456 (M-18), 442 (M-32), 438 (M-36). (Found C, 78.58; H, 11.35; $C_{31}H_{54}O_3$ requires C, 78.43; H, 11.46%).

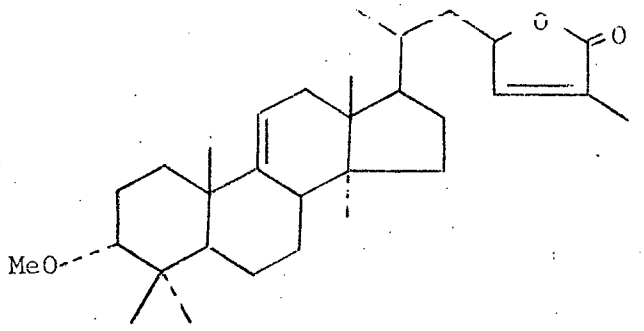
Bis-p-bromobenzoate of dihydrocyclograndisolide diol

Dihydrocyclograndisolide diol (41) (10 mgs.) and freshly crystallized p-bromobenzoyl chloride (15 mgs.) were dissolved in dry pyridine and left at room temperature for 2 days. The pyridine was removed in vacuo and the residue dissolved in methylene chloride and washed with water, 5% aqueous sodium bicarbonate solution, water and dried over sodium sulfate. Evaporation of the solvent and crystallization from petroleum ether the bis-p-bromobenzoate (42) as a white solid m.p. 154 - 156°C. IR (KBr) 1720 (ester carbonyl). NMR (60 MHz) 1.9 - 2.4 (8H, aromatic protons), 9.50 and 9.68 (2H, pair of doublets, J = 4 Hz, cyclopropane protons). Mass spectrum (m/e) 838, 840, 842 (M). (Found C, 64.10; H, 7.32; $C_{45}H_{60}O_5Br_2$ requires C, 64.29; H, 7.17%).

Investigations concerning the structure of abieslactone

Discussion

Abieslactone is a triterpene occurring in the bark and leaves of Abies mariesii Masters, a fir tree of northern Japan, and also in the North American Pacific silver fir [A. amabilis (Dougl.) Forbes] and Noble fir [A. procera Rehd.]. The arguments presented by the original investigators[28] for the structure of abieslactone as 30 are summarized in this section. For purposes of clarity in the present discussion, the published structure for abieslactone and its derivatives will be utilized initially while alternative structures are postulated subsequently wherever possible.



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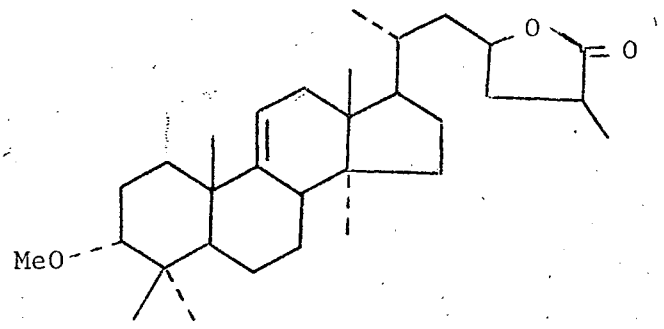
The molecular formula for abieslactone (30) established by elemental analysis and mass spectrometry was $C_{31}H_{48}O_3$. Of the three oxygen atom present, one could be assigned to a methyl ether group from a Zeizel determination and a three proton singlet at τ 6.73 in the NMR spectrum. The presence of an α,β-unsaturated-γ-lactone was indicated by the IR absorption at 1745 and 1660 cm^{-1} and the UV absorption maximum at

207.5 mμ (log ε 4.30 in EtOH).

The NMR spectrum exhibited signals attributed to six C-methyl groups (τ 8.98 - 9.08), a vinylic methyl as a triplet at τ 8.10, a methyl ether at τ 6.73, a narrow diffused triplet at τ 7.20, two one proton multiplets at τ 5.05 and τ 4.48, and a one proton quintet at τ 3.00. Spin decoupling showed that the vinylic methyl was coupled to both the olefinic proton at τ 3.00 and the multiplet at τ 5.05. The multiplet at τ 4.48 was coupled with protons in the methylene envelope (τ 7.61 and 7.81). The narrow diffused triplet was assigned as an equatorial proton geminal to the methyl ether.

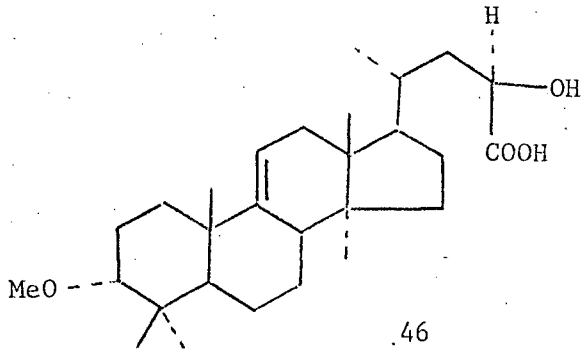
Mild hydrogenation of abieslactone in tetrahydrofuran with Pd/C catalyst afforded dihydroabieslactone (44). The IR spectrum revealed a lactone



44

carbonyl at 1770 cm^{-1}. The NMR spectrum was altered in that signals at τ 3.00, 5.03, and 8.10 had disappeared while a new one proton multiplet at τ 5.53 and a new three proton doublet at τ 8.73 now appeared. The τ 4.48 signal remained unchanged. Prolonged hydrogenation over Adams catalyst in acetic acid - ethyl acetate resulted in tetrahydroabieslactone (45) with loss of the τ 4.48 signal.

Oxidation of abieslactone with potassium permanganate acetic acid afforded a trisnor-hydroxy acid (46) as a result of cleavage of the unsaturated lactone ring. The configuration at C(23) in the trisnor-hydroxy acid, and hence in abieslactone, was established as R by measurement of the circular dichroism curves for the trisnor-hydroxy acid.



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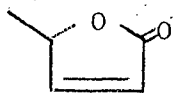

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Alkaline hydrolysis of abieslactone (Figure 15) gave, in analogy with angelicalactone (47)[77], a keto acid (48). The NMR spectrum now lacked the τ 3.00, 5.03, and 8.10 resonances but retained the τ 4.48 resonance. The acid function was esterified with diazomethane; the NMR spectrum still retained the τ 4.48 resonance plus a new three proton singlet at τ 6.30 for a methyl ester. Treatment of the keto ester 49 with boron trifluoride etherate and ethane dithiol followed by desulfurization with Raney nickel, gave deoxy ester (51). The τ 4.48 resonance was now shifted to a new position at τ 4.78. The resulting deoxy ester was treated with lithium aluminum hydride to give an alcohol (52) which was converted to the tosylate (53). Reduction of the tosylate with lithium aluminum hydride gave a compound (54) in which the oxygen functions of the side chain of abieslactone

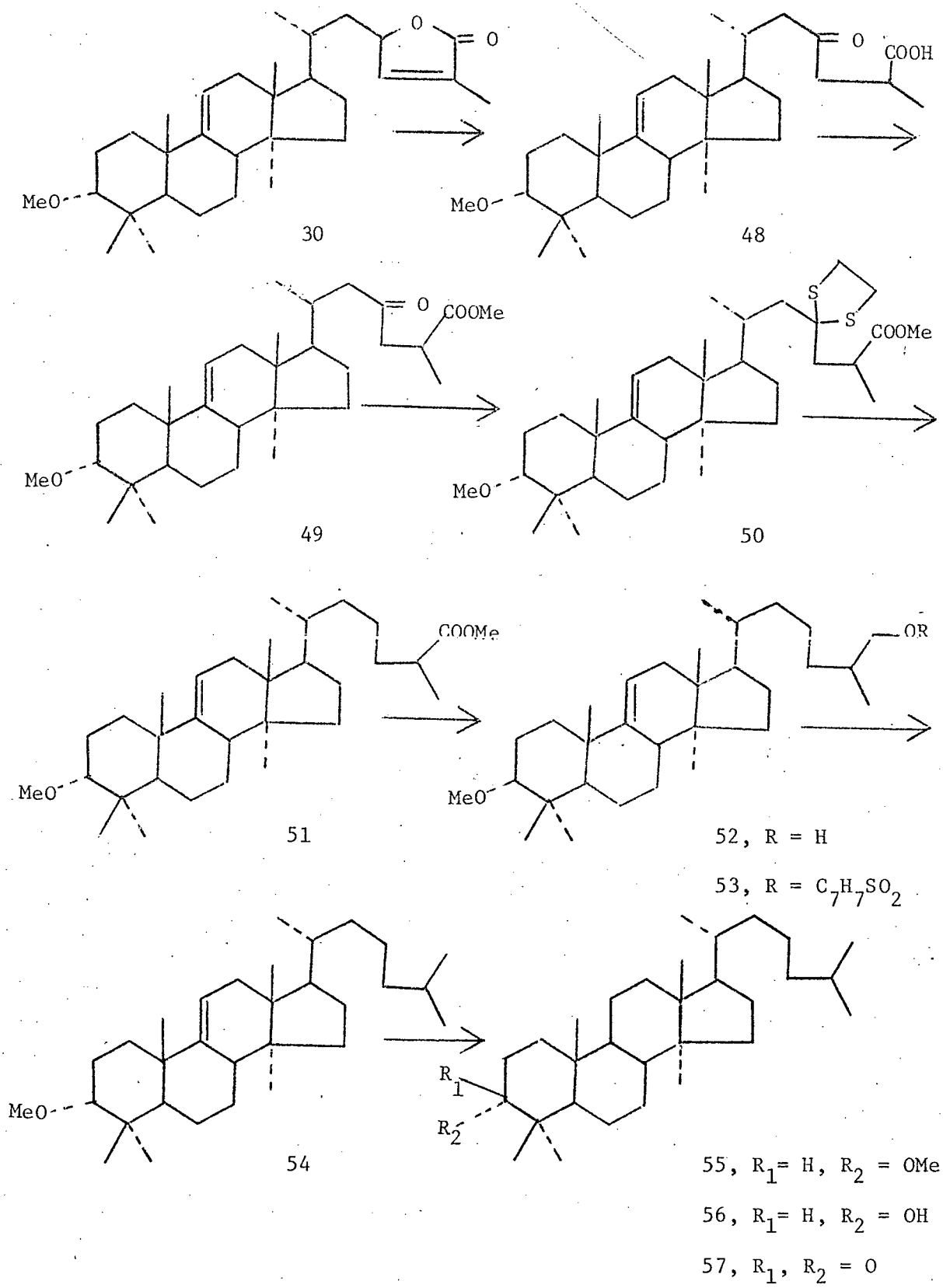
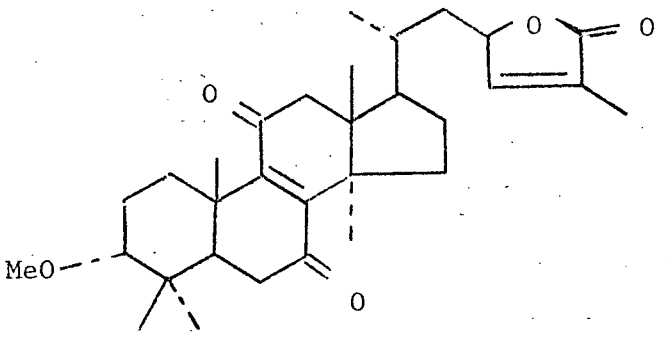


Figure 15. Degradation sequence of abieslactone.

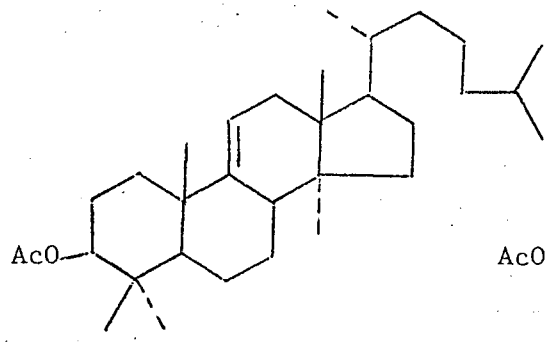
were completely removed. The NMR spectrum showed 8 C-methyl groups in the region τ 8.93 to 9.35, one O-methyl singlet at τ 6.70, the one proton diffused triplet at τ 7.20, and the olefinic proton at τ 4.75.

Hydrogenation of 54 gave a dihydro derivative, 55, whose NMR spectrum was devoid of the olefinic proton signal. Demethylation of the dihydro derivative was accomplished by hydrogen bromide in boiling acetic anhydride - acetic acid. The resulting compound, 56, was different from lanostan-3β-ol but when 56 was oxidized it was identical with lanostan-3-one (57). It was now clear that 56 was the C(3) epimer, lanostan-3α-ol. In turn this result also revealed that the various degradation products and abieslactone itself would possess the tetracyclic lanostane skeleton. This assumption is valid only if no major skeletal rearrangements occur during the degradation sequence leading to 57. Some comments concerning this point will be made later.

The position of the double bond was the one remaining uncertainty. Abieslactone could be oxidized with chromium trioxide in acetic acid to an ene-dione system (58). The UV spectrum (λ_{max}^{EtOH} 274, log ε 3.84) was



characteristic of steroids or triterpenes possessing the 8-ene-7,11-diketo chromophore.[46] The olefinic proton at τ 4.48 in the NMR spectrum of abieslactone was no longer present suggesting that the double bond in the skeleton of abieslactone had shifted to the C(8) - C(9) position in the course of the oxidation. Uyeo writes[28], "Of the two possibilities [7-ene or 9(11)-ene], the position of the double bond between C(7) - C(8) could be ruled out because of the fact that abieslactone and its derivative [54] showed no facile shift of the double bond in the ring system on treatment with mineral acids, analogous to lanost-9(11)-en-3β-yl acetate [dihydroparkeyl acetate, 34].[46,47,48,49] Compounds such as lanost-7-en-3β-yl acetate [59][50], euph-7-en-3β-yl acetate [60][51], and tirucall-7-en-3β-yl acetate [61][52] that contain the double bond between C(7) and C(8) are known to be readily converted with acids into compounds having the double bond at C(8) - C(9). Further, the fact that the isolated double bond can be hydrogenated under catalytic conditions parallels the behavior of lanost-9(11)-en-3β-ol, while lanost-7-en-3β-ol is resistant."



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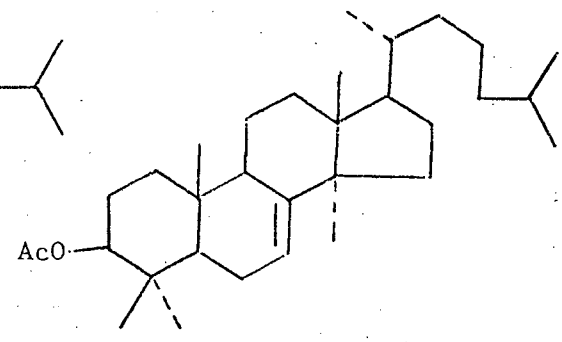

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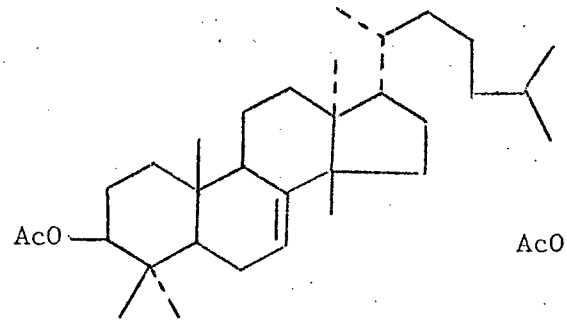

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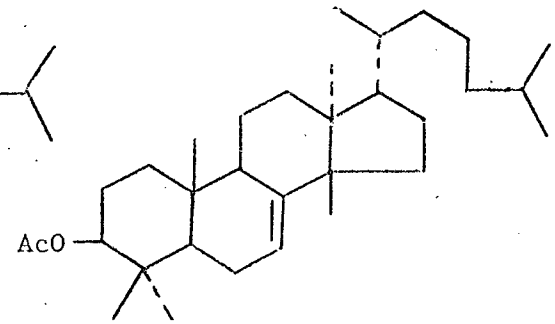

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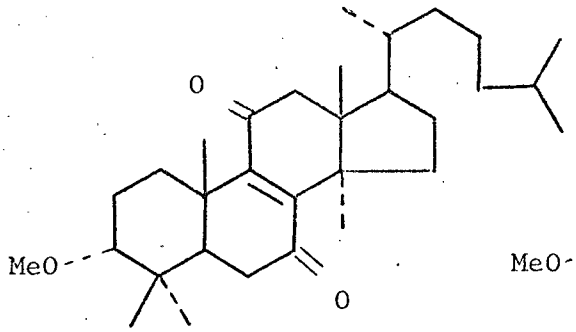
An observation that supported the view that the nuclear double bond must be in the C(9) - C(11) position was secured by oxidation of the 3α-methoxylanostene (54) with chromium trioxide in acetic acid which afforded two products after chromatography. The minor and less polar product was an ene-dione (62) whose UV spectrum was similar to the chromium trioxide product of abieslactone. The major and more polar compound was an α,β-unsaturated ketone (63) whose UV spectral properties



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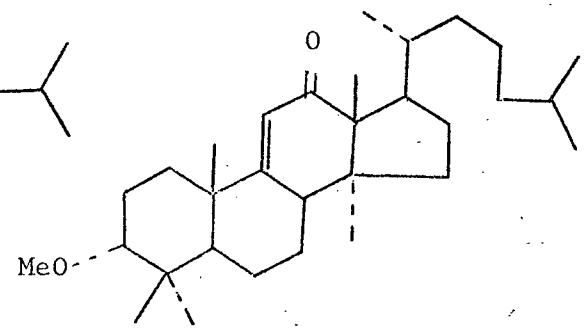

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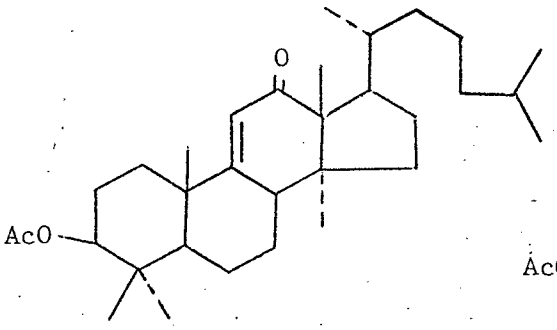
(λ_{max} 275 mμ, log ε 4.10) were like those of 3β-acetoxylanost-9(11)-en-12-one (64)[47], 3α-acetoxyarbor-9(11)-en-12-one (65)[53], and methyl 12-detodavallate (66)[54]. The ORD curve was like that of 3α-acetoxyarbor-9(11)-en-12-one (65)[53] and 3β-acetoxy-18α-olean-9(11)-en-12-one (67)[55].



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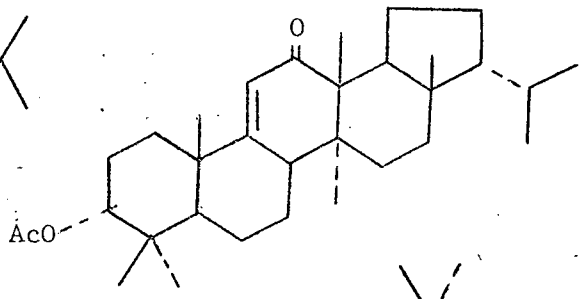

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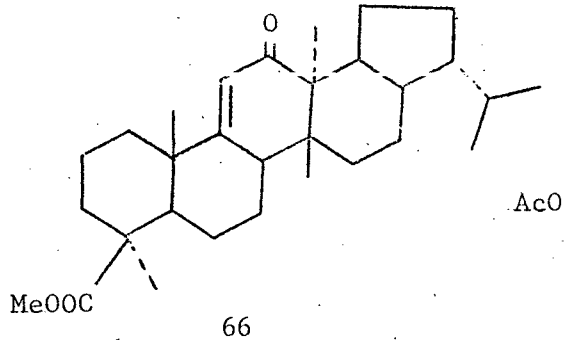

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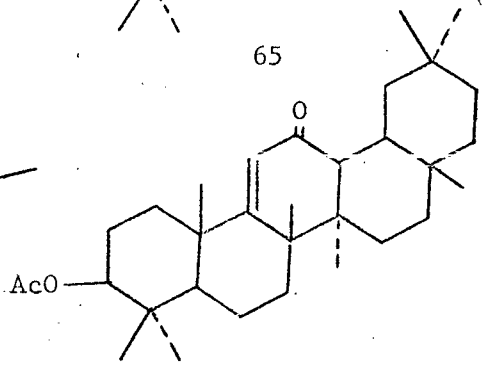

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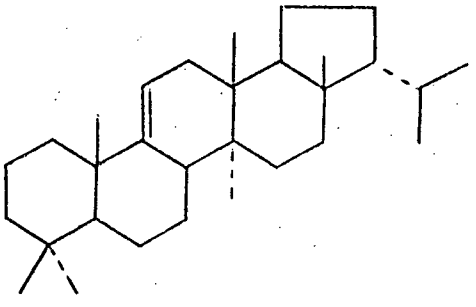
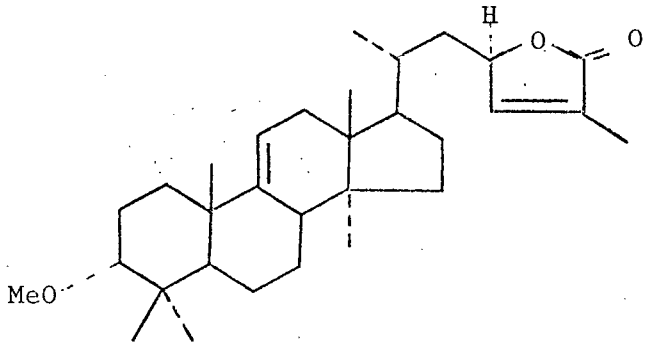
The mass spectrum of the 3α-methoxylanostene (54) was reported to show a similar cracking pattern to that of arborene (68)[53] which contains a double bond at C(9) - C(11).



68

On the basis of the above data the structure for abieslactone suggested by Uyeo and co-workers[28] is shown in 30 and the systematic name given is 3α-methoxylanosta-9(11),24-dien—27,23R-olide [(23R)-3α-methoxy-lanosta-9(11),24-diene-26,23-lactone].



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It will be remembered that 30 is the structure proposed for grandisolide in the previous section. When it was found that this substance was not identical with abieslactone careful re-evaluation of the chemistry of abieslactone was necessary.

This called for, in part, a larger amount of abieslactone than had been originally received from Professor Uyeo. Hergert[30] had reported its presence in the bark of silver fir [Abies amabilis (Dougl.) Forbes]. A supply of that bark was obtained through the kindness of the Forest Products Laboratory in Vancouver from the University of British Columbia Research Forest near Haney, British Columbia.

Extraction of the ground, air dried bark with chloroform or diethyl ether and evaporation of the solvent gave a brown residue. This residue was dissolved in hot ether and left to crystallize overnight at room temperature. The precipitate was filtered, washed with cold ether, and,

after drying, provided a light brown powder. TLC of this brown powder showed one main compound corresponding to an authentic sample of abieslactone obtained from Professor Uyeo, plus two minor spots which were both more polar than abieslactone. Recrystallization of this brown powder from ethyl acetate indicated by TLC that very little enrichment of abieslactone had occurred.

It was found that abieslactone could be purified by chromatography on neutral deactivated alumina. Elution with petroleum ether - benzene gave abieslactone as a white solid, while further elution with benzene - methylene chloride gave the second compound initially coded as AA_2. Finally elution with methylene chloride gave the third compound, AA_3. Compounds AA_2 and AA_3 will be discussed later.

The abieslactone obtained in this manner was crystallized twice from ethyl acetate to give a white crystalline solid m.p. 251 - 253°C (literature value 251 - 253°C).[28] A mixed melting point determination with authentic abieslactone showed no melting point depression. In addition, the TLC of both specimens were the same as were the IR spectra. The NMR spectrum of our product (Figure 16) had the three low field signals at τ 3.00, 4.48, and 5.05; a three proton singlet at τ 6.73; the diffused triplet at τ 7.20; the vinylic methyl at τ 8.10; and signals at τ 8.98 - 9.08 for six methyl groups. This spectrum was in agreement with the reported NMR spectrum of abieslactone.

The ORD curve (Figure 17) of abieslactone (in dioxane) had a trough $[\phi]_{224}$ - 49,000°. The CD curve (Figure 18) of abieslactone (in dioxane) had a weak positive peak $[\theta]_{250}$ + 450°, and a strong negative value with

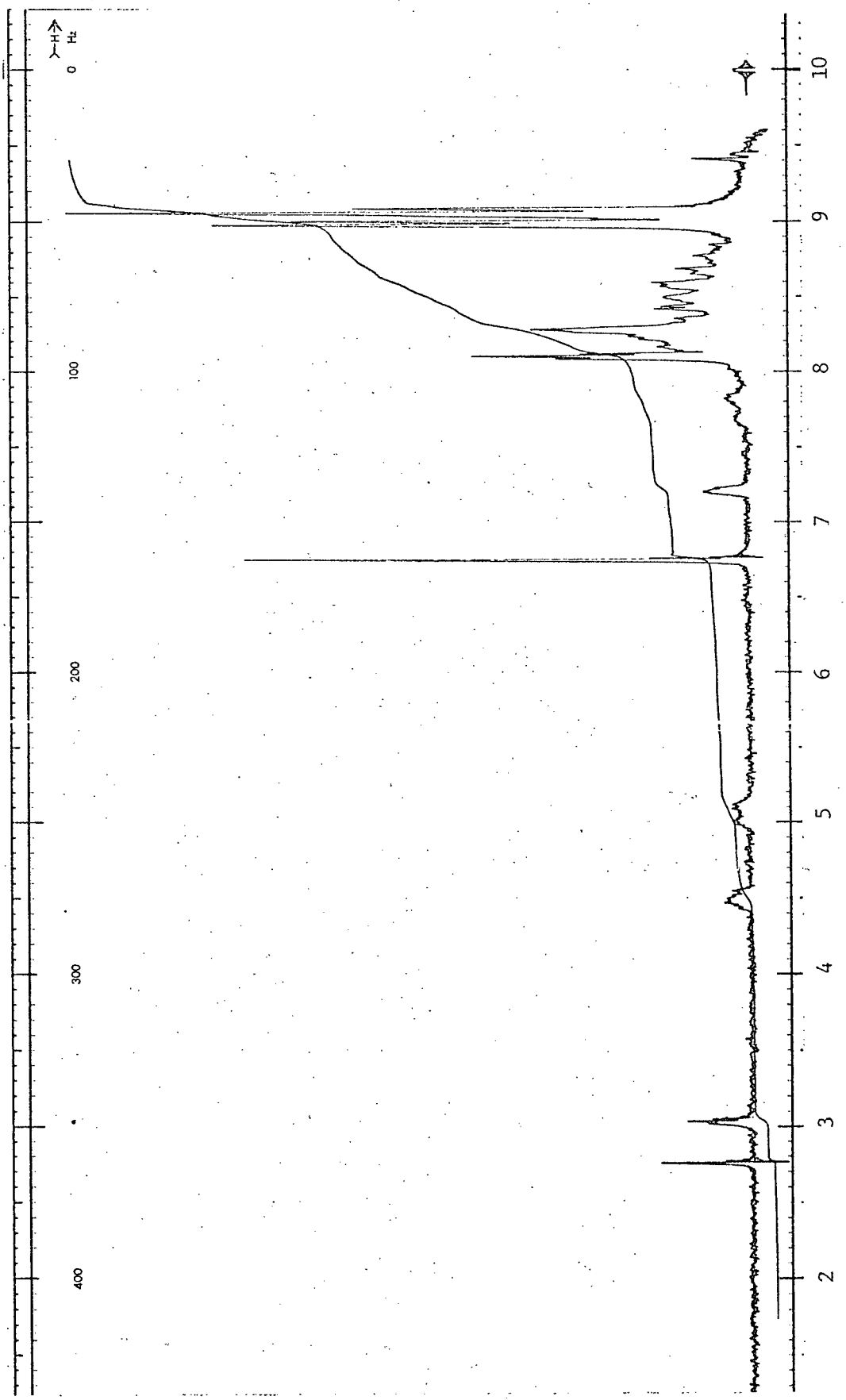


Figure 16. NMR spectrum of abieslactone.

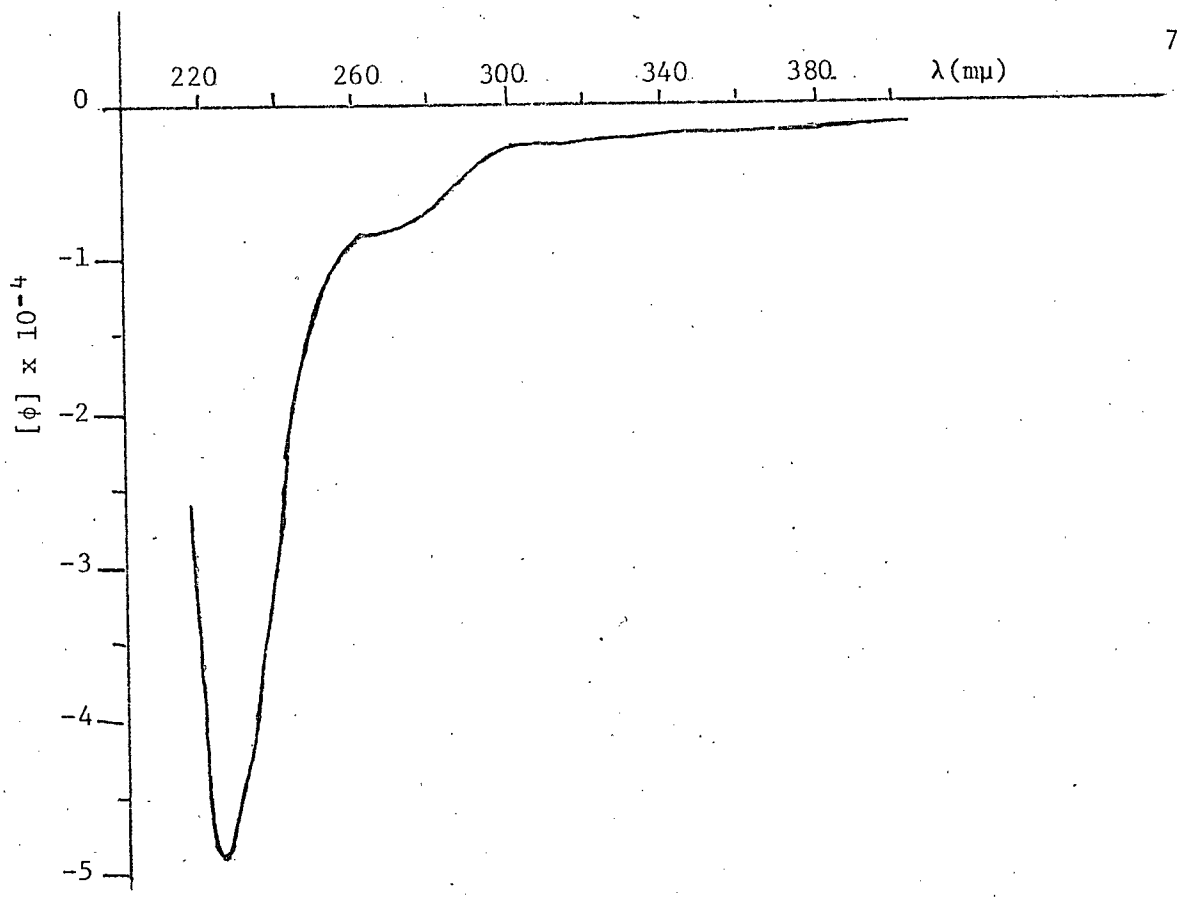


Figure 17. ORD curve of abieslactone.

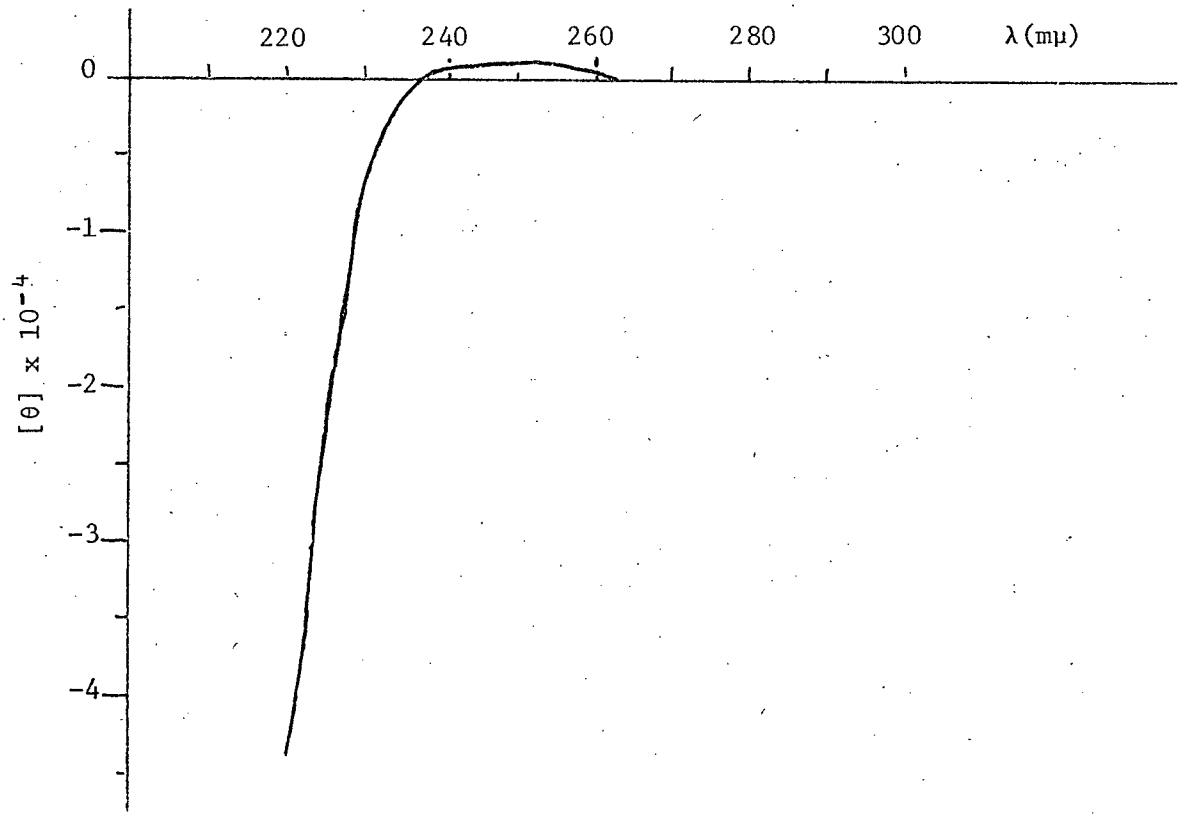


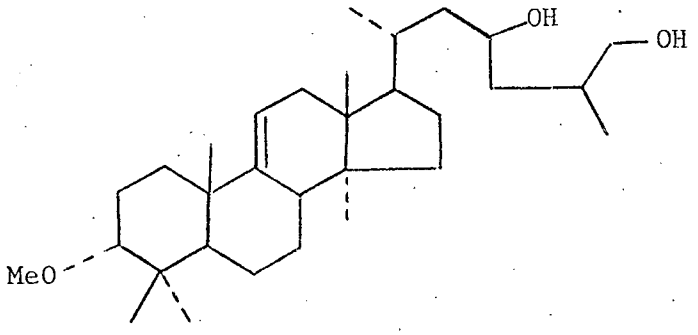
Figure 18. CD curve of abieslactone.

no minimum observed below 220 mμ ($[0]_{220}$ - 45,050°).

As a further check that the isolated abieslactone was indeed identical to the reported material, a derivative was prepared. The derivative (58), the 8-ene-7,11-diketone obtained by chromium trioxide oxidation, was selected for a number of reasons. The chromophore produced had characteristic spectral properties and its physical properties were known as it had been prepared in the original structural elucidation of abieslactone.

The derivative (58) was prepared and the product exhibited the expected UV absorption (λ_{max}^{MeOH} 274 log ε 3.85), IR absorption (1735, 1670 cm^{-1}), and a melting point of 217 - 219°C in agreement with the reported[28] value of 215 - 216°C.

In addition to the above derivative, dihydroabieslactone (44), tetrahydroabieslactone (45), and the diol (69) had the same melting points as reported[28].



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On the basis of the above work, it was concluded that the abieslactone isolated from A. amabilis was indeed identical with the reported substance obtained from A. mariesii.

The re-evaluation of the data for abieslactone started with the NMR

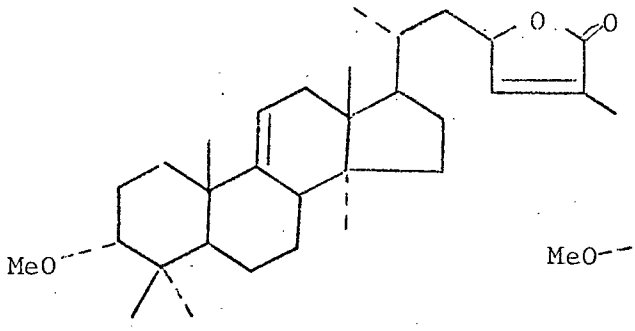
spectral results. In the last section it was demonstrated that the position of C-methyl groups in the triterpene series could be predicted with reasonable accuracy using certain empirical calculations.[39,40,41] An examination of Table II (page 28) shows that it is only the 3-keto group which affects the signals of the C(18), C(19), and C(32) methyl groups. The effect of a 3α-methoxyl substituent was assumed to be negligible in the previous study if the 3α-hydroxyl group was used as a model. Also it was assumed that the lactone ring would have negligible effect. The validity of these assumptions was eventually established when our previous work coupled with X-ray analysis left no doubt about the structures of cyclograndisolide and grandisolide.

One of the anomalies associated with the NMR spectrum of abieslactone is the unusually narrow range of resonance frequencies for the angular methyl groups. This point can be best illustrated by considering several examples from the published[28] chemistry of abieslactone and the present study.

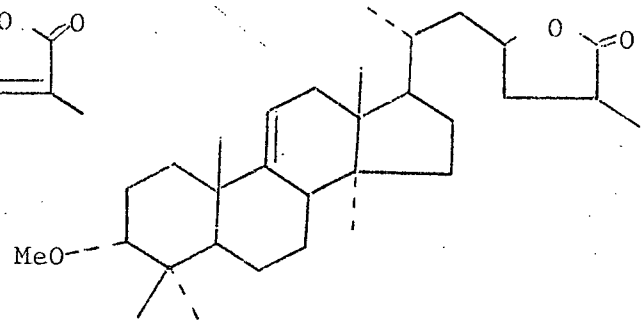
Compound	Resonance Frequency (τ units)					
abieslactone (30)	8.98	9.00	9.06	9.08		
dihydroabieslactone (44)	8.96	9.00	9.06	9.08		
keto acid (48)	8.73	8.84	8.95	9.00	9.05	9.09
keto ester (49)	8.76	8.87	8.95	9.03	9.07	
diol (69)	8.97	9.00	9.05	9.08		
grandisolide	8.95	9.02	9.07	9.12	9.29	9.36

Table VII. Range of resonance frequencies of the C-methyl groups.

Table VII illustrates that the lactone ring has little effect on the positions of the methyl signals. If the lactone ring was causing the C-methyls to absorb over a narrow range in abieslactone, this range would



30†



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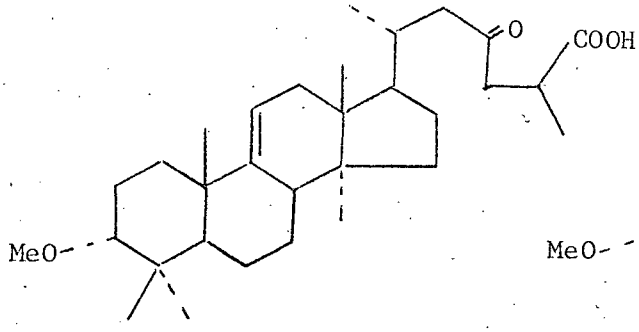

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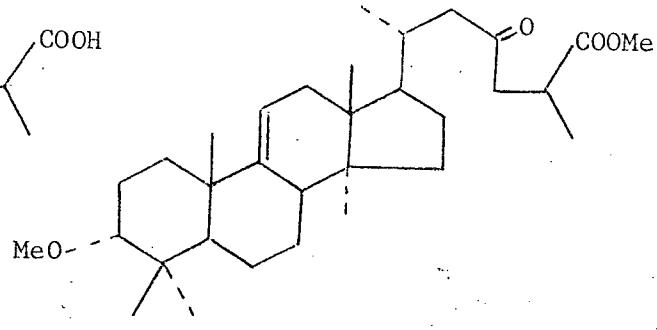

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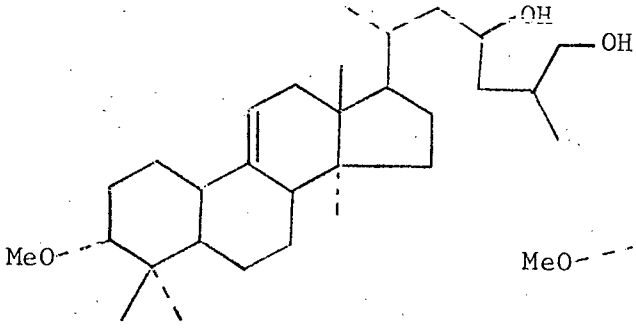

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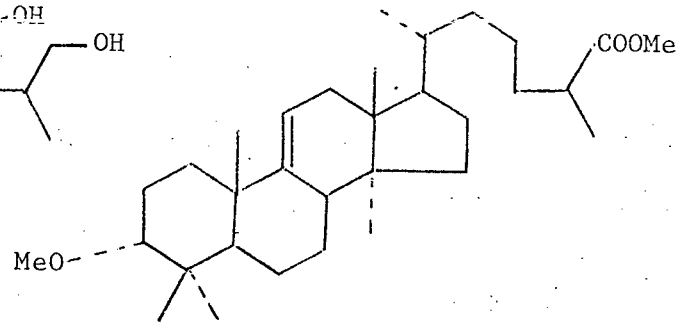

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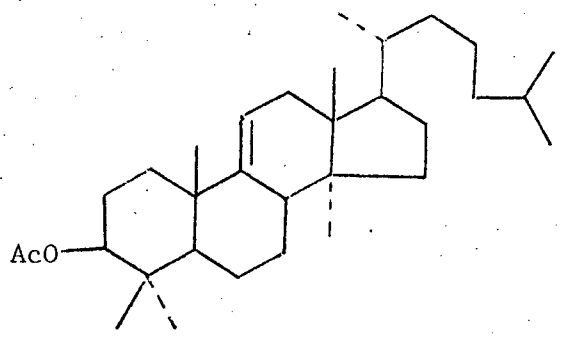

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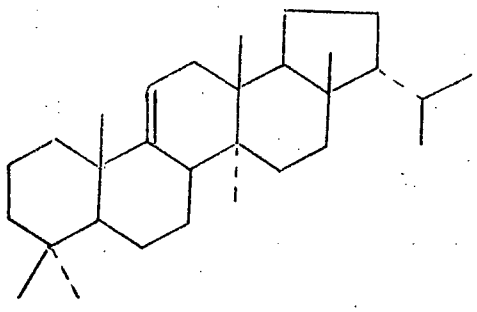

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† Structure 30 is the structure assigned to abieslactone by Uyeo[28]; a different compound, grandisolide, was shown to have this structure in the present study.

be expected to broaden as this functionality is altered. The range of dihydroabieslactone and the diol are essentially unchanged. The two signals to slightly lower field in the keto acid and keto ester can likely be assigned to methyl groups on the side chain which are now attached to carbons alpha or beta from an oxygenated carbon. Even in these derivatives the range of the resonance frequencies is anomalous in that the upper limit is never over τ 9.08. Comparison of all of these values with known C(9) - C(11) unsaturated lanostene derivatives (Table IV, page 29) or grandisolide requires that some structural feature in the abieslactone molecule lowers the resonance frequency of the methyl groups.

In addition to the mentioned anomalies in the NMR spectra there is an anomaly associated with the resonance frequency of the olefinic proton postulated to be at C(11) in abieslactone. Table VIII shows that abieslactone and the first four derivatives mentioned possess an olefinic proton signal which is considerably lower than in the C(9) - C(11) unsaturated triterpenes noted. Of further interest is the fact that the deoxy ester (51) obtained by reductive removal of the carbonyl function in 49 now shows

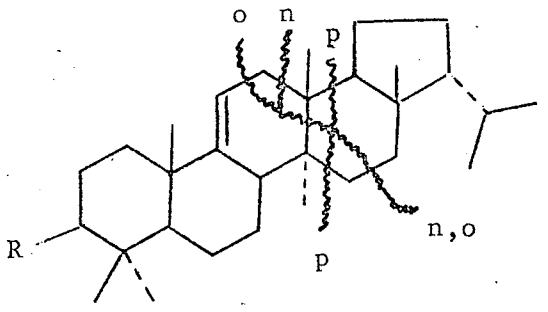
Compound	Resonance Frequency (τ units)
abieslactone (30)	4.48
dihydroabieslactone (44)	4.47
keto acid (48)	4.48
keto ester (49)	4.45
diol (69)	4.47
deoxy ester (51)	4.78
grandisolide	4.80
dihydroparkeyl acetate (34)	4.81
arborene (68)[53]	4.73

Table VIII. Comparison of resonance frequencies of olefinic proton in abieslactone series with some C(9) - C(11) unsaturated triterpenes.

absorption in a region consistent with a C(11) olefinic proton. One cannot help but wonder whether the conversion, 49→51, is not associated with double bond migration.

In view of the previously successful comparison of cyclograndisolide and cycloartenyl acetate, a mass spectrometric comparison of dihydroparkeyl acetate and grandisolide, both known members of the lanostane family possessing C(9) - C(11) unsaturation, with abieslactone was undertaken. The elemental composition of ions where given was determined by high resolution mass spectrometry.

The fragmentation of 9(11)-ene triterpenes is not well studied, the principle examples being arborene (68)[53] and arborenone (68a)[56]. In both cases the spectra are characterized by strong loss of methyl and a base



68, R = H_2

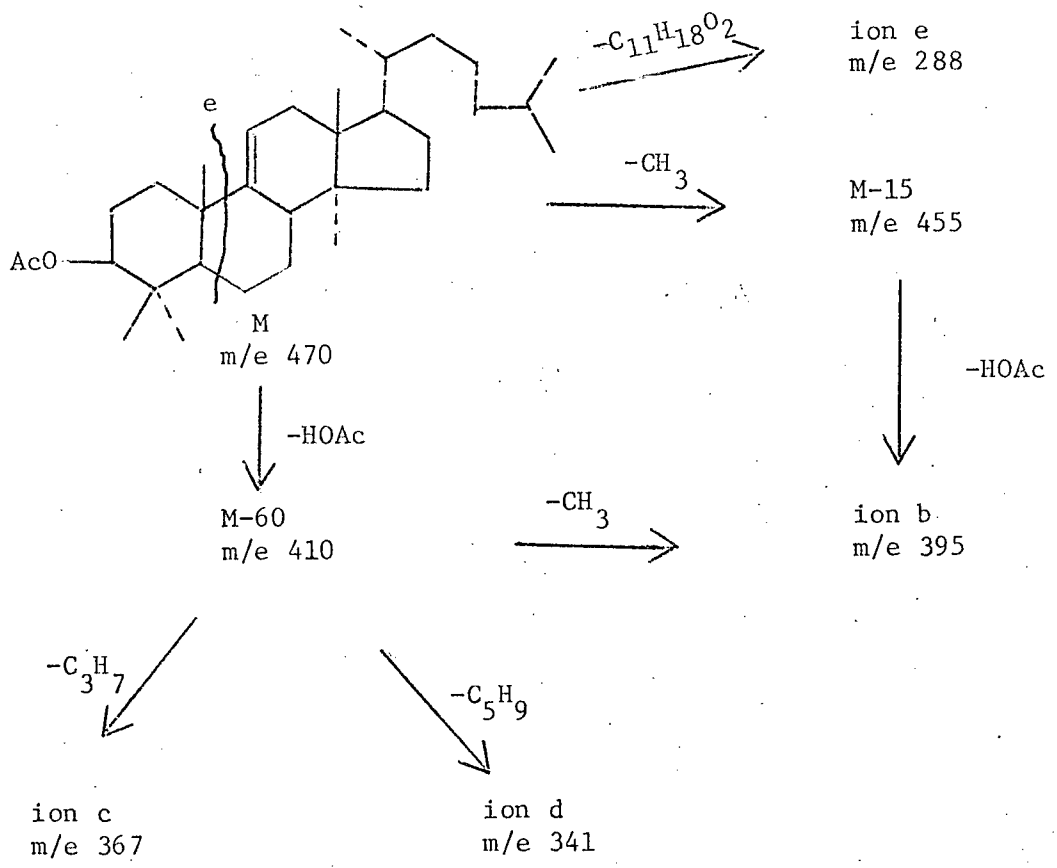
68a, R = O

peak corresponding to fragmentation mode n with smaller ions due to fragments from fissions via pathways o and p. However, triterpenes with double bonds in positions other than C(9) - C(11) also exhibited some of the same fragments[56] so care must be exercised in the interpretation.

Furthermore, application of these fragmentation pathways to the

present study is of doubtful validity. Fragmentations n, o, and p all involve cleavages in rings C and D. Arborene represents a pentacyclic triterpene which may fragment rather differently from the tetracyclic system portrayed in abieslactone.

The mass spectrum of dihydroparkeyl acetate (34) (Figure 19) shows a molecular ion at m/e 470. A loss of methyl gives a M-15 ion at m/e 455, which is the base peak, plus a metastable ion at m/e 440.5. A loss of acetic acid from the molecular ion gives ion a (m/e 410) plus a metastable ion at m/e 357.9. An ion b at m/e 395 can come from either the M-15 ion



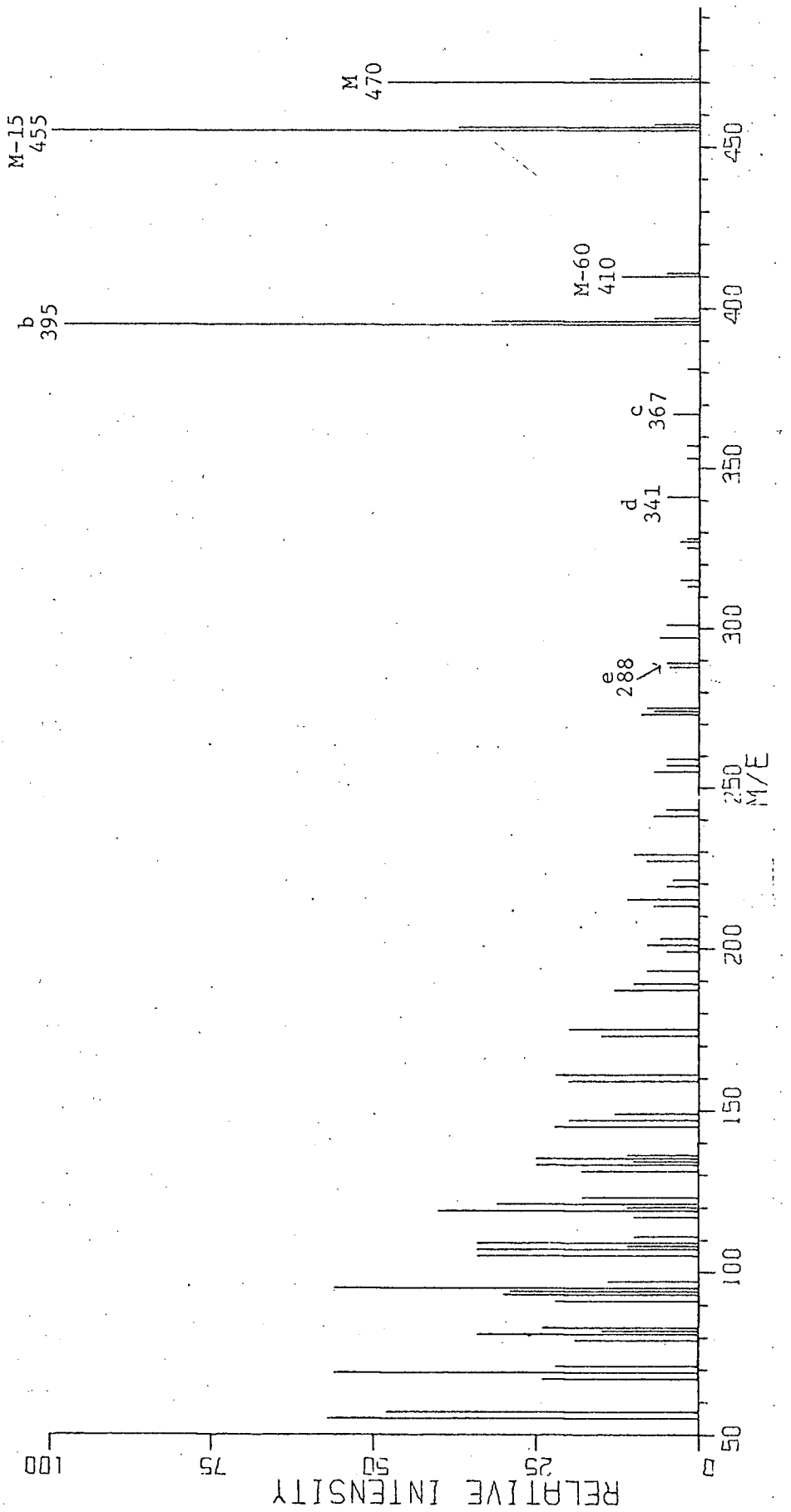
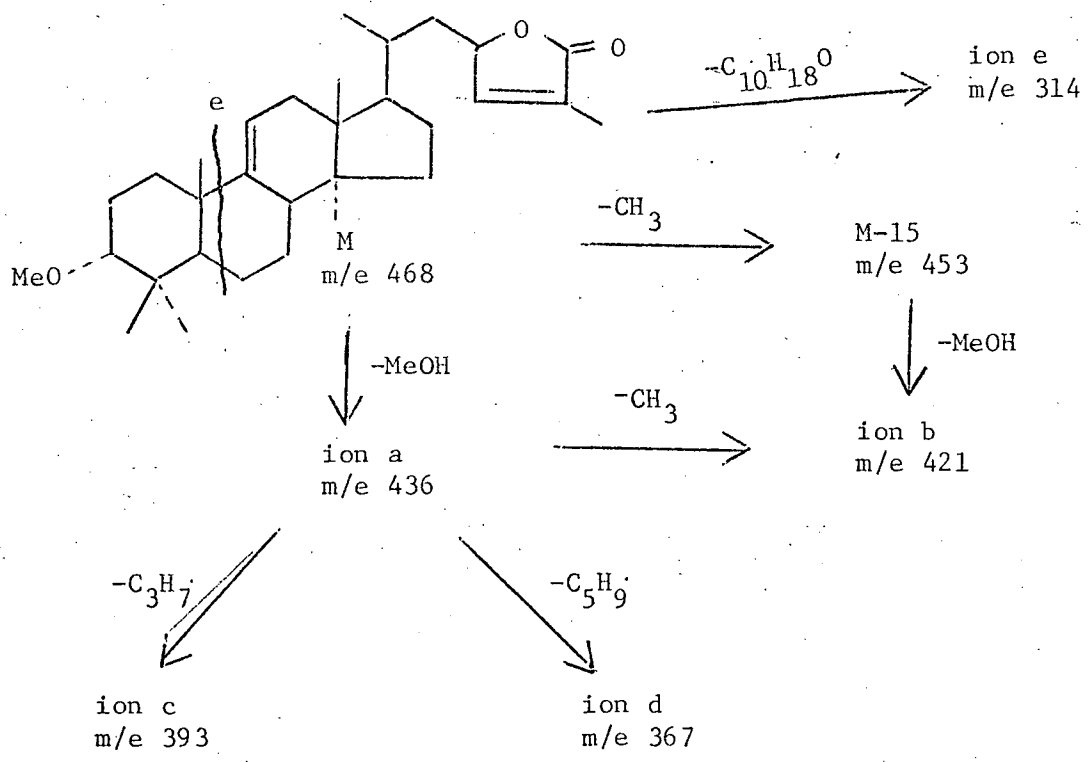


Figure 19. Mass spectrum of dihydroparkeyl acetate.

by loss of acetic acid as seen by a metastable ion at m/e 343.1 or from ion a by loss of methyl as seen by a metastable ion at m/e 380.6. Ion a loses $C_3H_7\cdot$ to give ion c at m/e 367 and a metastable at m/e 328.8. A peak at m/e 341 could correspond to loss of $C_5H_9\cdot$ from ion a.

An ion, e (m/e 288), could correspond to the loss of ring A as observed for cycloartenyl acetate and cyclograndisolide. This ion is approximately one fifth as intense in dihydroparkeyl acetate as it was in either of the cyclopropanoid triterpenes.

Grandisolide (30) obtained from cyclograndisolide was next compared in the mass spectrometer since, in addition to the 9,11 double bond, it possesses the unsaturated lactone side chain, the same as reported for abieslactone. The spectrum (Figure 20) shows a molecular ion (m/e 468), a M-15 ion (m/e 453), and ion a (m/e 436). As in dihydroparkeyl acetate,



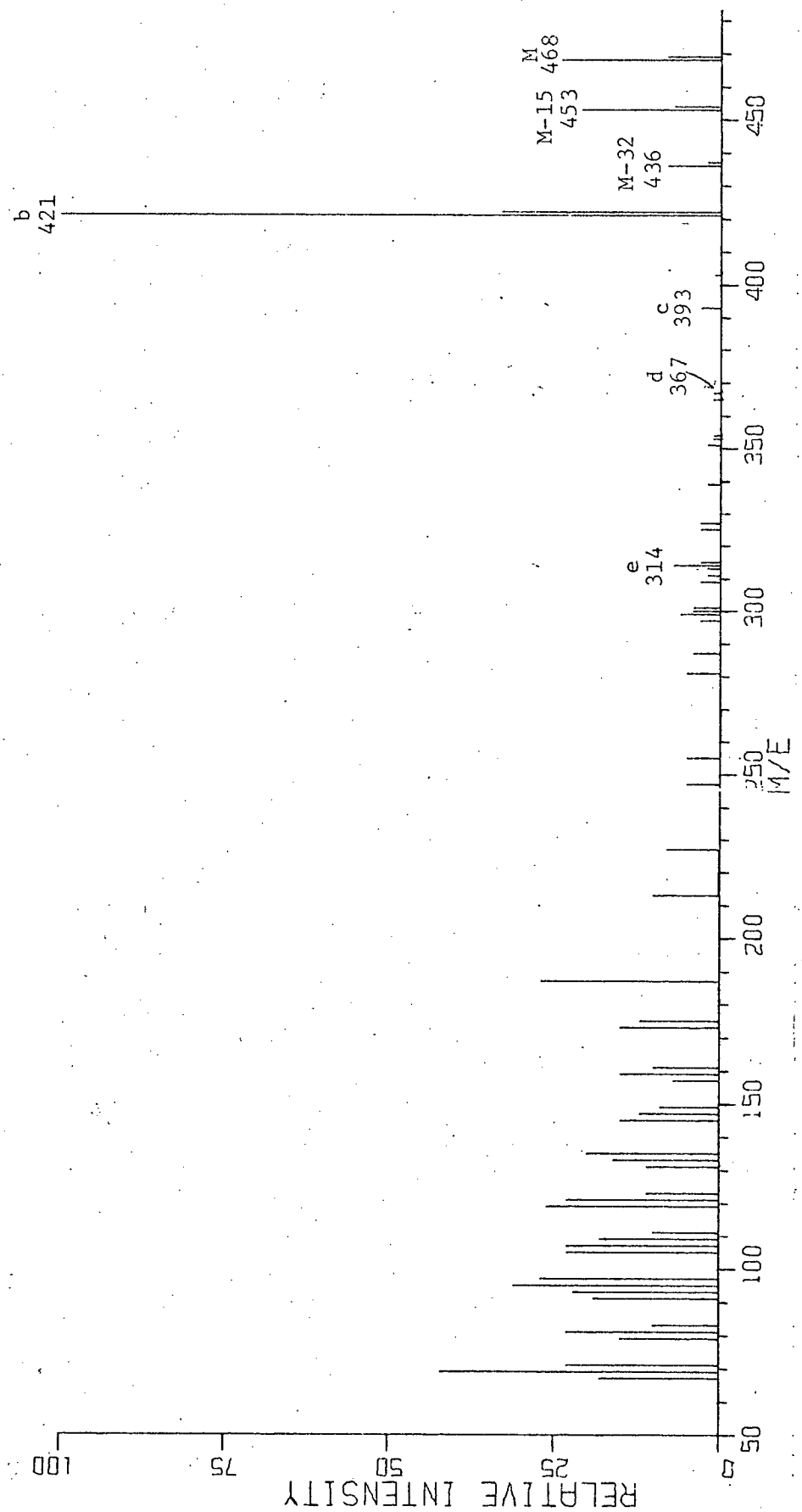
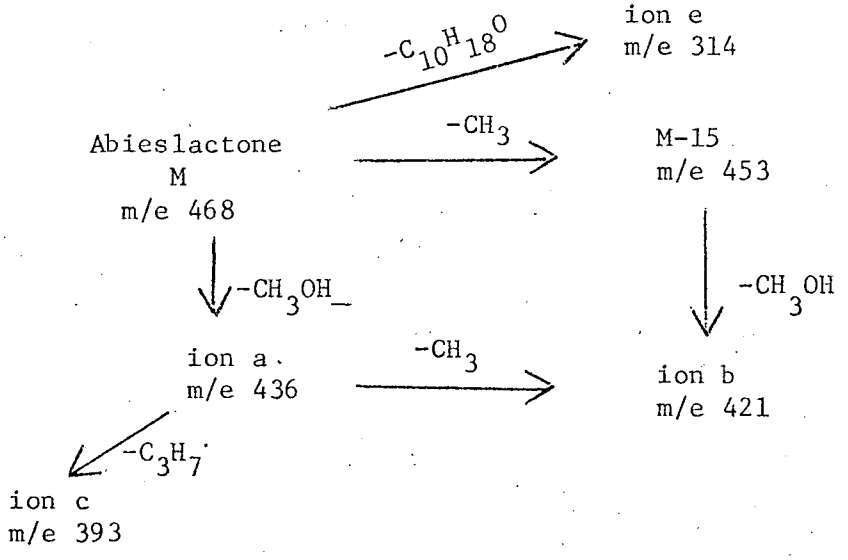


Figure 20. Mass spectrum of grandisolide.

ion a can lose methyl (ion b, m/e 421), $C_3H_7\cdot$ (ion c, m/e 393), and $C_5H_9\cdot$ (ion d, m/e 367). Ion e corresponding to loss of ring A is seen at m/e 314, just slightly more intense than ion e in dihydroparkeyl acetate.

Comparison of the mass spectra of dihydroparkeyl acetate and grandisolide reveals that the major difference is the relative intensities of the M-15 fragment (m/e 455 in the former and m/e 453 in the latter). The lactone side chain does not otherwise alter appreciably the fragmentation of the lanostane skeleton.

The mass spectrum of abieslactone (Figure 21) exhibits a molecular ion at m/e 468 with composition $C_{31}H_{44}O_3$. The M-15 ion is seen at m/e 453 ($C_{30}H_{41}O_3$) but, as in grandisolide, its relative intensity is 20% of the corresponding ion in dihydroparkeyl acetate. Loss of methanol from the molecular ion is seen as ion a at m/e 436 ($C_{29}H_{41}O_2$) while ion b at m/e 421 ($C_{28}H_{37}O_2$) again arises via loss of methanol from the M-15 ion or via loss of methyl from ion a. Furthermore, the ion a may lose $C_3H_7\cdot$ to give ion c at m/e 393 ($C_{27}H_{37}O_2$).



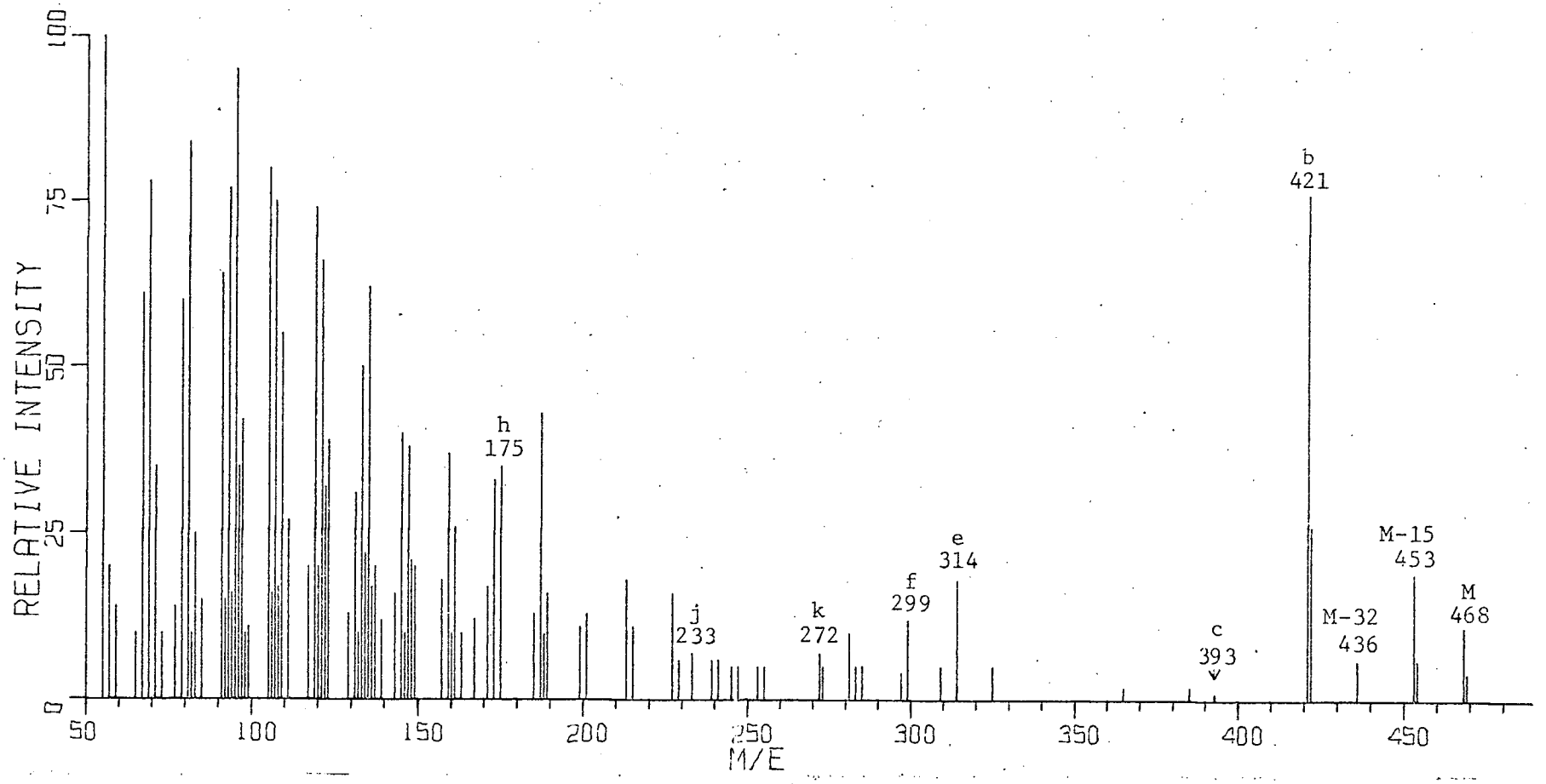
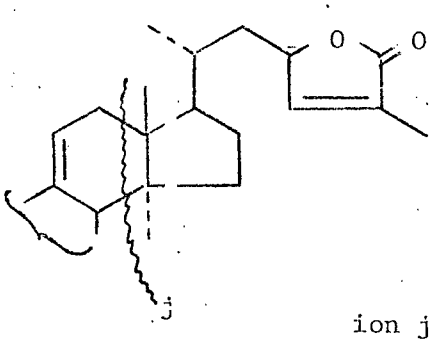


Figure 21. Mass spectrum of abieslactone.

Ion e (m/e 314), with elemental composition $C_{21}H_{30}O_2$, is approximately three fold more intense in abieslactone than in grandisolide. Ion f (m/e 299, $C_{20}H_{27}O_2$) arises from loss of methyl from ion e while ion h (m/e 175, $C_{13}H_{19}$) may represent fragmentation of the side chain from ion f.

Ion k (m/e 272, $C_{18}H_{24}O_2$) is seen in the mass spectrum of abieslactone along with ion j (m/e 233, $C_{15}H_{21}O_2$) corresponding to cleavage through ring D.



ion j
m/e 233

Although it is clear that definite conclusions concerning the structural differences between grandisolide and abieslactone cannot be made from the consideration of the mass spectral data, it is significant that the region below m/e 300 in abieslactone is appreciably altered when compared with that of grandisolide. In particular, ions k and j noted in abieslactone suggest a different orientation for the double bond in this substance.

In order to provide additional data concerning the structure of abieslactone it is necessary to consider first some results on two additional components isolated from Pacific silver fir.

The first component coded as AA_2, m.p. 236 - 238°C, had formula $C_{30}H_{44}O_3$. The IR spectrum had two carbonyl absorptions, the α,β-unsaturated-γ-lactone (1745 cm^{-1}) and a ketone (1705 cm^{-1}). The NMR spectrum had a quartet at τ 7.45 and this was assigned to protons adjacent to the ketone. There was no absorption for a methoxyl group while the C-methyl region contained signals at τ 8.90, 8.98, and 9.18 integrating for six methyl groups. The τ 9.18 signal was a singlet and integrated for one methyl group. Signals at τ 3.00, 5.02, and 8.10 were assigned to the same protons at C(24), C(23), and C(26) as in abieslactone. The olefinic proton absorbed at τ 4.40. The ORD curve (Figure 22) had a peak, $[\phi]_{317}$ + 1485°, and a trough at lower wavelengths, $[\phi]_{224}$ - 25,400°. The CD curve (Figure 23) had a peak, $[\theta]_{294}$ + 3,160° and a trough at lower wavelengths, $[\theta]_{215}$ - 35,510°.

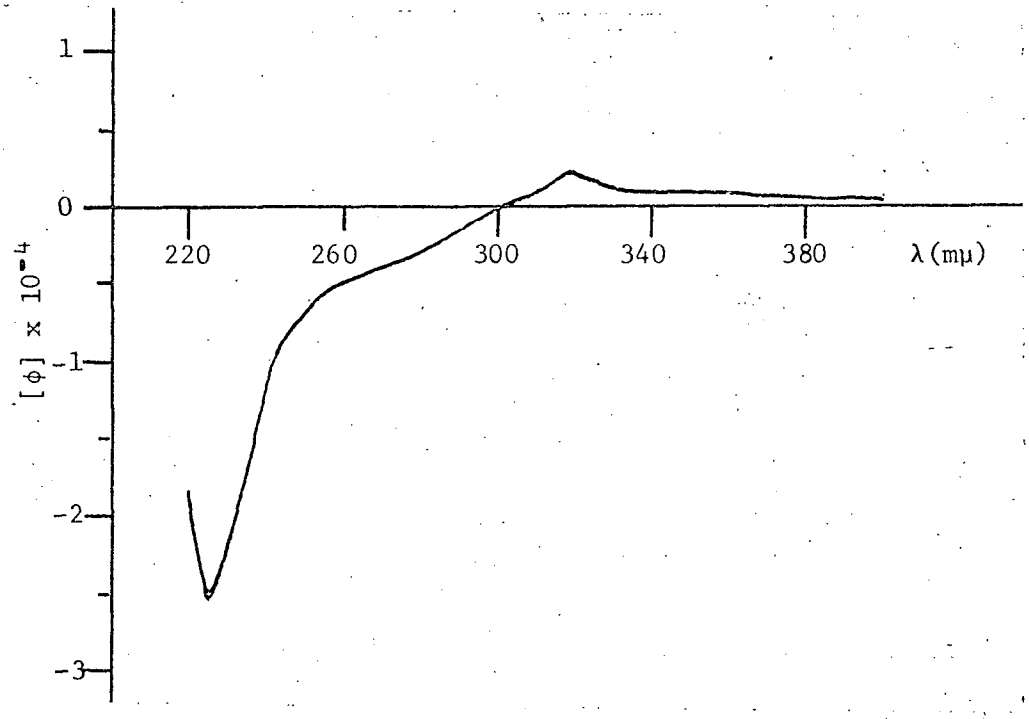


Figure 22. ORD curve of AA_2.

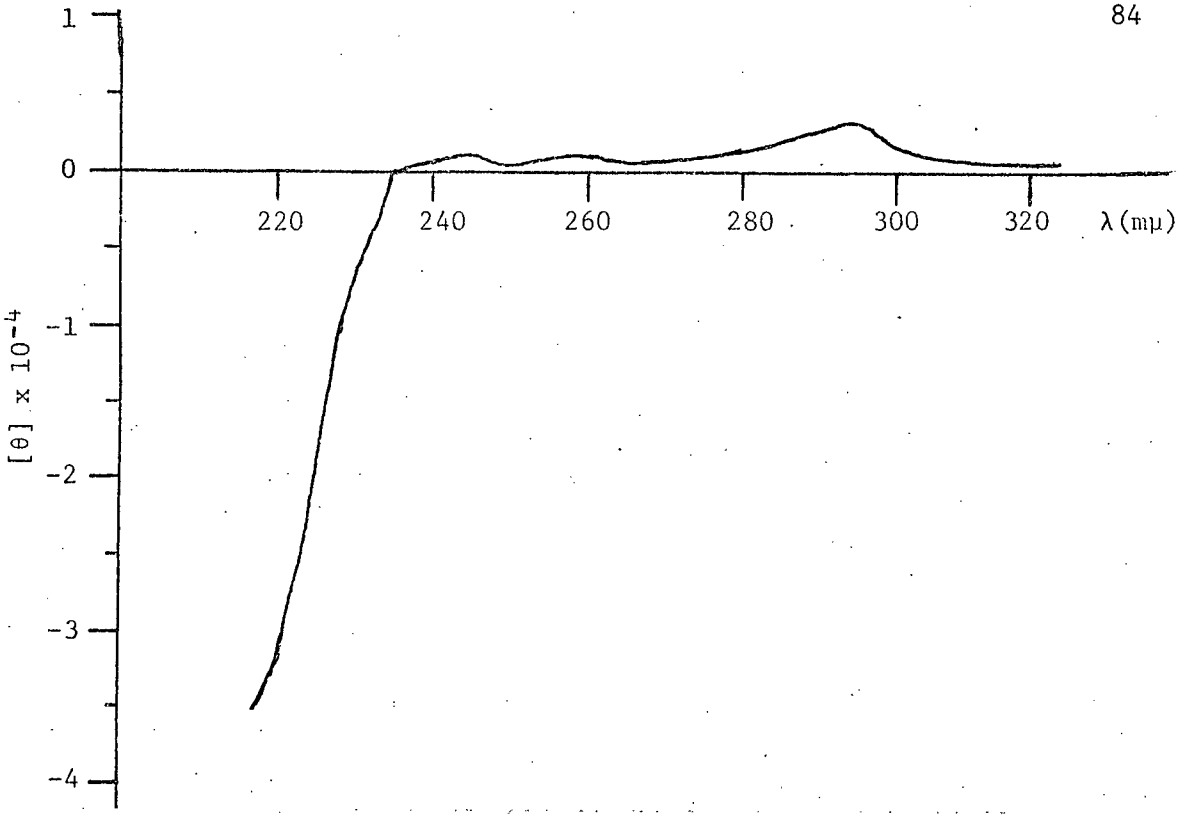


Figure 23. CD curve of AA_2.

The mass spectrum had the molecular ion at m/e 452 ($C_{30}H_{44}O_3$). A peak at m/e 314 was also present as well as the m/e 299 ion peak, both of which were of lesser relative intensity than in abieslactone.

The other minor component, AA_3 (m.p. 249 - 250°C, $C_{30}H_{46}O_3$), was the most polar of all three products from silver fir. The IR spectrum had hydroxyl absorption (3520 cm^{-1}) plus the lactone carbonyl. The NMR spectrum had signals at τ 3.02, 5.02, and 8.10 characteristic of the lactone system; a broadened signal at τ 6.6 assigned to a proton geminal to the hydroxyl; and C-methyl signals at τ 8.98, 9.00, 9.03, and 9.06 for six methyls. The olefinic proton was at τ 4.48 as in abieslactone. The mass spectrum had a molecular ion at m/e 454 ($C_{30}H_{46}O_3$). The ion peaks at m/e 314 and m/e 299 were both present and were of greater relative intensity than in AA_2 but slightly smaller than in abieslactone.

Oxidation of AA_3 with chromium trioxide in pyridine gave AA_2, suggesting that they differed only in oxidation level at one center. AA_3 upon methylation gave a product whose TLC, m.p., mixed m.p., and IR were identical to abieslactone. Thus AA_3 is 3-desmethylabieslactone and AA_2 is the 3-keto derivative of abieslactone.

Catalytic reduction of AA_2 in tetrahydrofuran with palladium on charcoal as catalyst gave dihydro AA_2. The NMR signals formerly associated with the lactone had disappeared with a new one proton multiplet at τ 5.5 now visible. The C-methyls absorbed at τ 8.96, 9.00, 9.02, and 9.20; the higher field resonance was still a three proton singlet. The ORD curve of dihydro AA_2 had a peak $[\phi]_{315}$ + 3055° and a trough $[\phi]_{275}$ - 3921° associated with the ketone plus a peak at 220 mμ ($[\phi]_{220}$ + 6110°) associated with the lactone ring. The CD curve had a maximum for the ketone, $[\theta]_{294}$ + 3170°, and the lactone had a positive curve with no maximum observed above 220 mμ ($[\theta]_{220}$ + 12,960°).

As was shown, AA_2 differs from abieslactone only in the nature of the functionality at C(3), with the nuclear double bond and the lactone in the same positions. If AA_2 is a 9(11)-ene system as is reported for abieslactone[28] then the ORD curve associated with the ketone function of AA_2 and dihydro AA_2 should be similar to other 3-keto-9(11)-ene triterpenes. The ORD curves of AA_2 and dihydro AA_2 are shown in Figure 24 along with the ORD curve of lanost-9(11)-en-3-one.[28,53]

From Figure 24 it can be seen that lanost-9(11)-en-3-one has a miniumum in the 320 mμ region. AA_2 and dihydro AA_2 have a maximum in this region. The data for the abieslactone series is at variance with the

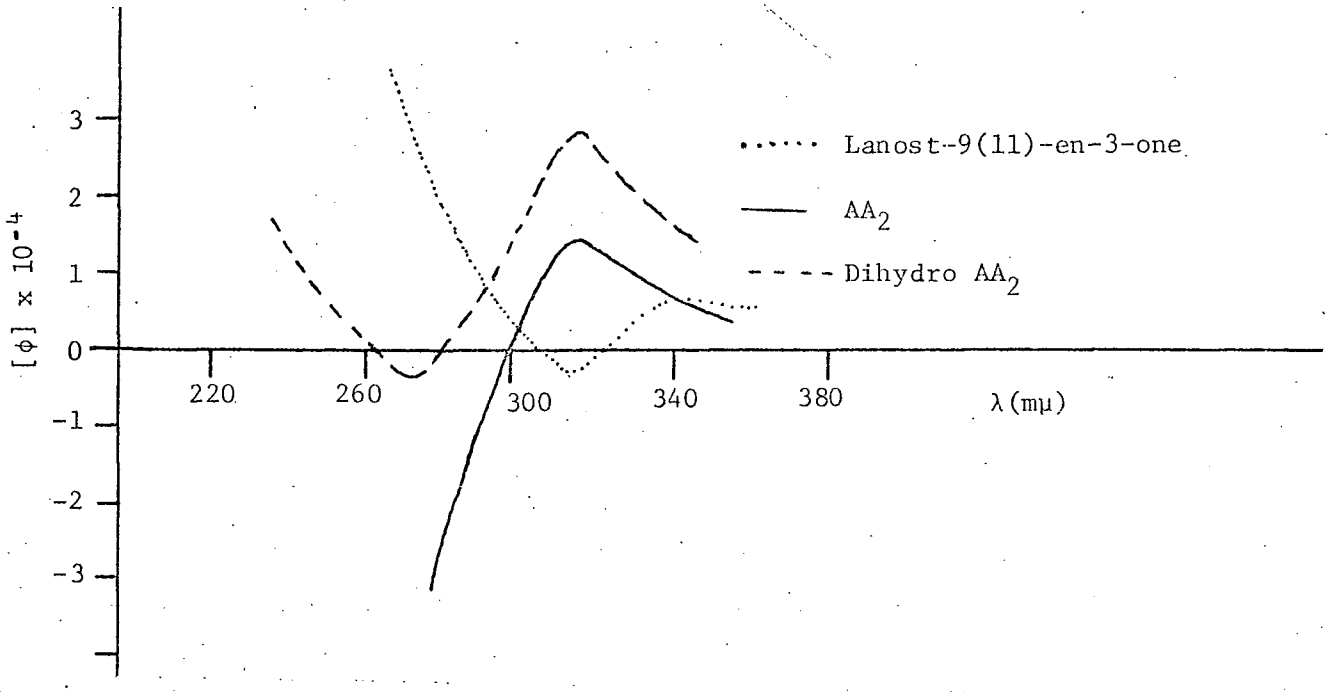


Figure 24. ORD curves of lanost-9(11)-en-3-one, AA_2, and dihydro AA_2.

reported results for known 3-keto-9(11)-ene triterpene systems. On this basis the position of the double bond in AA_2, AA_3, and, in turn, in abieslactone cannot be at C(9) - C(11).

The above discussion reveals that the NMR, ORD, and mass spectrometric studies of abieslactone and its derivatives present several anomalies. It is recognized that all three techniques are sensitive to the position of any olefinic linkages present in the system. In fact, it was the introduction of an olefinic linkage via the cyclopropane ring opening reaction which caused doubt to be cast on the structure of abieslactone.

For these reasons an examination of the chemistry of abieslactone was undertaken with particular reference to the assignment of the position of the nuclear double bond.

Five factors that were considered by the previous authors[28] in the placing of the double bond in the C(9) - C(11) position are:

(a) abieslactone is reducible by catalytic methods;

(b) abieslactone is stable to mineral acid;

(c) abieslactone upon chromium trioxide oxidation gave a 8-ene-7,11-diketone (58);

(d) a 3α-methoxylanostene obtained from degradation of abieslactone gave, upon chromium trioxide oxidation, a 9(11)-ene-12-ketone (63);

(e) a degradation product of abieslactone was compared to an authentic lanost-9(11)-ene derivative.

The double bond was thought to be part of the triterpene nucleus since it was not hydrogenated under mild conditions. It could be catalytically hydrogenated under more rigorous conditions. The common position of a trisubstituted nuclear double bond in a triterpene is between C(7) and C(8), although double bonds between C(9) and C(11) are known. The fact that the isolated double bond can be catalytically reduced parallels the reported behavior of lanost-9(11)-en-3β-ol, while lanost-7-en-3β-ol is resistant even under rigorous conditions.

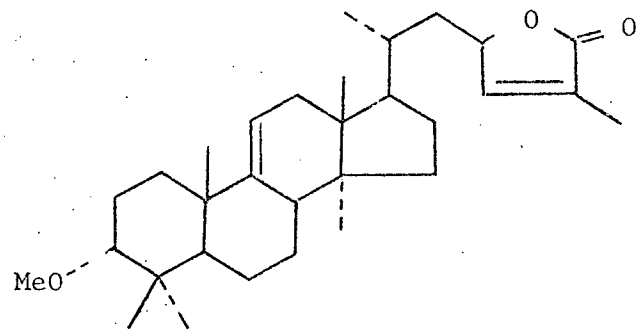
The originial investigators also report that abieslactone is stable to mineral acid. It is known that compounds such as lanost-7-en-3β-yl acetate[50], euph-7-en-3β-yl acetate[51], and tirucall-7-en-3β-yl acetate[52] that contain the double bond between C(7) and C(8) are readily converted into compounds having a double bond at C(8) - C(9). On the other hand, compounds like parkeyl acetate with a C(9) - C(11) double bond are stable to acid.

Furthermore, abieslactone was oxidized with chromium trioxide in aqueous acetic acid to a 8-ene-7,11-diketo derivative. This suggested to the original investigators that the C(9) - C(11) double bond was probable.

Before continuing with other evidence present in the original structural determination, some observations from the present study are in order. It was found in our work that under mild hydrogenation conditions abieslactone gave a dihydro derivative, and that under more rigorous conditions a tetrahydro derivative was formed. The oxidation of abieslactone with chromium trioxide gave a compound with the same properties as the 8-ene-7,11 diketone reported by the original investigators[28]. However, significantly different results were obtained upon treatment of abieslactone with acid.

Under anhydrous conditions, hydrogen chloride in chloroform isomerized abieslactone into a mixture of new products. The NMR spectrum of the product mixture had signals for the protons of the lactone ring indicating that the isomerization was not about that functionality. A new signal was observed for an olefinic proton at τ 4.8 with complete absence of the former olefinic proton signal at τ 4.4. In addition to the olefinic signal differences, the C-methyl groups now resonated over the range τ 8.94 to 9.34. The above NMR data is in good agreement with the values obtained for grandisolide (30) as mentioned previously.

Indeed, crystallization of the above product gave a white solid, m.p. 212 - 214°C, which was identical in every respect (mixed m.p., TLC, IR) with grandisolide (30). It was now certain that abieslactone can be isomerized with hydrogen chloride in chloroform into a C(9) - C(11) olefin.



30

If abieslactone is dissolved in acetic acid, it may be recovered unchanged with no changes in the NMR data or melting point being observed. However, dissolving abieslactone in one percent concentrated hydrochloric acid in acetic acid affected an isomerization but gave a different isomer ratio than before. In the previous isomerization there were two high field singlets at τ 9.30 and 9.34; in this case only one singlet at τ 9.30 was of appreciable intensity. As before the olefinic proton signal was non-integral. The NMR spectrum of the latter agrees with the calculated[39-41] NMR spectrum for a mixture of lanost-7-en-3α-ol and lanost-8-en-3α-ol.

Obtaining different isomer ratios with different acid is not unusual. In the Introduction it was seen that treatment of α-onocerin with protic acids gave β- and γ-onocerin.[21] Treatment of α-onocerin with a Lewis acid such as boron trifluoride gives γ-onocerin and serratenes.[20]

A cursory examination of the oxidation reaction of abieslactone with chromium trioxide seems to reveal no abnormalities. However, on closer examination this reaction is found to be atypical of C(9) - C(11) triterpene olefins. Oxidation of dihydroparkeyl acetate[49] or arborene[53] gives

the 9(11)-ene-12-keto derivative, none of the 8-ene-7,11-diketo derivative being reported. Compounds such as lanost-7-ene, lanost-8-ene, or lanosta-7,9(11)-diene can be oxidized[57,58] to the 8-ene-7,11-diketo derivative, but these systems were rejected by the original authors on the basis of previous arguments.

If one returns to the original arguments for the assignment of the double bond position, one must consider the degradation sequence described earlier. It is re-presented in tabular form in Figure 25. Also in Figure 25 the appropriate NMR data is listed and this will be discussed shortly.

In the degradation sequence a 3α-methoxylanostene was obtained which, on chromium trioxide oxidation, gave two products. The major product isolated was reported as a 9(11)-ene-12-keto derivative (63), while the minor product was a 8-ene-7,11-diketo derivative (62). It will be recalled that abieslactone gave only the 8-ene-7,11-diketo derivative. The observation that the methoxylanostene gives two products is inconsistent with the previous results. It also contradicts the precedents established for either 7-ene or 9(11)-ene systems.

The final piece of evidence presented for the C(9) - C(11) double bond is that the lanost-9(11)-en-3-one and lanost-9(11)-en-3β-yl acetate obtained from the degradation sequence were compared to authentic samples of those materials and found to be identical.

This result seems surprising since compounds like abieslactone and 3-keto abieslactone had spectral properties which were different from the observed or calculated values for C(9) - C(11) olefinic systems.

The NMR data for the compounds of the degradative sequence is presented

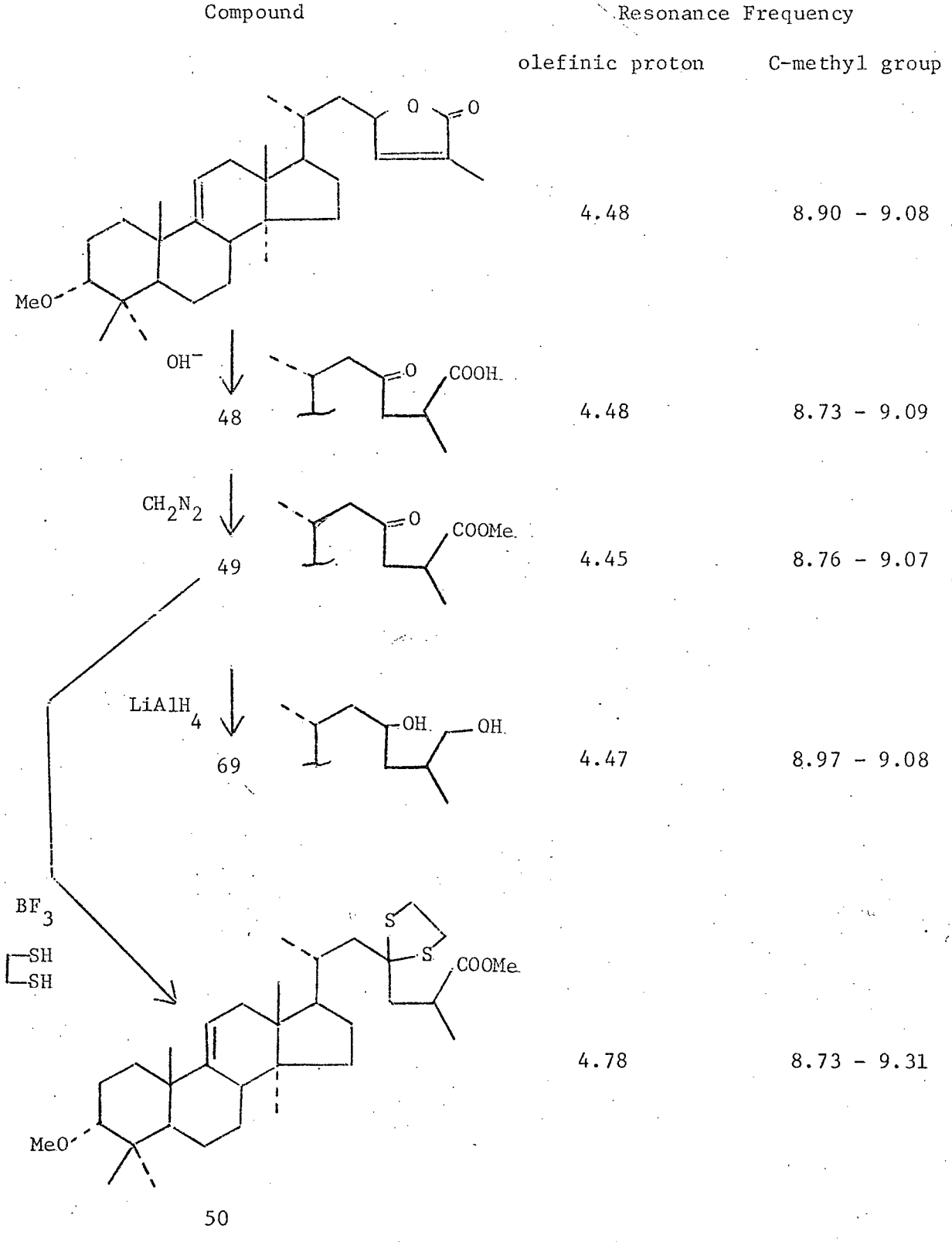


Figure 25. Degradation sequence of abieslactone.

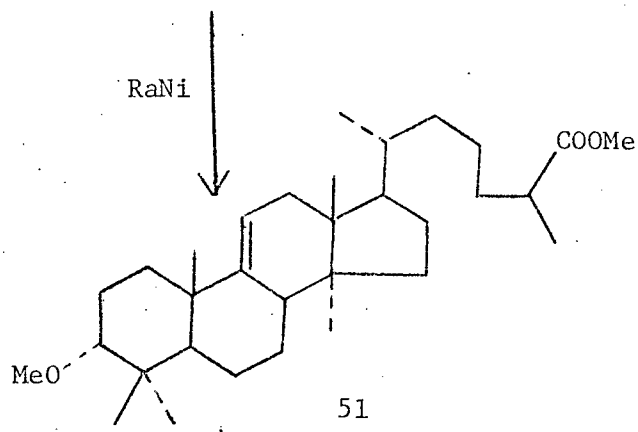
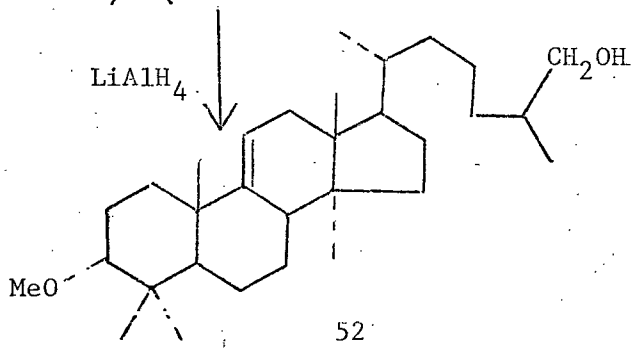
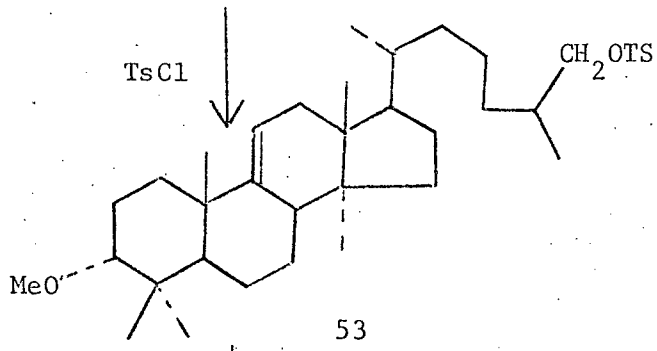
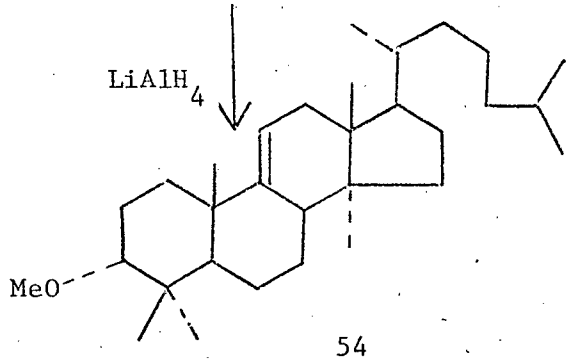
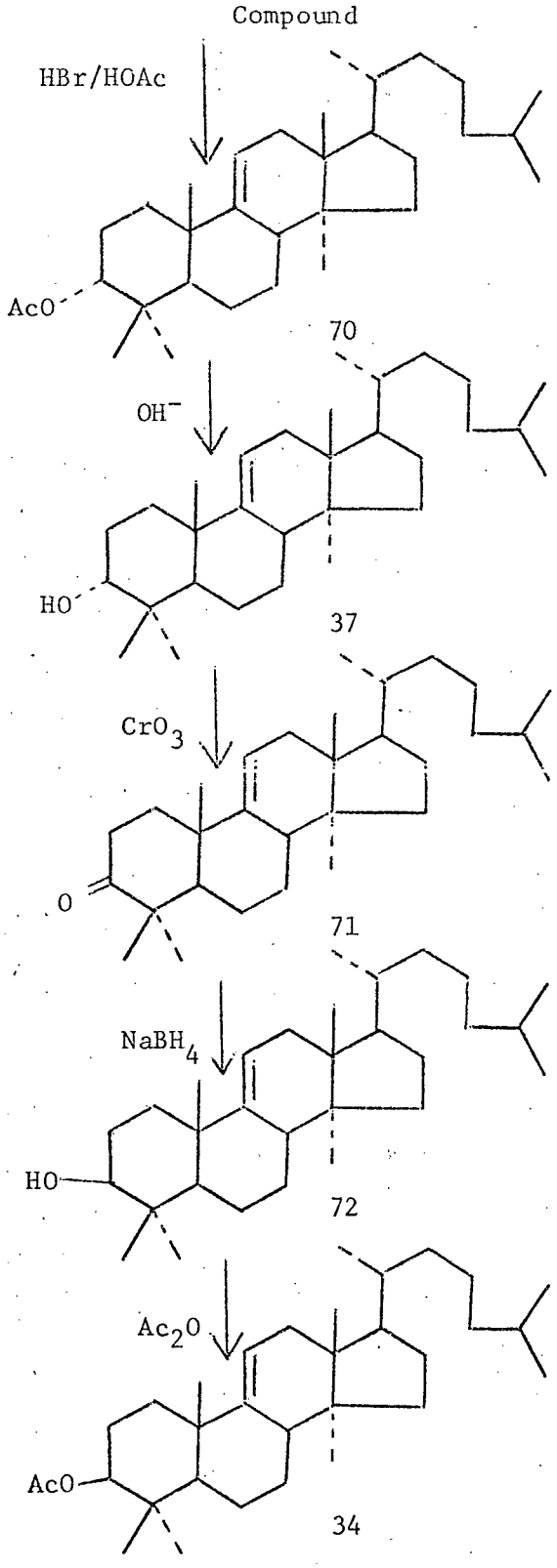
Compound	Resonance Frequency	
	olefinic proton	C-methyl group
 51	4.78	8.82 - 9.37
 52	4.77	8.93 - 9.35
 53		
 54	4.75	8.93 - 9.35

Figure 25. Degradation sequence of abieslactone (cont'd.).



Compound	Resonance Frequency	
	olefinic proton	C-methyl group
70	4.75	8.91 - 9.38
37	4.75	8.95 - 9.37
71	4.70	8.76 - 9.32
34	4.75	8.92 - 9.35

Figure 25. Degradation sequence of abieslactone (cont'd.).

in Figure 25. In the previous discussion the NMR data of abieslactone and keto acid (48), keto ester (49), and diol (69) has been described as anomalous for a 9(11)-ene system. The NMR data as presently reported for the thioketal (50) is in agreement with the "expected" NMR values of 9(11)-enes. The olefinic proton now absorbs at τ 4.78 in agreement with the position in other C(9) - C(11) olefins. The C-methyl groups range of absorption has an upper limit of τ 9.31, again in agreement with other C(9) - C(11) olefins. The NMR values for all products obtained later in the degradation sequence are also in agreement with the "expected" NMR results.

The dramatic changes observed in the NMR spectra of the thioketal and the subsequent compounds were not commented upon by the original investigators. The changes are definitely suggestive of a rearrangement reaction occurring during the preparation of the thioketal derivative to give the C(9) - C(11) olefin as the major isomer. This behavior parallels the observation reported earlier that hydrogen chloride in chloroform can convert abieslactone into a mixture of isomers. Indeed, if such a rearrangement did occur under the acidic conditions employed, the subsequent comparison of the degradation products with authentic samples of lanostene derivatives would not give direct evidence for the structure of abieslactone.

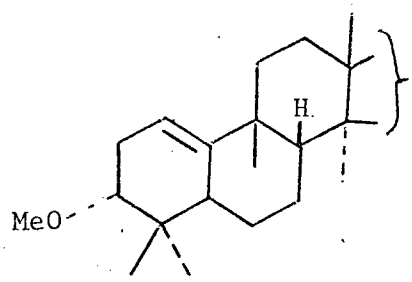
With this rearrangement in mind it is possible to explain the two products obtained in the oxidation of the methoxylanostene (54). Since the methoxylanostene is obtained in the later stages of the degradation sequence, that is, after the thioketal (50), it is likely a mixture of olefins. This mixture could easily explanin the oxidation products obtained.

Lack of sufficient quantities of abieslactone prevents further chemical work at this time on the structure of abieslactone. With the data presently available possible structures for abieslactone may be discussed.

Several factors must be taken into account for any proposed structure of abieslactone. The nuclear double bond must be trisubstituted. Further, cleavage of the double bond with osmium tetroxide/periodate gives a product containing lactone and aldehydo carbonyl groups as well as a ketonic carbonyl on an alicyclic system or a six member, or larger, ring. This requires that the double bond must <u>not</u> be exocyclic to a cyclopentane ring.

It is necessary that the proposed structure for abieslactone be able to rearrange to give a lanost-9(11)-ene derivative to account for the reported comparison with authentic lanostene derivatives. In keeping with other observed rearrangements of triterpenes or steroids, the migrating groups are usually axial and in a trans coplanar relationship. Keeping these requirements in mind, several possibilities may be presented.

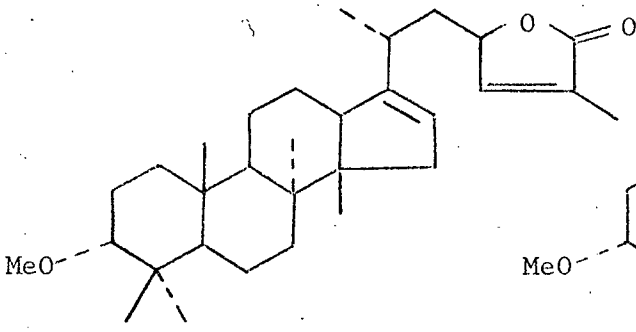
While it is possible to place the double bond at C(1) - C(10) as in partial structure 73, this feature is unlikely in view of the mass spectral



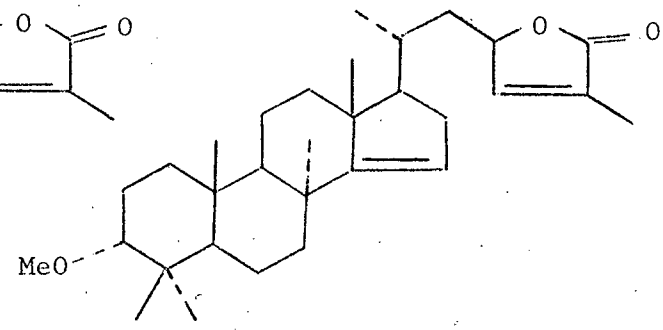
73

fragmentation. Ion e, mentioned previously, corresponding to loss of ring A would not be as feasible and, if it did occur, several migrations are needed before fragmentation to give an ion of correct elemental composition.

It is possible to place the double bond in ring D as in structures 74 and 75. Neither of these two structures can explain the facile loss of



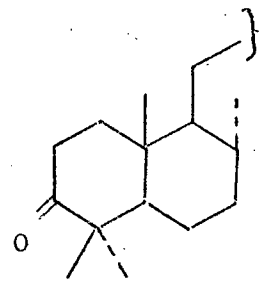
74



75

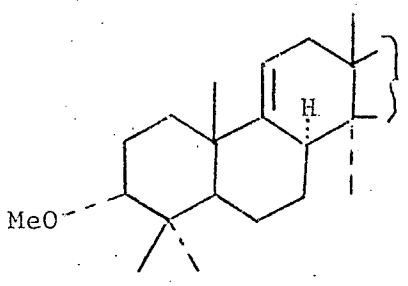
ring A in the mass spectrum of abieslactone unless rearrangement occurs before fragmentation. Structure 74 does not allow a rational explanation for the anomalies noted in the C-methyl group region of the NMR spectrum of abieslactone. Structure 75 on the other hand, has both the C(18) and C(32) methyl groups in a allylic position which may cause them to resonate at the lower field as observed.

The Cotton effect associated with the 3-ketone of 74 and 75 would be expected to be negative using the onocerin part structure 76 as the model.[59]



76

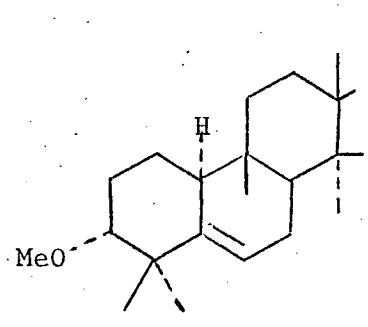
The double bond could be placed in ring C at C(9) - C(11) if the C(8) hydrogen is in the abnormal alpha configuration (structure 77). If C(8)



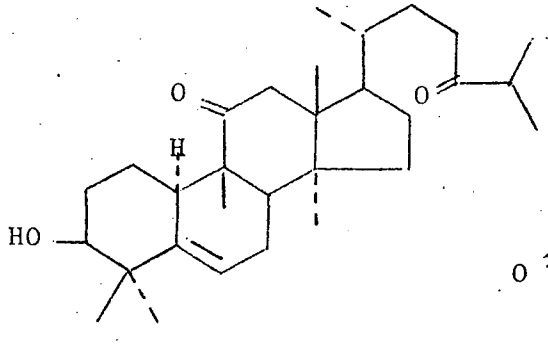
77

was beta, a lanost-9(11)-ene type system could be the result. It is known that such systems are stable to acidic reagents, that is, no double bond and/or no methyl group migrations occur. Also the Cotton effect associated with the 3-ketone of 77 would be expected to be negative based on the octant rule[60] and molecular models. The Cotton effect for the 3-keto derivative of abieslactone measured in this study is positive, so structure 77 is not likely on this basis.

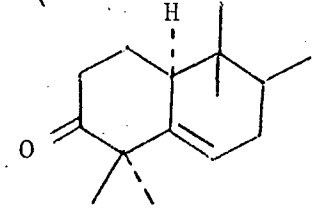
There are two positions in ring B for the double bond. It is possible to place it between C(5) - C(6) (structure 78) as is found in bryogenin (79).[61]



78

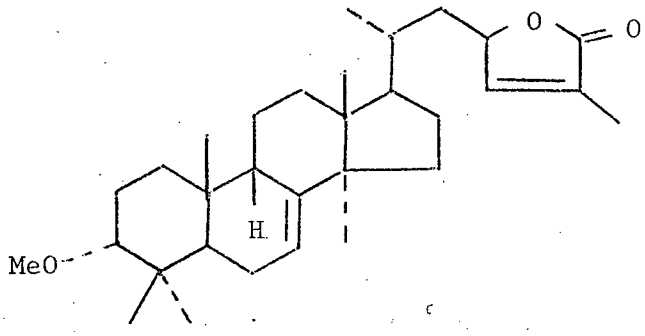


79



80

The Cotton effect associated with partial structure 80 is negative.[62] Similarly, the Cotton effect associated with the 3-keto derivative of 79 is negative, opposite to the observed Cotton effect for the 3-keto derivative of abieslactone. On this basis, structure 78 is an unlikely candidate.



81

The remaining possibility, 81, has a C(7) - C(8) double bond with the C(9) β hydrogen configuration. This latter assignment is necessary for two reasons: (a) if the C(9) α configuration prevailed, 81 would reveal chemical and physical properties characteristic of the lanost-7-ene system which is not the case with abieslactone; (b) in the isomerization from the 7-ene shown in 81 to the lanost-9(11)-ene system, the C(9) hydrogen in migrating to C(8) must remain on the β face of the molecule thereby requiring that the C(9) configuration is β in the original structure.

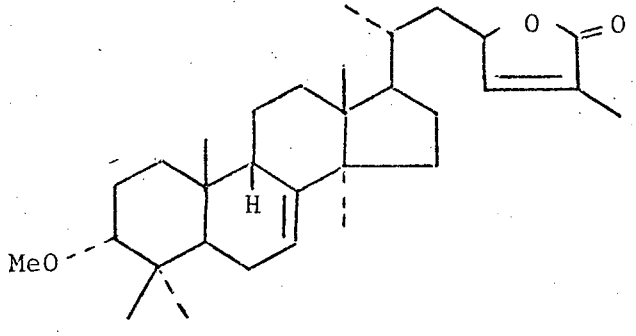
Structure 81 can also account for ion e in the mass spectrum of abieslactone since a retro Diels-Alder collapse of ring B would give an ion of correct composition without any further migrations being necessary.

Using molecular models and the octant rule, the 3-keto derivative of 81 should give a positive Cotton effect as is observed for the 3-keto derivative of abieslactone.

The observed NMR data of abieslactone is not as easily explained. The C(32) methyl group is allylic to the double bond and could be expected to resonate at lower field, however, this explanation does not account for the C(18) methyl signal unless its resonance frequency is altered by the C(9) β configuration proposed in 81. It is known that change of configuration at the ring junction may affect the resonance frequency of the angular methyl on the adjacent carbon.[63] The actual effect of the C(9) β configuration on the resonance frequencies is not known. Cycloartenyl acetate with the C(9) β configuration had the resonance frequencies of the C(18)/C(32) methyl groups assigned to signals at τ 9.10/9.03.[64] Some of this effect may be the result of deshielding by the cyclopropane but this again is not known.

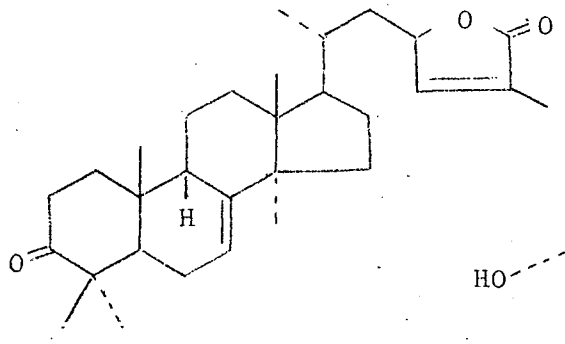
Chemically structure 81 is also favored, as oxidation with chromium trioxide could give the observed 8-ene-7,11-diketo system without rearrangement prior to oxidation.

X-ray analysis will be conducted on this molecule in order to settle the complete structure. At this time structure 81 would appear to be the best postulate for abieslactone which is then (23R)-3α-methoxy-9β-lanosta-7,24-diene-26,23-lactone.

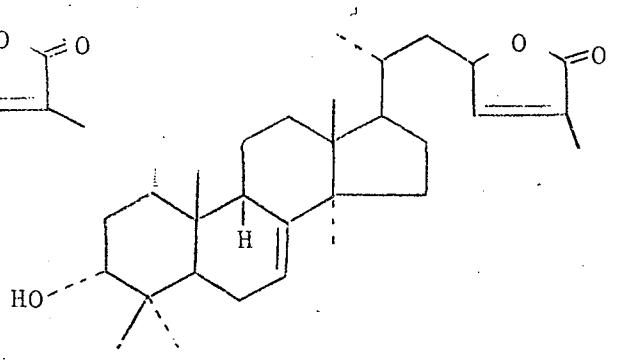


81

With the assignment of structure 81 to abieslactone, the structures of the two minor components isolated from Pacific silver fir may now be assigned. Compound AA_2, previously shown to be the 3-keto derivative of abieslactone, would be (23R)-3-oxo-9β-lanosta-7,24-diene-26,23-lactone (82). The other component, AA_3, was shown to be 3-desmethylabieslactone which may now be assigned as (23R)-3α-hydroxy-9β-lanosta-7,24-diene-26,23-lactone (83).



82



83

Experimental

Throughout this work Merck silica gel G with added fluorescent indicator was used as adsorbent in thin layer chromatography (TLC). The chromatograms, 0.3 mm. in thickness, were air dried and activated in an oven at 100°C for three hours. The chromatograms were developed in chloroform and sprayed with antimony pentachloride in carbon tetrachloride (1:2) unless otherwise noted.

For preparative layer chromatography a thicker layer (0.5 mm.) of adsorbent was utilized, with 0.01% Rhodamine 6G added as indicator.[34] Spraying with antimony pentachloride was done only along one edge or not at all as detection of bands was possible with ultraviolet light in most instances.

Column chromatography was performed on either Woelm silica gel or neutral alumina. The preferred adsorbent was deactivated alumina (Activity III) prepared by the addition of water as directed by the manufacturers. Except in larger scale work the solvents were distilled before use.

The nuclear magnetic resonance (NMR) spectra were measured in deuterochloroform at room temperature. The NMR spectra were obtained at either 60 MHz using a Jelco C-60, Varian A-60, or a Varian T-60 instrument or at 100 MHz using a Varian HA-100 instrument. The positions of all NMR resonances are given in the Tiers τ scale with tetramethylsilane as internal standard set at 10.0 units. For multiplets the τ values given represent

the center of the signal.

Mass spectra were measured on an Associated Electrical Industries MS 9 high resolution mass spectrometer or, where noted, on an Atlas CH 4 spectrometer. High resolution molecular weight determinations were determined on the MS 9 spectrometer.

Infrared (IR) spectra were measured on Perkin Elmer model 21, 137, or 457 instrument. The samples were usually measured as KBr pellets, however, some were measured in chloroform or neat. The positions of absorption maxima are quoted in wave numbers (cm^{-1}).

Ultraviolet (UV) absorptions were measured in methanol or ethanol on a Cary model 11 or model 15 spectrophotometer.

A Jasco model UV/ORD/CD 5 spectropolarimeter was used to measure the circular dichroism (CD) and optical rotatory dispersion (ORD) curves using dioxane as solvent.

Melting points were determined on a Kofler block and are uncorrected.

Elemental analyses were performed by Mr. P. Borda, University of British Columbia.

Isolation of triterpenes from Pacific silver fir

The bark of a Pacific silver fir growing in the University of British Columbia forest preserve near Haney, British Columbia was removed from the log and air dried. The dried bark was ground in a Wiley mill to pass through a 3 mm. sieve. The ground bark was extracted with chloroform for 18 hours in a large all glass Soxhlet extractor. The chloroform was evaporated to give a crude extract in a yield of 5.6% based on the weight of air dried bark extracted.

Ninety grams of crude extract were dissolved in 300 mls. of hot ether and left to cool whereupon 2.1 gms. of light brown powder could be collected by filtration. TLC of the light brown powder showed the presence of three compounds, the major one corresponded to an authentic sample of abieslactone received from Professor Uyeo.

Crystallization of triterpene mixture

The light brown powder (4.4 gms.) was dissolved in the minimum amount of refluxing ethyl acetate. The precipitate (2.2 gms.) was collected by filtration. Examination of the precipitate and the mother liquor by TLC showed very little, if any, enrichment of the desired abieslactone.

Column chromatography of triterpene mixture

The precipitate (2.2 gms.) was chromatographed on alumina (220 gms.). Elution with 50% benzene in petroleum ether (4500 mls.) gave abieslactone (1.5 gms.). Further elution with 50% methylene chloride in benzene (400 mls.) gave a compound coded AA_2 (75 mgs.). Elution with methylene chloride (800 mls.) gave a compound coded AA_3 (45 mgs.).

Abieslactone

Abieslactone obtained from the column was crystallized twice from ethyl acetate to give a white solid m.p. 251 - 253°C (literature m.p. 251 - 253°C[28]); mixed m.p. with authentic sample obtained from Professor Uyeo (Kyoto University, Kyoto) was 251 - 253°C. ORD (c, 0.0422) $[\phi]_{700}$ - 412°, $[\phi]_{589}$ - 520°, $[\phi]_{300}$ - 4,000°, $[\phi]_{250}$ - 12,900°, $[\phi]_{220}$ - 45,500°, $[\phi]_{216}$ - 26,600°. CD (c, 0.0422) $[\theta]_{265}$ 0°, $[\theta]_{260}$ + 225°, $[\theta]_{250}$ + 450°, $[\theta]_{240}$ + 290°, $[\theta]_{230}$ - 6,430°, $[\theta]_{220}$ - 45,050°. IR (KBr) 1745 (lactone carbonyl), 1660 (C=C). UV λ_{max}^{MeOH} 209 (log ε 4.30). NMR (100 MHz) 3.02 (1H, apparent triplet, J = 1.7 Hz, H-C=C-C=O), 4.48 (1H, multiplet, H-C=C),

5.05 (1H, multiplet, H-C-O), 6.72 (3H, singlet, OMe), 7.20 (1H, triplet, J = 1.8 Hz, equitorial H-C-OMe), 8.10 (3H, triplet, J = 1.7 Hz, vinylic methyl), and 8.90, 9.00, 9.06, 9.08 (6 C-methyls). Mass spectrum (m/e) 468 (M), 453 (M-15), 436 (M-32), 421 (M-47), and 314. (Found C, 79.50; H, 10.20; $C_{31}H_{48}O_3$ requires C, 79.44; H, 10.32%); high resolution: 468.357 $C_{31}H_{48}O_3$ requires 468.360, 453.333 $C_{30}H_{45}O_3$ requires 453.336, 436.330 $C_{36}H_{44}O_2$ requires 436.334, 421.310 $C_{29}H_{41}O_2$ requires 421.311, 393.278 $C_{27}H_{39}O_2$ requires 393.279, 339.232 $C_{23}H_{31}O_2$ requires 339.232, 314.224 $C_{21}H_{30}O_2$ requires 314.224, 299.203 $C_{20}H_{27}O_2$ requires 299.201, 272.177 $C_{18}H_{24}O_2$ requires 272.178, 233.155 $C_{15}H_{21}O_2$ requires 233.154, 175.149 $C_{13}H_{19}$ requires 175.149.

Isolation of AA_2

The fraction containing AA_2 from chromatography was crystallized from ethyl acetate to give a white solid m.p. 236 - 238°C. ORD (c, 0.0210) $[\phi]_{589}$ + 150°, $[\phi]_{400}$ + 262°, $[\phi]_{350}$ + 348°, $[\phi]_{330}$ + 701°, $[\phi]_{317}$ + 1485°, $[\phi]_{300}$ 0°, $[\phi]_{280}$ - 3,230°, $[\phi]_{260}$ - 4,950°, $[\phi]_{224}$ - 25,400°, $[\phi]_{220}$ - 18,950°. CD (c, 0.0210) $[\theta]_{330}$ 0°, $[\theta]_{325}$ + 106°, $[\theta]_{310}$ + 1,640°, $[\theta]_{294}$ + 3,160°, $[\theta]_{260}$ + 710°, $[\theta]_{250}$ + 781°, $[\theta]_{230}$ - 5,750°, $[\theta]_{215}$ - 35,510°, $[\theta]_{212}$ - 30,190°. IR (KBr) 1745 (lactone carbonyl), 1705 (ketone carbonyl). NMR (60 MHz) 3.00 (1H, triplet, J = 1.7 Hz, H-C=C-CO), 4.40 (1H, multiplet, H-C=C), 5.02 (1H, multiplet, H-C-O), 7.45 (2H, quartet, $-CH_2-CO$), 8.10 (3H, triplet, J = 1.7 Hz, vinylic methyl), 8.90, 8.98, 9.18 (6 C-methyls). Mass spectrum (m/e) 452 (M), 437 (M-15), and 314. (Found C, 79.52; H, 9.70; $C_{30}H_{44}O_3$ requires C, 79.64; H, 9.73%).

Isolation of AA_3

The fraction containing AA_3 from chromatography was crystallized from ethyl acetate to give a white solid m.p. 249 - 250°C. IR (KBr) 3520 (OH), 1760,1745 (lactone carbonyl); ($CHCl_3$) 1755 (lactone carbonyl). NMR (60 MHz) 3.02 (1H, triplet), 4.48 (1H, multiplet), 5.02 (1H, multiplet), 6.6 (1H, multiplet, H-COH), 8.10 (3H, triplet), 8.98, 9.00, 9.03, 9.06 (6 C-methyls). Mass spectrum (m/e) 454 (M), 439 (M-15), 436 (M-18), and 314. (Found C, 79.11; H, 10.07; $C_{30}H_{46}O_3$ requires C, 79.25; H, 10.20%).

8-ene-7,11-diketo derivative of abieslactone

Chromium trioxide (230 mgs.) in 90% acetic acid (10 mls.) was added slowly into a solution of abieslactone (258 mgs.) in hot acetic acid (40 mls.) at 55°C. Stirring was continued at 60°C for 4.5 hours and then the solvent was evaporated in vacuo. The residue was dissolved in ether, washed with aqueous sodium carbonate and water, and dried over sodium sulfate. Evaporation of the ether gave a yellow solid (300 mgs.) that was chromatographed on silica gel (15 gms.). Elution with chloroform gave a yellow solid (200 mgs.). Crystallization from methanol gave a yellow solid m.p. 210 - 215°C. Re-chromatography of the crystalline product on silica gel (10 gms.) gave the 8-ene-7,11-diketo derivative (58) as a yellow solid which was twice crystallized from methanol, m.p. 217 - 219°C (literature m.p. 215 - 216°C[28]). IR (KBr) 1740 (lactone carbonyl), 1678 (ketone carbonyl). UV λ_{max}^{MeOH} 274 mμ (log ε 3.85). NMR (60 MHz) 3.02 (1H, triplet, J = 1.7 Hz), 5.05 (1H, multiplet), 6.72 (3H, singlet, OMe), 8.10 (3H, triplet, J = 1.7 Hz, vinylic methyl), and 8.69, 8.83, 9.05, 9.18 (6 C-methyls).

Dihydroabieslactone (44)

Abieslactone (50 mgs.) in tetrahydrofuran (15 mls.) was hydrogenated over 10% palladium on charcoal (50 mgs.) at room temperature for 2 hours. The catalyst was removed by filtration and the filtrate evaporated to give a white solid. Crystallization from ethyl acetate gave a white solid m.p. 216 - 218°C (literature m.p. 219 - 221°C[28]). IR (KBr) 1770 (lactone carbonyl); ($CHCl_3$) 1765 (lactone carbonyl). NMR (60 MHz) 4.47 (1H, multiplet, H-C=C), 5.53 (1H, multiplet, H-C-O), 6.72 (3H, singlet, OMe), 7.20 (1H, triplet, J = 1.7 Hz, equatorial H-C-OMe), 8.74 (3H, doublet, J = 6.3 Hz, CH_3-CH=CO), and 8.96, 9.00, 9.06, 9.08 (6 C-methyls). Mass spectrum (m/e) 470 (M), 455 (M-15), 438 (M-32), 423 (M-47) and 316. (Found C, 78.95; H, 10.66; $C_{31}H_{50}O_3$ requires C, 79.10; H, 10.71%).

Tetrahydroabieslactone (45)

Abieslactone (50 mgs.) in acetic acid (30 mls.) was hydrogenated over Adams catalyst (15 mgs.) at room temperature for 40 hours. The catalyst was filtered off and the solvent evaporated to give a white solid. Crystallization from methylene chloride - methanol then hexane gave a white solid m.p. 226 - 228°C (literature m.p. 230 - 231°C[28]). IR (KBr) 1770 (lactone carbonyl). NMR (60 MHz) 5.50 (1H, multiplet, H-C-O), 6.72 (3H, singlet, OMe), 7.20 (1H, triplet, J = 1.7 Hz, H-C-OMe), and 8.98, 9.03, 9.10, 9.15 (6 C-methyls). Mass spectrum (m/e) 472 (M), 457 (M-15), 440 (M-32), 425 (M-47). (Found C, 78.84; H, 11.18; $C_{31}H_{52}O_3$ requires C, 78.76; H, 11.09%).

Lithium aluminum hydride reduction of dihydroabieslactone

Dihydroabieslactone (25 mgs.) in tetrahydrofuran (10 mls.) was stirred

with lithium aluminum hydride (10 mgs.) for 18 hours. A small amount of water was added and the solvent was evaporated. The residue was carefully acidified with dilute hydrochloric acid and extracted with ether. The extract was washed with water, dried (sodium sulfate) and evaporated to give a white residue. Column chromatography on alumina (1 gm.) of the residue and elution with ether gave the diol (69); crystallization from petroleum ether - ether gave white needles m.p. 161 - 162°C (literature m.p. 161 - 163°C[28]). IR (KBr) 3350 (OH). NMR (60 MHz) 4.47 (1H, multiplet, H-C=C), and 8.97, 9.00, 9.05, 9.08 (6 C-methyls). Mass spectrum (m/e) 474 (M), 459 (M-15), 456 (M-18), 442 (M-32), 438 (M-36). (Found C, 78.36; H, 11.36; $C_{31}H_{54}O_3$ requires C, 78.43; H, 11.46%).

Methylation of AA_3

AA_3 (8 mgs.) was dissolved in methylene chloride (5 mls.) and cooled thoroughly in an ice bath. A few mls. of ethereal diazomethane was added followed by a catalytic amount of dry aluminum chloride. Fresh diazomethane solution was added over a period of 3 hours to maintain a yellow color in the solution. The excess diazomethane was destroyed with a drop of dilute acetic acid. The solution was filtered and the filtrate washed with water and dried (sodium sulfate); evaporation gave a solid (15 mgs.) which was purified by preparative layer chromatography. The band with R_f 0.40 was collected. NMR of this band showed methoxy (6.72) and olefinic proton (4.48) as in abieslactone. Crystallization from ethyl acetate gave a white solid m.p. 249 - 250°C, mixed m.p. with abieslactone 249 - 250°C. IR was superimposable with that of abieslactone.

Oxidation of AA_3

AA_3 (20 mgs.) in dry pyridine (3 mls.) and chromium trioxide (15 mgs.) were stirred at room temperature for 3 days. The solvent was removed in vacuo and the residue dissolved in methylene chloride and washed with water, dried (sodium sulfate), and evaporated. Crystallization from ethyl acetate gave a white solid m.p. 236 - 238°C, mixed m.p. with AA_2 236 - 238°C. IR and TLC were identical with those of AA_2.

Dihydro AA_2

AA_2 (30 mgs.) was dissolved in tetrahydrofuran (10 mls.) and hydrogenated over 10% palladium on charcoal (15 mgs.) for 2 hours. The catalyst was removed by filtration and filtrate evaporated. Crystallization from ethyl acetate gave a white solid m.p. 223 - 225°C. ORD (c, 0.0208) $[\phi]_{700}$ + 283°, $[\phi]_{589}$ + 388°, $[\phi]_{350}$ + 1,440°, $[\phi]_{315}$ + 3,055°, $[\phi]_{285}$ 0°, $[\phi]_{275}$ - 392°, $[\phi]_{265}$ 0°, $[\phi]_{220}$ + 6,110°, $[\phi]_{218}$ + 4,360°. CD (c, 0.0208) $[\theta]_{330}$ 0°, $[\theta]_{294}$ + 3,170°, $[\theta]_{250}$ + 360°, $[\theta]_{220}$ + 12,960°. IR ($CHCl_3$) 1760 (lactone carbonyl). NMR (60 MHz) 4.40 (1H, multiplet), 5.50 (1H, multiplet), 8.73 (3H, doublet), and 8.96, 9.00, 9.02, 9.20 (7 C-methyls). (Found C, 79.04; H, 10.32; $C_{30}H_{46}O_3$ requires C, 79.25; H, 10.20%).

Treatment of abieslactone with acetic acid

Abieslactone (25 mgs.) was dissolved in acetic acid (20 mls.) and heated at 50°C for 4 hours. The solvent was evaporated and the residue dissolved in methylene chloride and washed with water, sodium bicarbonate solution and dried (sodium sulfate). Evaporation and crystallization gave a white solid m.p. 250 - 252°C, mixed m.p. with abieslactone 250 - 252°C. The NMR spectrum was the same as that of abieslactone.

Treatment of abieslactone with 1% concentrated hydrochloric acid in acetic acid

Abieslactone (20 mgs.) was dissolved in 1% concentrated hydrochloric acid in acetic acid (by volume) (15 mls.) and heated at 50°C for 2 hours. The solvent was evaporated and the residue dissolved in methylene chloride and washed with water, sodium bicarbonate solution and dried (sodium sulfate). Evaporation gave a white solid. NMR (60 MHz) 3.02 (1H, triplet, J = 1.7 Hz), 4.81 (non-integral, multiplet, H-C=C), 5.05 (1H, multiplet, H-C-O), 6.72 (3H, singlet, OMe), 7.20 (1H, triplet, J = 1.7 Hz, equitorial H-C-OMe), 8.10 (3H, triplet, vinylic methyl), and 8.94, 9.00, 9.05, 9.07, 9.12, 9.30 (6 C-methyls).

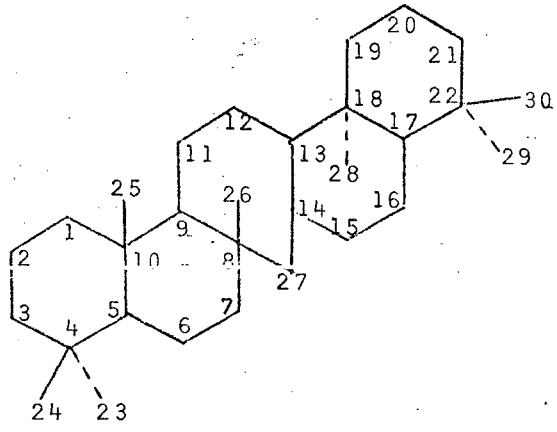
Cleavage of double bond in dihydroabieslactone

Dihydroabieslactone (30 mgs.) was dissolved in ether (5 mls.) containing pyridine (0.2 mls.) and osmium tetroxide (50 mgs.) was added. The solution was left for 10 days at room temperature. The solution was then saturated with hydrogen sulfide gas and the black solution filtered through celite with thorough washing of the filter cake with chloroform. The filtrate was evaporated first on a rotatory evaporator under reduced pressure and later with a mechanical pump. The residue was dissolved in ether (5 mls.) and periodic acid (30 mgs.) was added. The solution was stirred for 18 hours before being extracted with water and aqueous sodium bicarbonate solution. The ethereal layer was dried over sodium sulfate and evaporated to dryness. IR ($CHCl_3$) 1760 (lactone carbonyl), 1720 (aldehydo carbonyl), 1705 (ketone carbonyl).

Structural Studies on W_4 from Western White Spruce

Discussion

It was known from earlier work in our laboratories[25,26] that triterpenes of the serratane family (84) were constituents in at least one species of the genus Picea (spruce). The presence or absence of these novel triterpenes in other species of the same genus would be of chemotaxonomic interest.



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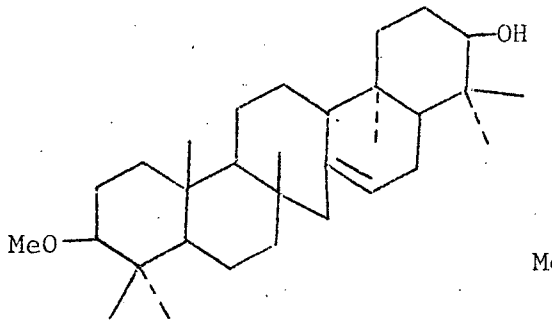
The two major triterpenes which had been isolated from Sitka spruce [Picea sitchensis][25] were 3β-methoxyserrat-14-en-21β-ol (24) and the corresponding 3α-methoxy isomer (25). The minor constituents of the bark included the first reported[25] isolation of the double bond isomer, 3α-methoxyserrat-13-en-21β-ol (28). With authentic samples of these and other serratenes available for comparison, the study of Western white spruce [P. glauca (Moench) Voss. var. albertiana (S. Brown) Sarg.] and Engelmann spruce [P. engelmannii Parry] was undertaken.



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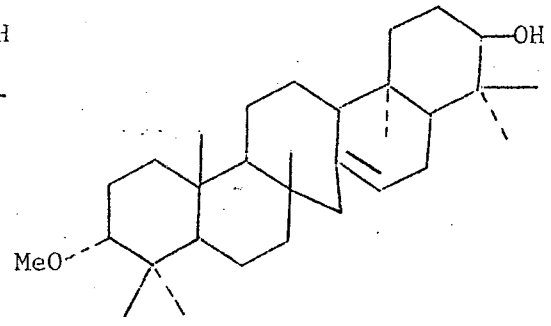

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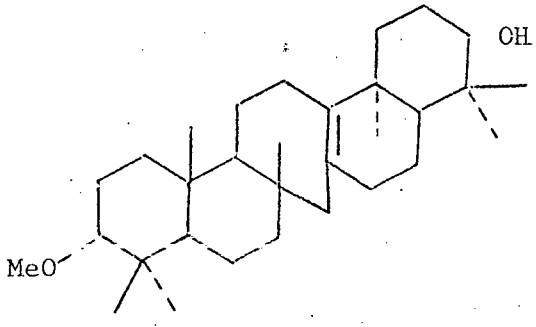

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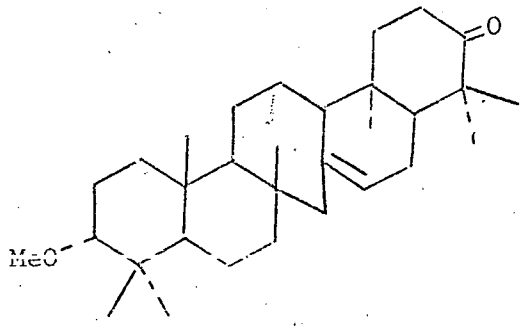

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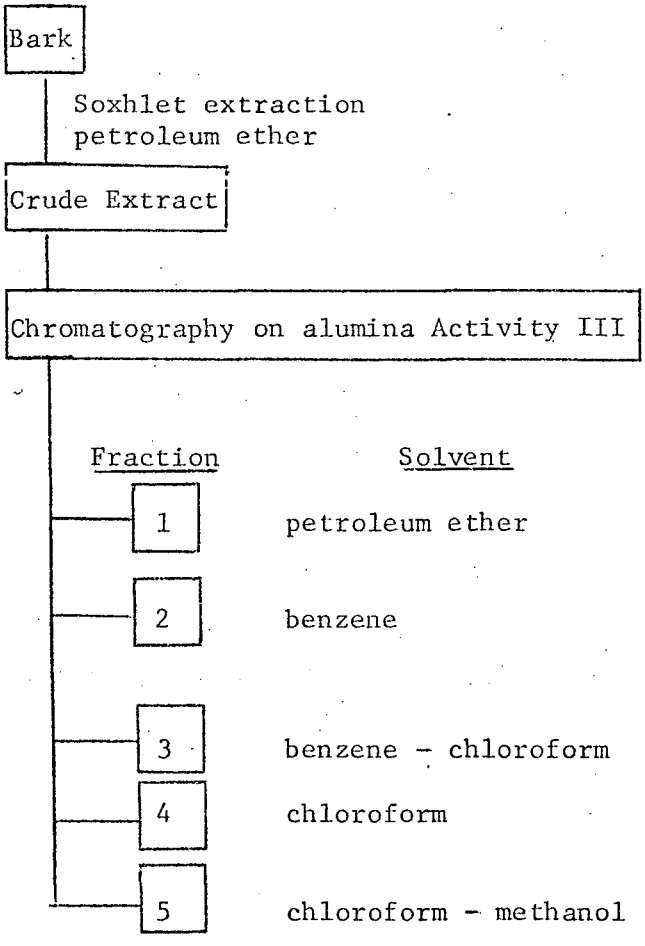
Earlier investigation of the bark extracts of Western white spruce had been conducted by Drs. Gletsos and Gladstone in these laboratories.[65] By a combination of column and thin layer chromatography, they had succeeded in isolating three triterpenes, all found previously in Sitka spruce. The first compound was found to be identical to 3α-methoxyserrat-14-en-21β-ol (25). The second compound was shown to be identical to the corresponding ketone, 3α-methoxyserrat-14-en-21-one (26). The third compound was epimeric with the first and was shown to be 3β-methoxyserrat-14-en-21β-ol (24).

A fourth triterpene code named W_4, a ketone, was isolated but was of undetermined structure.

The present work describes the isolation and structural studies of this new triterpene.

The ground, air dried bark of Western white spruce was extracted in a Soxhlet extractor with petroleum ether. Evaporation of the petroleum ether solution left a crude extract as a brown gummy solid.

The crude extract was chromatographed on a large column of alumina (Figure 26). The first fraction eluted was evaporated to give a yellowish,

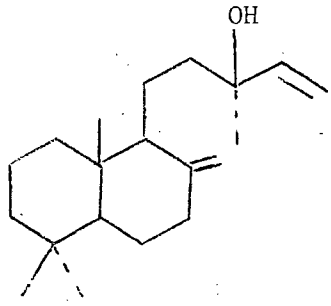


Fraction	Solvent	Compounds
1	petroleum ether	hydrocarbons, sterol and wax esters
2	benzene	3α-methoxyserrat-14-en-21β-ol, 3α-methoxyserrat-14-en-21-one, W_4, and epimanool
3	benzene - chloroform	fatty alcohol, fatty ester
4	chloroform	3β-methoxyserrat-14-en-21β-ol and β-sitosterol
5	chloroform - methanol	unidentified polar components

Figure 26. Typical purification sequence of components from Western white spruce bark.

low melting wax. The TLC properties of this fraction suggested that it was non-polar and did not contain any of the desired W_4.

The second fraction was eluted with benzene and removal of solvent gave a gummy solid. TLC analysis showed that this fraction contained W_4 plus two of the other known triterpenes, 3α-methoxyserrat-14-en-21β-ol (25) and 3α-methoxyserrat-14-en-3-one (26). In addition a fourth compound was present whose TLC behavior was like that of epimanool (29) or manool (31).



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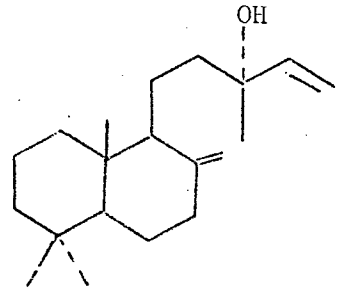

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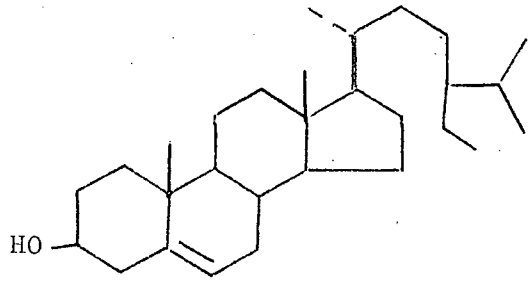
The third fraction was eluted with benzene - chloroform. This fraction appeared to be mainly fatty ester, fatty alcohol.

The fourth fraction eluted with chloroform gave a solid upon evaporation. TLC investigation of this fraction using two different solvent systems revealed the presence of β-sitosterol (1) and 3β-methoxyserrat-14-en-21β-ol (24) by comparison with authentic samples.



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The last fraction consisted of chloroform - methanol and methanol washings of the column. TLC investigation showed few distinct spots but showed that the fraction contained mainly polar compounds.

Fraction 2 from the above chromatography was chromatographed on Activity I alumina (Figure 27). Elution with benzene gave a fraction 6 containing W_4 and the known 3α-methoxyserrat-14-en-21-one (26). Further elution with chloroform (Fraction 7) gave manool or epimanool plus 3α-methoxyserrat-14-en-21β-ol.

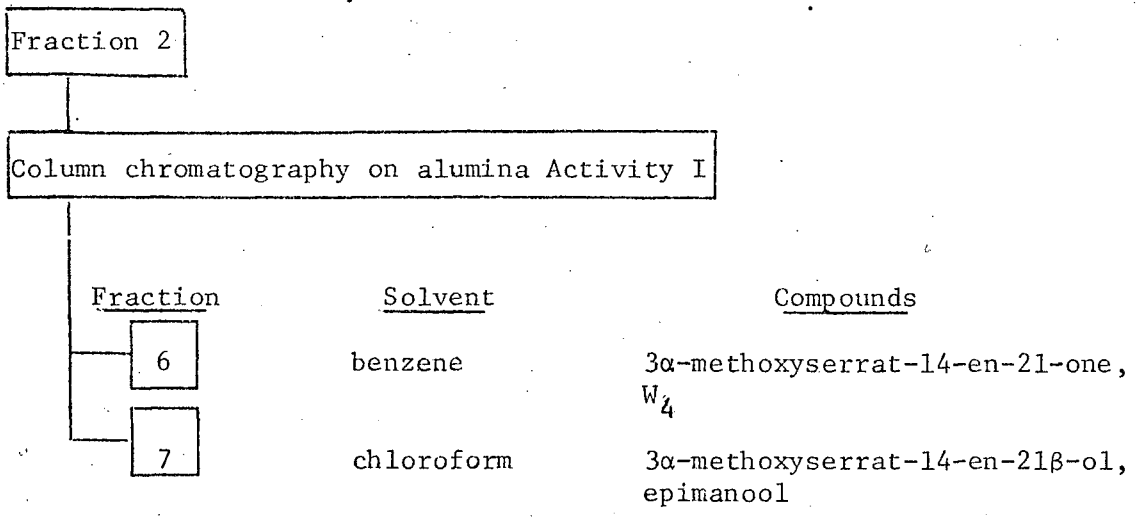


Figure 27. Purification of Fraction 2.

The ketone fraction (6) was chromatographed on alumina (Figure 28). Initial elution with petroleum ether gave a fraction (8) which had TLC and NMR properties characteristic of a fatty ester. Further elution with petroleum ether - benzene gave Fraction 9 whose TLC showed the presence of 3α-methoxyserrat-14-en-21-one by comparison with an authentic sample. Continued elution with the same solvent gave Fraction 10

containing an unidentified oily component, which on TLC analysis was free of the desired W_4.

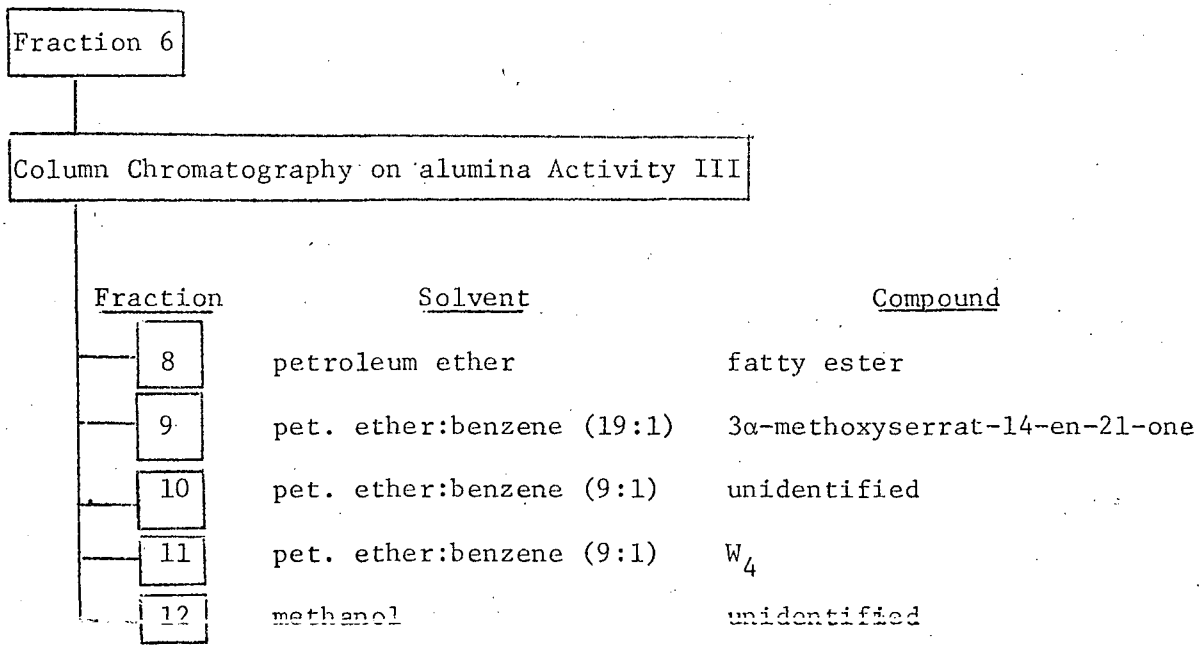


Figure 28. Purification of Fraction 6.

Continued elution with petroleum ether:benzene (19:1) gave Fraction 11 containing W_4. A methanol wash of the column gave only base line material (Fraction 12) when examined by TLC.

The initial NMR spectrum of Fraction 11 (m.p. 190 - 225°C) containing W_4 showed the resonance for an O-methyl group at τ 6.65 but it had a slight shoulder to it. When this region was examined on an expanded scale one large O-methyl resonance was seen plus a small signal. Several recrystallizations of a portion of this fraction provided a crystalline product (m.p. 200 - 230°C) which, upon sublimation, provided further purification (m.p. 215 - 225°C). Finally, additional crystallizations

from ethanol and then sublimation gave an analytical sample as a white solid, m.p. 227 - 229°C. Elemental analysis and high resolution mass spectrometry of this material established the molecular formula $C_{31}H_{50}O_2$.

The NMR spectrum (Figure 29) of this compound had resonances for an O-methyl group (τ 6.65); a one proton quartet (τ 7.30, J = 5 and 11 Hz); a two proton quartet (τ 7.55, J = 6 and 8 Hz); and signals at τ 8.92, 8.99, 9.02, 9.12, 9.16, 9.17, and 9.23 integrating for seven methyl groups. The τ 7.30 quartet was assigned to an axial proton geminal to the O-methyl group. This assignment was based on the coupling constants Jaa = 11 Hz and Jae = 5 Hz. Similar coupling was observed in 3β-methoxyserratene derivatives previously isolated in our laboratories.[25]

The IR spectrum of W_4 had absorption at 1700 cm^{-1} (C=O), 1665 cm^{-1} (C=C), and 1100 cm^{-1} (O-Me). The ORD curve (Figure 30) showed a peak at 306 mμ ([φ] = +8800°) and a trough at 276 mμ ([φ] = +2160°).

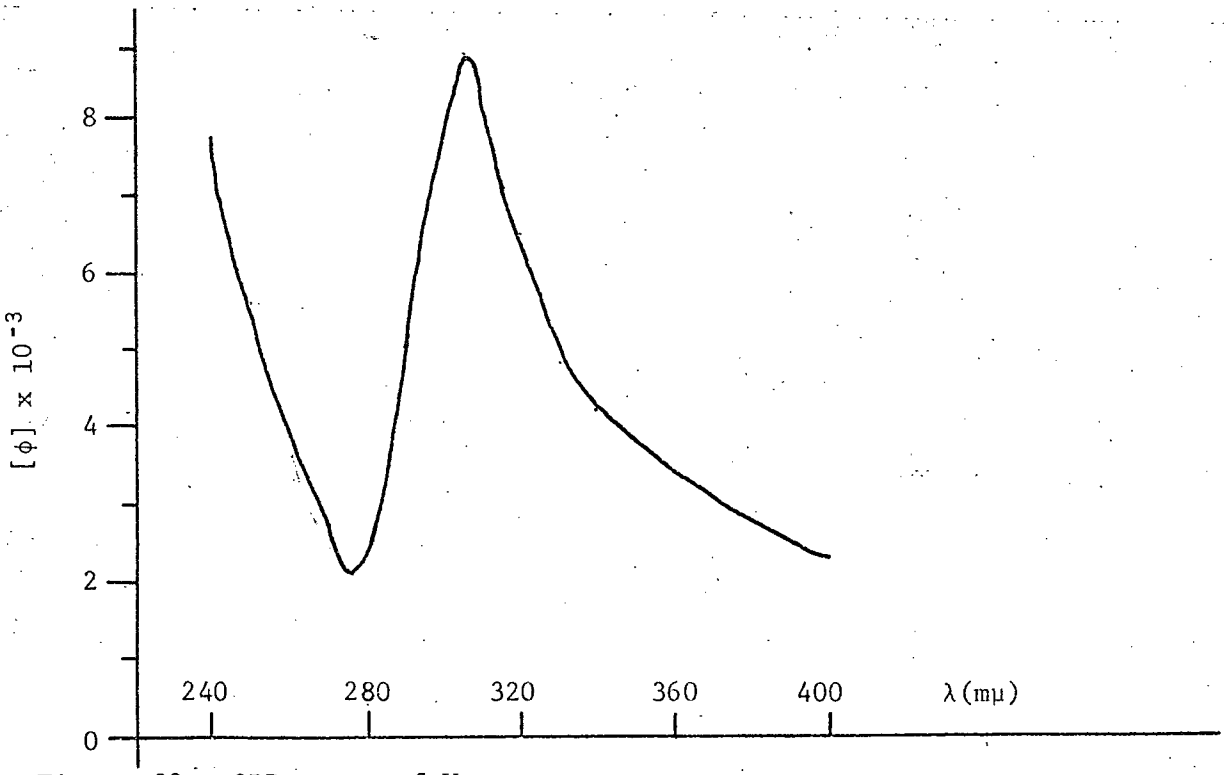


Figure 30. ORD curve of W_4.

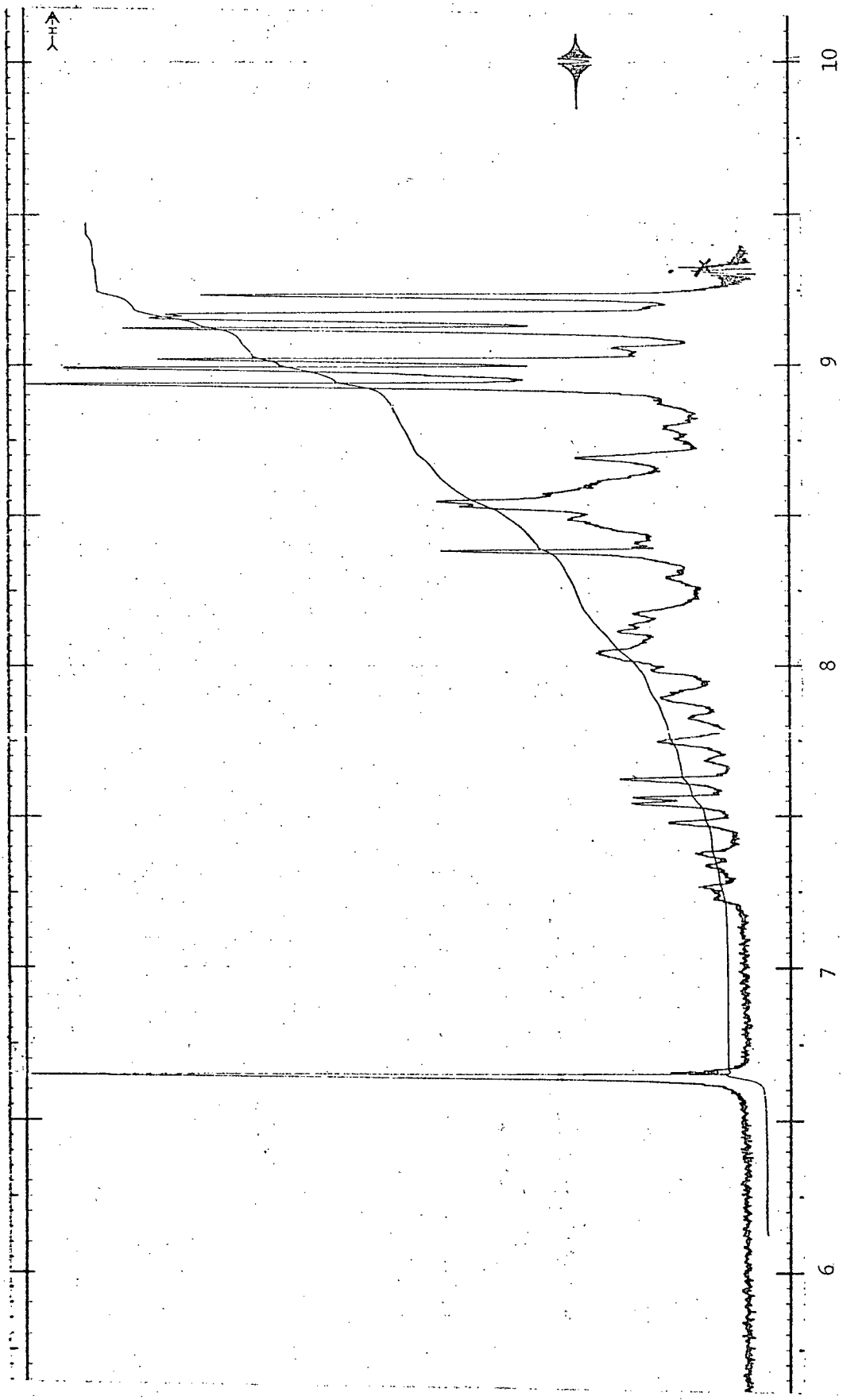
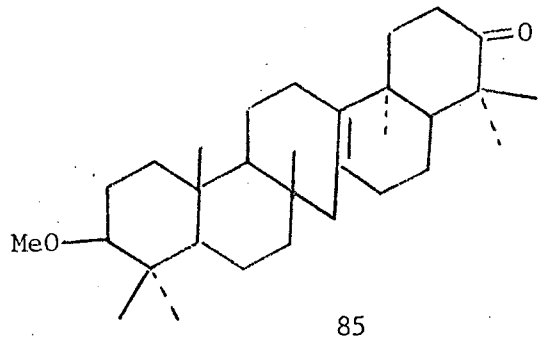


Figure 29. NMR spectrum of W_4.

The mass spectrum showed a molecular ion at m/e 454. Other strong ion peaks at m/e 439, 422, and 407 were assigned to loss of methyl, methanol, and both methyl and methanol from the molecular ion. Weaker ions were found at m/e 221, 203, and 189.

A priori this spectral data suggested a possible structure for W_4. The occurrence of only seven C-methyl groups in the NMR is typical of the serratane family of triterpenes which was known to occur in the same genus. The degree of unsaturation is seven, five in the pentacyclic skeleton, one in the carbonyl; the remaining degree of unsaturation would then be present as a tetrasubstituted double bond since no olefinic protons were observed in the NMR spectrum. The natural occurrence of another serrat-13-ene derivative had been reported from our laboratories earlier,[25] so the double bond was tentatively assigned this position in a serratene skeleton. The ORD characteristics of both serrat-13-en-3-one and serrat-13-en-21-one were known[19] and the ORD curve of W_4 was of the same shape as the ORD curve reported[19] for a serrat-13-en-21-one derivative. The O-methyl ether function was on a secondary carbon atom since there was only one proton geminal to it and its most probable location was at C(3). With these results in hand, the postulated structure of W_4 would then be 3β-methoxy-serrat-13-en-21-one (85). This compound had not been previously reported



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but was accessible from known serrat-14-ene derivatives.

When Inubushi[19] was investigating the chemistry of the serrat-14-enes, he found that by treatment with acid the corresponding serrat-13-enes were produced. In the case of the 21-ketones, the formation of the serrat-13-enes was very nearly quantitative. The desired compound for isomerization, 3β-methoxyserrat-14-en-21-one, had been isolated[25] in small amounts but the corresponding 21β-hydroxy compound was more abundant both in this study and in previous work on Sitka spruce.[25]

Thus the ketone obtained from the Jones oxidation of 3β-methoxyserrat-14-en-21β-ol was subjected to treatment by a mixture of sulphuric acid and acetic acid at room temperature[19] to give a new ketone, m.p. 241 - 243°C. The NMR spectrum of the new product showed an O-methyl signal at τ 6.67 and seven C-methyl groups (τ 8.90, 8.96, 9.05, 9.07, 9.16, 9.23, 9.26). There was no NMR signal for the olefinic proton suggesting complete isomerization of the olefinic linkage to the tetrasubstituted position. The IR spectrum had bands at 1700 cm^{-1} (C=O) and 1100 cm^{-1} (OMe), while the ORD curve had a peak at 304 mμ ([φ] = +6230°) and a trough at 270 mμ ([φ] = +2035°). The mass spectrum had a molecular ion at m/e 454 with peaks of low intensity at m/e 439, 422, and 407 for loss of methyl, methanol, and both methyl and methanol from the molecular ion. Reasonably intense peaks were also seen at m/e 221, 203, and 189.

The above data was entirely consistent with the expected oxidation and isomerization product, 3β-methoxyserrat-13-en-21-one (85). It will be remembered that the data for W_4 also suggested that structure. However, when both sets of data are compared significant differences exist. The

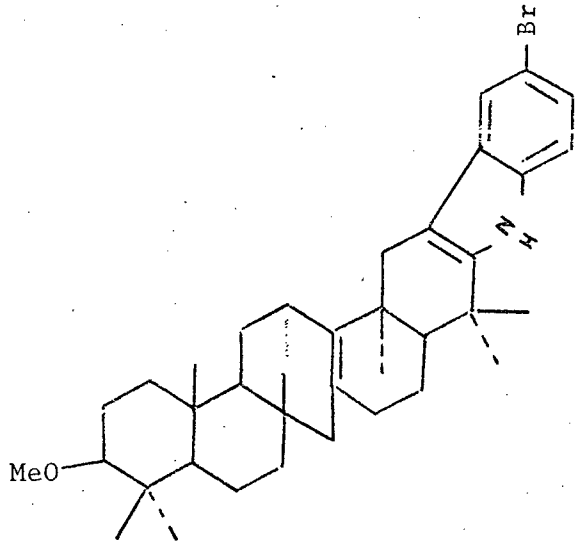
positions of the C-methyl groups in the NMR spectrum of each compound were different. The ORD curve of each compound, while of the same shape, always had W_4 with greater intensity. In addition, the IR spectrum of W_4 had a C=C stretching frequency at 1665 cm^{-1} while the synthetic compound did not have this absorption. The mass spectra had similar fragmentations but differed significantly in intensities.

With two different compounds, both of which could be assigned the same structure on evidence obtained so far, the problem became more difficult. Skeletal rearrangements or methyl migrations, both well known processes in triterpene chemistry, could not be readily excluded in the double bond isomerization reaction. In fact, it was possible that W_4 had the proposed structure while the acid isomerized product had suffered more changes in structure than suspected.

The most conclusive way to determine each structure would be by X-ray analysis. Since each was a ketone, the derivative selected was a p-bromophenylhydrazone. Each ketone was individually reacted with p-bromophenylhydrazine hydrochloride in ethanol containing acetic acid. The synthetic ketone was reacted first and the derivative was crystallized from ethanol as pale yellow needles, m.p. 204 - 207°C (dec.). Ultraviolet absorption at 233, 293, and 302 mμ suggested that the expected hydrazone had reacted further to give a bromoindole derivative. The elemental formula, $C_{37}H_{52}NOBr \cdot C_2H_5OH$, determined by elemental analysis indicated that one molecule of ethanol was associated with each molecule of the bromoindole derivative in the crystal.

The X-ray analysis of the crystalline bromoindole derivative was

performed by Dr. F.H. Allen of this department.[66] The crystals were orthorhombic, $\underline{a}$ = 10.20, $\underline{b}$ = 11.12, $\underline{c}$ = 31.23 Å, space group $P2_12_12_1$, with four formula units of $C_{37}H_{52}NOBr \cdot C_2H_5OH$ in the unit cell. The intensities of 2105 reflections with $2\theta \leq 100°$ were measured on a Datex-automated GE XRD6 diffractometer using CuKα radiation and a $\theta - 2\theta$ scan. The structure 86 was determined for the bromoindole using Patterson and Fourier techniques together with careful restrained least-squares refinement of portions of the structure as they emerged from the electron density map, the final $\underline{R}$ being 0.086 for 1459 observed reflections. The absolute configuration depicted in 86 was determined by the X-ray fluorescence technique[76].



86

The X-ray analysis showed that the bromoindole had been formed in the reaction. More important to the present study was the fact that the parent ketone was indeed the expected 3β-methoxyserrat-13-en-21-one (85) and no skeletal rearrangements had occurred.

In the crystalline state the X-ray analysis showed that the seven

member ring C in 86 adopts a chair conformation with some out-of-plane distortions about the C(13) - C(14) olefinic bond. The plane through the terpenoid nucleus is slightly concave to minimize the methyl - methyl interactions, a common feature in steroidal systems[67].

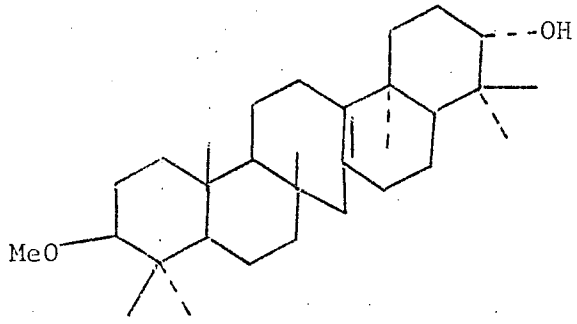
When W_4 was reacted with p-bromophenylhydrazine in the same manner as before, the product, a dark solid, was obtained in low yield. Attempts to crystallize the product from ethanol gave a dark solid that did not have a sharp melting point but rather decomposed at temperatures above 300°C. This solid when examined was not suitable for X-ray analysis.

The difficulty in obtaining a suitable crystalline derivative for X-ray analysis from W_4 prompted another approach. A portion of W_4 was reduced with sodium borohydride in methanol to give a mixture of alcohols which were purified by preparative layer chromatography. The NMR spectrum of the major alcohol had signals for the O-methyl (τ 6.66), a proton geminal to the hydroxyl (τ 6.84, J = 7 and 9 Hz, quartet), a proton geminal to the methoxyl (τ 7.32, J = 4 and 10 Hz, quartet), and C-methyl groups (τ 9.02, 9.04, 9.12, 9.18, 9.24, seven methyls). The IR spectrum had lost the carbonyl band found in the ketone and now had a new absorption at 3495 cm^{-1} for the OH stretching frequency.

A small portion of the major reduction product referred to as W_4 alcohol was treated with bromoacetyl chloride and sodium bromoacetate in benzene to give the bromoacetate derivative of W_4. This derivative was submitted for X-ray analysis. These crystals seemed suitable for X-ray analysis and much data was gathered on this material. However, several months were spent on this problem before it was decided that the data

collected could not be solved with methods currently available.

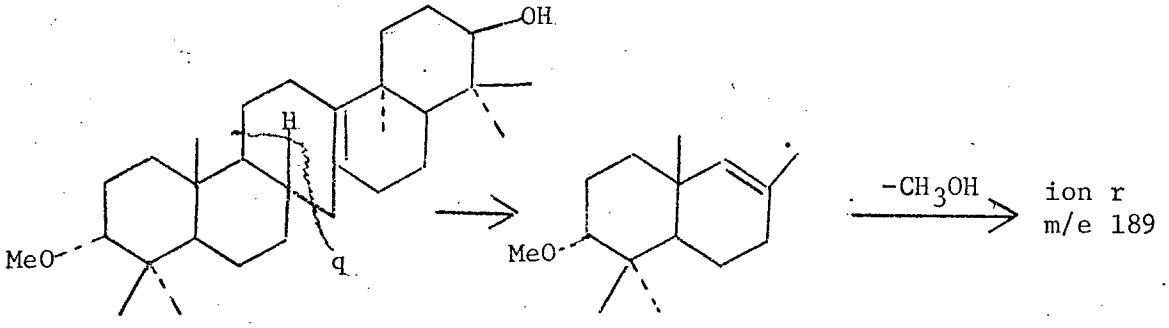
While the above X-ray analyses were being done, the mass spectra of W_4 alcohol and 3β-methoxyserrat-13-en-21α-ol (87) were examined. The mass



87

spectra of several serratenes had been previously studied in our laboratories.[68] Recently, serratene derivatives of unknown structure had been examined in the mass spectrometer and the data had provided structural information.[69]

The mass spectrum of 3α-methoxyserrat-13-en-21β-ol (28) was available and had been partially analyzed.[68] The major fragment ions of interest to the present study are located at m/e 221, 203, and 189. It was suggested that the ion at m/e 221 originates from rings A and B as a fragment q.



ion q
m/e 221

Ion r (m/e 189) is produced by loss of methanol from the m/e 221 ion. A metastable peak observed at m/e 161.6 tends to confirm this process.

Ion s (m/e 203, $C_{15}H_{23}$) was prominent in both 3α-methoxyserrat-13-en-21-one and 3α-methoxyserrat-13-21β-ol.[68] This ion is not as prominent in serrat-14-ene derivatives and for this reason it was felt[68] that it is diagnostic of serrat-13-ene derivatives; however, the origin of this ion was not postulated.

The mass spectra of 3β-methoxyserrat-13-en-21α-ol and W_4 alcohol were determined and are presented in Figures 32 and 33 respectively. The elemental composition of ions where given were determined by high resolution mass spectrometry.

As expected the mass spectrum of 3β-methoxyserrat-13-en-21α-ol (87) resembles that of 3α-methoxyserrat-13-en-21β-ol. The molecular ion of 87 is seen at m/e 456. A partial fragmentation pattern is given in Figure 31. The molecular ion may lose methyl (M-15, m/e 441), water (M-18, m/e 438), and methanol (M-32, m/e 424). Ion t (m/e 423) would correspond to loss of methyl and water from the molecular ion. Loss of both methyl and methanol from the molecular ion would account for ion u (m/e 409). Ion v (m/e 391) would correspond to loss of methyl, water, and methanol from the molecular ion.

As in the reported[68] spectrum of 3α-methoxyserrat-13-en-21β-ol, ions q, s, and r at m/e 221, 203, and 189 respectively are again prominent. A metastable ion at m/e 161.6 suggests that ion q (m/e 221, $C_{15}H_{25}O$) loses methanol to give ion r (m/e 189, $C_{14}H_{21}$).

It was found in this study that a decomposition of ion x (m/e 235,

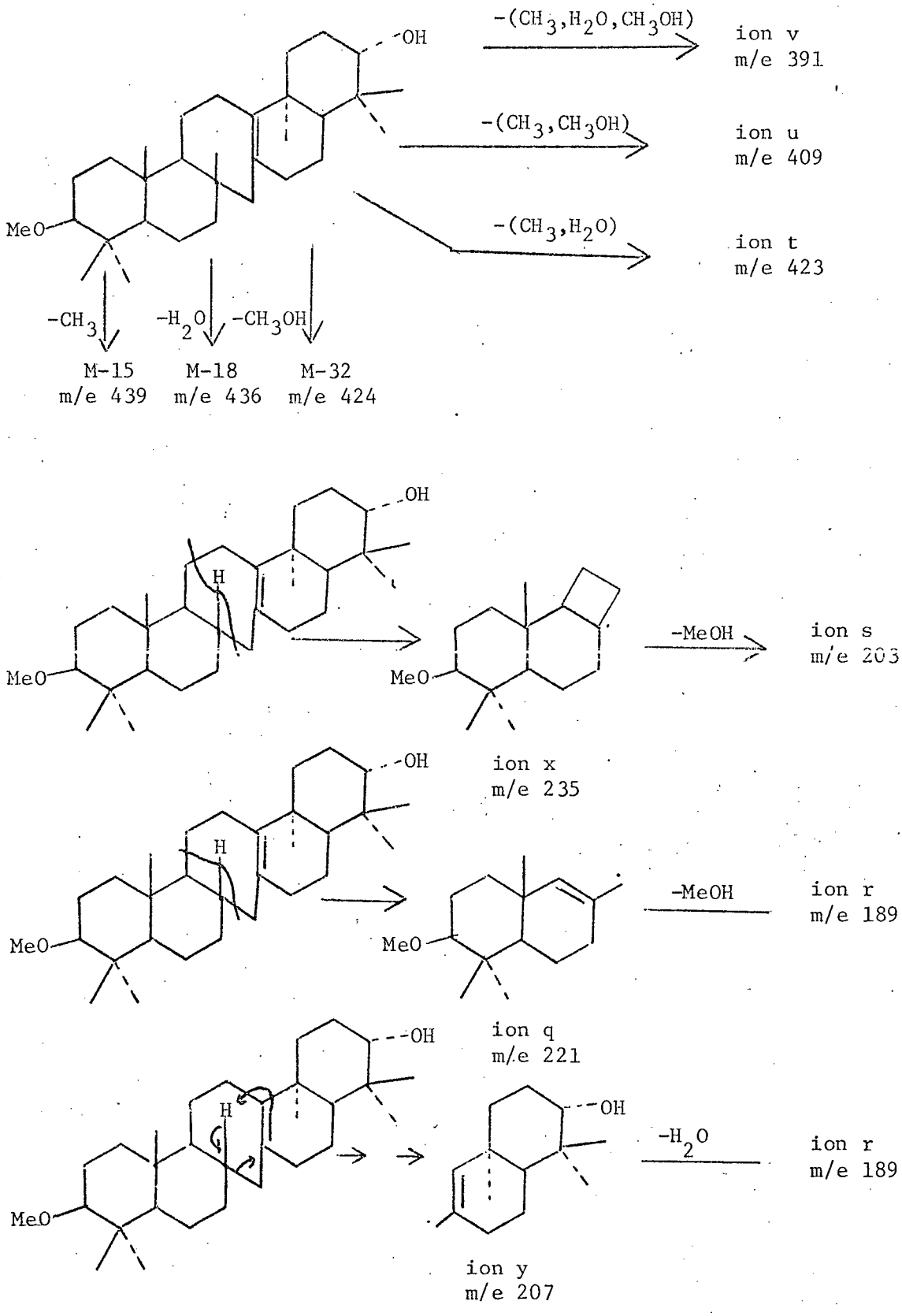


Figure 31. Fragmentation pattern of 3β-methoxyserrat-13-en-21α-ol.

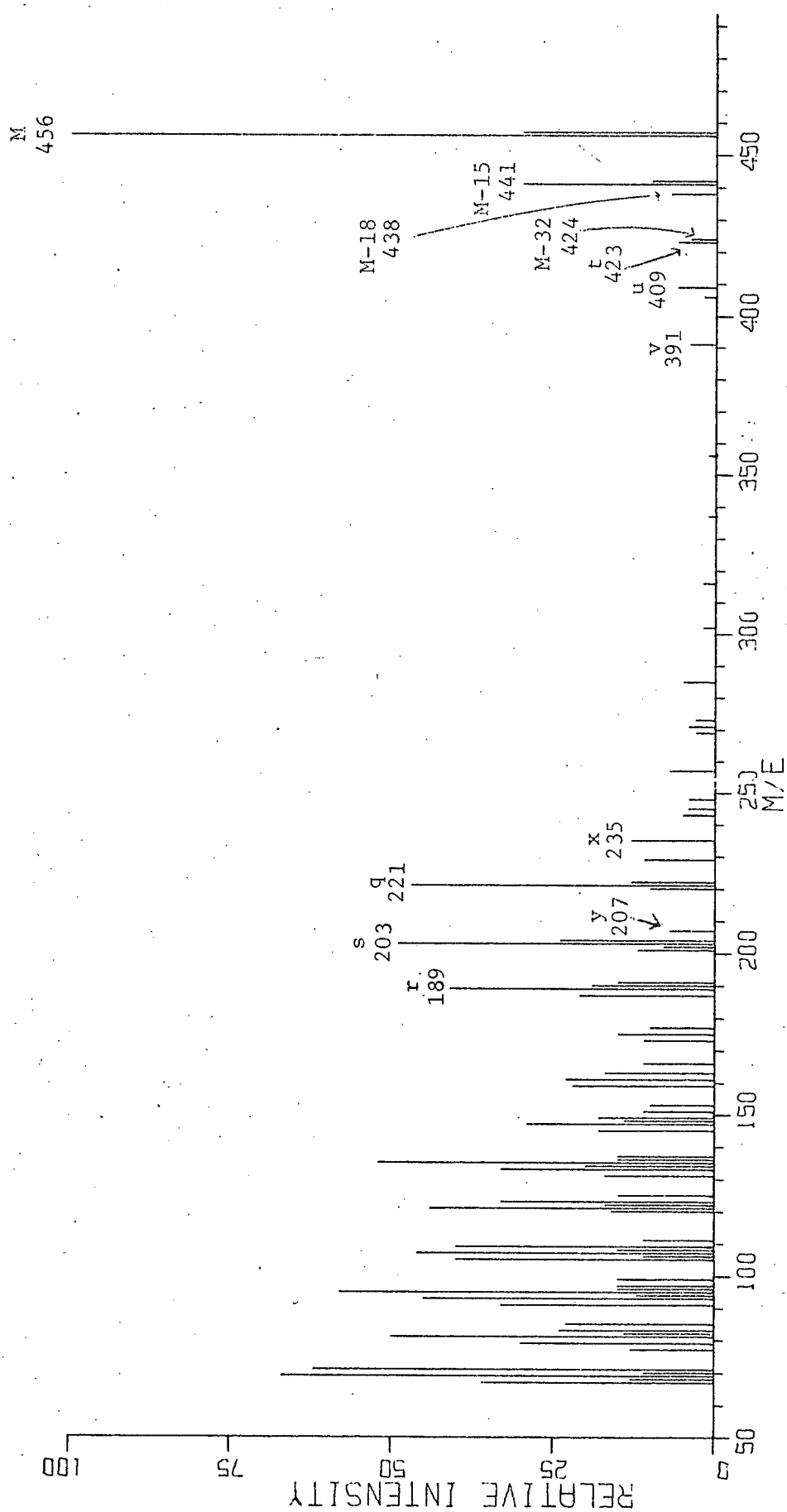


Figure 32. Mass spectrum of 3β-methoxyserrat-13-en-21α-ol.

$C_{16}H_{27}O$) by loss of methanol to give ion s (m/e 203, $C_{15}H_{23}$) could account for a metastable ion at m/e 175.4. Previously[68] an observed metastable ion at m/e 175.4 was assigned to the m/e 203 ion losing 14 mass units to give the m/e 189 ion. A loss of 14 mass units is not a favored process.

An ion y (m/e 207, $C_{14}H_{23}O$) is thought to contain rings D and E as there is a metastable ion at m/e 172.8 for the loss of water to give ion r (m/e 189).

The high mass region of the mass spectrum of W_4 alcohol (Figure 33) has many of the same peaks as the mass spectrum of 3β-methoxyserrat-13-en-21α-ol (87). In fact the general fragmentation pattern given in Figure 31 for 87 applies equally well to W_4 alcohol. The molecular ion is seen at m/e 456; a M-15 ion at m/e 441, a M-18 ion at m/e 438, and a M-32 ion at m/e 424 are also present. Ion t (m/e 423) may arise by loss of water and methyl as before. Ion u at m/e 409 would arise by loss of both methyl and methanol from the molecular ion and ion v (m/e 391) by loss of methyl, water, and methanol from the molecular ion.

While this region of the spectra is comparable, the relative intensity of several peaks is very much different. The M-15 ion of W_4 alcohol is more intense than in 87. Loss of methanol gives a M-32 ion which is four times more intense in W_4 alcohol than in 87. Furthermore, loss of both methyl and methanol gives ion u (m/e 409) which is six times more intense in W_4 alcohol than in 87.

A facile loss of a methyl group is often suggestive of an allylic methyl group. In this case, however, 3β-methoxyserrat-13-en-21α-ol (87) would contain a methyl group in the same position as W_4 alcohol if the latter is a serrat-13-ene derivative as thought. The reason, although not

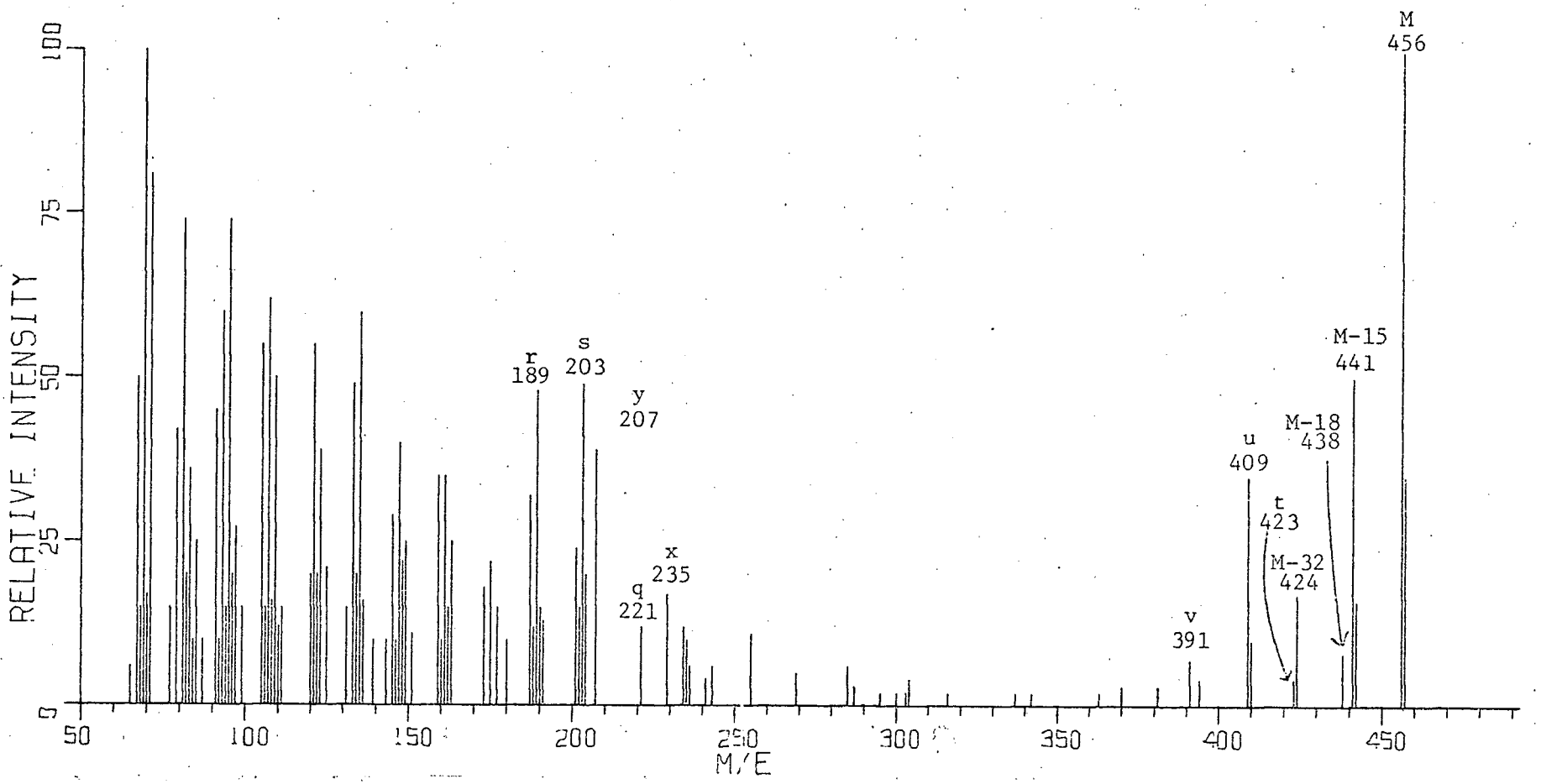


Figure 33. Mass spectrum of W_4 alcohol.

known, may be accounted for if there was greater steric crowding about the methyl group(s) lost in W_4 alcohol as opposed to 87.

The differences observed in the loss of methanol is puzzling. On the basis of NMR spectra the methoxyl groups in both compounds should have the same β orientation. A more qualitative observation is that the TLC properties of these compounds are the same, either as the parent ketone or as the alcohol. From previous work in our laboratories it was found that the 3α-methoxyserratene derivatives had different TLC properties from the 3β-methoxyserratene derivatives.[70] It would seem for both of these reasons that both compounds have a 3β methoxyl substituent.

Earlier in this thesis the mass spectra of several lanosterol derivatives were presented. It can be seen from these spectra that configurational and/or conformational changes in the molecule can affect the facility of the loss of the C(3) functionality. This effect has also been reported in the case of 5α- and 5β-steroids.[71]

Returning to the spectra of W_4 alcohol, attention is directed to the ions at m/e 235 (x), 221 (q), 207 (y), 203 (s), and 189 (r). All these ions were seen in the spectrum of 87. As before ion x (m/e 235, $C_{16}H_{27}O$) can lose methanol to give ion s (m/e 203, $C_{15}H_{23}$) and a metastable ion at m/e 175.4. Both the ions at m/e 235 and 203 are approximately the same realtive intensity as they were in 3β-methoxyserrat-13-en-21α-ol (87).

As in the spectrum of 87, ion q (m/e 221, $C_{15}H_{25}O$) can lose methanol to give ion r (m/e 189, $C_{14}H_{21}$). In comparison with the previous spectrum ion q is about a factor of five less intense than before.

In contrast to this observation, ion y (m/e 207, $C_{14}H_{23}O$) is now a

factor of five more intense than in the previous spectrum. As before, ion y can lose water to give ion r (m/e 189) and a metastable ion at m/e 172.7.

The origin of the m/e 221 ion has been postulated[68] to involve a hydrogen transfer from the C(26) methyl group to C(11) with rupture of the C(9) - C(11) bond. For this type of fragmentation to occur it is necessary for the methyl group to be sterically close to C(11). The postulated origin of ion y (m/e 207), on the other hand, requires a hydrogen transfer to C(13) from the C(26) methyl group and, as could be expected, the proper spatial arrangement is requisite again.

In the normal serratene skeleton the C(26) methyl group is β-oriented which, on the basis of molecular models, would seem to place it closer to C(11) than to C(13). On this basis fragmentation leading to ion q (m/e 221) could be expected to be more favored than fragmentation leading to ion y (m/e 207).

If the C(26) methyl group is α-oriented, a change in conformation of ring C occurs. Now the C(26) methyl group is closer to C(13) and farther away from C(11) than it was in the normal serratenes. The expected result of this situation would be an increase in the m/e 207 ion at the relative expense of the m/e 221 ion.

Although the β-orientation of the C(26) methyl group prevails in the normal serratane family, the α isomer could be expected on biogenetic grounds. The biosynthesis of the serratenes has not been studied but is thought to occur via the onocerins.[20] Protonation of C(26) in

α-onocerin (11) followed by ring closure and loss of a proton from C(13) could give serrat-13-ene-3β,21α-diol (88) directly (Figure 34).

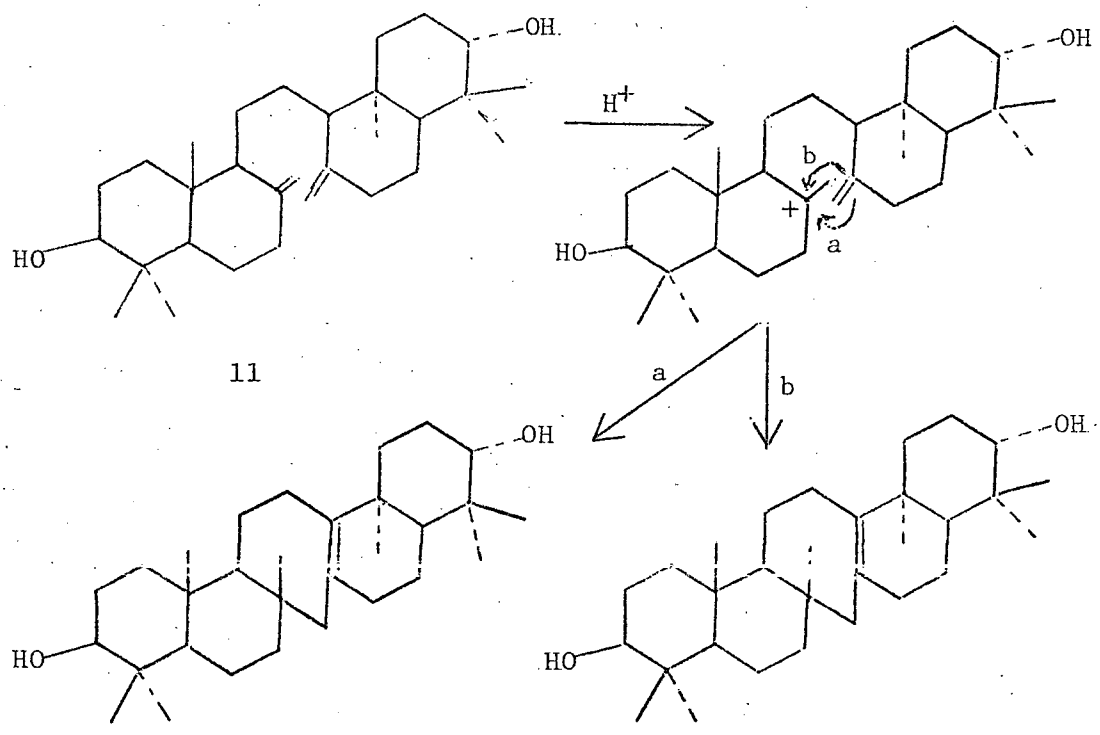
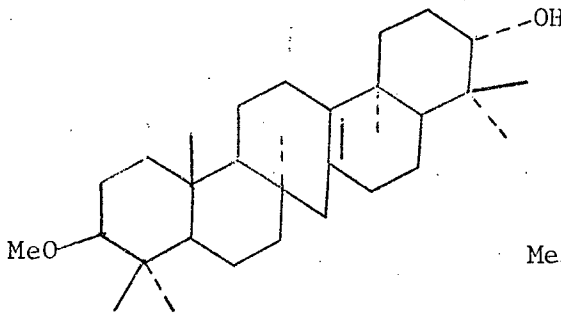


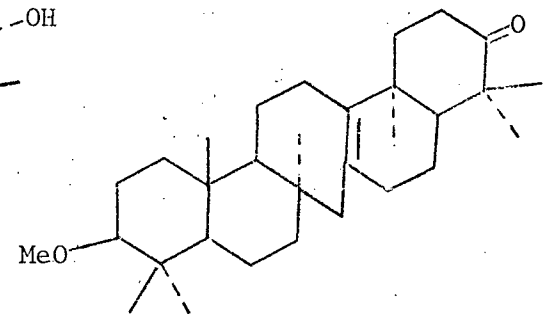
Figure 34. Postulate for the biosynthesis of 8α- and 8β-serrat-13-ene derivatives.

However, protonation at C(26) makes C(8) planar and subsequent completion of the reaction may lead to the α-orientation for the C(26) methyl group as in 8α-serrat-13-ene-3β,21α-diol (89), thereby allowing structure 90 to be postulated for W_4 alcohol. This postulate for the structure of W_4 skeleton is compatible with all evidence so far obtained. The ORD curve of W_4 suggests that rings D and E are the same as found in

serrat-13-en-21-one derivatives. The NMR spectrum suggests seven C-methyl groups and a β methoxyl group on a secondary carbon atom. The formation of ions at m/e 221 and m/e 207 in the mass spectrum would also be explained by this system. Furthermore, the conformational and configurational changes may be the factor responsible for the more intense M-15 and M-32 ions observed for W_4 alcohol.



90



91

The above evidence does not prove the structure of W_4 alcohol, but would suggest that W_4 alcohol is 3β-methoxy-8α-serrat-13-en-21α-ol (90). In turn the parent ketone, W_4, would be 3β-methoxy-8α-serrat-13-en-21-one (91).

Experimental

Throughout this work Merck silica gel G with added fluorescent indicator was used as adsorbent in thin layer chromatography (TLC). The chromatograms, 0.3 mm. in thickness, were air dried and activated in an oven at 100°C for three hours. The chromatograms were developed in chloroform unless stated otherwise. Detection of compounds was done by spraying with antimony pentachloride in carbon tetrachloride (1:2) unless otherwise noted.

For preparative layer chromatography a thicker layer of adsorbent (0.5 mm.) was utilized with 0.01% Rhodamine 6G added as indicator[34]. Spraying with antimony pentachloride solution was done only along one edge or not at all as detection of bands was possible with ultraviolet light in most instances.

Column chromatography was usually performed on Woelm neutral alumina. The preferred adsorbent was deactivated alumina (Activity III) prepared by the addition of water as directed by the manufacturers. In column chromatography of the crude extract where large quantities of adsorbent were used, Shawinigan alumina was deactivated by addition of 3% of a 10% acetic acid solution. Except in large scale column chromatography, the solvents were distilled before use.

The nuclear magnetic resonance (NMR) spectra were measured in deuterochloroform at room temperature. The NMR spectra were obtained at either 60 MHz using a Jelco C-60, Varian A-60, or a Varian T-60 instrument or at 100 MHz using a Varian HA-100 instrument. The positions of all NMR reso-

nances are given in the Tiers τ scale with tetramethylsilane as internal standard set at 10.0 units. For multiplets the τ values given represent the center of the signal.

Mass spectra were measured on an Associated Electrical Industries MS 9 high resolution mass spectrometer or, where noted, on an Atlas CH 4 spectrometer. High resolution molecular weight determinations wer determined on the MS 9 spectrometer.

Infrared (IR) spectra were measured on Perkin Elmer model 21, 137, or 457 instrument. The samples were usually measured as KBr pellets, however, some were measured in chloroform or carbon tetrachloride or neat. The positions of absorption maxima are quoted in wave numbers (cm^{-1}).

A Jasco model UV/ORD/CD 5 spectropolarimeter was used to measure the optical rotatory dispersion (ORD) curves using methanol as solvent.

Melting points were determined on a Kofler block and are uncorrected.

Elemental analyses were performed by Mr. P. Borda, University of British Columbia.

Extraction of Western white spruce

The bark for this study was obtained from a Western white spruce tree growing in the Prince George region of British Columbia. The bark was air dried and ground in a Wiley mill to pass through a 3 mm. sieve. Air dried bark (1,850 gms.) was extracted with petroleum ether for 18 hours in a large glass Soxhlet extractor. Evaporation of the solvent gave a crude extract (40 gms.) as a thick, brown, gummy wax.

Column chromatography of crude extract

Crude extract (80 gms.) was applied in petroleum ether (2 l.) to the top of a column prepared from deactivated Shawinigan alumina (5 lbs.).

Elution of the column was with various solvents as below.

Fraction	Solvent (volume, l.)	Wt.(gms.)	Compounds
1	petroleum ether (14)	8.0	hydrocarbons, sterol and wax esters
2	benzene (13)	5.6	3α-methoxyserrat-14-en-21β-ol, 3α-methoxyserrat-14-en-21-one, W_4, (epi)-manool
3	20% chloroform in benzene (8)	2.9	fatty ester, fatty alcohol
4	chloroform (5)	4.2	3β-methoxyserrat-14-en-21β-ol, β-sitosterol
5	50% methanol in chloroform (2) methanol (4)	13.0	unidentified components

Fraction 1

A portion of Fraction 1 when examined by TLC showed the presence of at least three compounds (R_f 0.57, 0.67, and 0.72). This fraction had no components with chromatographic properties like W_4 and was not further examined.

Fraction 2

A portion of Fraction 2 when examined by TLC showed the presence of at least three compounds: (epi)manool (R_f 0.29), 3α-methoxyserrat-14-en-21β-ol (R_f 0.36), and W_4 (R_f 0.42) when compared with authentic samples.

Fraction 3

A portion of Fraction 3 was compared with authentic lignocerol alcohol ($C_{24}H_{49}OH$) and showed the same R_f of 0.25. IR (film) 3300 (OH). NMR (60 MHz) 8.75 (broadened singlet). Fraction 3 seemed to be mainly fatty alcohol and was not further examined.

Fraction 4

A portion of Fraction 4 when examined by TLC showed the presence of at

least two compounds, 3β-methoxyserrat-14-en-21β-ol (R_f 0.17) and β-sitosterol (R_f 0.22), when compared to authentic samples.

Fraction 5

A portion of Fraction 5 when examined by TLC showed only polar compounds and was not examined further in this study.

Column chromatography of Fraction 2

Fraction 2 (10.6 gms.) was dissolved in 50% benzene in petroleum ether (100 mls.) and applied to the top of a column prepared from alumina (Activity I, 600 gms.). Elution with benzene (1.5 l.) gave a yellow oily material which was examined by TLC and found to be free of the desired W_4. Further elution with benzene (3.5 l.) gave Fraction 6 (3.9 gms.) containing W_4 and 3α-methoxyserrat-14-en-21-one by TLC examination. Elution with chloroform gave Fraction 7 (6.5 gms.) containing 3α-methoxyserrat-14-en-21β-ol and (epi)manool by TCL examination. Washing the column with methanol gave, upon evaporation, a brown residue which was seen to be polar material by TLC examination and was not further investigated.

Isolation of W_4

Fraction 6 (3.9 gms.) was chromatographed on alumina (Activity III, 300 gms.). Elution with petroleum ether (3.5 l.) gave Fraction 8 (800 mgs.) as a yellowish waxy solid (R_f 0.76). IR ($CHCl_3$) 1725 (ester carbonyl). NMR (60 MHz) 8.75 (broadened singlet).

Elution with 5% benzene in petroleum ether (1.5 l.) gave Fraction 9 (415 mgs.) containing 3α-methoxyserrat-14-en-21-one by TLC comparison with authentic sample.

Elution with 10% benzene in petroleum ether (2 l.) gave an unidentified

oil (Fraction 10) which was not further examined in this study.

Continuing to elute with 10% benzene in petroleum ether gave the desired W_4 (1,100 mgs.) in Fraction 11 when examined by TLC.

Eluting with methanol (1 l.) gave only polar or baseline material when examined by TLC.

Properties of W_4

A portion of Fraction 11 (600 mgs.) was crystallized from ethyl acetate to give a white solid m.p. 190 - 225°C. NMR (60 MHz) 6.65 (3H, singlet with slight shoulder at 6.67, OMe). Several re-crystallizations from ethyl acetate provided a crystalline product m.p. 200 - 230°C which, upon sublimation provided further purification, m.p. 215 - 225°C. Additional crystallizations from ethanol and the sublimation gave an analytical sample m.p. 227 - 229°C. ORD (c, 0.0201) $[\phi]_{650}$ + 677°, $[\phi]_{589}$ + 677°, $[\phi]_{400}$ + 2,300°, $[\phi]_{350}$ + 3,790°, $[\phi]_{330}$ + 4,870°, $[\phi]_{320}$ + 6,500°, $[\phi]_{310}$ + 8,266°, $[\phi]_{306}$ + 8,800°, $[\phi]_{300}$ + 7,860°, $[\phi]_{280}$ + 2,430°, $[\phi]_{276}$ + 2,160°, $[\phi]_{250}$ + 5,690°. IR (KBr) 1700 (ketone carbonyl), 1665 (C=C), 1100 (OMe). NMR (100 MHz) 6.65 (3H, singlet, OMe), 7.30 (1H, quartet, J = 5 and 11 Hz, axial H-C-OMe), 7.55 (2H, quartet, J = 6 and 8 Hz, $-CH_2CO-$), and 8.92, 8.99, 9.02, 9.12, 9.16, 9.17, 9.23 (7 C-methyls). Mass spectrum (m/e) 454 (M), 439 (M-15), 422 (M-32), 407 (M-47), 221, 203, and 189. (Found C, 81.74; H, 11.23; $C_{31}H_{50}O_2$ requires C, 81.88; H, 11.08%; high resolution 454.379 $C_{31}H_{50}O_2$ requires 454.381; 439.356 $C_{30}H_{47}O_2$(M-15) requires 439.357.

3β-methoxyserrat-13-en-21-one (85)

Jones reagent was prepared by dissolving chromium trioxide (2.668 gms.) in concentrated sulfuric acid (2.13 mls.) and diluting to 10 mls. with

water in a volumetric flask.

3β-methoxyserrat-14-en-21β-ol (24) (140 mgs.) was dissolved in acetone (50 mls.) and was oxidized with Jones reagent (0.2 mls.). After 30 minutes the solution was filtered and the filtrate evaporated to dryness. This solid was dissolved in a mixture of acetic acid (15 mls.) and concentrated sulfuric acid (1 ml.) and left for 18 hours. The acid mixture was poured onto crushed ice; after the ice had melted the white solid was extracted with methylene chloride. The methylene chloride extract was washed with water, 5% sodium bicarbonate solution, saturated salt solution and dried over sodium sulfate. Evaporation of the solvent gave 120 mgs. of yellowish solid. This solid was chromatographed on alumina (Activity I, 10 gms.). Prolonged elution with petroleum ether gave a few mgs. of pale yellow oil. Elution with benzene gave a white solid; crystallization from ethyl acetate and sublimation gave analytical sample m.p. 241 - 243°C. ORD (c, 0.0201) $[\phi]_{650}$ + 670°, $[\phi]_{589}$ + 670°, $[\phi]_{400}$ + 1,762°, $[\phi]_{350}$ + 2,843°, $[\phi]_{330}$ + 3,795°, $[\phi]_{320}$ + 4,740°, $[\phi]_{310}$ + 5,967°, $[\phi]_{304}$ + 6,230°, $[\phi]_{290}$ + 3,650°, $[\phi]_{270}$ + 2,035°, $[\phi]_{250}$ + 5,014°. IR (KBr) 1700 (ketone carbonyl), 1100 (OMe). NMR (100 MHz) 6.67 (3H, singlet, OMe), 7.30 (1H, quartet, J = 5 and 11 Hz, axial H-C-OMe), 7.55 (2H, quartet, J = 6 and 8 Hz, $-CH_2-CO-$), and 8.90, 8.96, 9.05, 9.07, 9.16, 9.23, 9.26 (7 C-methyls). Mass spectrum (m/e) 454 (M), 439 (M-15), 422 (M-32), 407 (M-47), 221, 203, and 189. (Found C, 81.65; H, 11.14; $C_{31}H_{50}O_2$ requires C, 81.88; H, 11.08%).

Bromoindole derivative (86)

3β-methoxyserrat-13-en-21-one (25 mgs.) was dissolved in warm ethanol (5 mls.) containing a few drops of acetic acid; p-bromophenylhydrazine

hydrochloride (100 mgs.) was added and the solution refluxed for 10 hours and then left at room temperature for a further 10 hours. The ethanolic solution was poured into water and the precipitate collected by filtration The precipitate was crystallized twice from ethanol to give 86 as yellow needles m.p. 204 - 207°C (dec.). IR (KBr) 3280 (NH). UV λ_{max}^{MeOH} 233 mμ (log ε 3.64), 293 mμ (log ε 2.81), 302 mμ (log ε 2.69). (Found C, 71.30; H, 9.09; Br, 12.38; $C_{37}H_{52}NOBr \cdot C_2H_5OH$ requires C, 71.24; H, 9.06; Br, 12.50%).

Reaction of W_4 with p-bromophenylhydrazine

W_4 (25 mgs.) was dissolved in warm ethanol (5 mls.) containing a few drops of acetic acid; p-bromophenylhydrazine hydrochloride (100 mgs.) was added and the solution refluxed for 10 hours and left at room temperature for another 10 hours. The ethanolic solution was poured into water and the precipitate collected by filtration. The precipitate was crystallized from ethanol to give a small amount of dark solid which decomposed on heating over 300°C with no sharp melting point.

W_4 alcohol

W_4 (100 mgs.) was dissolved in methanol (30 mls.) and was reduced with sodium borohydride (300 mgs.) over a period of 2 hours. Excess sodium borohydride was destroyed with a few drops of dilute hydrochloric acid and the solvent was evaporated to leave a white paste. This paste was dissolved in water and chloroform and the chloroform layer was washed with water and dried over sodium sulfate. Evaporation of the solvent gave 110 mgs. of white solid which was applied to a preparative layer chromatogram and developed twice in chloroform. The top band when extracted

from the adsorbent gave 15 mgs. of orange-red solid; TLC showed this to be a mixture of at least two compounds. The lower band when extracted from the adsorbent gave 80 mgs. of orange-red solid. The 80 mgs. of solid was flushed through a short column of alumina (5 gms.) using benzene as the eluant. Evaporation of the benzene gave a white solid (65 mgs.). Crystallization from ethanol gave an analytical sample m.p. 299 - 300°C. IR (KBr) 3495 (OH). NMR (100 MHz) 6.66 (3H, singlet, OMe), 6.84 (1H, quartet, J = 7 and 9 Hz, axial H-C-OH), 7.32 (1H, quartet, J = 4 and 10 Hz, axial H-C-OMe), and 8.95, 9.02, 9.04, 9.12, 9.18, 9.24 (7 C-methyls). Mass spectrum (m/e) 456 (M), 441 (M-15), 438 (M-18), 424 (M-32), 423 (M-33), 409 (M-47), 391 (M-65), 235, 221, 207, 203, and 189. (Found C, 81.65; H, 11.16; $C_{31}H_{52}O_2$ requires C, 81.52; H, 11.48%); high resolution 456.396 $C_{31}H_{52}O_2$ requires 456.397, 235.206 $C_{16}H_{27}O$ requires 235.206, 221.190 $C_{15}H_{25}O$ requires 221.191, 207.174 $C_{14}H_{23}O$ requires 207.175, 203.178 $C_{15}H_{23}$ requires 203.180, 189.164 $C_{14}H_{21}$ requires 129.164.

Bromoacetate derivative of W_4 alcohol

W_4 alcohol (15 mgs.) was dissolved in benzene (10 mls.) and sodium bromoacetate (50 mgs.) was added and stirring was started. Bromoacetyl chloride (30 mgs.) was diluted with benzene (0.5 mls.) and added dropwise. The flask was firmly stoppered and stirring was continued for 2 days. The benzene was washed with water, 5% sodium bicarbonate solution, water and dried over sodium sulfate. Evaporation of the benzene left a white solid which was crystallized from methylene chloride - hexane to give white crystals m.p. 245 - 246°C. IR (KBr) 1725 (C=O). NMR (60 MHz) 6.13 (2H, singlet, $OCCH_2Br$), 6.63 (3H, singlet, OMe). Mass spectrum (m/e)

578 and 576 (M).

3β-methoxyserrat-13-en-21α-ol (87)

3β-methoxyserrat-13-en-21-one (35 mgs.) was dissolved in methanol (20 mls.) and reduced over a period of 2 hours with sodium borohydride (50 mgs.). Excess sodium borohydride was destroyed with a few drops of dilute hydrochloric acid and the solution evaporated to dryness to give a white paste. The paste was dissolved in water and chloroform and the chloroform solution washed with water and dried over sodium sulfate. Evaporation gave a white solid which was purified by preparative layer chromatography. The main band of material was extracted with chloroform to give an orange-red solid which was flushed through a short alumina column with benzene as the eluant to give a white solid. An analytical sample was obtained by crystallization from methanol, m.p. 267 - 268°C. IR (KBr) 3450 (OH). NMR (60 MHz) 6.66 (3H, singlet, OMe), and 8.95, 9.01, 9.09, 9.13, 9.17 (7 C-methyls). Mass spectrum (m/e) 456 (M), 441 (M-15), 438 (M-18), 424 (M-32), 423 (M-33), 409 (M-47), 391 (M-65), 235, 221, 207, 203, and 189. (Found C, 81.46; H, 11.84; $C_{31}H_{52}O_2$ requires C, 81.52; H, 11.48%); high resolution 456.397 $C_{31}H_{52}O_2$ requires 456.397, 235.205 $C_{16}H_{27}O$ requires 235.206, 221.190 $C_{15}H_{25}O$ requires 221.191, 207.174 $C_{14}H_{23}O$ requires 207.175, 203.179 $C_{15}H_{23}$ requires 203.180, 189.165 $C_{14}H_{21}$ requires 189.164.

Chemosystematic studies on Engelmann spruce

Discussion

Investigations of the bark extractives of Sitka and Western white spruce had revealed the presence of readily isolable amounts of methoxyserratene derivatives. It was of taxonomic interest to see if the occurrence of methoxyserratenes was a chemosystematic feature of the genus Picea. For this reason a third member of the genus was examined.

Engelmann spruce [Picea engelmannii Parry] is common throughout the interior mountain region of southern and central British Columbia. It often forms hybrids with white spruce in British Columbia but grows in pure stands in Colorado. The bark of Engelmann spruce for this study was obtained from a region near Fort Collins, Colorado.

The bark as obtained was air dried and ground so it would pass through a 3 mm. sieve. A portion of the ground bark was continuously extracted in a large Soxhlet apparatus with petroleum ether. Upon evaporation of the solvent a brown gummy crude extract was obtained in 3% yield based on the weight of the air dried bark extracted.

Following the petroleum ether extract, the bark was extracted with benzene to give, upon evaporation, a dark residue amounting to 1% of the weight of the original air dried bark. Finally, the bark was extracted with methanol to give a syrupy residue corresponding to 21% of the weight of the original air dried bark. The benzene and methanol extracts were not further examined in this study.

A portion of the petroleum ether extract was chromatographed on deactivated alumina (Figure 35). The first fraction was eluted with

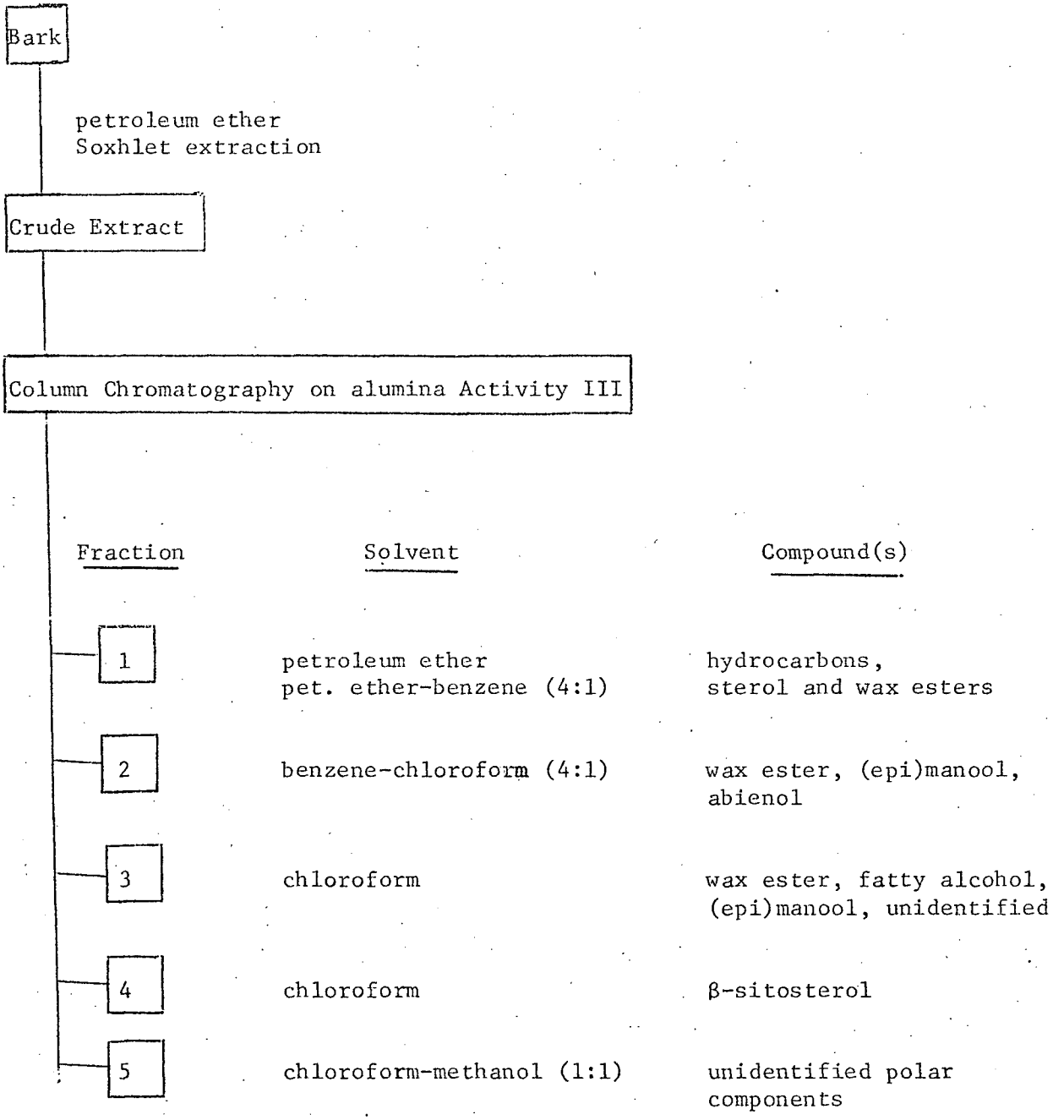


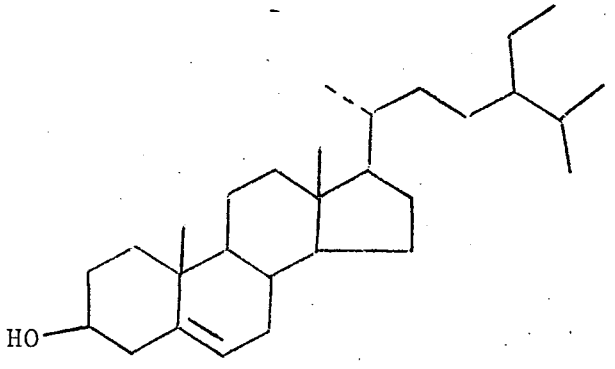
Figure 35. Separation sequence of components of Engelmann spruce bark.

petroleum ether and petroleum ether - benzene to give only oily material. The TLC of this fraction revealed very non-polar materials in the nature of hydrocarbons and wax or sterol esters.

The second fraction was eluted with benzene - chloroform to give a syrupy fraction. Thin layer chromatography showed at least three compounds (wax ester, (epi)manool, and abienol) were present.

The third fraction was eluted with chloroform to give a waxy solid. The TLC of this fraction showed the presence of several components much like the components of the second fraction.

The fourth fraction eluted with chloroform gave a white solid. Comparison of this material with authentic β-sitosterol (1) by TLC using two different solvent systems suggested they were identical. Crystallization of this substance gave a white solid m.p. 139 - 140°C (β-sitosterol melts at 139 - 140°C[72]); a mixed melting point of 139 - 140°C was observed proving their identity.



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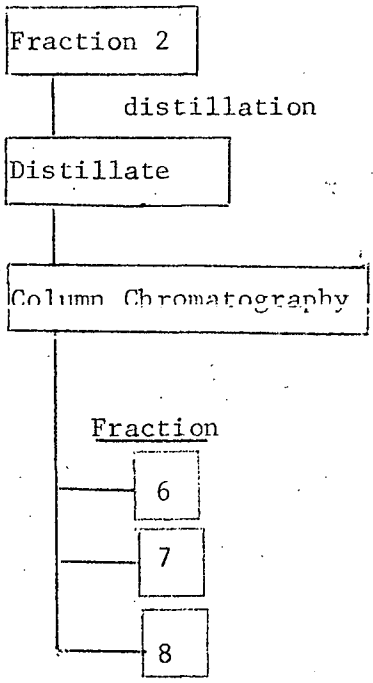
The fifth fraction was the column washings and contained the polar material. Examination of this fraction by TLC failed to detect any compounds of similar chromatographic properties to the sought after methoxyserratene derivatives.

Both Fractions 2 and 3 had TLC properties similar to the methoxyserratene derivatives isolated from other spruce species.

A portion of Fraction 2 (Figure 36) was distilled in vacuum to give



Fraction	Solvent	Compounds
6	petroleum ether	hydrocarbons
7	pet. ether:benzene (19:1)	(epi)manool, abienol
8	pet. ether:benzene (19:1)	(epi)manool, abienol

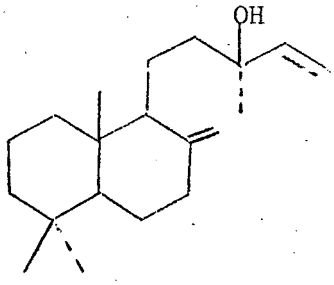
Figure 36. Purification of Fraction 2.

a clear yellow distillate. The residue was dark brown and contained only base line materials on a TLC chromatogram and was not examined further.

The distillate was chromatographed on alumina to afford a partial

separation. Petroleum ether eluted Fraction 6 containing the least polar compound. Evaporation of the solvent gave a waxy semi-solid. The IR spectrum had no peaks for hydroxyl or carbonyl functions. The NMR spectrum had none of the distinguishing features of the methoxyserratene derivatives isolated in other spruce species.

Further elution with petroleum ether - benzene gave mixtures of the two more polar compounds in the distillate. The faster running of the two compounds had TLC properties like manool (31) or epimanool (29). The slower running compound had TLC properties like abienol (92).



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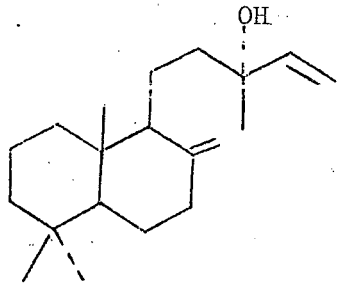

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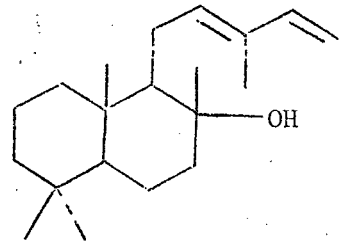


92

Fraction 7 was enriched in the faster running component, while Fraction 8 was enriched in the slower running component. A NMR spectrum of Fraction 7 showed signals at τ 3.15 (quartet, J = 17 and 11 Hz); τ 4.10 (quartet, J = 17 and 11 Hz); τ 4.5 (triplet, J = 7 Hz); τ 4.85 (quartet, J = 17 and 1 Hz); τ 4.93 - 5.10 (multiplet); τ 5.22, 5.55 (broadened singlets); and singlets at τ 8.76, 9.15, 9.22, and 9.34. Signals and couplings at

τ 4.10, 4.85, 4.93 - 5.10, 5.22, 8.76, 9.15, 9.22, and 9.34 are all in agreement with the reported NMR spectrum of manool[73] or epimanool. The other signals mentioned were of lesser intensity. Signals at τ 3.15, 4.5, and 4.93 - 5.10 were assigned to the olefinic protons of abienol on the basis of the reported spectrum[74]. The NMR spectrum of Fraction 8 had the same signals as the NMR spectrum of Fraction 7 in the olefinic region with C-methyl groups at τ 8.84, 9.14, 9.18, and 9.22. The position of these methyl groups in the NMR spectrum is in agreement with the reported spectrum of abienol[74].

Vapor phase chromatography of the above mixture gave peaks with retention time of 13.5 and 15.2 minutes. Injection of manool or epimanool gave a peak with a retention time of 13.5 minutes; abienol gave a peak with retention time of 15.2 minutes.

The mixture was not further purified as it appeared clear that neither of these compounds was the sought after methoxyserratene derivative.

The third fraction was dissolved in warm acetone for crystallization (Figure 37). The precipitate was collected and chromatographed on alumina. The initial fraction (9) gave an oily wax which exhibited no absorption for either hydroxyl or carbonyl in the infrared spectrum. Fraction 10 gave a low melting waxy substance. The IR spectrum of this fraction had carbonyl absorption at 1725 cm^{-1}. The NMR spectrum had a dominant peak at τ 8.7 as a broadened singlet and a signal of small intensity at τ 9.1. Upon amplification, a τ 7.7 signal was seen as a triplet (J = 6 Hz) and a τ 5.9 signal was seen as a triplet (J = 6 Hz). It is not thought that these two triplets are coupled to each other but rather represent

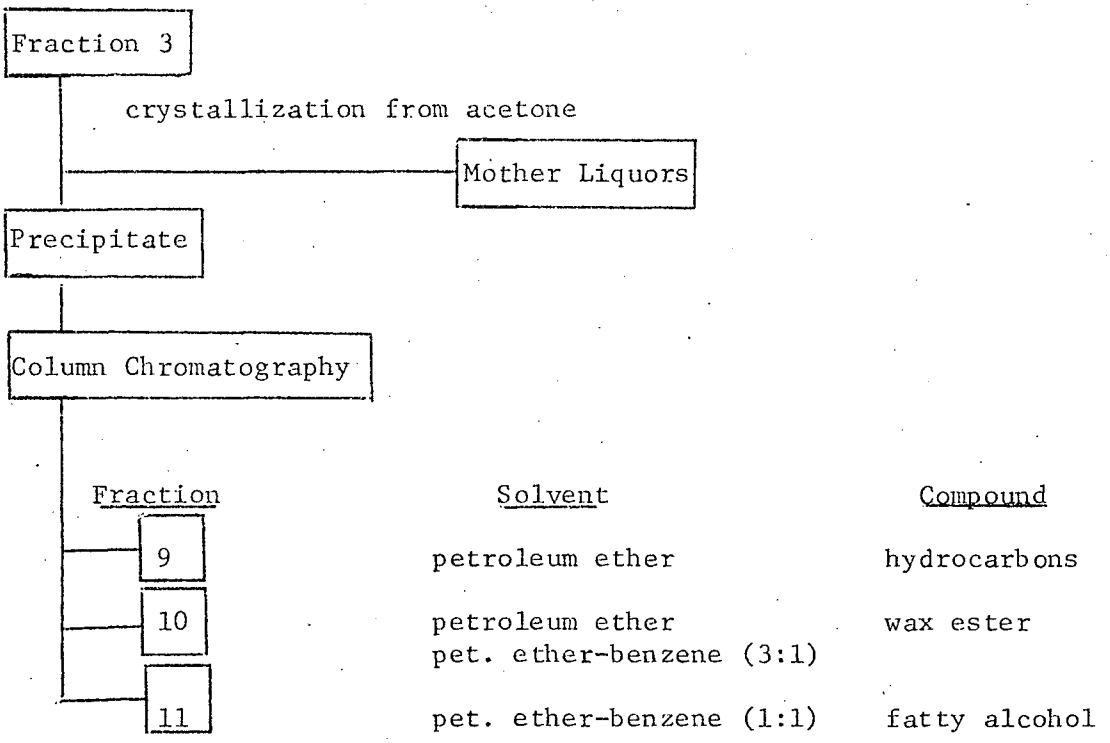


Figure 37. Purification of precipitate from Fraction 3.

methylenes adjacent to the ester function of a wax ester. Fraction 11 had a weak hydroxyl absorption at 3570 cm^{-1} in the IR spectrum. The NMR spectrum again had the dominating signal at τ 8.7; a weak resonance at τ 9.1; and, upon amplification, weak signals as triplets at τ 5.9, 6.4, and 7.7. TLC of the material showed that the main component had the same properties as an authentic fatty alcohol, in this case lignocerol [CH_3-$(CH_2)_{22}$-CH_2OH]. The minor component had properties like the wax ester of Fraction 10.

The mother liquors of Fraction 3 were separated by chromatography on alumina (Figure 38). Fraction 12 gave a compound that had the same properties as manool or epimanool. Further elution gave Fraction 13

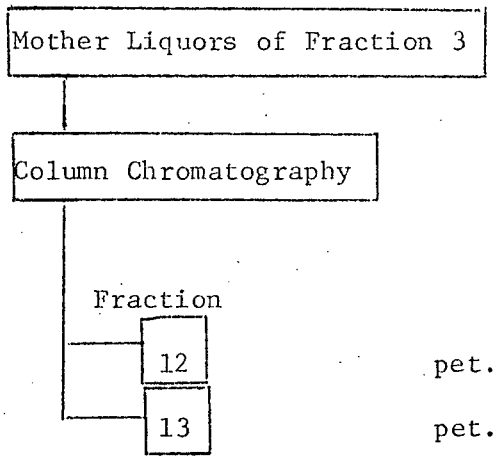


Figure 38. Purification of mother liquors of Fraction 3.

which was oily and contained at least two major and two minor components. Separation of the mixture on preparative TLC gave four bands (R_f 0.70, 0.55, 0.45, and 0.37). The first two bands were present in small amounts and were waxy. The NMR spectrum of the R_f 0.70 band had a dominant peak at τ 8.7 with a small signal centered at τ 9.1, as found in early fractions containing fatty ester or alcohol. The NMR spectrum of the R_f 0.55 band again had the τ 8.7 peak typical of the fatty esters or alcohols and was not further examined. The NMR spectrum of the R_f 0.45 and 0.37 bands were reminiscent of the NMR spectrum of the mixture of (epi)manool and abienol isolated earlier. On the basis of their NMR spectra and physical characteristics, it was felt that these compounds were not the sought after methoxyserratene derivatives.

While both Sitka[25] and Western white spruce had contained easily isolable quantities of methoxyserratene derivatives, none were detected in the above cursory examination. Repeating the examination on a fresh portion of crude petroleum ether extract failed again to detect any of

the sought for methoxyserratene derivatives.

I.H. Rogers of the Forest Products Laboratory, who had done much of the earlier work on Sitka spruce,[25] examined the petroleum ether extract and various chromatographic fractions. This examination also failed to detect any of the serratenes which were in relative abundance in the other spruces examined.

Two possible reasons may be advanced for the apparent absence of serratenes in Engelmann spruce. It may be that Engelmann spruce, because of its phytochemical background, does not synthesize these triterpenes. The other possible reason involves the anatomical structure of the bark.

In the original study[25] of Sitka spruce the crude plant material was hand picked to collect the cork layer from the bark of overmature trees. This portion of the bark apparently has an enrichment of serratenes, although serratenes have been isolated from whole bark of Sitka and Western white spruce in our laboratories. The bark of Engelmann spruce is generally quite thin.[78] In this study the bark had been removed from the log and broken into small chunks prior to being received. The hand sorting of this bark was not possible nor was it felt necessary before grinding and extraction.

In summary, it may be stated that this survey did not detect any of the serratenes in Engelmann spruce which were previously isolated from Sitka or Western white spruce.

Experimental

Throughout this work Merck silica gel G with added fluorescent indicator was used as adsorbent in thin layer chromatography (TLC). The chromatograms, 0.3 mm. in thickness, were air dried and activated in an oven at 100°C for three hours. The chromatograms were developed in chloroform unless stated otherwise. Detection of compounds was done by spraying with antimony pentachloride in carbon tetrachloride (1:2) unless otherwise noted.

For preparative layer chromatography a thicker layer (0.5 mm.) of adsorbent was utilized with 0.01% Rhodamine 6G added as indicator[34]. Spraying with antimony pentachloride solution was done only along one edge or not at all as detection of bands was possible with ultraviolet light in most instances.

Column chromatography was usually performed on Woelm neutral alumina. The preferred adsorbent was deactivated alumina (Activity III) prepared by the addition of water as directed by the manufacturers. In column chromatography of the crude extract where large quantities of adsorbent were used, Shawinigan alumina was deactivated by addition of 3% of a 10% acetic acid solution. Except in large scale column chromatography the solvents were distilled before use.

The nuclear magnetic resonance (NMR) spectra were measured in deuterochloroform at room temperature. The NMR spectra were obtained at either 60 MHz using a Jelco C-60 or Varian A-60 instrument or at 100 MHz using a Varian HA-100 instrument. The positions of all NMR resonances are given

in the Tiers τ scale with tetramethylsilane as internal standard set at 10.0 units. For multiplets the τ values given represent the center of the signal.

Infrared (IR) spectra were measured on Perkin Elmer model 21 or 137 instrument. Samples were measured in KBr pellets, in chloroform or carbon-tetrachloride solution or neat. The position of absorption maxima are given in wave numbers (cm^{-1}).

Melting points were determined on a Kofler block and are uncorrected.

Extraction of Engelmann spruce bark

The bark for this study was obtained from an Engelmann spruce tree growing in the Fort Collins region of Colorado. The bark was air dried and ground in a Wiley mill to pass through a 3 mm. sieve. Air dried bark (1,866 gms.) was extracted with petroleum ether for 18 hours in a large glass Soxhlet extractor. Evaporation of the petroleum ether gave a crude petroleum ether extract (67.1 gms.) as a brown, gummy, semi-solid. The bark was left in the extraction thimble and extracted with benzene for 18 hours. The benzene extract was evaporated to give a crude benzene extract (21.2 gms.). Finally the bark was extracted with methanol for 24 hours. Evaporation of the methanol gave a crude methanol extract (375 gms.). The benzene and methanol extracts were not further examined in this study.

Column chromatography of crude extract

Crude petroleum ether extract (43 gms.) was dissolved in petroleum ether (2 l.) and applied to the top of a column prepared from Shawinigan alumina (5 lbs.) deactivated by addition of a 3% (67.5 mls.) of a 10% aqueous acetic acid solution. Elution with petroleum ether (6 l.) and

20% benzene in petroleum ether (15 1.) gave Fraction 1 (4.5 gms.). TLC suggested that Fraction 1 was non-polar material in the nature of hydrocarbons and sterol or wax esters.

Further elution with 20% chloroform in petroleum ether (6 1.) gave Fraction 2 (1.9 gms.) whose TLC properties suggested abienol, (epi)manool, and wax ester.

Elution with chloroform (8 1.) gave Fraction 3 (1.8 gms.) whose TLC properties suggested (epi)manool, fatty alcohol, and two other components.

Elution with chloroform (8 1.) gave Fraction 4 (3.8 gms.) containing β-sitosterol by TLC comparison.

Finally, elution with 50% methanol in chloroform (3 1.) gave polar compounds (5.1 gms.) as Fraction 5. Fraction 5 was not further examined in this study.

Distillation of Fraction 2

Fraction 2 (550 mgs.) was placed in a bulb for hot box distillation at a temperature of 140°C and a pressure of 0.07 mm. of mercury. A pale yellow oil was collected (416 mgs.) as the distillate; the residue was a brown solid (125 mgs.).

The distillate contained 3 components when examined by TLC (R_f 0.79, 0.39, and 0.32). The residue was mainly polar materials on the base line of the chromatogram with a trace of the distillate still present.

Chromatography of the distillate of Fraction 2

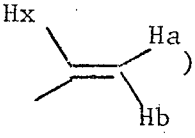
The distillate of Fraction 2 (400 mgs.) was chromatographed on alumina (40 gms.). Elution with petroleum ether (300 mls.) gave a waxy semi-solid (50 mgs.) as Fraction 6. Elution with 5% benzene in petroleum

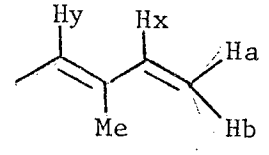
ether (300 mls.) gave Fraction 7 (223 mgs.) as an oil. Continuing to elute with the same solvent (200 mls.) gave Fraction 8 (118 mgs.) as an oil.

Properties of Fraction 6

Fraction 6 was a waxy semi-solid which contained a major component (R_f 0.79) and a trace of a second component (R_f 0.65) when examined by TLC. IR (neat) 2950 (CH), 1440 (CH_2), 1380 (CH_3), and 970 (olefinic C-H). NMR (60 MHz) 8.24 (3H, singlet), 8.44 (3H, singlet), 8.52 (3H, singlet), and 9.12, 9.16, 9.22 (3 C-methyls).

Properties of Fraction 7

Fraction 7 was an oil which contained a major component at R_f 0.39 and a second component at R_f 0.32 when examined by TLC. The faster running component had the same TLC properties as (epi)manool (R_f 0.39), while the second component had the same TLC properties as abienol (R_f 0.32). IR (film) 3330 (OH). NMR (100 MHz) showed signals attributable to (epi)-manool at: 4.12 (1H, quartet, J = 11 Hz and 17 Hz, Hx in system 

), 4.85 (1H, quartet, J = 17 Hz and 1 Hz, Hb), 5.05 (1H, quartet, J = 11 Hz and 1 Hz, Ha), 5.22, 5.51 (2H, pair of broadened singlets, exocyclic methylene), 8.76 (3H, singlet, allylic methyl), and 9.13, 9.21, 9.34 (3 C-methyls). In addition, signals attributable to abienol were seen at: 3.15 (1H, quartet, J = 11 Hz and 17 Hz, Hx in system 

), 4.54 (1H, triplet, J = 7 Hz, Hy), 4.93 - 5.10 (2H, multiplet, Ha and Hb), 8.21 (3H, broadened singlet, vinylic methyl), 8.84 (3H, singlet, CH_3-COH),

and 9.14, 9.18, 9.22 (3 C-methyls). Vapor phase chromatography of Fraction 7 on a 3% SE 30 column (8 ft.) at 205°C gave peaks with retention time of 13.5 and 15.2 minutes. Injection of (epi)manool gave a peak with retention time of 13.5 minutes; abienol had a retention time of 15.2 minutes.

Fraction 8

Fraction 8 was an oil which contained a major component at R_f 0.32 and a second component at R_f 0.39 when examined by TLC. The major component had TLC properties like abienol (R_f 0.32); the minor component had properties like (epi)manool (R_f 0.39). IR (film) 3335 (OH). NMR (100 MHz) showed signals attributable to abienol and (epi)manool as in the NMR spectrum of Fraction 7. Vapor phase chromatography on a 3% SE 60 column (8 ft.) at 205°C gave peaks with retention time 13.5 and 15.2 minutes; under the same conditions (epi)manool and abienol had retention times of 13.5 and 15.2 minutes respectively.

Crystallization of Fraction 3

Fraction 3 (1.8 gms.) was taken up in warm acetone (35 mls.) and left to crystallize for one day. The precipitate was filtered, washed with cold acetone and dried to give a yellowish wax (307 gms.). The filtrate was evaporated to give 1.4 gms. of gummy oil.

Chromatography of precipitate from Fraction 3

The precipitate (307 mgs.) was chromatographed on alumina (30 gms.). Elution with petroleum ether (50 mls.) gave Fraction 9 as an oily wax. The IR had no absorption for either hydroxyl or carbonyl functions. Elution with petroleum ether (50 mls.) and 25% benzene in petroleum ether (600 mls.) gave Fraction 10 as a low melting waxy substance. IR (film) 1725. NMR

(60 MHz) 5.9 (triplet, J = 6 Hz, $-CH_2-OCOR$), 7.7 (triplet, J = 6 Hz, $-OCOCH_2$), 8.7 (broad singlet, $-CH_2-$), 9.1 (multiplet, $-CH_3$). On this basis Fraction 10 was thought to be fatty ester and not further examined. Fraction 11 was eluted with 50% benzene in petroleum ether (360 mls.) to give a low melting wax. TLC revealed two components, a major component at R_f 0.14 and a minor component at R_f 0.48. IR (film) 3570. NMR (60 MHz) 5.9 (triplet, J = 6 Hz, $-CH_2-OCOR$), 6.4 (triplet, J = 6 Hz, $-CH_2-OH$), 7.7 (triplet, J = 6 Hz, $-OCO-CH_2-$), 8.7 (broad singlet, $-CH_2-$), 9.1 (multiplet, $-CH_3$). The spectral data suggested the Fraction 11 was mainly fatty alcohol with some fatty ester from Fraction 10 as a contaminant.

Chromatography of mother liquors from Fraction 3

A portion of the mother liquors from Fraction 3 (0.4 mgs.) was chromatographed on alumina (125 gms.). Elution with 10% benzene in petroleum ether (1,000 mls.) gave Fraction 12 (102 mgs.) as an oil. Further elution with the same solvent (1,500 mls.) gave Fraction 13 (170 mgs.) as an oil.

Properties of Fraction 12

Fraction 12 was a gummy oil that contained two components by TLC (R_f 0.39 and 0.32). Comparison with (epi)manool (R_f 0.39) and abienol (R_f 0.32) suggested identity.

Preparative layer chromatography of Fraction 13

A portion of Fraction 13 (90 mgs.) was applied to a preparative layer chromatogram and developed in chloroform to reveal four bands (R_f 0.70, 0.55, 0.45, and 0.37). The first two bands (R_f 0.70 and 0.55) were present in small amounts. Their NMR spectra were dominated by a broadened singlet

at 8.7 suggestive of the fatty ester or fatty alcohol components in early fractions.

The band with R_f 0.45 was a viscous oil. IR (film) 3425 (OH). NMR (60 MHz) 3.00 (unresolved multiplet), 4.20 (unresolved multiplet), 4.95 (singlet), 5.15 (unresolved multiplet), and 8.98, 9.10, 9.15 (singlets).

The band with R_f 0.37 was also a viscous oil. IR (film) 3390 (OH). NMR (60 MHz) 4.50 (unresolved multiplet), 4.95 (singlet), 8.72 (singlet).

Purification of Fraction 4

A portion of Fraction 4 (500 mgs.) was crystallized from ethanol to give a white solid m.p. 139 - 140°C, mixed m.p. with β-sitosterol 139 - 140°C, (literature m.p. 139 - 140°C[72]). A second crop of white crystals was collected m.p. 137 - 139°C. TLC on silica gel with chloroform as solvent showed only 1 compound with R_f 0.14 (β-sitosterol, R_f 0.14): with 25% ethyl acetate in benzene as developing solvent the white solid and β-sitosterol both had identical properties (R_f 0.30). NMR (60 MHz) 4.66 (1H, multiplet, H-C=C), 6.50 (1H, multiplet, H-C-OH), 8.35 (1H, singlet, H-O-C-; exchangable with D.O), 8.98 - 9.27 (6 C-methyls).

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PART II

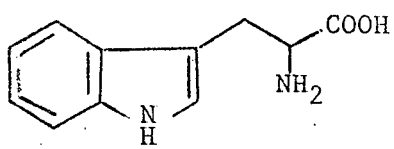
STUDIES RELATED TO SYNTHESIS AND BIOSYNTHESIS OF INDOLE ALKALOIDS

Introduction

In the last century Serturner recognized the basic character of morphine and referred to it as a vegetable alkali. Meisner later proposed the term alkaloid for such vegetable alkalis. Among the earliest known alkaloids were the indole alkaloids. In a recent compilation by Hesse some five hundred of these bases have been reported from about three hundred plants.[1]

The study of the biosynthesis of the indole alkaloids has intrigued and interested workers in this field for many years. Early studies on the biosynthesis were based on natural compounds with structural similarities to the proposed intermediate and on chemical reactions which were thought to be of biogenetic significance. With the availability of radioactive isotopes these hypotheses could be tested.

A common structural feature of many indole alkaloids, the β-(2-amino-ethyl)-indole moiety suggested the intermediacy of tryptophan (1) or tryptamine (2). In fact radioactive labelled tryptophan has been shown to be incorporated into a number of indole alkaloids including vindoline (3)[2,3,4], catharanthine (4)[2,4], ajmalicine (5)[4], vincaminoreine (6)[5], vincamine (7)[6], and minovine (8)[5].



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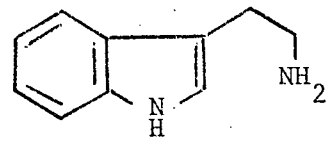

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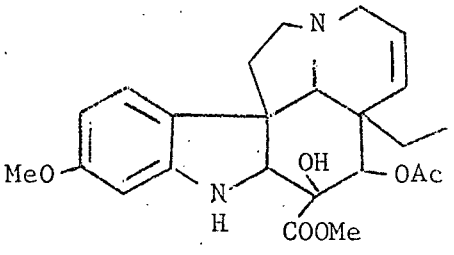

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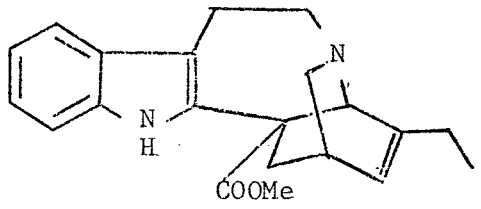

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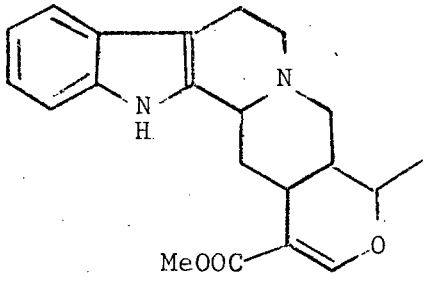

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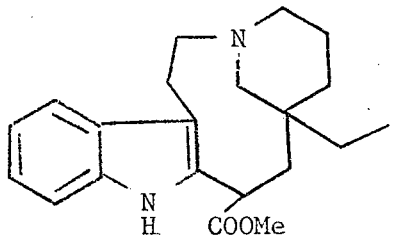

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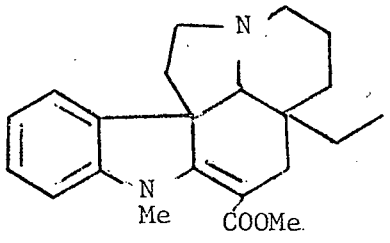

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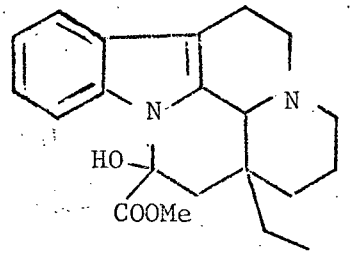

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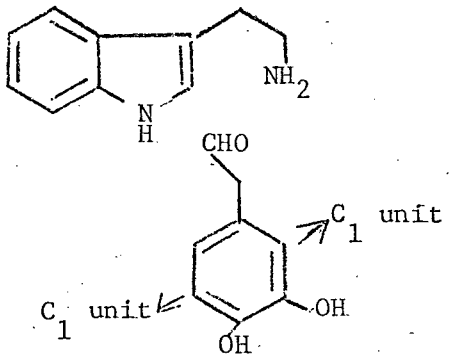
In contrast to the acceptance of the origin of the "tryptophan" portion, the origin of the non-tryptophan portion was the subject of considerable discussion with a number of theories emerging.

The earliest theory due to Barger[7]- Hahn[8,9]-Robinson[10]-Woodward[11,12] suggested the intermediacy of a dihydroxyphenylacetaldehyde (9), or equivalent, plus two C_1 units. This theory predicted the biosynthesis of yohimbine (10) as shown:



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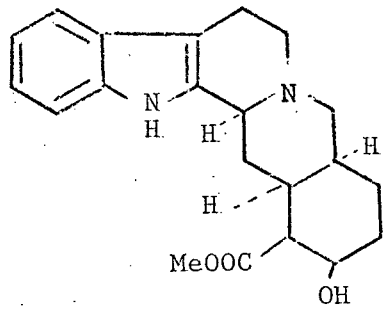

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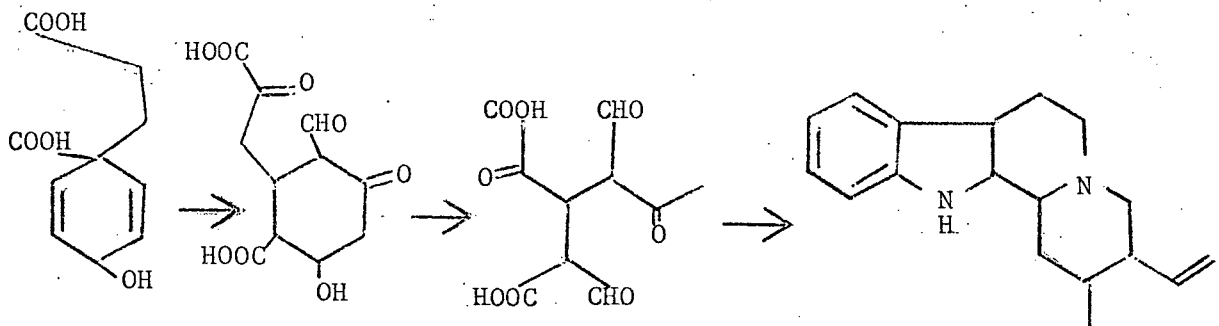
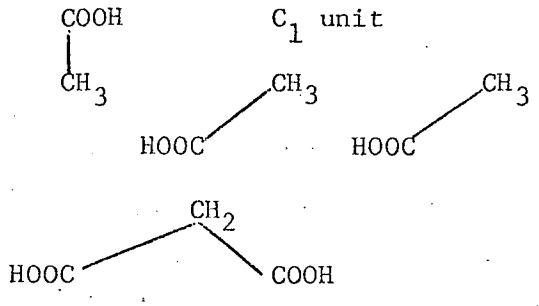
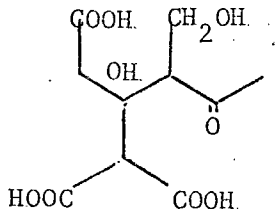
That theory had a number of deficiencies and prompted Wenkert[13,14] to suggest a new possibility. His initial postulate involved the intermediacy of a hydrated prephenic acid (11). Later[15] this was altered so prephenic acid itself was utilized. Condensation with a one carbon unit and various rearrangements would afford the seco-prephenate-formaldehyde (SPF) unit (12). This unit would then condense in a subsequent step to give corynantheine (13).



11 12 13

A third theory[16-19] proposed that the non-tryptophan portion had an acetate background. Here three acetate units, a malonate unit, and a one carbon fragment would condense to give the proposed intermediate 14. However, this hypothesis could not be substantiated experimentally and was subsequently withdrawn.





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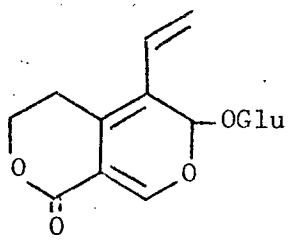
Yet another proposal was advanced by Wenkert[13-15] and Thomas[20] based on structural relationships. They suggested that the non-tryptophan portion of the indole alkaloids was monoterpenoid in origin. This suggestion followed the discovery of several monoterpenes, for example gentiopicrin (15), bakankosin (16), swertiamarin (17), and genipin (18) which all have a slightly masked form of the seco-prephenate-formaldehyde unit.



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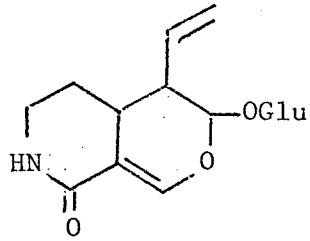

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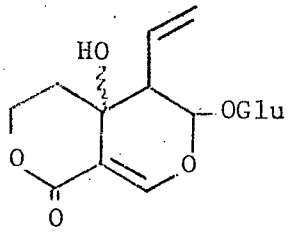

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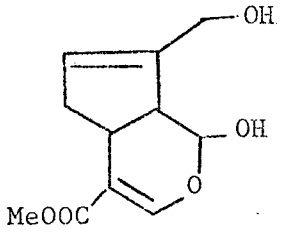

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When Wenkert[15] advanced his monoterpenoid hypothesis, he also suggested a sequence for the formation of the Aspidosperma and Iboga families of indole alkaloids. Condensation of tryptophan with the SPF unit followed by the appropriate reactions as outlined in Figure 1 could lead to these two families.

Attention should be drawn to some ideas presented in the Wenkert proposal. It can be seen that this proposal suggests that more than one family can arise from the same precursor, for example 19. The acrylic acid intermediates 20 and 23 are important intermediates and will be mentioned later in this thesis. The third feature is the transannular cyclization of 21 to 22 and 24 to 25. The transannular cyclization reaction is a facile reaction *in vitro*[21] and, on the basis of Wenkert's postulate, should occur *in vivo* as well. However, when the appropriate experiments were performed[5] no evidence for an *in vivo* cyclization was found. Also the reverse ring opening process is known *in vitro*[5] but does not appear to be operational in the biosynthesis.[5] This discrepancy does not, however, seriously affect the overall proposal.

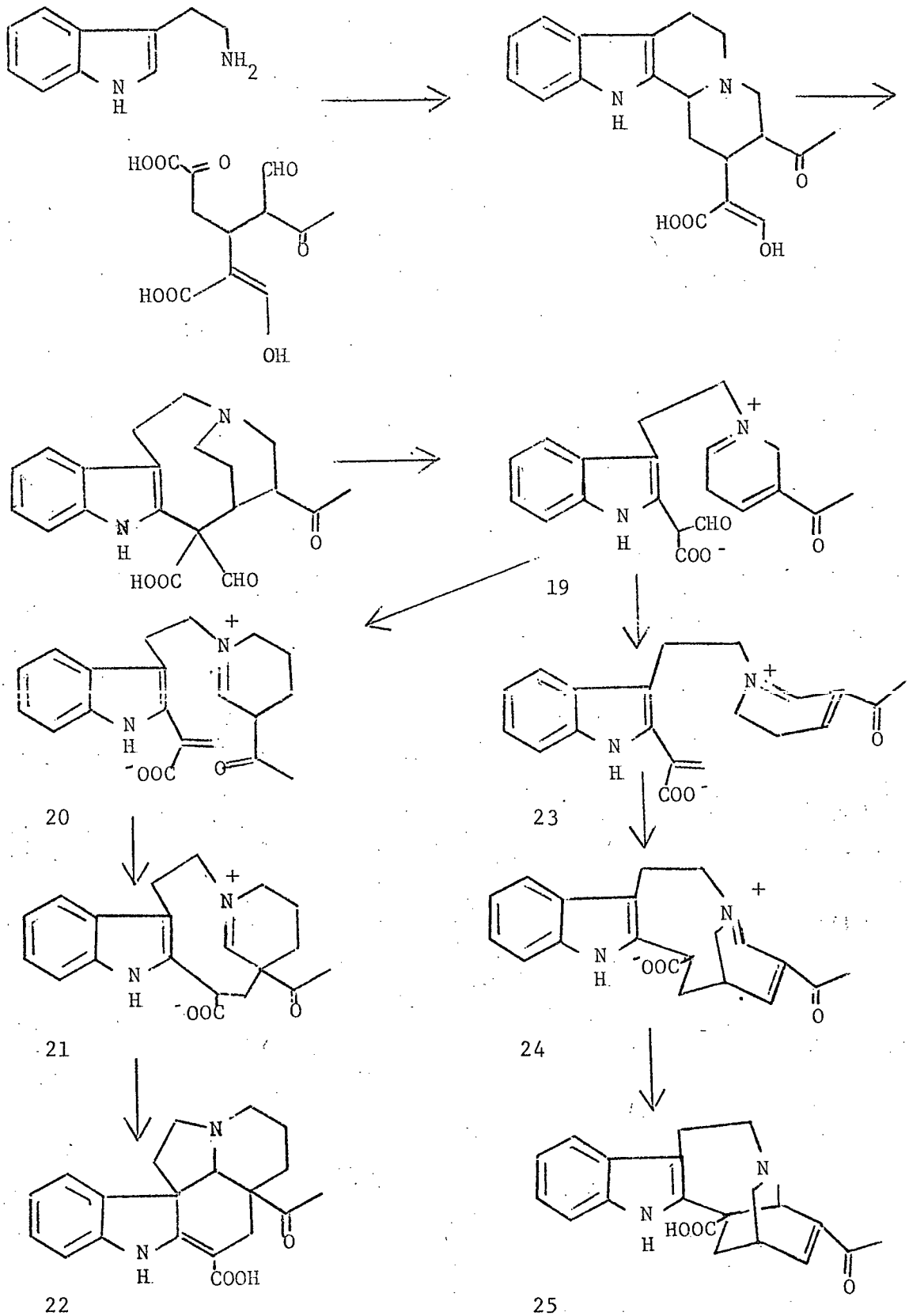
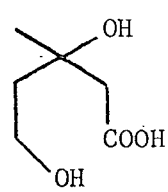


Figure 1. Wenkert's proposal for the biosynthesis of Aspidosperma and Iboga alkaloids.

If the monoterpene hypothesis is correct the established precursor of terpenes, mevalonic acid (26), would be expected to be incorporated. Early experiments[18] were unable to detect the incorporation of mevalonic acid. In 1965 Scott and co-workers[22] reported the successful incorporation of mevalonic acid into vindoline (3). Subsequent publications by several groups[23-26] showed that the non-tryptophan portion of indole alkaloids was derived from specifically labelled mevalonic acid. The monoterpene hypothesis was further corroborated when geraniol (27) was shown to be incorporated into vindoline, catharanthine, and ajmalicine in Vinca rosea plants.[27-30] These three alkaloids are representative of the three major families of indole alkaloids: Aspidosperma, Iboga, and Corynanthe families respectively.

Further supporting evidence for the monoterpene hypothesis was provided by Battersby[31] who reported the incorporation of loganin (28) into vindoline, catharanthine, and ajmalicine in Vinca rosea plants. Subsequent reports[32-34] confirmed these results and extended them to Rauwolfia serpentia[33] plants. Degradation of the alkaloids suggested the formal transformations as illustrated in Figure 2. Loganin has also been isolated from Vinca rosea plants,[31,35] satisfying a requirement for a true precursor.



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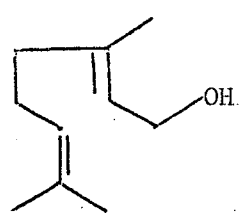

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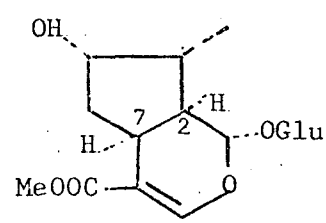

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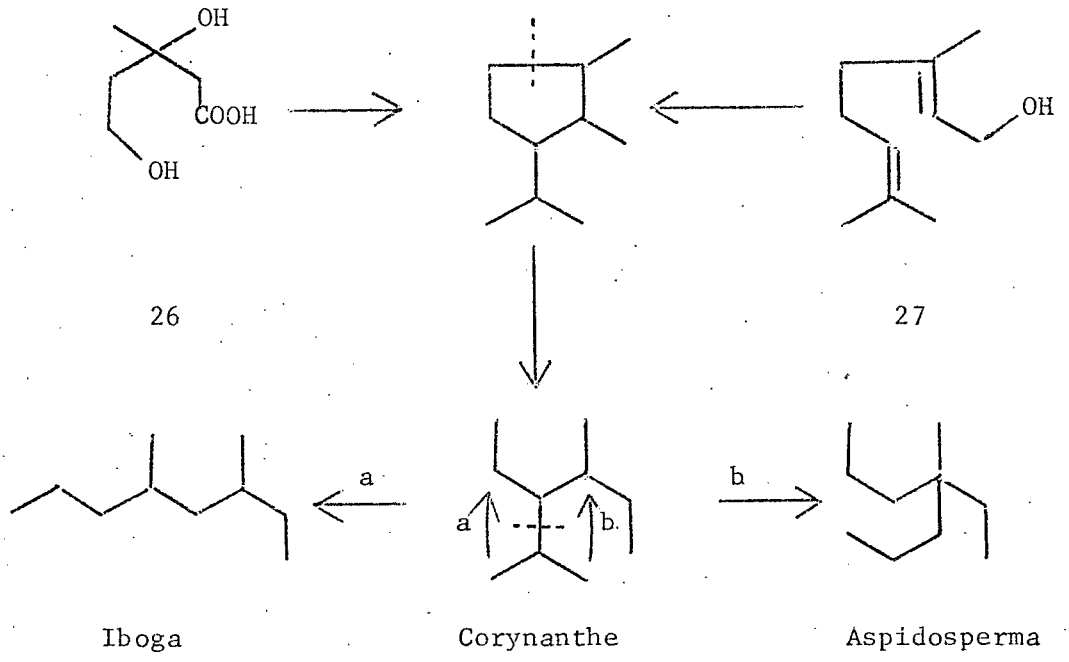
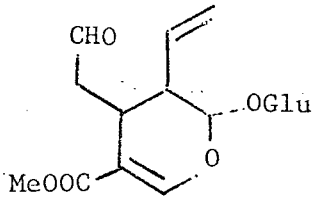


Figure 2. Formal transformation of the monoterpene unit.

In an extension of the incorporation studies, Battersby reported *in vivo* experiments[36] which impose strict requirements on the formation of loganin and its conversion into the three major classes of indole alkaloids. From the study of doubly labelled specimens he concluded that: (a) the stereospecificity established for the formation of the two geraniol double bonds in other biological systems[37] also holds good in *Vinca rosea*; (b) the configuration of C(7) in loganin is determining for the stereochemistry of the corresponding center in ajmalicine and by extension for other Corynanthe and Strychnos compounds; (c) the stereochemical correlations of C(2) in loganin with the corresponding center in ajmalicine is fortuitous, the observed loss of a proton from this position supports the idea of an enamine intermediate.

The discovery that loganin is a precursor of the indole alkaloids prompted detailed study of the later stages of the biosynthesis. Both Wenkert[15] and Thomas[20] suggested that the indole alkaloids are derived from a cyclopentane monoterpene by some process involving cleavage of the cyclopentane ring. Battersby[33] recognized that a cleavage compound, seco-loganin (29), was found in a masked form in menthiafolin (30). Thus mild



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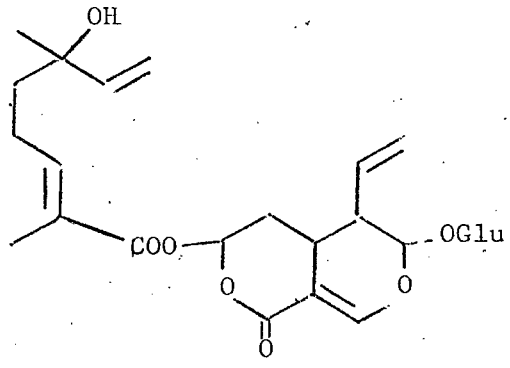

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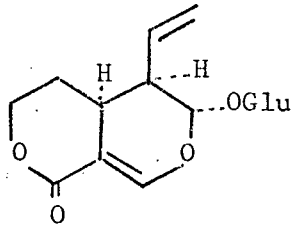
alkaline hydrolysis of menthiafolin, followed by careful acidification and esterification by diazomethane afforded seco-loganin. With seco-loganin available Battersby[38] was able to prove that it is: a constituent of Vinca rosea plants; biosynthesized from loganin; a specific precursor of representative examples of the Iboga, Corynanthe, and Aspidosperma families.

The incorporation of sweroside (31) into vindoline has been



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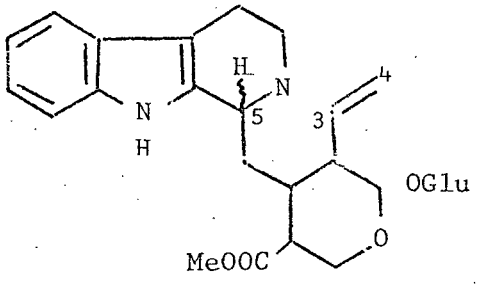
demonstrated in Vinca rosea.[39] It is likely that sweroside enters the direct pathway by biological conversion into seco-loganin. Evidence for an early nitrogen containing intermediate in the biosynthesis was obtained with the discovery of strictosidine (32) (stereochemistry not established) in Rhazya species.[40] Its presence was subsequently demonstrated in Vinca rosea plants[41] by dilution analysis starting with radioactive tryptophan and loganin.

The in vitro condensation of tryptamine and seco-loganin[41] produced two bases, vincoside (33) and isovincoside (34), which have the same gross



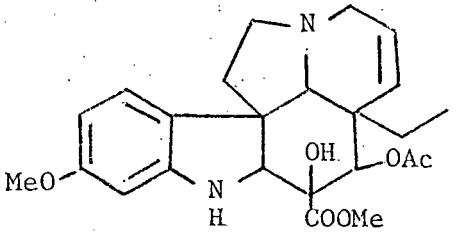
32, strictosidine

33, $C_5H\alpha$: vincoside

34, $C_5H\beta$: isovincoside

35, $C_5H\alpha$, C(3) - C(4) double bond reduced: dihydrovincoside

structure as strictosidine. It is likely that one of these two bases is identical to strictosidine. While both vincoside and isovincoside have been isolated from Vinca rosea, only vincoside is involved in the biosynthesis of vindoline (3)[41,42], catharanthine (4)[41,42], ajmalicine (5)[41,42], and



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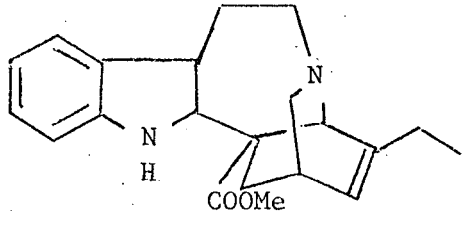

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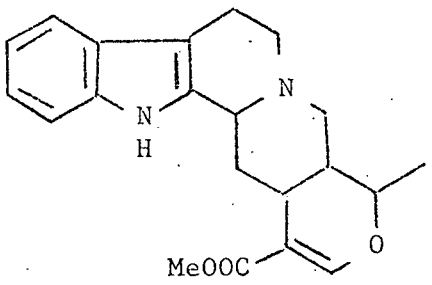

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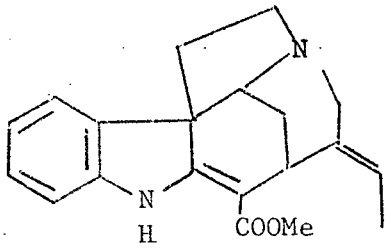

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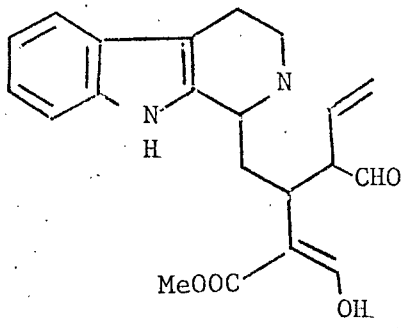
akuammicine (36)[42], the latter being an example of a Strychnos alkaloid. Dihydrovincoside (35) produced by reduction of the ethylenic side chain was not incorporated into the three representative alkaloids usually examined.[41]

The biological conversion of vincoside into the Aspidosperma and Iboga bases necessitates rearrangement of the skeleton. Earlier work proved that this was an intramolecular process. Cleavage of the glucose moiety of vincoside results in an aglucone which should be in equilibrium with, or convertible to, the aldehyde (37). Ring closure could lead to corynantheine aldehyde (38) and/or geissoschizine (39).



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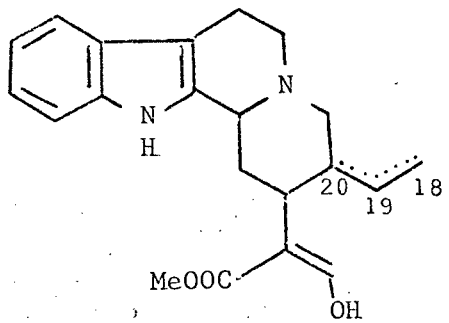
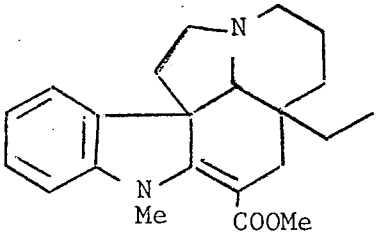


38, 18(19)-ene, corynanthine aldehyde

39, 19(20)-ene, geissoschizine

Early experiments[36,43] with mature Vinca rosea plants had shown no incorporation of corynantheine aldehyde into the alkaloids examined. Geissoschizine, however, was shown to be present in Vinca rosea plants and also was incorporated into ajmalicine (5), vindoline (3), catharanthine (4), and akuammicine (36).[42] In Vinca minor L shoots geissoschizine was incorporated into vincamine (7) and minovine (8).[44]



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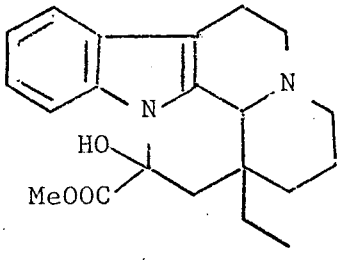

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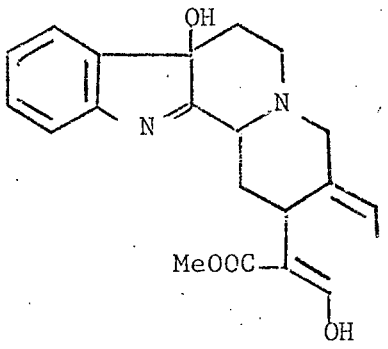
A different approach to the study of the biosynthesis was adopted by Scott.[43,45,46] It was known that seeds of Vinca rosea were essentially devoid of alkaloidal content. Thus in growing Vinca rosea plants from seeds it should be possible to observe the sequential formation of alkaloidal material. Using this technique[46], vincoside (33) was among the first alkaloids isolated at 26 hours after germination. Between 28 and 40 hours five alkaloids were isolated: corynantheine aldehyde (38), geissoschizine (39), a β-hydroxy indolenine (40), a diol (41), and geissoschizine oxindole (42). While corynantheine aldehyde was not incorporated in mature Vinca rosea plants, it, along with geissoschizine, was incorporated into seedlings. The significance of the last three alkaloids isolated in the 28 to 40 hour time period will be discussed later.



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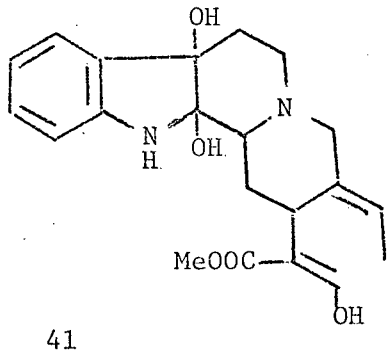

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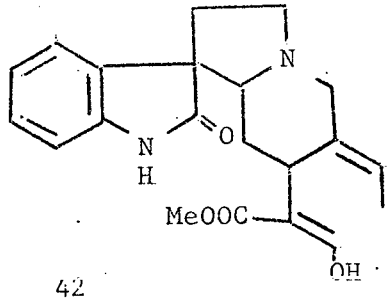

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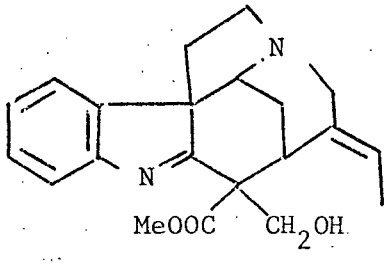
Still working on sequential studies, Scott[41] found that the next alkaloids isolated (40 - 50 hours) were pre-akuammicine (43), akuammicine (36),



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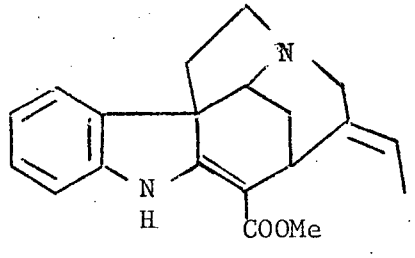

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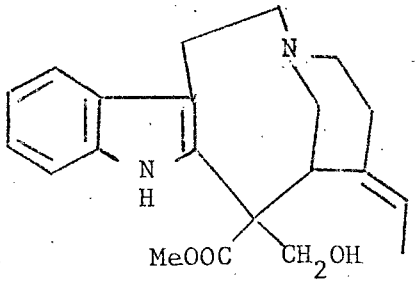

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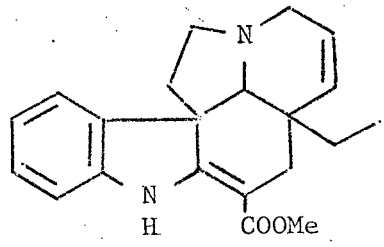

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stemmadenine (44), and tabersonine (45). The occurrence of pre-akuammicine is a significant development since it represents an intermediate between geissoschizine (Corynanthe), akuammicine (Strychnos), and stemmadenine (Corynanthe-Strychnos). Pre-akuammicine is a key member since it retains all ten carbons of geraniol, yet can also suffer loss of a single carbon atom necessary for Strychnos alkaloids and, by rearrangement, generates Aspidosperma or Iboga alkaloids.

Three processes have been suggested for the biosynthesis of pre-akuammicine (Figure 3). Wenkert[15,47] suggested a one electron oxidative coupling to give a strictamine type derivative, 46. Precedent for the rearrangement of compounds such as 47 to the Strychnos representative akuammicine (36) is available[48] so that the indoline (48) or its reduced form, pre-akuammicine (43), could be reached by such a mechanism. An alternative to this process, also with <u>in vitro</u> analogy, is α protonation of the indole nucleus followed by α to β rearrangement[49] as shown in Figure 3. A third possibility is oxidation of geissoschizine to a β-hydroxyindolenine (45) followed by formation of geissoschizine oxindole (42).[50] This latter could react via the imino ether (49) to give

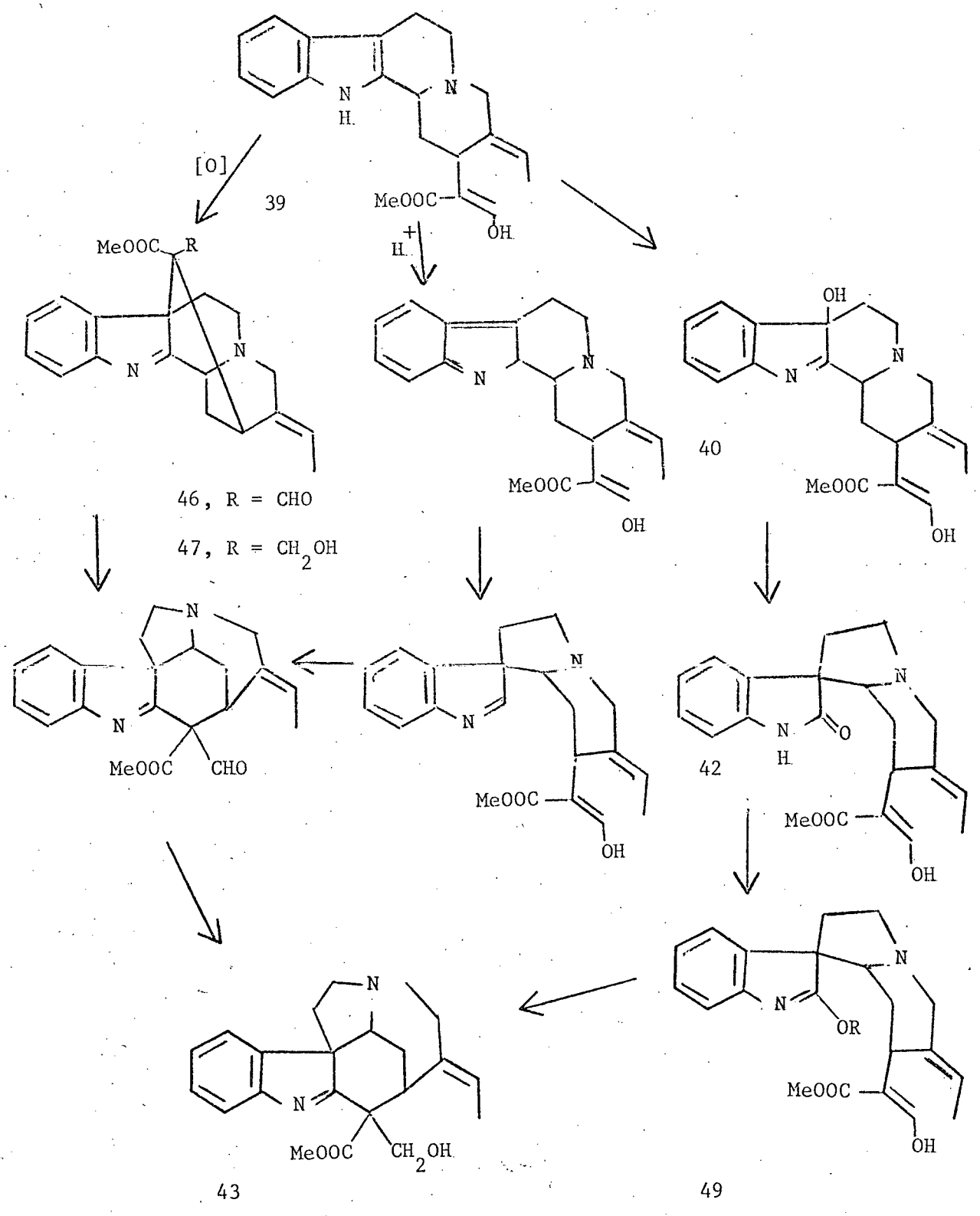


Figure 3. Proposals for the biosynthesis of pre-akuammicine.

pre-akuammicine (43). As mentioned previously, Scott[46] found the β-hydroxyindolenine and geissoschizine oxindole in his sequence studies and showed that Vinca rosea seedlings were able to incorporate the oxindole (42) into akuammicine and vindoline. Thus the oxindole hypothesis has been shown to be a viable process in Vinca rosea.

The occurrence of pre-akuammicine, stemmadenine, and tabersonine at approximately the same time is interesting. It can be seen formally that reduction of pre-akuammicine can lead to stemmadenine. Scott[50] found, in vitro, sodium borohydride reduction of pre-akuammicine afforded akuammicine (36) and stemmadenine (44). In fact, stemmadenine appears to be the next intermediate as it is incorporated into catharanthine, vindoline, and tabersonine in Vinca rosea seedlings.[50] In Vinca minor vincamine and minovine are labelled starting from labelled stemmadenine.[51]

The conversion of stemmadenine to tabersonine is still speculative. It is thought[5] that the exocyclic double bond of stemmadenine migrates to give a new compound, iso-stemmadenine (50), with an endocyclic double bond (Figure 4). Iso-stemmadenine can, with the aid of the lone pair of electrons on nitrogen, expel the hydroxide group to give an acrylic ester derivative (51). The iminium system can, by loss of a proton and ring closure, give tabersonine (45); or, it is also possible that acrylic ester (51) may lose a proton without cyclization to give a new dihydropyridine acrylic ester (52) (Figure 5). This derivative may then close in a Diels-Alder fashion to give tabersonine (45) or, by another Diels-Alder reaction using both double bonds in the dihydropyridine, to produce catharanthine (4).

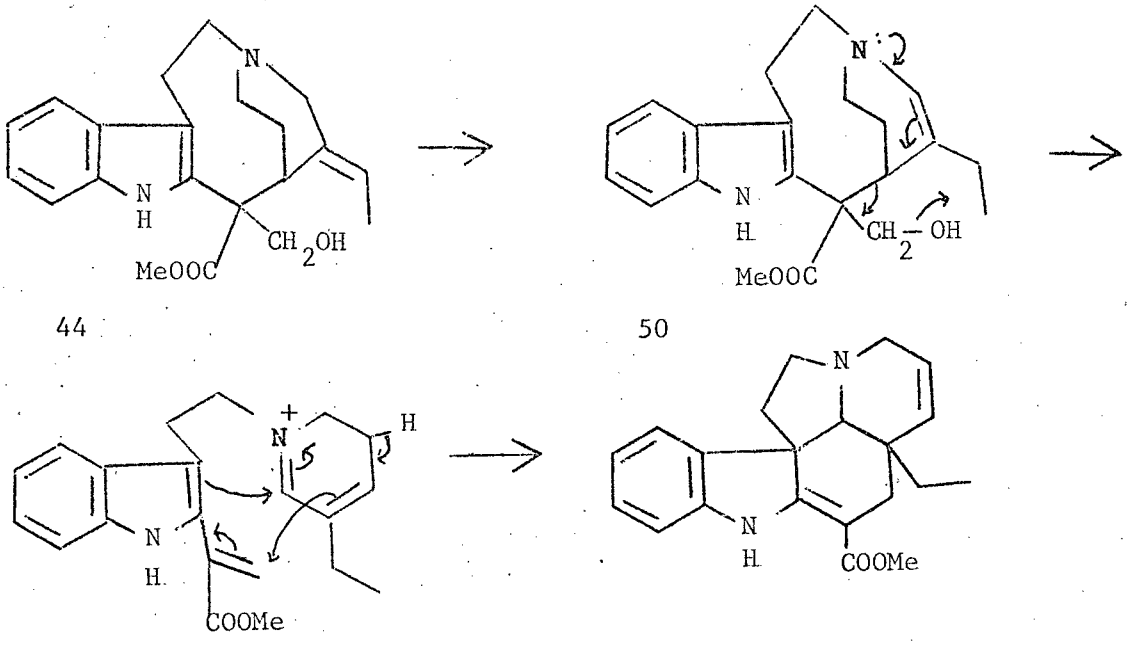


Figure 4. Postulated biosynthesis of tabersonine (45).

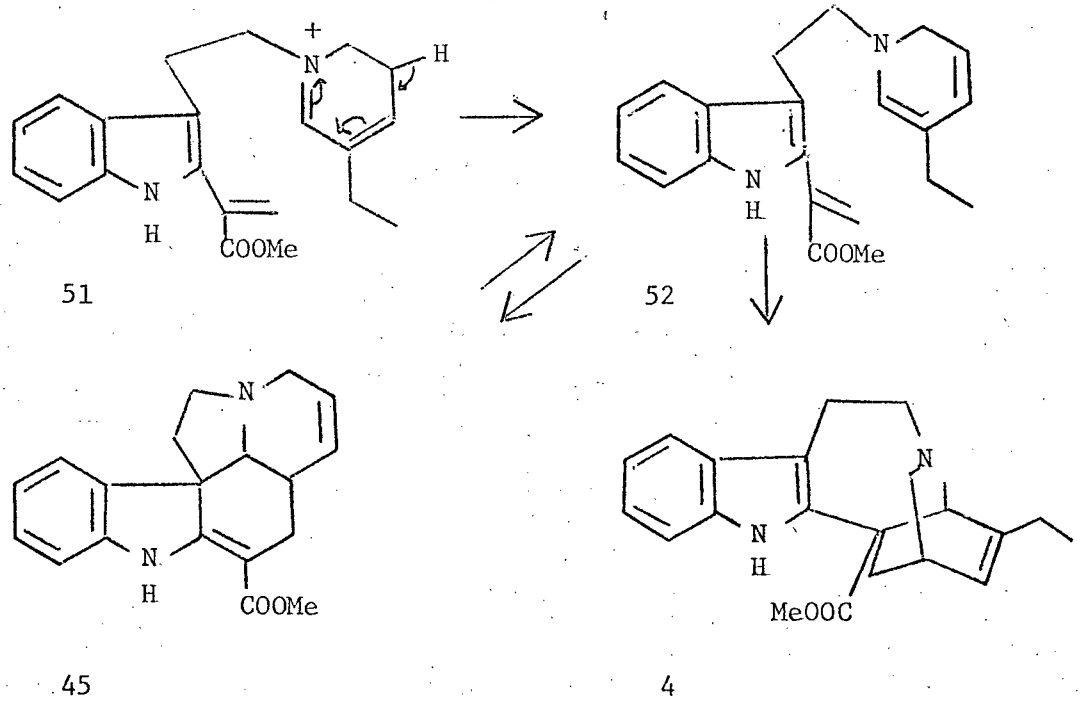
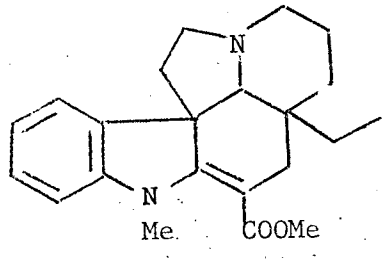


Figure 5. Postulated biosynthesis of catharanthine (4).

Tabersonine has been incorporated by Vinca rosea plants[4] and seedlings[43] into both vindoline and catharanthine. The conversion of tabersonine into catharanthine is interesting since it formally requires a partial reversal to acrylic ester (52). In Vinca minor, tabersonine is incorporated into vincamine (7) and minovine (8).[52]



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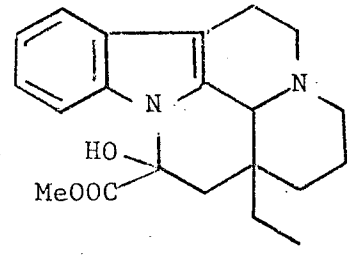

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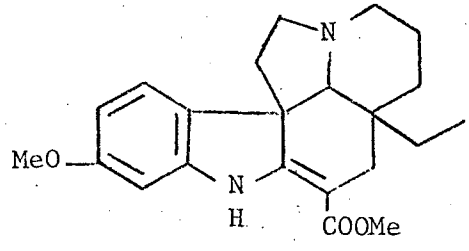
The detection of 11-methoxytabersonine (53)[41] as a time intermediate between the occurrence of tabersonine and vindoline is expected since that methyl ether grouping is necessary for vindoline (3).

The acrylic ester derivatives 51 or 52 remain still to be detected in the biological system although derivatives of them have been detected. The derivatives tetrahydrosecodine (54) and dihydrosecodine (55) have been



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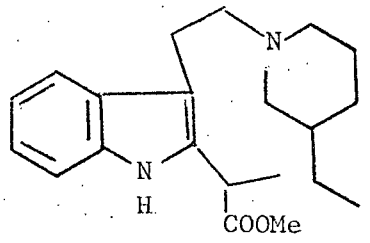
detected in Rhazya stricta[53] and tetrahydrosecodin-17-ol (56) has been detected in Rhazya orientalis[53]. Battersby[54] has detected dihydrosecodin-17-ol (57) in Vinca rosea plants. Both Battersby[56] and Kutney[57] have



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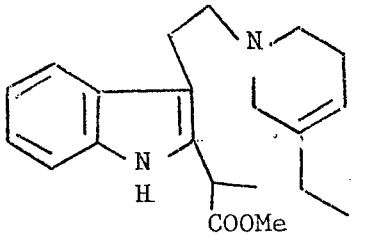

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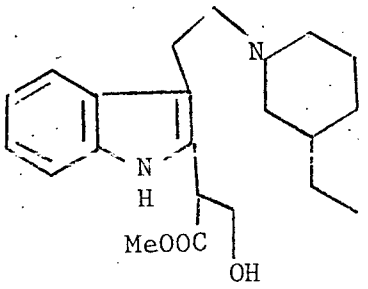

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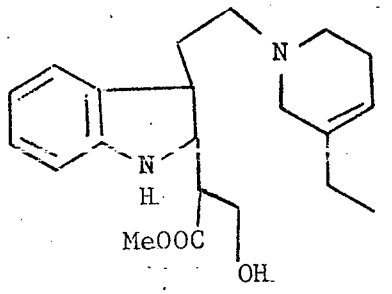

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found that dihydrosecodin-17-ol (57) was not a precursor in Vinca rosea and Vinca minor. Work on this aspect is continuing and synthetic steps leading to acrylic ester derivatives will be described in this thesis.

A summary of the biosynthetic sequence is found in Figure 6.

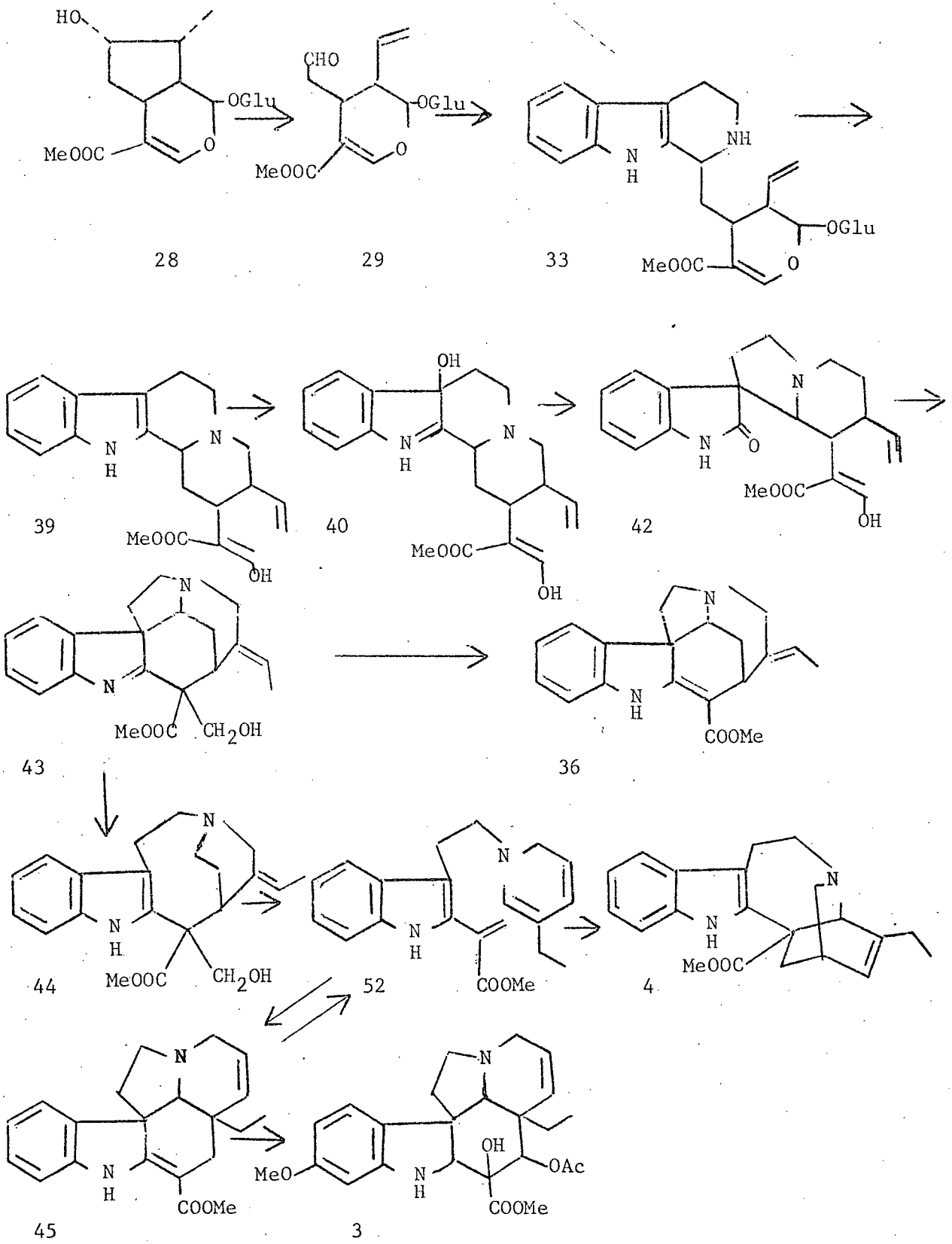


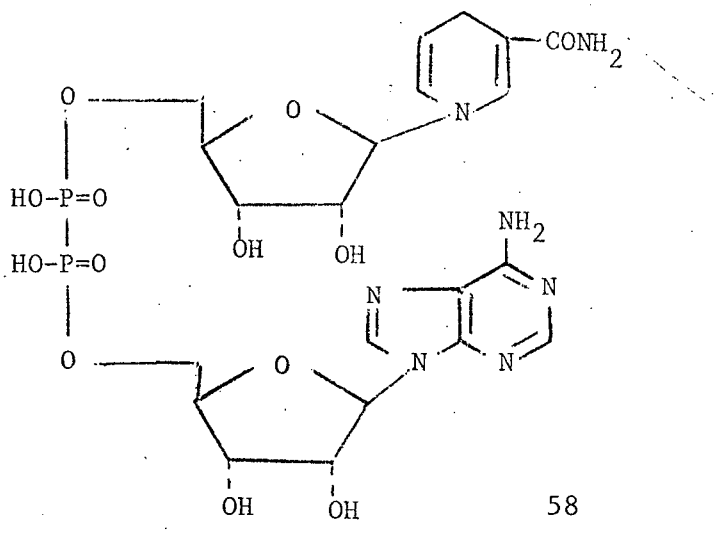
Figure 6. Summary of pathway from loganin to indole alkaloids.

Discussion

Acrylic ester - dihydropyridine derivatives were postulated as bio-intermediates in indole alkaloid biosynthesis. Efforts to synthesize intermediates of that type, or derivatives thereof, plus some biosynthetic studies of a possible precursor will be discussed.

The synthesis of a derivative containing both acrylic ester and dihydropyridine functionalities is fraught with difficulties either real or imagined. The first difficulty was foreseen in the acrylic ester portion. Acrylic acid polymers were prepared in the early 1870's with polymerization of esters noted shortly after. Acrylic esters polymerized under the influence of heat, light, oxygen, or peroxides. On the basis of these results it was felt that the acrylic ester function should be elaborated at a late stage in the synthesis. It was also undesirable to have the reactive acrylic ester moeity undergo reaction when it was submitted to precursor studies in the plant system. To circumvent this possibility a hydrated form of the acrylic ester was the synthetic goal. This choice allows for a possible chemical dehydration either prior to the biosynthetic studies or *in vivo* by an enzymatic process.

The other difficulty to be considered was the instability of dihydropyridine systems.[57] They are known to oxidize to the corresponding pyridine readily, contact with atmospheric oxygen will frequently be sufficient. Some dihydropyridines which have substituents capable of conjugation with the unsaturated system of the dihydropyridine are relatively stable. The reduced form of nicotinamide adenine dinucleotide (NADH, 58)



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may be cited as such a case. Two choices were available: the use of an acetyl group at the C(3) position of the dihydropyridine; or the use of an ethyl group at this position but in a tetrahydropyridine system. The first possibility, while it may give a relatively stable dihydropyridine, would have an oxygen function in the two carbon side chain, a feature not found in the alkaloids being examined. The second possibility, using the ethyl side chain, circumvents that drawback but gives an undesired oxidation level in the heterocyclic ring. The second choice was subsequently selected with the understanding that an in vivo oxidation may be necessary.

The overall sequence is outlined in Figure 7. The synthesis of 2-carboethoxy-3-(β-chloroethyl)-indole (60) had been previously designed in our laboratories but was modified slightly in the present case. The sodium salt of diethyl malonate was treated with 1-chloro-3-bromopropane to give diethyl γ-chloropropylmalonate (59). In the previous synthetic scheme, dry powdered benzenediazonium chloride was added to the sodium salt of 59 and the resulting adduct refluxed in acidic ethanol to produce the indole derivative 60. In this work, the benzenediazonium chloride

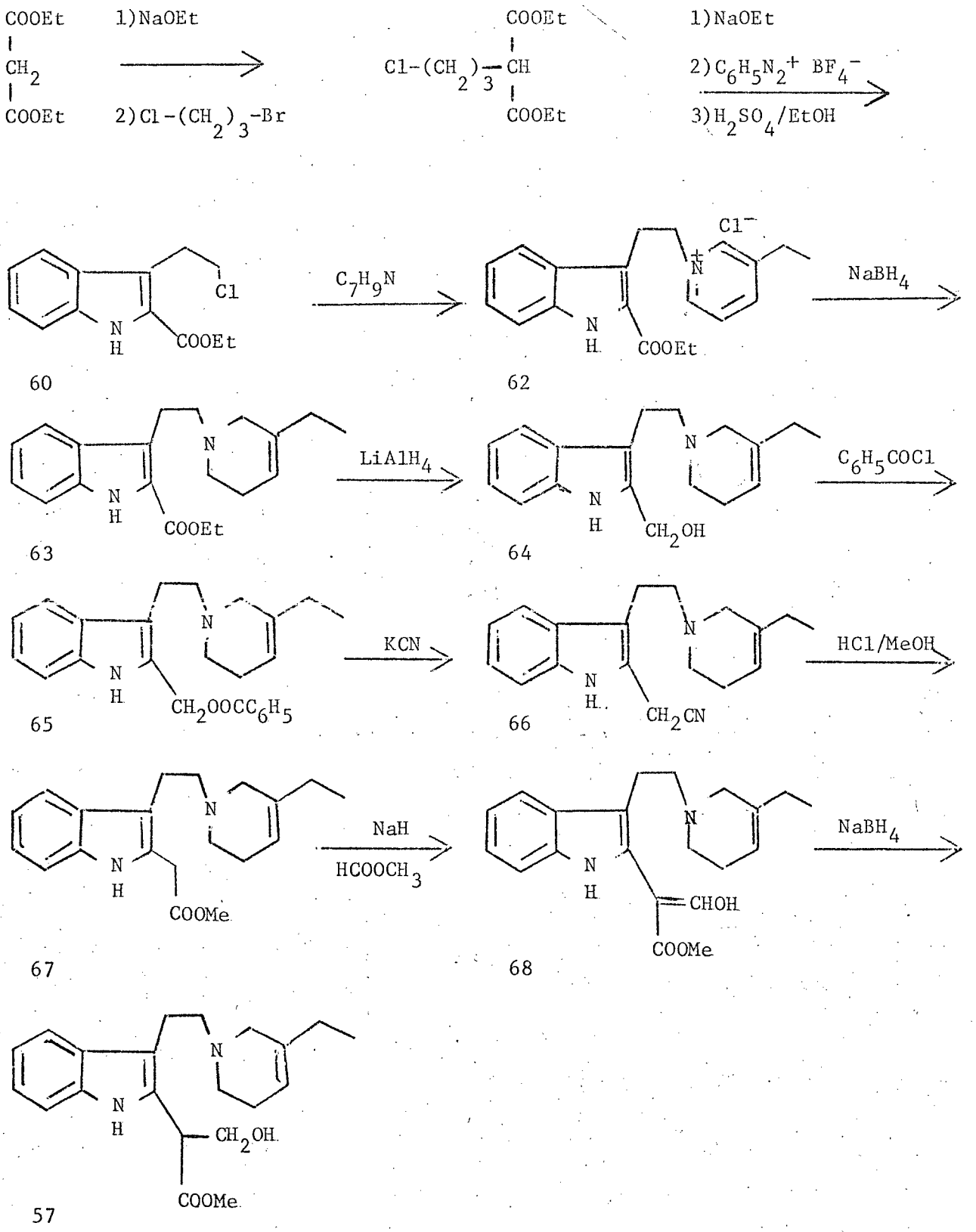


Figure 7. Synthesis of 16,17-dihydrosecodin-17-ol (57).

was replaced with the corresponding fluoroborate salt which is less sensitive to shock and thermally more stable. The indole derivative prepared in this way was identical to the product isolated previously and it was obtained in higher overall yield.

The required 3-ethylpyridine (61) obtained by Wolff-Kishner reduction of 3-acetylpyridine, was condensed with the indole derivative 60 to give N-[β{3(2-carboethoxyindolyl)}ethyl]-3-ethylpyridinium chloride (62) as a white crystalline solid.

Reduction of the pyridinium chloride (62) in cold methanol with sodium borohydride gave N-[β{3(2-carboethoxyindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (63). Lithium aluminum hydride reduction in refluxing tetrahydrofuran gave, after chromatography, N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (64). The NMR spectrum had a broadened singlet for one olefinic proton (τ 4.45); a singlet for the hydroxymethylene (τ 5.19); a well resolved quartet for the methylene group of the ethyl side chain (τ 8.02, J = 6.5 Hz); and a triplet for the methyl group of the ethyl side-chain (τ 8.98, J = 6.5 Hz); all in good agreement with structure 64.

The alcohol (64) could be benzoylated in dry pyridine with benzoyl chloride to give N-[β{3(2-benzoxymethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (65). This derivative so obtained could be used directly in the next reaction. A solution of the benzoate (65) in dimethylformamide containing a large excess of potassium cyanide was slowly heated to a maximum of 120°C for two hours and this mixture upon work up gave N-[β{3(2-cyanomethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (66)

in varying yields. The NMR spectrum of the nitrile had a two proton singlet for the methylene carrying the nitrile absorbing at τ 6.12.

The nitrile (66) was readily hydrolyzed to N-[β{3(2-carbomethoxymethyl-indolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (67) in methanol with concentrated hydrochloric acid. A less satisfactory procedure was alkaline hydrolysis of the nitrile (66) and subsequent esterification. Both methods gave the same ester (67). The ester (67) could be alkylated in benzene using methyl formate and sodium hydride as the base. The resulting enol (68) was not isolated but was reduced directly with sodium borohydride in methanol under carefully controlled conditions to give 16,17-dihydrosecodin-17-ol (57).

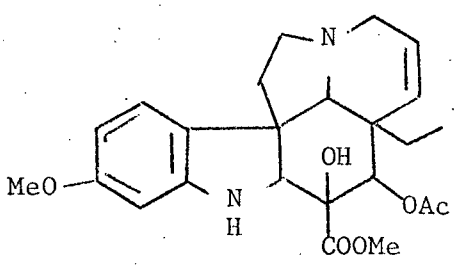
The NMR spectrum of 57 exhibited signals for the olefinic proton (τ 4.61), the hydroxymethyl group (τ 6.02), the methyl ester (τ 6.38), and the methylene group (τ 8.10) and methyl group (τ 9.04) of the ethyl side chain. This data was in full agreement for 16,17-dihydrosecodin-17-ol (57).

With a possible precursor now available, attention was focused on providing a radioactive substance necessary for biosynthetic investigations. A method had been developed in our laboratories for exchanging the aromatic protons of the indole system for tritium atoms. The method involves transferring tritiated trifluoroacetic acid, prepared from equal molar quantities of trifluoroacetic anhydride and tritiated water, in a vacuum system to the alkaloidal material. After a period of one or two days the acid is removed in a vacuum system and the labelled alkaloid neutralized and isolated.

The above procedure had been used many times to prepare labelled

derivatives with no difficulties being encountered. In the present case DL-tryptophan was labelled in this manner. However when 16,17-dihydro-secodin-17-ol (57) was subjected to these conditions none of the labelled alcohol (57) could be detected by TLC. The alcohol had evidently decomposed during this treatment. Successful labelling could, however, be carried out on the ester (67) prior to alkylation. The labelled ester thus obtained could then be alkylated and reduced as before to give a product identical to 57 except for the enrichment of tritium in the aromatic portion.

Earlier investigators in our laboratories had developed a method for the incorporation of various substances into Vinca rosea L. plants using a cotton wick in the stem of the living plant. In order to ensure that the experimental technique was satisfactory and to make sure that the age of the plant was suitable for biosynthetic investigations, the tritiated DL-tryptophan prepared previously was fed. After nine days, the optimum conditions as determined by earlier investigations, the plants were macerated and extracted for alkaloids. Chromatography of the crude alkaloidal extract on alumina gave, among other alkaloids, vindoline (3), catharanthine (4), and ajmalicine (5). The labelled alkaloids were diluted with cold



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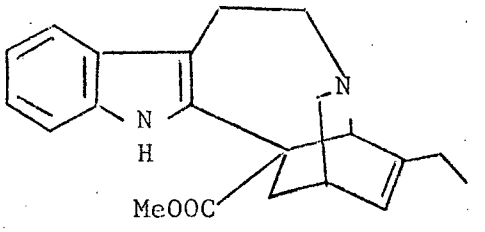

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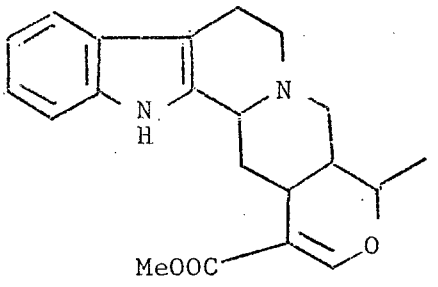


5

or unlabelled alkaloids and were repeatedly crystallized either as the free base or as the hydrochloride salt to constant activity. The percentage incorporations observed for this experiment are shown in Table I.

Compound Fed	Percent Incorporation		
	catharanthine	vindoline	ajmalicine
[ar-^{3}H]-DL-tryptophan	0.980	0.155	0.312
[ar-^{3}H]-alcohol (57)	0.0007	inactive	0.0004

Table I. Results of incorporation of synthetic intermediates into V. rosea L. plants.

The successful incorporation of tryptophan indicated that the experimental method was satisfactory and that the age of the plants selected was suitable for biosynthetic investigation.

The synthetic alcohol (57) was tested in the same manner. The results after work up, chromatography, and crystallization to constant activity are given above in Table I.

Although a very small incorporation was observed, it was impossible to tell if this was evidence of true incorporation or if it was simply the

result of an aberrant biosynthetic pathway. Several months later Professor Battersby reported[54] that his laboratories had found evidence for the alcohol (57) in V. rosea plants. They too had tested the compound as a precursor and had found very slight or no incorporation.[55]

At the same time as alcohol (57) was being tested in V. rosea L. other members of our laboratories were testing the compound in V. minor L. The test period in V. minor is two days and in the time it took to obtain results in V. rosea several testings could be done on V. minor. The detailed observations will be reported elsewhere, but essentially the isolated alkaloids were inactive or possibility with a trace of activity.

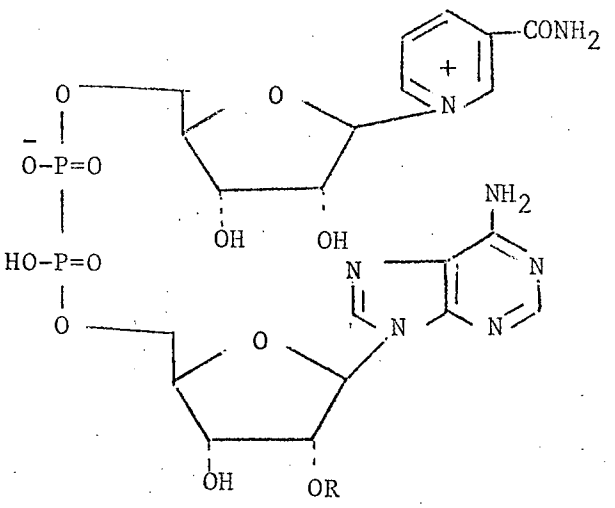
With the apparent failure of the alcohol (57) to act as an efficient precursor for the indole alkaloids, it was necessary to redirect our synthetic efforts.

It will be recalled that the desired system was an acrylic ester - dihydropyridine derivative. The compound just tested differed in oxidation level in both the acrylic ester and the heterocyclic portions of the molecule. Alteration of the oxidation level in the ester portion was investigated by other members of our laboratories and will be reported elsewhere.

Although the postulated intermediate was a dihydropyridine derivative, it was still considered to be too labile to be isolated and submitted for biosynthetic evaluation. In this situation it seemed that the corresponding pyridinium salt may provide the solution.

Pyridinium salts are found in vivo and are known to be reduced enzymatically to dihydropyridines. Two well known examples of pyridinium

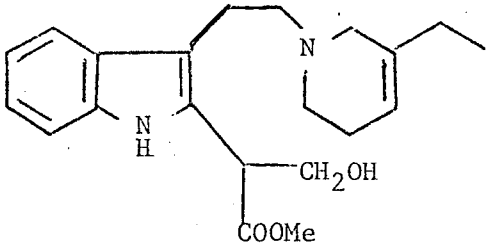
salts which may be reduced are the co-enzymes nicotinamide adenine dinucleotide (NAD, 69) and the phosphorylated form (NADP, 70).



69, R = H

70, R = PO_3H_2

A pyridinium salt had been prepared in the above synthetic sequence but its oxidation level could not be preserved in later synthetic steps. It was necessary in that instance to reduce the ester function to an alcohol under conditions which also would reduce the pyridinium salt. Even if the pyridinium salt had been preserved at this step, it would not likely survive either the nitrile formation or alkylation steps. The tetrahydropyridine system seemed to overcome these difficulties as this was the oxidation level which had been used previously and found stable. What was necessary then was to find conditions for oxidation of the tetrahydropyridine (57) to pyridinium salt (71).



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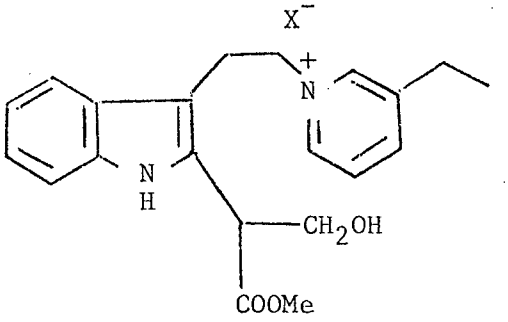
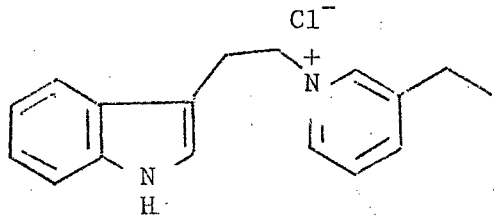


71

To study this sequence a model pyridinium salt N-[β(3-indolyl)ethyl]-3-ethylpyridinium bromide (72) was prepared by the condensation of tryptophyl bromide and 3-ethyl pyridine. The pyridinium bromide was reduced with sodium borohydride as before to give N[β(3-indolyl)ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (73).



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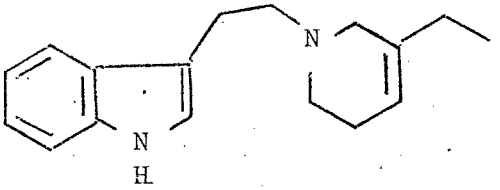
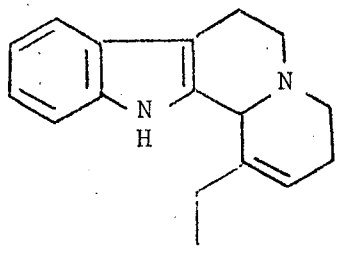


73

Mercuric acetate is a common oxidant in alkaloid chemistry and was the reagent selected for these oxidations. Thus compound 73 was added to a solution of 2% aqueous acetic acid containing mercuric acetate and the disodium salt of ethylenediaminetetraacetic acid (EDTA)[58,59] and maintained at a temperature of 100°C for one and a half hours. After cooling the precipitated mercurous acetate was filtered off and the solution extracted with methylene chloride. The resultant product, obtained as a brown residue, consisted of several components and its weight corresponded to 70% overall recovery of organic material. The major component was separated by preparative layer chromatography and gave a typical indolic ultraviolet absorption spectrum (λ_{max} 294, 283, 274, and 230 mμ). The NMR spectrum had, among other signals, the NH resonance (τ 2.21), four aromatic protons

(τ 2.45 - 3.05), and one olefinic proton (τ 4.38). Low resolution mass spectrometry revealed a molecular ion at m/e 252. The structure suggested by this data is 74. Another structural possibility possessing this molecular weight could be the dihydropyridine derivative 75. However, the stability of the product in air, the lack of sufficient aromatic and olefinic protons in the NMR spectrum, and the typical indolic UV spectrum would suggest that this alternative (75) is unlikely.



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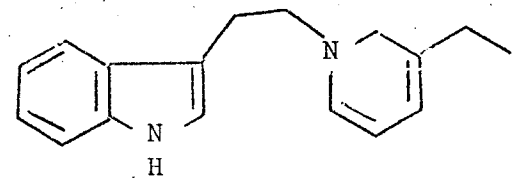


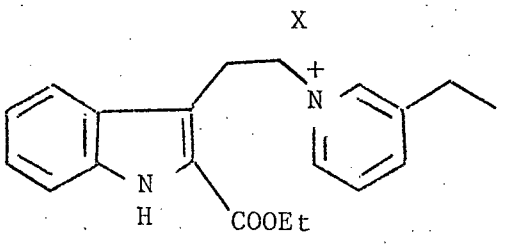
75

The aqueous portion of the above oxidation reaction mixture was treated with hydrogen sulfide gas to precipitate the excess mercuric salt used as an oxidant. The precipitate was removed by filtration through Celite and the filtrate evaporated to dryness to give a yellow solid. This solid was mainly the added EDTA salt plus some organic compound which could not be separated.

The next mercuric acetate oxidation experiment was changed in several ways. First, the model compound was changed to the previously prepared 2,3-disubstituted indole derivative 63 in an effort to minimize cyclization

onto the indole ring. Also, the EDTA salt was eliminated in this case. It was well known that alkaloids had been oxidized with mercuric acetate without the addition of EDTA salt although its addition was shown to decrease the possibility of carbon - carbon bond fission.[59] Further, the temperature was changed to room temperature and the solvent changed from aqueous acid to ethanol in an effort to perform the reaction under the mildest conditions possible.

The sought for pyridinium salt should exist in the oxidation solution as the acetate 76. As an aid to recognition N-[β{3(2-carboethoxyindolyl)} ethyl]-3-ethylpyridinium acetate (76) was prepared by treatment of a solution of the pyridinium chloride 62 with a solution of silver acetate. The resulting silver chloride precipitated and was removed by filtration to give crude pyridinium acetate 76 upon evaporation.



62, X = Cl^-

76, X = OAc^-

The tetrahydropyridine derivative 63 was oxidized with mercuric acetate in ethanol at room temperature for four days. Removal of the mercurous acetate and treatment with hydrogen sulfide as before gave, after filtration, a yellow solution. Concentration of this solution

and examination by TLC showed some starting material and both faster and slower running compounds, plus some material still at the origin of the chromatogram. None of the compounds had similar TLC properties to the desired pyridinium acetate. Separation of the mixture on preparative layer chromatography and examination of each fraction, including the material at the origin, by UV showed that none of the fractions had the absorption of the pyridinium system 76. Repeating the oxidation reaction using the same conditions failed to produce the desired pyridinium salt.

Since there had been a lack of success in the previous mercuric acetate oxidations, the conditions were varied in a further study. In these cases, the temperature and period of reaction were both varied. In one case oxidation was allowed to proceed at 35°C for 18 hours, in another at 50°C for four hours. In both experiments the yield of mercurous acetate was about 90% of the theoretical amount, yet it was not possible to detect any pyridinium acetate 76. Other experiments in glacial acetic acid and in 10% aqueous acetic acid, left for three days at room temperature, gave approximately 60% of the theoretical amount of mercurous acetate but again none of the desired material was found.

Concurrently with the mercuric acetate oxidation experiments other synthetic work was being done. The possibility of using a 3-acetylpyridine as one of the condensing units was interesting. The acetyl side chain may stabilize a dihydropyridine intermediate sufficiently to allow isolation and subsequent biosynthetic studies. The other interesting point is that compounds with an acetyl side chain had been proposed by Wenkert[15] as possible intermediates and would be accessible for biosynthetic evaluation

if 3-acetylpyridine were used in the initial condensation.

Condensation of 2-carboethoxy-3(β-chloroethyl)indole (60) prepared earlier with the ethylene ketal of 3-acetylpyridine, gave N-[β{3(2-carboethoxyindolyl)}ethyl]-3-acetylpyridinium chloride ethylene ketal (77) as a light gray powder (Figure 8).

Sodium borohydride reduction of pyridinium chloride 77 in methanol at 0°C followed by careful work up gave N-[β{3(2-carboethoxyindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine ethylene ketal (78) as a white crystalline solid. The NMR spectrum of 78 contained a four proton multiplet (τ6.14) for the protons of the ethylene ketal protecting group.

The carboethoxy derivative 78 was reduced in tetrahydrofuran and the desired N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine ethylene ketal (79) purified by column chromatography on alumina.

The ketal alcohol 79 was then subjected to the same homologation steps as performed previously. Treatment of the ketal alcohol with benzoyl chloride gave N-[β{3(2-benzoxymethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine ethylene ketal (80).

The benzoate group in compound 80 was then displaced by cyanide ion to give N-[β{3(2-cyanomethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine ethylene ketal (81). This compound was recognizable by the characteristic nitrile absorption in the IR spectrum at 2255 cm^{-1}.

Hydrolysis of the ketal nitrile 81 was accomplished in a methanolic solution saturated with hydrogen chloride gas. Purification by column chromatography on alumina afforded N-[β{3(2-carbomethoxymethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine (82). The IR spectrum of this

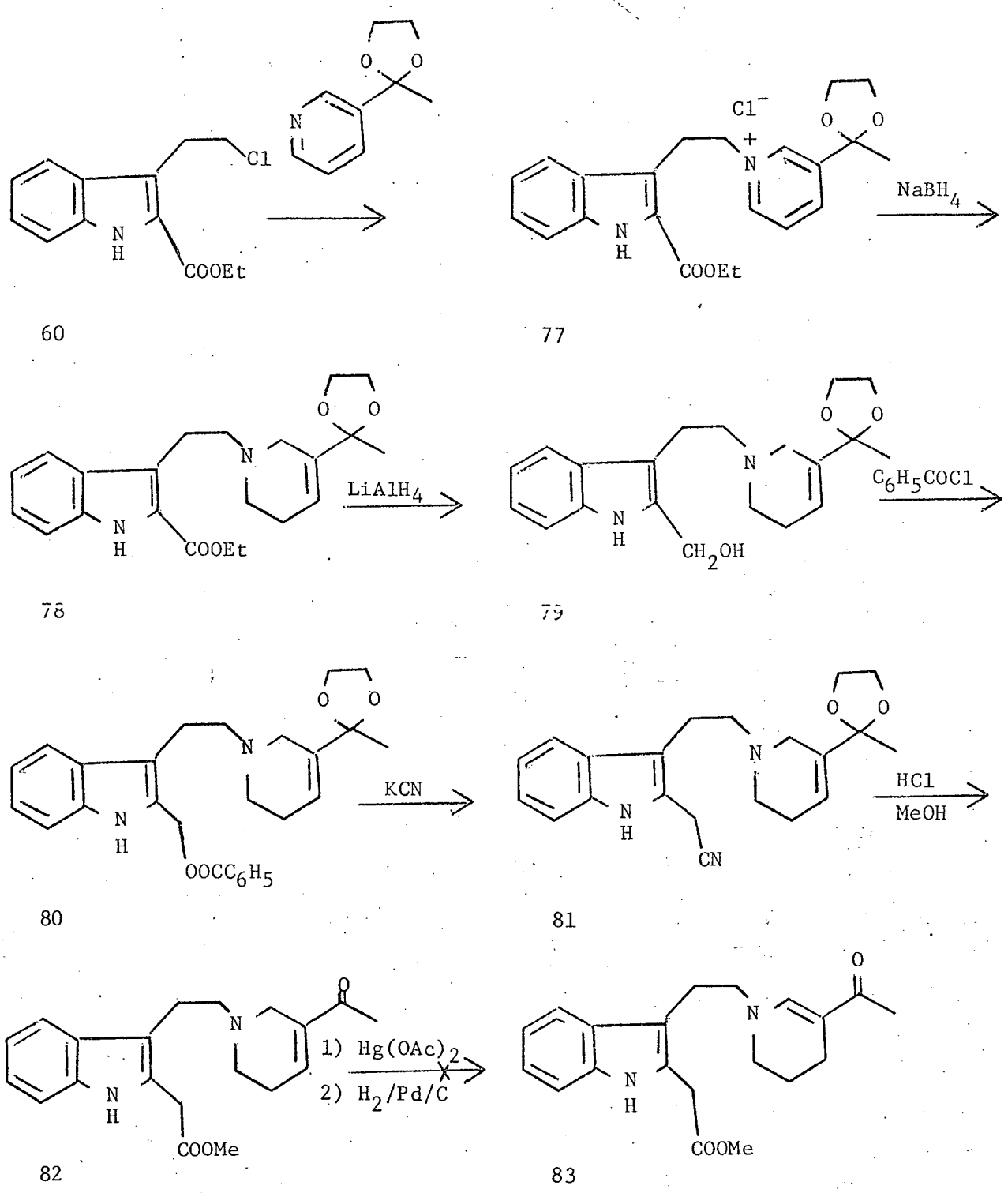


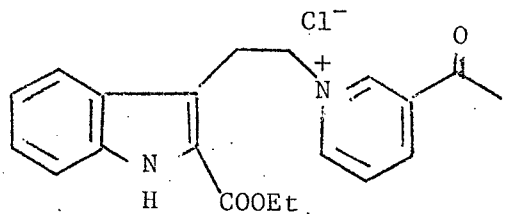
Figure 8. Attempted synthesis of vinylogous amide 83.

compound had two carbonyl absorptions (1727 and 1652 cm^{-1}) for an ester function and an α,β-unsaturated ketone. The NMR spectrum had resonances for one olefinic proton (τ 3.17), a methyl group of the ester function (τ 6.33), and a methyl group of a ketone (τ 7.75) in full agreement with structure 82.

When this synthetic sequence was conceived, it was intended to oxidize the unsaturated ketone 82 to the corresponding pyridinium system and then catalytically reduce[47] the latter to the vinylogous amide 83. Hopeful that this might still be the case, even though the concurrently conducted oxidation reactions in the ethyl series were unsuccessful, the keto ester 82 was subjected to the oxidation reaction. . The oxidation mixture, worked up as before, was immediately subjected to catalytic reduction. The IR spectrum of the crude product obtained from the latter reaction was not in accord with the expected data[47] for the amide while attempts to purify the products resulted in great losses of material.

The overall lack of success with the oxidation reactions necessitated yet another approach. Condensation of 2-carboethoxy-3-(β-chloroethyl)-indole (60) with 3-acetylpyridine gave, in early preparations, a very dark solid which tended to become gummy upon standing in air. Later condensations performed at a lower temperature gave lower yields of N-[β{3(2-carboethoxy-indolyl)}ethyl]-3-acetylpyridinium chloride (84) but the product now was an orange - red crystalline solid. Hydrogenation of the salt with palladium catalyst gave N-[β{3(2-carboethoxyindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahdyropyridine (85) quickly recognized by its characteristic spectroscopic properties. Thus the next step in the sequence required reduction of the

ester while leaving the amide intact.



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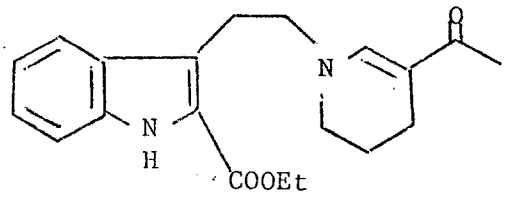
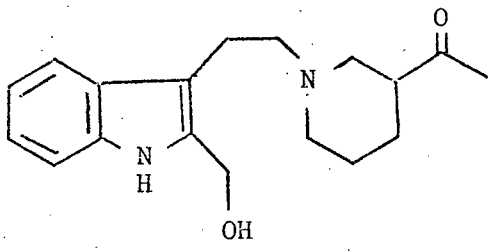


85

Use of lithium aluminum hydride in tetrahydrofuran at 0°C as the reducing agent gave complete disappearance of starting material within 15 minutes. Work up and chromatography on alumina gave one main compound. The IR spectrum had one carbonyl absorption at 1710 cm^{-1} suggesting a saturated ketone. The NMR spectrum had lost the signals for the olefinic and methyl group protons of the amide chromophore and had new signals at τ 5.32 for the hydroxymethyl group and a singlet for the saturated ketone at τ 7.90 thereby suggesting N[β{3(2-hydroxymethylindolyl)}ethyl]-3-acetylpiperidine (86).



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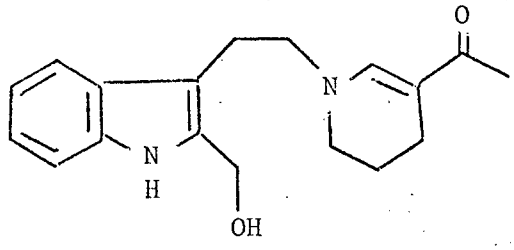


87

If the lithium aluminum hydride reduction was started at -30°C and the temperature slowly raised to 0°C over a few hours some N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine (87) could be isolated in about 10% yield. In this instance some starting material as well as keto alcohol 86 were also obtained. The presence of 87 indicated that the ester group was being reduced preferentially to the vinylogous amide.

When the reduction was done with lithium borohydride in tetrahydrofuran at 0°C no reaction was observed after three hours. Allowing the solution to warm to room temperature for one and a half hours seemed to be without effect. However, at reflux temperature the major product was again the keto alcohol 86 and not the desired amide alcohol 87.

Sodium borohydride will not normally reduce the ester carbonyl although there are reports of esters being reduced with this reagent.[60] Thus to a cold (0°C) methanol solution of the amide ester 85 sodium borohydride was added and the temperature slowly raised to reflux over a two hour period. However no reduction products could be detected. Repeating the reaction but maintaining the temperature at 0°C for one hour before warming again failed to give any indication of reduction.

Although the amide alcohol 87 could be obtained from some of the reductions, the small amounts isolated precluded the usefulness of this synthetic approach.

The next approach has, of yet, not given the product desired for biosynthetic investigations but has allowed synthesis to proceed closer to that objective than has other syntheses reported thus far. The

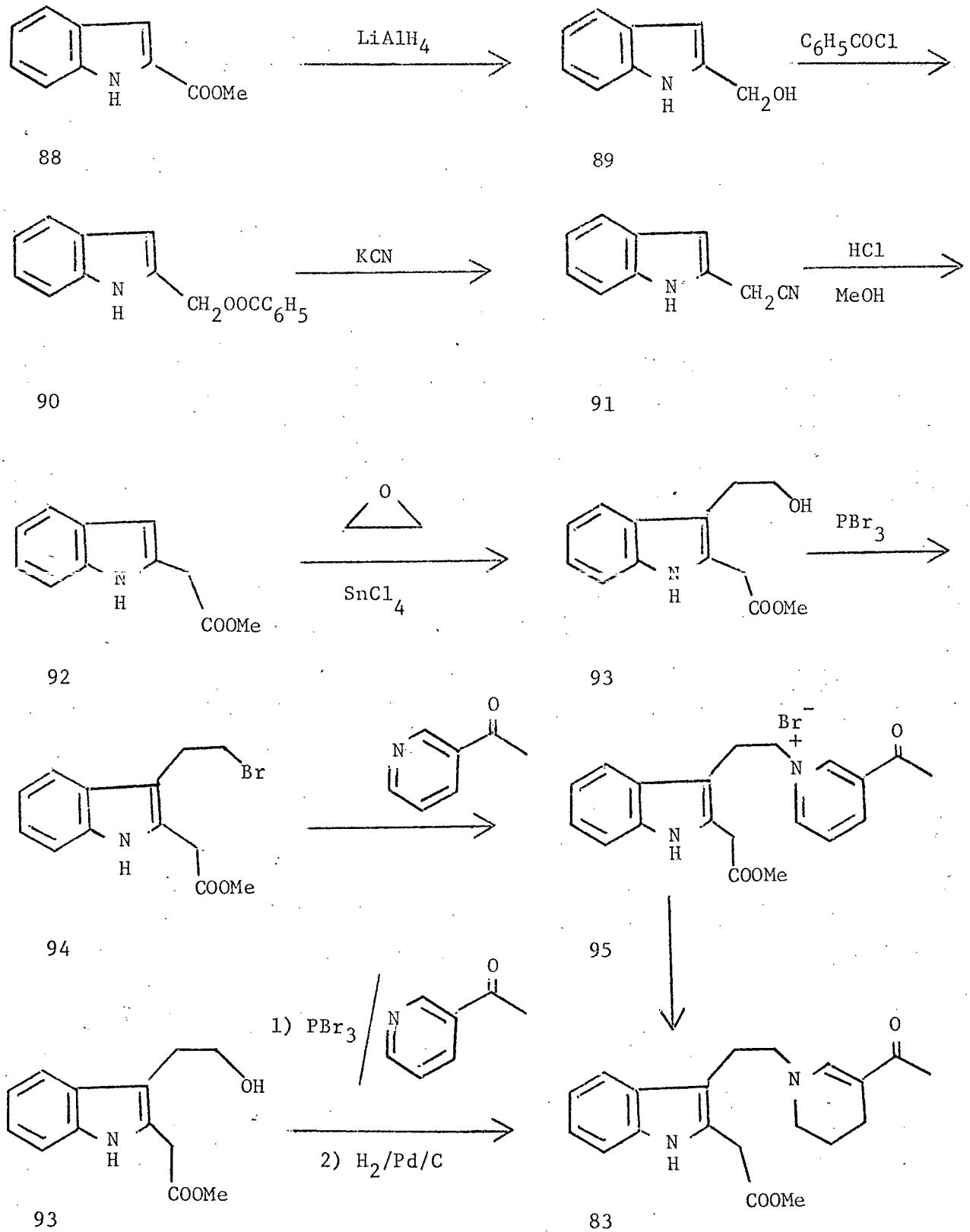
synthetic approach is outlined in Figure 9.

Methyl indole-2-carboxylate (88) was reduced with lithium aluminum hydride to give 2-hydroxymethylindole (89). After chromatography and crystallization the alcohol was obtained as a white solid in 70% yield.

Use of the same homologation sequence as before allowed extension of the C(2) substituent by one carbon. Thus treatment of 2-hydroxymethylindole with benzoyl chloride gave, in near quantitative yields, 2-benzoxymethylindole (90). Displacement of the benzoate group by cyanide ion resulted in the formation of 2-cyanomethylindole (91) as a white crystalline solid.

Formation of the desired carbomethoxy function was accomplished by hydrolysis of the nitrile 91 in methanol saturated with hydrogen chloride gas. The resulting methyl indole-2-acetate (92) was recognizable by its IR absorption at 1719 cm^{-1} for the ester carbonyl. The NMR spectrum had the C(3) proton of the indole moiety at τ 3.68 and the methyl ester group at τ 6.32.

The necessary ethyl bridge at C(3) of the indole nucleus could be inserted under relatively mild conditions using ethylene oxide and stannic chloride.[61] The yield of methyl 3(β-hydroxyethyl)indole-2-acetate (93) was only moderate but some starting material (92) could be recovered and re-used in subsequent reactions. The NMR spectrum had new signals at τ 6.25 and 7.12 for the two methylene groups of the hydroxyethyl side chain. Significantly the signals formerly assigned to the C(3) proton of the indole nucleus was now absent, suggesting substitution had occurred at C(3) as indicated.



88 89 90 91 92 93 94 95 93 83

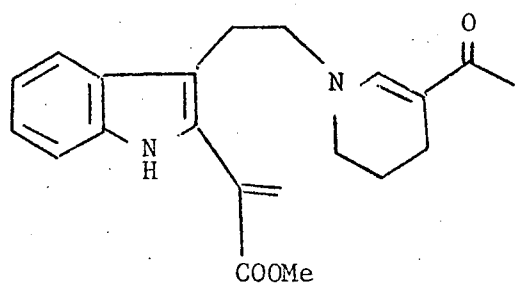
Figure 9. Syntheses of vinylogous amide 83.

Treatment of 93 with phosphorous tribromide gave methyl 3(β-bromoethyl)indole-2-acetate (94) as an oil which decomposed on storage and hence was condensed with 3-acetylpyridine immediately. The resulting pyridinium bromide 95 was formed but was contaminated with the excess of 3-acetylpyridine. The pyridinium bromide 95 could be catalytically reduced to give the desired N-[β{3(2-carbomethoxymethylindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine (83).

A more satisfactory procedure for the preparation of 83 was achieved with phosphorous tribormide in 3-acetylpyridine. The product of this reaction was not isolated but immediately subjected to hydrogenation to yield the same vinylogous amide 83 as before but in much better yield.

The vinylogous amide 83 obtained by either procedure had absorption in the IR spectrum for the ester carbonyl (1720 cm^{-1}) and the amide carbonyl (1610 cm^{-1}). The NMR spectrum had a singlet for one olefinic proton (τ 3.15), a three proton singlet for the carbomethoxy group (τ 6.38), and a three proton singlet for the methyl of the acetyl group (τ 8.20).

With the synthesis of the vinylogous amide now established, other members of the laboratory are investigating methods for the synthesizing of the possible bio-intermediate 96 in indole alkaloid biosynthesis.



96

Experimental

Throughout this work Woelm neutral alumina or Merck silica gel G with added fluorescent indicator were used as adsorbent for thin layer chromatography (TLC). The chromatograms, 0.3 mm. in thickness, were air dried and activated in an oven at 100°C for three hours. For preparative layer chromatography a thicker layer (0.5 mm.) of adsorbent was used. The chromatograms were developed in a variety of solvents. Compounds were detected using antimony pentachloride in carbon tetrachloride (1:2).

Column chromatography was usually performed on Woelm neutral alumina deactivated to Activity III according to the manufacturers' direction.

Infrared (IR) spectra were measured on a Perkin Elmer model 21, 137, or 457 instrument. Samples were measured either as KBr pellets or in chloroform solution. The position of absorption maxima are given in wave numbers (cm^{-1}).

Ultraviolet (UV) spectra were measured in methanol or ethanol on a Cary model 11 or model 15 instrument. The position of absorption maxima (λ_{max}) are given in millimicrons (mμ).

Nuclear magnetic resonance (NMR) spectra were measured in deuterochloroform, unless otherwise noted, at either 60 MHz using a Jelco C-60, a Varian A-60, or a Varian T-60 or at 100 MHz using a Varian HA-100 instrument. The chemical shifts are given in the Tiers τ scale with reference to tetramethylsilane as internal standard set at 10.0 units.

Mass spectra were measured on an Associated Electrical Industries MS 9 high resolution mass spectrometer or on an Atlas CH 4 spectrometer.

High resolution molecular weight determinations were determined on the MS 9 spectrometer.

Melting points were determined on a Kofler block and are uncorrected.

Elemental analyses were performed by Mr. P. Borda, University of British Columbia.

Radioactivity was measured with a Nuclear-Chicago Mark 1 Model 6860 Liquid Scintillation counter in counts per minute (cpm). The radioactivity of a sample in disintegrations per minute (dpm) was calculated using the counting efficiency which was determined for each sample by the external standard technique[62] utilizing the built-in barium 133 gamma source. The radioactivity of the sample was determined using a scintillator solution made up of the following components: toluene (1 litre); 2,5-diphenyloxazole (4 gms.); and 1,4-bis[2-(5-phenyloxazolyl)]benzene (50 mgs.). In practice a sample of an alkaloid as the free base was dissolved in benzene (1 ml.) in a counting vial or, in the case of an alkaloidal salt, the sample was dissolved in methanol (1 ml.) in a counting vial. Then, in both cases, the volume was made up to 15 mls. with the above scintillator solution. For each sample counted the background was determined for the counting vial to be used by filling the vial with one of the above scintillator solutions and counting to determine the background cpm. The counting vial was emptied, refilled with sample to be counted and the scintillator solution, and counted. The difference in cpm between the background count and the sample count was used for subsequent calculations.

Diethyl γ-chloropropylmalonate

A solution of diethylmalonate (160 gms.) and 1,3-chlorobromopropane

(160 gms.) in anhydrous ether (200 mls.) was added in one portion to a solution of sodium ethoxide prepared by dissolving sodium (24 gms.) in dry ethanol (350 mls.). The reaction was maintained at 35°C for 4 hours and then allowed to stand at room temperature for 24 hours. The reaction mixture was poured in water (1,000 mls.) and extracted with ether. The ether extract was washed with water, saturated sodium chloride solution, dried over sodium sulfate, and concentrated under reduced pressure. The resulting oil was distilled (110°C at 1 mm.) to give a clear, colorless liquid (120 gms.). IR (film) 1730 (COOEt). NMR (60 MHz) 5.75 (4H, quartet, J = 7 Hz, $-COO\underline{CH}_2CH_3$), 6.56 (2H, triplet, J = 4 Hz, $-\underline{CH}_2Cl$), 6.75 (1H, triplet, J = 4 Hz, $-\underline{CH}(COOEt)_2$), 8.10 (4H, multiplet, $ClCH_2\underline{CH}_2\underline{CH}_2$), 8.80 (6H, triplet, J = 7 Hz, $-COOCH_2\underline{CH}_3$).

Benzenediazonium fluoroborate

Aniline hydrochloride (180 gms.) was dissolved in water (275 mls.) and concentrated hydrochloric acid (140 mls.) in a 3 litre beaker. This solution was cooled to -5°C in an ice - salt bath and sodium nitrite (69 gms.) in water (150 mls.) was added dropwise so that the temperature remained below 5°C. No more sodium nitrite solution was added when a drop of the aniline solution gave a blue coloration when tested on potassium iodide - starch paper. A solution of 48% fluoroboric acid (183 mls.) was cooled to 0°C and added slowly to the diazonium salt solution. Precipitation was immediate but stirring was maintained for 10 minutes after all the fluoroboric acid had been added. About one half of the precipitate was transferred to a sintered glass funnel and sucked as dry as possible with the aid of a rubber sheet. The vacuum was removed and the precipitate

was washed thoroughly with ice cold water (50 mls.) and sucked dry as before. In a similar manner the precipitate was washed with ice cold methanol (25 mls.) and, finally, ether (50 mls.). The precipitate was transferred to a tared beaker and dried in a vacuum desiccator containing anhydrous calcium sulfate for 18 hours. The remaining half of the precipitate was collected and stored in a similar manner. Combined weight of the benzenediazonium fluoroborate was 126 gms.

2-carboethoxy-3(β-chloroethyl)indole (60)

In a 5 litre, 3 neck flask sodium metal (14.7 gms.) was dissolved in dry ethanol (1,000 mls.) and γ-chloropropylmalonate (147 gms.) was added and the mixture stirred for 30 minutes at room temperature under a slight positive pressure of nitrogen. The solution was cooled to -10°C. Benzenediazonium chloride (126 gms.) was transferred in portions (~20 gms.) to an Elemmyer flask and then added slowly and cautiously through a short length of Gooch tubing to the stirred ethanolic solution keeping the solution below -5°C. After all the benzenediazonium fluoroborate had been added, the solution was stirred for 2 more hours at -5°C and then stored for 18 hours at -5°C.

The solution was then poured into water (1,500 mls.) and the red oil separated. The aqueous solution was thoroughly extracted with chloroform and the chloroform washings combined with the red oil. This chloroform solution was washed thoroughly with water, saturated salt solution, dried over sodium sulfate, and concentrated to a dark red oil.

The dark red oil was dissolved in ethanol (1,000 mls.) and concentrated sulfuric acid (200 mls.) was added slowly with stirring. The

mixture was refluxed for 12 hours then cooled and poured onto ice and neutralized. This mixture was extracted with chloroform thoroughly and the chloroform extract washed with water, saturated salt solution, dried over sodium sulfate, and concentrated under reduced pressure to give a brown solid (140 gms.).

This brown solid was dissolved in benzene and applied to a column of Shawinigan alumina (3 kgs.) deactivated by the addition of 3% (90 mls.) of a 10% acetic acid solution. Elution with benzene gave initially a red oil and finally the desired product as a crystalline solid. Further elution with chloroform eluted more of the desired product. The crystalline fractions were combined and crystallized from benzene - petroleum ether to give 29 gms. of white crystalline 2-carboethoxy-3(β-chloroethyl)indole (60) m.p. 130 - 132°C.

3-ethylpyridine (61)

A mixture of 3-acetylpyridine (60 gms.), 85% hydrazine hydrate (90 mls.), potassium hydroxide (50 gms.), and triethylene glycol (400 mls.) was heated under nitrogen for 2 hours at a bath temperature of 130°C. The solution was cooled and gradually reheated with a take off condensor to a bath temperature of 200°C. During this time the distillate (175 mls.) was collected. The distillate was dissolved in ethyl ether and washed with water, dried over sodium sulfate, and evaporated to a colorless liquid. Distillation of this liquid and collection of the fraction boiling at 60°C at 17 mm. gave 3-ethylpyridine as a colorless liquid. IR (film) no carbonyl band. NMR (60 MHz) 1.58 (2H, multiplet, aromatic), 2.70 (2H, multiplet, aromatic), 7.38 (2H, quartet, J = 7 Hz, $-C\underline{H}_2CH_3$), 8.82 (3H, triplet, J = 7 Hz, $-CH_2C\underline{H}_3$)

N-[β{3(2-carboethoxyindolyl)}ethyl]-3-ethylpyridinium chloride (62)

2-carboethoxy-3(β-chloroethyl)indole (2.9 gms.) in 3-ethylpyridine (12 mls.) was heated under nitrogen in a Carius tube at 120°C for 18 hours. The reaction mixture was cooled and titurated with ether and the pyridinium chloride 62 collected by filtration (3.7 gms.) m.p. 87 - 89°C. IR (KBr) 1680 (COOEt). UV 233, 297. NMR (CD_3OD) 5.63 (2H, quartet, J = 7 Hz, $CH_3-\underline{CH_2}-O-$), 7.34 (2H, quartet, J = 7.5 Hz, $CH_3\underline{CH_2}-C$), 8.60 (3H, triplet, J = 7 Hz, $\underline{CH_3}-CH_2O$), and 8.96 (3H, triplet, J = 7.5 Hz, $\underline{CH_3}CH_2C$).

N-[β{3(2-carboethoxyindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (63)

The pyridinium chloride 62 (4.1 gms.) was dissolved in methanol (300 mls.) and triethylamine (3 mls.). The solution was cooled in an ice - salt bath to -5°C and sodium borohydride (7 gms.) was added in small portions to keep the temperature below 0°C. Four hours after the addition of the sodium borohydride, the solution was evaporated to near dryness, water was added (~50 mls.) and dilute hydrochloric acid was added until the mixture was acidic. After 15 minutes the solution was basified with sodium bicarbonate and extracted with chloroform. The chloroform extract was washed with water, dried over sodium sulfate, and evaporated to give 63 as a slightly yellow oil (4 gms.) which was one spot on TLC. IR ($CHCl_3$) 1680 (COOEt).

N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (64)

The carboethoxy derivative 63 (4 gms.), dissolved in tetrahydrofuran (20 mls.), was added over 30 minutes to a suspension of lithium aluminum hydride (4 gms.) in tetrahydrofuran (250 mls.). The mixture was refluxed

under nitrogen for two hours then cooled in an ice water bath. Water (4 mls.) was added dropwise, followed by 15% sodium hydroxide solution (4 mls.), and finally water (12 mls.) was added. The precipitate was filtered off and washed and the filtrate evaporated. The residue was re-dissolved in chloroform and the chloroform solution washed with water, dried over sodium sulfate, and evaporated. This extract was chromatographed on alumina (50 gms.) and elution with benzene then chloroform gave the alcohol 64 (2.5 gms.) m.p. 108 - 110°C. IR (KBr) 3340, 3180. UV (log ε) 284 (3.81), 292 (3.73). NMR (100 MHz) 4.45 (1H, broad singlet, H-C=C), 5.19 (2H, singlet, $C\underline{H}_2$-OH), 8.02 (2H, quartet, J = 6 Hz, CH_3-$C\underline{H}_2$-), and 8.98 (3H, triplet, J = 6 Hz, $C\underline{H}_3CH_2$). (high resolution found 284.184 $C_{18}H_{24}N_2O$ requires 284.188).

N-[β{3(2-benzoxymethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (65)

The alcohol 64 (2.1 gms.) was dissolved in dry pyridine (25 mls.), cooled to 0°C, and the benzoyl chloride (7 mls.) added dropwise. After 3 hours the reaction mixture was diluted with water and basified with sodium bicarbonate. The benzoate 65 was extracted with chloroform and the solution washed with water, dried over sodium sulfate, and evaporated. Chromatography of the crude benzoate (2.5 gms.) on alumina and crystallization from methylene chloride - petroleum ether gave 65 as a white solid m.p. 110 - 112°C. IR (KBr) 1720 ($-CH_2OCO\phi$). UV (log ε) 224 (4.60), 274 (3.94), 284 (3.96), 293 (3.84). NMR (100 MHz) 1.4 (1H, broad singlet, NH), 2.1 - 3.1 (9H, aromatic protons), 4.6 (3H, multiplet, H-C=C and $-CH_2$-O), 7.40 (2H, quartet, J = 6 Hz, $CH_3C\underline{H}_2$-), 9.00 (3H, triplet, J = 6 Hz, $C\underline{H}_3CH_2$-). (Found C, 77.05; H, 7.29; N, 7.05; $C_{24}H_{28}N_2O_2$ requires C, 77.27;

H, 7.28; N, 7.21%; high resolution 388.215 $C_{24}H_{28}N_2O_2$ requires 388.216).

N-[β{3(2-cyanomethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (66)

The benzoate 65 (1.1 gms.) was dissolved in N,N-dimethylformamide; potassium cyanide (5 gms.) was added. The mixture was stirred at room temperature for 45 minutes and the temperature was slowly raised to 120°C for 3 hours. After cooling and addition of water the solution was extracted with methylene chloride. The methylene chloride was washed with water, dried over sodium sulfate, and evaporated to a thick oil. Chromatography on alumina (50 gms.) and elution with benzene and then with 25% methylene chloride in benzene gave nitrile 66 (460 gms.). Crystallization from methylene chloride - petroleum ether gave an analytical sample m.p. 135 - 137°C. IR ($CHCl_3$) 2210 (CN). UV (log ε) 221 (4.69), 274 (3.84), 281 (3.85), 291 (3.77). NMR (60 MHz) 4.55 (1H, broad singlet, H-C=C), 6.12 (2H, singlet, $C\underline{H}_2CN$), 8.97 (3H, triplet, J = 7 Hz, $C\underline{H}_3CH_2$-). (Found C, 77.65; H, 7.86; N, 14.16; $C_{19}H_{23}N_3$ requires C, 77.75; H, 7.92; N, 14.36%; high resolution 293.186 $C_{19}H_{23}N_3$ requires 293.189).

N-[β{3(2-carbomethoxymethylindolyl)}ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (67)

The nitrile 66 (360 mgs.) was dissolved in methanol (35 mls.) and concentrated hydrochloric acid (35 mls.) was added. After 3 days the solution was concentrated, neutralized with dilute ammonium hydroxide, and extracted with methylene chloride. The methylene chloride extract was washed with water, dried over sodium sulfate, and evaporated. Chromatography on alumina (20 gms.) and elution with chloroform gave ester 67 as an oil (107 mgs.). IR ($CHCl_3$) 1720 (COOMe). UV (log ε) 224 (4.42), 274 (3.87),

284 (3.91), 292 (3.83). NMR (100 MHz) 4.55 (1H, broad singlet, H-C=C), 6.22 (2H, singlet, $C\underline{H}_2COOMe$), 6.30 (3H, singlet, $COOCH_3$), 8.98 (3H, triplet, J = 7 Hz, $C\underline{H}_3CH_2$-). (high resolution found 326.202 $C_{21}H_{26}N_2O_2$ requires 326.199).

16,17-dihydrosecodin-17-ol (69)

The ester 67 (450 mgs.) was dissolved in dry benzene (15 mls.) and dried by distillation of benzene (3 mls.). Sodium hydride (500 mgs. of suspension) was washed with benzene (2x10 mls.) suspended in benzene (5 mls.), and methyl formate (500 mgs.) was distilled from phosphorous pentoxide directly into the sodium hydride suspension; then the benzene solution containing the ester 67 was added dropwise. The mixture was heated to 35°C for 90 minutes then cooled to 0°C. Methanol (3 mls.) was added followed by ice cold water and the mixture acidified with 2N hydrochloric acid and basified with sodium bicarbonate solution. The reaction product was extracted with chloroform and the chloroform extract washed with water, dried over sodium sulfate, and evaporated to give crude enol 68 (450 mgs.).

The enol 68 (190 mgs.) was dissolved in methanol (15 mls.), cooled to -10°C in an ice - salt bath, and sodium borohydride (200 mgs.) was added. After 1 hour at -10°C the methanol was evaporated and water added. The mixture was extracted with chloroform and the chloroform extract washed with water, dried over sodium sulfate, and evaporated. The crude product was chromatographed on alumina (5 gms.) and elution with ether gave 16,17-dihydrosecodin-17-ol (57) (150 mgs.), m.p. 131 - 132°C. IR (KBr) 1725 (ester). UV (log ε) 292 (3.86), 284 (3.93), 274 (3.88), and

223 (4.49). NMR (100 MHz) 1.22 (1H, broad singlet, NH), 2.4 - 3.1 (4H, aromatic protons), 4.63 (1H, broad singlet, H-C=C), 6.02 (2H, singlet, $C\underline{H}_2OH$), 6.38 (3H, singlet, COOMe), 8.10 (2H, quartet, J = 6 Hz, $C\underline{H}_2CH_3$), and 9.04 (3H, triplet, J = 6 Hz, $CH_2C\underline{H}_3$). (high resolution found 356.207 $C_{21}H_{28}O_3N_2$ requires 356.209).

Trifluoroacetic acid-^{3}H

Trifluoroacetic anhydride (1.17 gms., 5.55 mmole) was added to water-^{3}H (0.10 g., 5.55 mmole, 100 mcurie/g) using a vacuum transfer system. The resulting trifluoroacetic acid-^{3}H (1.27 gms., 0.9 mc/mmole) was stored under an atmosphere of nitrogen at -10°C until required.

Extraction of alkaloids from Vinca rosea Linn

The following procedure was developed in order to extract and purify the alkaloids of Vinca rosea Linn plants. This procedure was used for all extractions of V. rosea L. plants and was scaled according to the wet weight of plants used.

V. rosea L. plants were obtained from the greenhouse at the University of British Columbia. The plants (30 gms., wet weight) were mascerated with methanol in a Waring Blender, filtered and re-mascerated until the filtrate was colorless. This green filtrate (combined volume was 300 mls.) was evaporated to dryness, the residue taken up in 2N hydrochloric acid (200 mls.) and washed with benzene (3 x 100 mls.). The combined benzene extracts were back extracted with 2N hydrochloric acid (2 x 50 mls.). The combined aqueous phases were made basic with 15N ammonium hydroxide and extracted with chloroform (3 x 100 mls.). The combined chloroform extracts were washed with water (100 mls.), dried over sodium sulfate and evaporated to give a brown oil (100 mgs.).

The oil was dissolved in benzene - methylene chloride (1:1, 1 ml.) and chromatographed on alumina (10 gms.). The column was eluted successively with petroleum ether, benzene, chloroform, and methanol; fractions of 10 mls. were taken. The later benzene - petroleum ether (1:1) fractions were combined and crystallized from methanol affording catharanthine (5 mgs.), the benzene fractions were combined and crystallized from methanol affording ajmalicine (2.5 mgs.), and the initial benzene - chloroform (4:1) fractions were combined and crystallized from ether giving vindoline (2.9 mgs.). When required, the hydrochloride salt of catharanthine and vindoline was formed by blowing hydrogen chloride gas on the surface of an ethereal solution of the alkaloid; catharanthine hydrochloride was crystallized from methanol, whereas vindoline hydrochloride was crystallized from acetone. The hydrochloride salt of ajmalicine was prepared by adding concentrated hydrochloric acid (1 drop) to a concentrated methanolic solution of the alkaloid and ajmalicine hydrochloride was crystallized from methanol.

Tritium labelled radioactive alkaloids for biosynthesis studies

The following procedure is typical for the formation of all the radioactive precursors utilizing tritium in the aromatic portion of the alkaloid molecule.

Trifluoroacetic acid-^{3}H (0.5 g., 0.9 mc/mmole) was added to DL-tryptophan (40 mgs.) using a vacuum transfer system. The solution was allowed to stand under an atmosphere of nitrogen at room temperature for 24 hours. The trifluoroacetic acid-^{3}H was removed using a vacuum transfer system. Concentrated ammonium hydroxide solution (10 mls.) was carefully

added to the residue and the organic components extracted with dichloromethane (10 x 15 mls.). The organic extract was washed with water (10 mls.), dried over sodium sulfate, and concentrated to dryness under reduced pressure to afford [ar-^{3}H]-DL-tryptophan (31 mgs., 7.6 x 10^{6} dpm/mg.).

Feeding of [ar-^{3}H]-DL-tryptophan

[ar-^{3}H]-DL-tryptophan (11.387 mgs., 8.63 x 10^{7} dpm) was dissolved in 0.1N acetic acid and the solution administered to V. rosea plants (wet weight 48.3 gms.) by the cotton wick method. After 9 days under intermittent fluorescent lamp illumination, the alkaloids were isolated and purified by chromatography to give catharanthine (7.00 mgs.), ajmalicine (5.68 mgs.), and vindoline (5.03 mgs.). After dilution with cold alkaloids and crystallization to constant activity the following incorporations were obtained: catharanthine (0.980%), ajmalicine (0.312%), and vindoline (0.155%).

[ar-^{3}H]-16,17-dihydrosecodin-17-ol

(a) 16,17-dihydrosecodin-17-ol (57) (50 mgs.) was treated as before with trifluoroacetic acid-^{3}H. Work up as before revealed decomposition of 57.

(b) Carbomethoxy derivative 67 (150 mgs.) was treated with trifluoroacetic acid-^{3}H as before. Work up gave [ar-^{3}H]-carbomethoxy derivative 67 which could be converted into [ar-^{3}H]-16,17-dihydrosecodin-17-ol by the described procedure.

Feeding of [ar-^{3}H]-16,17-dihydrosecodin-17-ol (57)

[ar-^{3}H]-16,17-Dihydrosecodin-17-ol (57) (8.0 mgs., 1.97 x 10^{7} dpm) was fed to V. rosea plants (wet weight 75 gms.) as before. After chroma-

tography, dilution with cold alkaloids, and crystallization to constant activity the following incorporations were obtained: catharanthine (0.0007%), ajmalicine (0.0004%), and vindoline (inactive).

Tryptophyl bromide

A solution of phosphorus tribromide (0.44 mls.) in ether (10 mls.) was added dropwise to an ice cold solution of tryptophol (2 gms.) dissolved in ether (200 mls.). The reaction was stirred for 16 hours with ice cooling for the first 6 hours. The supernatant was decanted, washed with sodium bicarbonate solution, water, and dried over sodium sulfate. Removal of the solvent yielded the product as white crystals (2.46 gms.), m.p. 100 - 102°C (literature m.p. 90 - 95°C[63]).

N-[β(3-indolyl)ethyl]-3-ethylpyridinium bromide (72)

Tryptophyl bromide (2.46 gms.) was heated under nitrogen at 80°C for 16 hours with 3-ethylpyridine (8 mls.). The supernatant was decanted and the solid titurated with ether and suction dried to give 72 as a yellow solid (3.6 gms.). UV 290, 282 (shoulder), 267.

N-[β(3-indolyl)ethyl]-3-ethyl-1,2,5,6-tetrahydropyridine (73)

Pyridinium bromide 72 (2 gms.) was dissolved in methanol (75 mls.) and triethylamine (2 mls.) was added. The solution was cooled to 0°C and sodium borohydride (7 gms.) was added in portions in order to keep the temperature below 5°C. After 2 hours the solution was diluted with water and evaporated to a sticky paste which was acidified with dilute hydrochloric acid, basified with sodium bicarbonate, and extracted with chloroform. The chloroform extract was washed with water, dried over sodium sulfate, and chromatographed on alumina to give 73 as an oil (1.3 gms.).

NMR (60 MHz) 2.23 - 3.13 (5H, aromatic protons), 4.51 (1H, triplet, J = 2 Hz, H-C=C), 8.98 (3H, triplet, J = 6.5 Hz, $\underline{CH_3}CH_2$-).

Mercuric acetate oxidation of 73

Tetrahydropyridine 73 (300 mgs.), ethylenediaminetetraacetic acid (EDTA, 800 mgs.), sodium hydroxide (200 mgs.), and mercuric acetate (800 mgs.) were dissolved in 2% acetic acid (45 mls.). The solution was maintained at a temperature of 100°C for one and a quarter hours. The solution was extracted with methylene chloride and the extract was washed with water, dried over sodium sulfate, and evaporated to give a brown oil (213 mgs.). A portion of this oil was purified by preparative layer chromatography to give 74 as a brown - orange solid. UV 230, 274, 283, 294. NMR (100 MHz) 2.21 (1H, broad singlet, NH), 2.45 - 3.05 (4H, aromatic protons), 4.38 (1H, multiplet, H-C=C), and 8.83 (3H, triplet, J = 7 Hz, $\underline{CH_3}CH_2$-). Mass spectrum m/e 252.

The aqueous layers of the extract were combined and saturated with hydrogen sulfide gas. The solution was filtered through Celite. Concentration of the filtrate to near dryness resulted in precipitation of EDTA which was filtered off. Further concentration resulted in more EDTA being precipitated and filtered off. The filtrate was evaporated to dryness to give a yellow solid. Attempts to wash the organic products out of this solid with methanol were unsuccessful.

N-[β{3(2-carboethoxyindolyl)}ethyl]-3-ethylpyridinium acetate (76)

Pyridinium chloride 62 (50 mgs.) was dissolved in ethanol (5 mls.) and an ethanolic solution of silver acetate was added dropwise until a drop failed to produce fresh precipitate. The precipitate was filtered

off and the filtrate evaporated to dryness to give 76 as a brown oil which later solidified. NMR (60 MHz) 0.65 (1H, NH); 1.06, 1.40 (2H, multiplets, N-H); 2.05 - 3.01 (6H, aromatic protons); 5.70 (2H, quartet, J = 7 Hz, $-C\underline{H}_2-OCO-$); 7.42 (2H, quartet, J = 7 Hz, $CH_3C\underline{H}_2-C$); 8.63 (3H, triplet, J = 7 Hz, $C\underline{H}_3CH_2-O$); 9.01 (3H, triplet, J = 7 Hz, $C\underline{H}_3CH_2-C$).

Oxidation of 63 in ethanol at room temperature

Tetrahydropyridine 63 (71 mgs.) and mercuric acetate (310 mgs.) were dissolved in ethanol (25 mgs.). Stirring was continued for 4 days under nitrogen. The mercurous acetate (140 mgs.) was filtered off, the filtrate saturated with hydrogen sulfide gas, and the solution filtered through Celite. The filtrate was evaporated (75 mgs.) and compared to the desired pyridinium acetate 76 on TLC. No spot corresponding to 76 could be detected.

A portion of the filtrate was separated on a preparative layer chromatogram (alumina, $CHCl_3$/MeOH) and four bands of material were examined. The first band (R_f 0.65) was less polar than 76 and its UV revealed absorption at 290 mμ and a shoulder at 334 mμ. The second band (R_f 0.50) was less polar than 76 and had UV absorption maxima at 315 mμ. The third band corresponded to starting material. The fourth band examined remained at the origin of the chromatogram and had a maxima at 320 mμ in the UV spectrum.

Oxidation of 63 at 35°C in ethanol

Tetrahydropyridine 63 (100 mgs.) and mercuric acetate (350 mgs.) in ethanol (10 mls.) were heated at 35°C for 18 hours under nitrogen. Mercurous acetate (245 mgs.) was filtered off and the reaction mixture worked up as before. TLC failed to detect any of the desired 76.

Oxidation of 63 at 50°C in ethanol

Tetrahydropyridine 63 (100 mgs.) and mercuric acetate (350 mgs.) in ethanol were heated at 50°C for 4 hours under nitrogen. Mercurous acetate (233 mgs.) was filtered off and the reaction worked up as before. TLC failed to detect any of the desired pyridinium acetate 76.

Oxidation of 63 in acetic acid

Tetrahydropyridine 63 (100 mgs.) and mercuric acetate (350 mgs.) were dissolved in glacial acetic acid (10 mls.) and stirred under nitrogen for 3 days at room temperature. The mercurous acetate (130 mgs.) was filtered off and the reaction worked up as before. TLC failed to detect any of the desired pyridinium acetate 76.

Oxidation of 63 in 10% acetic acid

Tetrahydropyridine 63 (93 mgs.) and mercuric acetate (325 mgs.) in 10% acetic acid (10 mls.) were stirred under nitrogen for 3 days at room temperature. Mercurous acetate (157 mgs.) was filtered off and the reaction worked up as before. TLC failed to detect any of the desired pyridinium acetate 76.

3-acetylpyridine ethylene ketal

A solution of 3-acetylpyridine (104 gms.), ethylene glycol (80 mls.), and p-toluene sulfonic acid hydrate (175 gms.) in benzene (400 mls.) was refluxed for 18 hours with a Dean - Stark apparatus to remove water. The mixture was poured into excess sodium bicarbonate solution, the layers separated, and the aqueous phase extracted with benzene. The combined benzene layers were washed with sodium bicarbonate solution, water, dried over sodium sulfate, and evaporated to give 150 gms. of oil. This oil

when distilled through a short Vigeraux column at 120°C at 20 mm. gave 3-acetylpyridine ethylene ketal (120 gms.). NMR (60 MHz) 1.23 (1H, doublet, J = 2 Hz, C_2-H), 1.47 (1H, quartet, J = 4 and 2 Hz, C_6-H), 2.25 (1H, multiplet), 2.77 (1H, multiplet), 6.15 (4H, multiplet, ketal), 8.37 (3H, singlet, CH_3).

N-[β{3(2-carboethoxyindolyl)}ethyl]-3-acetylpyridinium chloride ethylene ketal (77)

In a Carius tube 2-carboethoxy-3(β-chloroethyl)indole (60) (5.2 gms.) and 3-acetylpyridine ethylene ketal (15 mls.) was heated for 16 hours at 125°C. The solution was cooled, dissolved in methanol, filtered, and evaporated. Tituration with ether produced a gray precipitate (7.5 gms.), m.p. 232 - 233°C. IR (KBr) 1703 (COOEt). UV (log ε) 296 (4.16), 272 sh (3.85), 265 sh (3.80), 227 (4.38), 222 (4.32). NMR (CD_3OD) 6.15 (4H, multiplet, ketal), 8.62 (3H, singlet, $COCH_3$). (Found C, 63.50; H, 6.15; N, 6.52; $C_{22}H_{25}N_2O_4Cl$ requires C, 63.38; H, 6.04; N, 6.72%).

N-[β{3(2-carboethoxyindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine ethylene ketal (78)

The pyridinium chloride 77 (5.6 gms.) in methanol (300 mls.) with added triethylamine (3 mls.) was cooled to 0°C in an ice - salt bath under nitrogen. Sodium borohydride (18 gms.) was added in small portions such that the temperature remained at 0°C. After the addition was complete the solution was stirred for 2 hours and the methanol then slowly evaporated under reduced pressure with a bath temperature of 25°C. Addition of water to this paste followed by extraction with methylene chloride, washing with water, drying over potassium carbonate, and evaporating, gave a

yellow oil (5 gms.) which was crystallized from petroleum ether - methylene chloride to give 78 as a white solid, m.p. 129 - 131°C. IR (KBr) 3320 (NH), 1678 (COOEt). UV (log ε) 290 (4.47), 253 (4.59), 210 (4.15). NMR (60 MHz) 0.78 (1H, NH), 4.08 (1H, broad singlet, H-C=C), 6.14 (4H, multiplet, ketal), 8.50 (3H, singlet, CH_3CO).

N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine ethylene ketal (79)

The carboethoxy derivative 78 (4.8 gms.) in tetrahydrofuran (50 mls.) was added dropwise to a stirred suspension of lithium aluminum hydride in tetrahydrofuran (300 mls.) at 0°C. The solution was refluxed for 2 hours, cooled to 0°C, and the excess hydride destroyed by dropwise addition of saturated potassium carbonate solution. The resulting solids were separated by filtration and washed with chloroform. The filtrate was dried over potassium carbonate and evaporated. The alcohol 78 was purified by chromatography on alumina (250 gms.) by elution with benzene, 50% benzene in chloroform, and chloroform. Crystallization from petroleum ether - methylene chloride gave 79 as a white solid m.p. 114 - 116°C. IR (KBr) 3200 (OH). UV (log ε) 294 (3.95), 268 (4.02), 255 sh (3.98), 248 (4.66), 212 sh (4.45). NMR (60 MHz) 4.15 (1H, multiplet, H-C=C), 6.20 (4H, singlet, ketal), 8.60 (3H, singlet, CH_3-C). (Found C, 70.21; H, 7.64; N, 8.05; $C_{20}H_{26}N_2O_3$ requires C, 70.15; H, 7.65; N, 8.18%; high resolution 342.193 $C_{20}H_{26}N_2O_3$ requires 342.194).

N-[β{3(2-benzoxymethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine ethylene ketal (80)

The alcohol 79 (2.8 gms.) in tetrahydrofuran (20 mls.) with triethylamine (2.28 mls.) was cooled to 0°C and benzoyl chloride (1.14 mls.) was

added dropwise. After 3 hours at 0°C saturated sodium carbonate solution was added dropwise and the tetrahydrofuran decanted, dried over sodium carbonate, and evaporated to give crude benzoate (3.6 gms.). Purification by column chromatography on alumina (150 gms.) and elution with methylene chloride followed by crystallization gave benzoate 80, m.p. 147 - 148°C. IR (KBr) 1713. UV (log ε) 285 (4.53), 260 (4.66), 224 (5.33). NMR (60 MHz) 1.35 (1H, broad singlet, NH), 1.9 - 2.9 (9H, multiplets, aromatic protons), 4.12 (1H, broad singlet, H-C=C), 6.16 (4H, multiplet, ketal protons), 8.54 (3H, singlet, CH_3-C). (Found C, 72.4; H, 6.6; N, 6.2; $C_{27}H_{30}N_2O_4$ requires C, 72.6; H, 6.8; N, 6.3%).

N-[β{3(2-cyanomethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine ethylene ketal (81)

The benzoate 80 (2.4 gms.) in N,N-dimethylformamide (75 mls.) with potassium cyanide (4 gms.) was stirred at room temperature for 1 hour; the temperature was raised to 110°C over 45 minutes and maintained at that temperature for a further 45 minutes. The reaction was cooled, diluted with water, and extracted with chloroform. The organic layer was washed with water (3 times), saturated potassium carbonate, and dried over potassium carbonate. Evaporation and chormatography on alumina (200 gms.) with methylene chloride as eluant gave nitrile 81 (1.6 gms.). IR ($CHCl_3$) 2255 (CN). NMR (60 MHz) 4.12 (1H, broad singlet, H-C=C), 6.12 (4H, multiplet, ketal protons), 6.23 (2H, singlet, CH_2CN), 8.52 (3H, singlet, CH_3-C).

N-[β{3(2-carboethoxymethylindolyl)}ethyl]-3-acetyl-1,2,5,6-tetrahydropyridine (82)

The nitrile 81 (750 mgs.) was dissolved in absolute methanol (20 mls.)

and water (0.2 mls.) and dry hydrogen chloride gas was bubbled into the solution for 45 minutes. After 48 hours at room temperature, the methanol was evaporated, the residue neutralized with saturated sodium carbonate solution and extracted with methylene dichloride. The organic phase was washed with water, dried over sodium sulfate, and evaporated to give crude ester (660 mgs.). Column chromatography on alumina (50 gms.) and elution with methylene chloride gave keto ester 82 (230 mgs.) as an oil. IR (film) 3400 (NH), 1727 (COOMe), and 1652 (>CO). NMR (60 MHz) 1.50 (1H, broad singlet, NH), 3.17 (1H, broad singlet, H-C=C), 6.29 (2H, singlet, CH_2COO), 6.33 (3H, singlet, $COOCH_3$), 7.75 (3H, singlet, CH_3CO).

Mercuric acetate oxidation of keto ester 82

Keto ester 82 (52 mgs.) and mercuric acetate (200 mgs.) in methanol (15 mls.) were stirred under nitrogen for 36 hours at room temperature. Mercurous acetate (103 mgs.) was filtered off and hydrogen sulfide gas bubbled into the filtrate. This filtrate was re-filtered through Celite and evaporated to dryness. Fresh methanol (10 mls.), triethylamine (0.5 mls.), and 10% palladium on charcoal (25 mgs.) were hydrogenated at atmospheric pressure and room temperature for 48 hours. The catalyst was filtered off and the filtrate evaporated to give an oil (35 mgs.). IR ($CHCl_3$) 1754, 1724, and 1681. Chromatography on alumina (5 gms.) and elution with chloroform and the methanol gave an oil (5 mgs.). IR ($CHCl_3$) 1750, 1722, and 1685.

N-[β{3(2-carboethoxyindolyl)}ethyl]-3-acetylpyridinium chloride (84)

2-carboethoxy-3(β-chloroethyl)indole 60 (2.15 gms.) in 3-acetylpyridine (6 mls.) was heated under nitrogen in a Carius tube at 110°C for 18 hours

after which time the reaction mixture was titurated with ether and the pyridinium chloride collected by filtration as an orange powder. Careful re-crystallization from methanol - ether gave 84, m.p. 172 - 174°C. IR (KBr) 1702 (ester), 1691 (ketone). UV (log ε) 295 (4.36), 268 sh (3.98), 227 (4.55), 221 (4.54). NMR (100 MHz, DMSO - d_6) 0.61 (1H, broad singlet, $C_{21}H$); 0.97 (1H, doublet, J = 6 Hz, $C_{61}H$); 1.12 (1H, doublet, J = 8 Hz, $C_{41}H$), 1.90 (1H, quartet, J = 8 and 6 Hz, $C_{51}H$); 2.52, 2.60, 2.80, 3.09 (4H, aromatic protons); 4.97 (2H, multiplet, CH_2-N); 5.78 (2H,quartet, J = 6.5 Hz, O-$C\underline{H}_2CH_3$); 6.29 (2H, multiplet, CH_2-Ind.); 7.44 (3H, singlet, CH_3CO); 8.70 (3H, triplet, J = 6.5 Hz, O-CH_2-$C\underline{H}_3$).

N-[β{3(2-carboethoxyindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine (85)

The pyridinium chloride 84 (500 mgs.) was hydrogenated at room temperature and atmospheric pressure in ethanol (35 mls.) and triethylamine (1 ml.) with 10% palladium on charcoal (100 mgs.) until uptake ceased (48 hours). The catalyst was filtered off and the filtrate evaporated to dryness. The residue was dissolved in chloroform and extracted with pH3 buffer (3 x 50 mls.) and pH2 acid (0.01N HCl, 3 x 25 mls.), dried over sodium sulfate, and evaporated to give crude amide 85. Chromatography on alumina (25 gms.) and elution with chloroform gave amide 85 as a yellow oil (215 mgs.). IR ($CHCl_3$) 1706 (ester); 1626, 1553 (amide). UV (log ε) 305 (4.63), 257 (4.52), and 211 (4.47). NMR (60 MHz) 0.47 (1H, broad singlet, NH), 2.42 (1H, multiplet, aromatic protons), 2.80 (3H, multiplet, aromatic protons), 3.02 (1H, singlet, H-C=C), 5.65 (2H, quartet, J = 7.1 Hz, -O$C\underline{H}_2CH_3$), 7.80 (3H, singlet, $COCH_3$), 8.63 (3H, triplet, J = 7.1 Hz, $OCH_2C\underline{H}_3$).

N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-acetylpiperidine (86)

The amide 85 (40 mgs.) in tetrahydrofuran (10 mls.) was cooled to 0°C and lithium aluminum hydride (5 mgs.) was added. After 15 minutes TLC showed absence of starting material. After addition of a few drops of saturated potassium carbonate the solution was filtered and the filtrate dried over sodium sulfate and evaporated. Chromatography on alumina (5 gms.) gave 86 as an oil. IR ($CHCl_3$) 1710 (ketone). NMR (60 MHz) 5.32 (2H, singlet, $C\underline{H}_2OH$), 7.90 (3H, singlet, CH_3CO).

N-[β{3(2-hydroxymethylindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine (87)

The amide 85 (105 mgs.) in tetrahydrofuran (15 mls.) was cooled to -30°C in a dry ice - carbon tetrachloride bath and lithium aluminum hydride (10 mgs.) was added. After 1 hour at -30°C the temperature was slowly raised to 0°C and the reaction worked up as before. Chromatography on alumina (10 gms.) and elution with chloroform gave amide alcohol 87 (11 mgs.). IR ($CHCl_3$) 1625, 1550 (amide). NMR (60 MHz) 3.05 (1H, singlet, H-C=C), 5.33 (2H, singlet, $C\underline{H}_2OH$), and 7.80 (3H, singlet, $COCH_3$).

Reduction of 85 with lithium borohydride

The amide 85 (40 mgs.) in tetrahydrofuran was cooled to 0°C and lithium borohydride (5 mgs.) added. After 3 hours at 0°C TLC showed that only starting material was present. Allowing the solution to warm to room temperature for one and a half hours seemed to be without effect. The solution was refluxed for 1 hour and worked up as before. TLC showed that the major product was keto alcohol 86 with a trace amount of amide alcohol 87 present.

Reduction of 85 with sodium borohydride

The amide 85 (45 mgs.) in methanol (10 mls.) was cooled to 0°C and sodium borohydride (10 mgs.) was added and the solution slowly warmed and finally refluxed for 2 hours. TLC examination showed that no reduction had taken place. The solution was cooled to 0°C and fresh sodium borohydride (10 mgs.) was added. The temperature was maintained at 0°C for 1 hour before warming to reflux. TLC examination revealed the presence of only starting material.

2-hydroxymethylindole (89)

Methyl indole-2-carboxylate (88) (10 gms.) in tetrahydrofuran was added dropwise to a stirred suspension of lithium aluminum hydride (4 gms.) in tetrahydrofuran (250 mls.) at 0°C. After addition the solution was refluxed for 2 hours, cooled, and excess hydride decomposed by addition of saturated potassium carbonate solution. The solids were removed by filtration and washed with methylene dichloride. The organic solution was washed with saturated potassium carbonate solution, dried over sodium sulfate, and evaporated to yield crude alcohol 89. Chromatography on alumina (250 gms.) and elution with chloroform gave, after crystallization from benzene, the alcohol 89 (5.8 gms.), m.p. 73 - 74°C. IR (KBr) 3380 (OH). UV (log ε) 290 (3.81), 281 (3.97), 271 (3.98), 218 (4.64). NMR (60 MHz) 1.66 (1H, broad singlet, NH), 3.77 (1H, doublet, J = 3 Hz, C_3H), 5.48 (2H, singlet, $C\underline{H}_2OH$), 7.04 (1H, singlet, OH).

2-benzoxymethylindole (90)

The alcohol 89 (5 gms.) in tetrahydrofuran (100 mls.) and triethylamine (9.3 mls.) was cooled to 0°C and benzoyl chloride (4.8 mls.) was

added dropwise. After 3 hours at 0°C saturated potassium carbonate was added, followed by methylene chloride. The water layer was separated and washed with methylene dichloride. The organic layers were combined, dried over sodium sulfate, and evaporated. Column chromatography on alumina (250 gms.) and elution with benzene gave benzoate 90 (8.4 gms.). Crystallization from benzene gave a white solid m.p. 128 - 129°C. IR (KBr) 3355 (NH), 1700 (ester). UV (log ε) 290 (3.90), 282 (4.14), 270 (4.19), 217 (4.79). NMR (60 MHz) 1.01 (1H, broad singlet, NH), 1.95 (2H, multiplet, aromatic protons), 2.3 - 3.0 (7H, multiplets, aromatic protons), 3.40 (1H, doublet, J = 3 Hz, C_3H), 4.52 (2H, singlet, $C\underline{H}_2O$).

2-cyanomethylindole (91)

The benzoate 91 (5.7 gms.) was dissolved in N,N-dimethylformamide (130 mls.) and potassium cyanide (7.5 gms.). After stirring at room temperature for 1 hour the temperature was slowly raised to 80°C over a 1 hour period and maintained at that temperature for 3 hours. After cooling to room temperature methylene dichloride and water were added. The layers were separated and the water layer washed with fresh methylene dichloride. The organic layers were combined, washed with water, dried over sodium sulfate, and evaporated. The residual N,N-dimethylformamide was removed by freeze drying and the solid residue chromatographed on alumina. Elution with benzene gave nitrile 91 (2.8 gms.). Crystallization from benzene gave a white solid m.p. 102 - 103°C. IR (KBr) 3370, 3320 (NH); 2270, 2245 (CN). UV (log ε) 298 (3.94), 277 (4.09), 265 (4.15), 217 (4.83). NMR (60 MHz) 1.82 (1H, broad singlet, NH), 2.4 - 3.0 (4H, multiplet, aromatic protons), 3.56 (1H, doublet, J = 3 Hz, C_3H), 6.10 (2H, singlet,

CH_2CN). (Found C, 76.83; H, 5.00; N, 17.94; $C_{10}H_8N_2$ requires C, 76.92 H, 5.12; N, 17.95%).

Methyl indole-2-acetate (92)

The nitrile 91 (2.1 gms.) in methanol (200 mls. absolute with 1% water added) was treated with hydrogen chloride gas and allowed to stand at room temperature for 48 hours. After evaporation of the methanol, sodium bicarbonate solution was added and the mixture extracted with chloroform. The chloroform was washed with water, dried over sodium sulfate, and evaporated. Chromatography on alumina (100 gms.) and elution with benzene gave the acetate 92 (2.1 gms.). Crystallization from benzene - petroleum ether gave a white solid m.p. 71 - 72°C. IR (KBr) 3350 (NH), 1719 (ester). UV (log ε) 288 (3.92), 279 (4.01), 270 (4.04), 217 (4.69). NMR (60 MHz) 1.43 (1H, broad singlet, NH), 3.68 (1H, doublet, J = 3 Hz, C_3H), 6.29 (2H, singlet, $C\underline{H}_2COO$), and 6.32 (3H, singlet, $COOCH_3$). (Found C, 69.55; H, 5.80; N, 7.42; $C_{11}H_{11}NO_2$ requires C, 69.83; H, 5.86; N, 7.40%).

Methyl 3(β-hydroxyethyl)indole-2-acetate (93)

The acetate 92 (1.0 gm.) in carbon tetrachloride (100 mls.) was cooled to 0°C and ethylene oxide (0.4 mls.) added. The solution was cooled to -15°C and stannic chloride (0.65 mls.) in carbon tetrachloride (20 mls.) was added dropwise. After the addition was complete stirring was continued for 20 minutes keeping the temperature below 0°C. Chloroform (35 mls.) and saturated sodium carbonate (16 mls.) were added rapidly keeping the temperature below 10°C. The organic layer was separated and the aqueous layer washed with ether. The combined organic

layers were dried and evaporated. The resulting oil was chromatographed on alumina (50 gms.). Elution with benzene gave starting acetate 92 (460 mgs.) and elution with chloroform - methanol gave alcohol 93 (465 mgs.) as a brown oil. IR ($CHCl_3$) 3603 (OH), 3453 (NH), 1732 (ester). UV 292, 284, 276 sh, and 224. NMR (60 MHz) 1.20 (1H, broad singlet, NH), 6.25 (2H, triplet, J = 6.7 Hz, $\underline{CH_2}OH$), 6.32 (2H, singlet, CH_2COO), 6.40 (3H, singlet, $COOCH_3$), 7.12 (2H, triplet, J = 6.7 Hz, Ind.-CH_2-), and 7.40 (1H, broad singlet, OH).

Methyl 3(β-bromoethyl)indole-2-acetate (94)

To the alcohol 93 (340 mgs.) in ether (30 mls.) at 0°C, phosphorous tribromide (60 µls) in ether (10 mls.) was added dropwise. The mixture was left overnight at 0°C and then poured into saturated sodium carbonate solution. The layers were separated and the aqueous layer extracted with ether. The combined ether extracts were washed with water, dried over sodium sulfate, and evaporated. The crude bromide 94 was chromatographed on alumina (10 gms.) and elution with benzene gave 94 as a yellow oil (190 mgs.). IR ($CHCl_3$) 3450 (NH), 1721 (ester). UV 325, 292, 284, 272 sh, and 213. NMR (60 MHz) 1.40 (1H, broad singlet, NH), 6.26 (2H, singlet, $\underline{CH_2}COO$), 6.60 (4H, multiplet, Ind.-CH_2CH_2Br), 6.30 (3H, singlet, $COOCH_3$).

N-[β{3(2-carbomethoxymethylindolyl)}ethyl]-3-acetylpyridinium bromide (95)

The tryptophyl bromide derivative 94 (190 mgs.) in 3-acetylpyridine was heated to 100°C for 3 hours. Tituration with ether produced the pyridinium bromide 95 as a yellow solid (205 mgs.). UV 289, 267, and 220.

N-[β{3(2-carbomethoxymethylindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine (83)

(a) Pyridinium bromide 95 (175 mgs.) in ethanol (50 mls.) containing triethylamine (0.5 mls.) was hydrogenated over 10% palladium on charcoal (80 mgs.) for 48 hours. The catalyst was filtered off, the ethanol evaporated, and the residues dissoved in chloroform. The chloroform was extracted wtih pH3 buffer (3 x 25 mls.) and pH2 acid (2 x 25 mls.); after drying over sodium sulfate the chloroform was evaporated and chromatographed on alumina (10 gms.). Elution with chloroform gave the amide 83 (34 mgs.). IR ($CHCl_3$) 3450 (NH), 1720 (ester), and 1610 (amide), 1560 (C=C). UV 294 sh, 286 sh, and 223. NMR (100 MHz) 0.98 (1H, broad singlet, NH), 3.15 (1H, singlet, H-C=C), 6.35 (2H, singlet, $\underline{CH_2}COO$), 6.38 (3H, singlet, $COOCH_3$), 8.20 (3H, singlet, $COCH_3$).

(b) To the alcohol 93 (20 mgs.) in 3-acetylpyridine (5 mls.) at 0°C, phosphorous tribromide (37 µls.) was added. After addition the temperature was raised to 85°C and maintained there for 6 hours. After cooling and titurating with ether, the brown solids (600 mgs.) were filtered off and dried. The solids were dissoved in ethanol (100 mls.), filtered, and hydrogenated over 10% palladium on charcoal (100 mgs.) for 48 hours. The catalyst was filtered off, the ethanol evaporated, and the residues dissolved in chloroform. The chloroform solution was extracted with pH3 buffer (3 x 50 mls.), pH2 solution (2 x 50 mls.), dried over sodium sulfate, and evaporated. Chromatography on alumina (3 gms.) and elution with chloroform gave the amide 83 (59 mgs., 37% overall) which was identical to that obtained by method (a).

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