

CATIONIC REARRANGEMENTS AND CYCLISATIONS OF DITERPENES

by

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B.Sc. Tech. University of Manchester, 1964

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Chemistry

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SIMON FRASER UNIVERSITY

March, 1971

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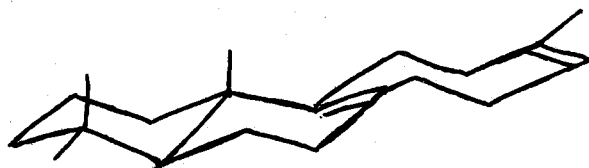
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ABSTRACT

The biogenesis of the tetracarbo-cyclic diterpenes is considered to involve cyclisation via a bicyclic C-13 carbonium ion. This species has been generated in the laboratory by acid treatment of manool and Δ 13-manool and found to give, under the relatively mild conditions of refluxing acetic acid, a 1:1 mixture of Δ 13-manool acetate and olefins. The olefin mixture consisted mainly of labdatrienes with smaller amounts of three classes of cyclised products. Ring closure between C-13 and C-17 gave approximately equal amounts of tri-cyclic α -vinyl isopimaric and β -vinyl pimaric Δ 7, Δ 8 and Δ 8(14) dienes together with a product of backbone rearrangement in each series. Each of the dienes was found to be stable under the reaction conditions, indicating that the backbone rearrangement occurred by transfer of the migrating functions along the backbone of the molecule without participation of the intermediate olefins. The third type of cyclised product was a hitherto unknown cycloocta-1,5-diene derivative, which we call 8,13-burnabadiene, generated by cyclisation between C-15 and C-17.



8,13-Burnabadiene

Under the more vigorous conditions of refluxing formic acid, the formation of labdatrienes was precluded and the yields of the initially cyclised pimaradienes and isopimaradienes, the backbone rearranged products and burnabadiene increased. In this reaction the ratio of Δ^{13} -manool formate to olefins was 1:7. The initial dienes and the backbone rearranged products were interconverted by the reaction conditions showing that backbone rearrangement is reversible. A tetracyclic product, hiban-14 α -formate was also isolated and in addition was formed in quantitative yield when 8,13-burnabadiene was subjected to the reaction conditions. Deuterium labelling of Δ^{13} -manool at C-14 showed that hiban-14 α -formate was indeed formed via such a carbon skeleton.

A synthetic route to 8,13-burnabadiene is discussed.

ACKNOWLEDGMENT

The author wishes to express his sincere thanks to his Research Director, Dr. A. C. Oehlschlager, for guidance and advice during the course of this work.

Appreciation is also extended to:

Dr. J. W. ApSimon for a sample of sandaracopimaric acid;

Dr. O. E. Edwards for samples of 8,15-pimaradiene, 8,15-isopimaradiene and for the NMR spectra of pimaradiene and sandaracopimaradiene;

Dr. L. Westfelt for a sample of isopimaradiene;

The faculty, staff and students of the chemistry department of Simon Fraser University for providing comradeship, encouragement and helpful discussions;

Miss I. McLeod for typing the manuscript of this dissertation;

And the National Research Council of Canada for providing generous support for this work in the form of a Post-Graduate Scholarship.

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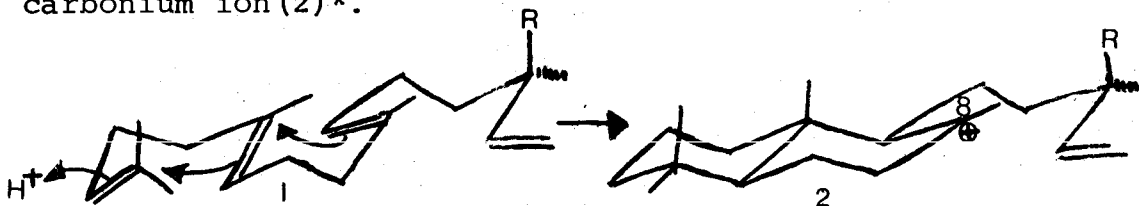
CHAPTER 1
INTRODUCTION

The biogenesis of the naturally occurring compounds known as the terpenes is considered to involve cationic cyclisations of acyclic precursors to give cyclic progenitors. Reasonable cationic rearrangements of these initially formed intermediates can account for the gross carbon structure of most of the terpenoids encountered in Nature. Often a single plant or animal extract will contain terpenes of apparently grossly different structures, which, on close examination, are seen to represent products of neutralisation, elimination and oxidation of the various carbocyclic intermediates reasonably involved in the biogenesis of the carbon skeleton of the most highly cyclised and rearranged component of the extract.

A number of the proposed cyclisations and rearrangements have been imitated in the laboratory by generating the appropriate carbonium ions in suitable skeletons and these cationic in vivo transformations and their in vitro analogues have been the subject of a number of recent reviews.¹⁻⁶

The principle cyclisations and rearrangements considered to occur in the biogenesis of the C₂₀ isoprenoids known as the diterpenes and the in vitro analogues which had been reported prior to the start of this work will be reviewed briefly.

The initial step of the biogenesis of carbocyclic diterpenes is the cyclisation of geranylinalyl pyrophosphate (1, R=pyrophosphate) or its Δ^{13} allylic isomer geranylgeranyl pyrophosphate to the bicyclic C-8 carbonium ion (2)*.



The involvement of the acyclic precursor (1) is supported by the isolation⁷ from the essence of jasmin of geranylinalyl alcohol, isophytol (its hexahydro derivative) and phytol, the analogous hexahydro alcohol derived from geranylgeranyl alcohol and by the incorporation⁸ of geranylgeranyl pyrophosphate into the tetracyclic diterpene kaurene (23 see below). In addition there have been performed a large number of mineral acid catalysed cyclisations of 1,5-dienes^{3,4,9-15} each of which portrays the essential features of the above reaction i.e. the con-

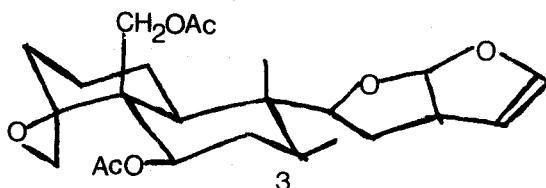
*Although a fully developed carbonium ion is probably not present in vivo, it is the most convenient way of representing the biological equivalent of this and subsequent cationic structures.

It is possible for an enantiomeric form of 1 to cyclise to an enantiomer of 2 and in fact compounds resulting from further reaction of both forms are well known. In order to simplify correlations between different skeletons, most structures are drawn here with the 10B methyl configuration although some (erythroxydiols, trachylobanes, gibberellins) have so far only been encountered in the 10 α methyl series.

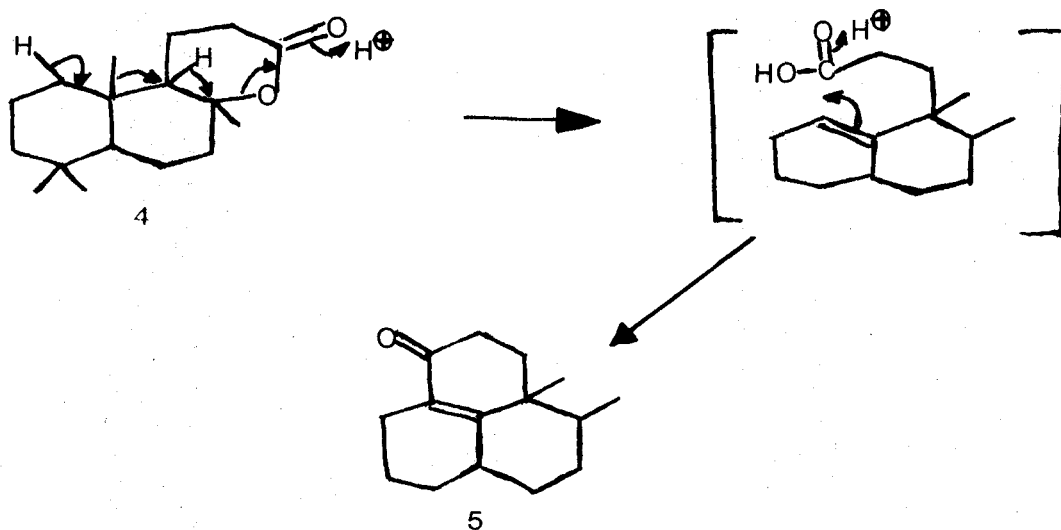
certed cyclisations of isoprenoid 1,5-dienes proceed to give cyclohexyl systems by trans anti-parallel addition to the double bonds.

The bicyclic ion (2) possesses the carbon skeleton of the class of diterpenes known as the labdanes, which are considered to derive directly from it.

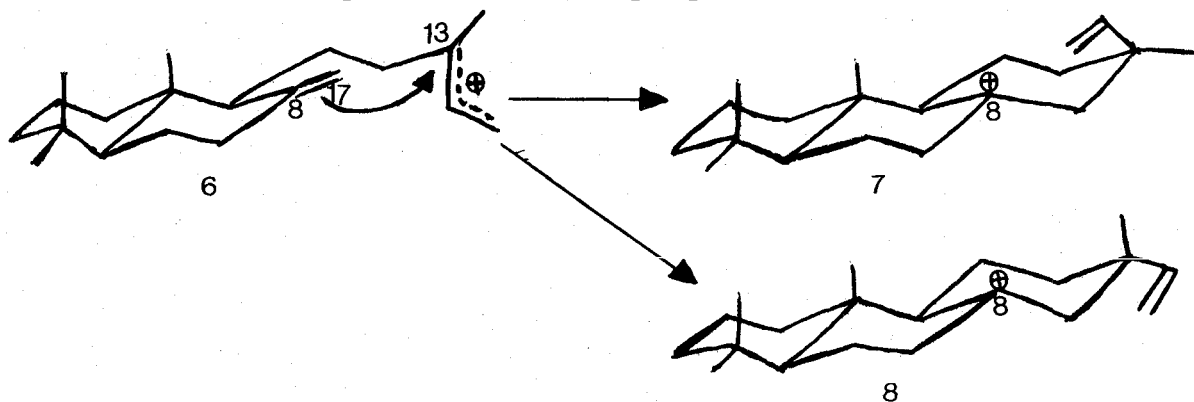
The bicyclic ion may, however, undergo a sequence of 1,2 shifts in vivo, known as a backbone rearrangement. This leads to the carbon skeletons of the re-arranged labdanes, of which clerodien¹⁶ (3) is an example.



The formation of (5) upon treatment of (4) with sulphuric acid¹⁷ is an example of this type of reaction in vitro.

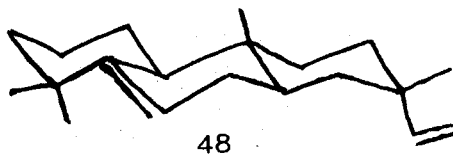
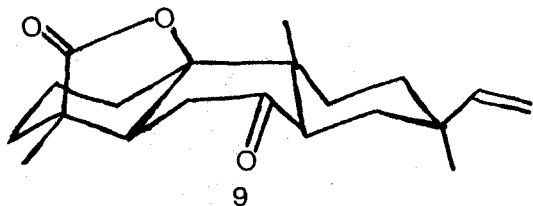


Diterpenes possessing the pimarane (7) and isopimarane (8) skeletons are considered to be formed from an 8(17)-labdene precursor (6) by cyclisation to C-13.

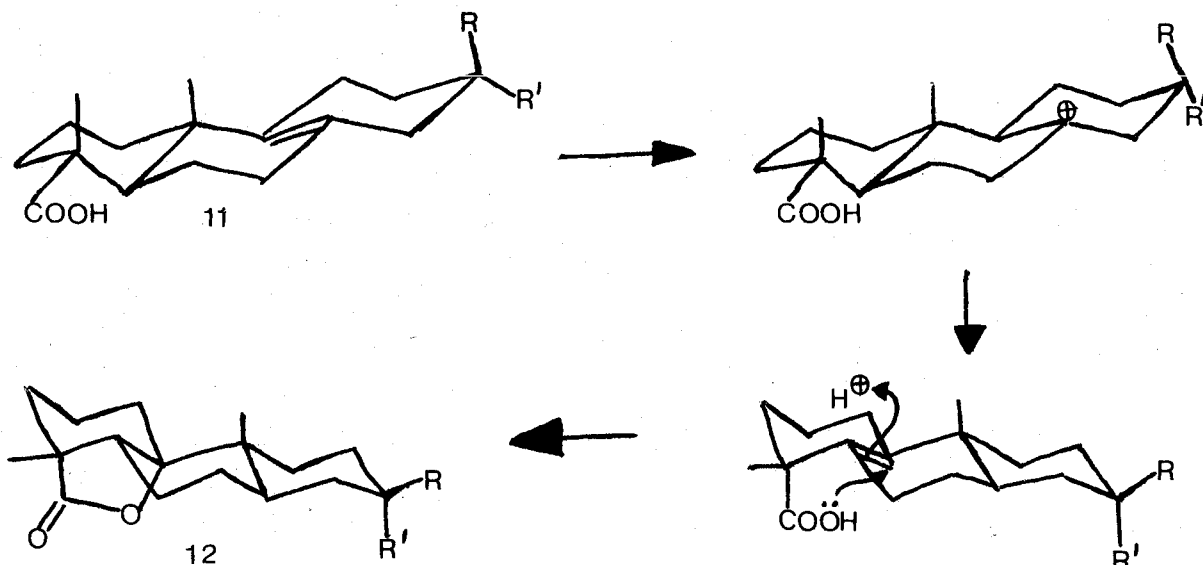


In each case a tricyclic C-8 carbonium ion is considered likely and this can lead directly to the known members of these groups. At the commencement of this work there was only one report¹⁸ of this cyclisation in vitro.

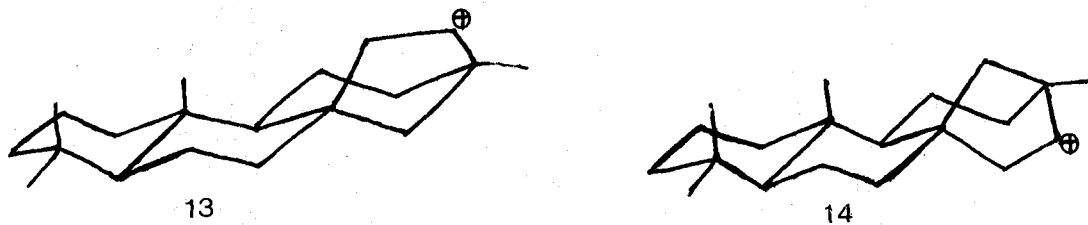
Biologically induced backbone rearrangements in a fashion similar to that described for the labdanes would lead to the rearranged skeletons of rosenonolactone (9) and rimuene (48).



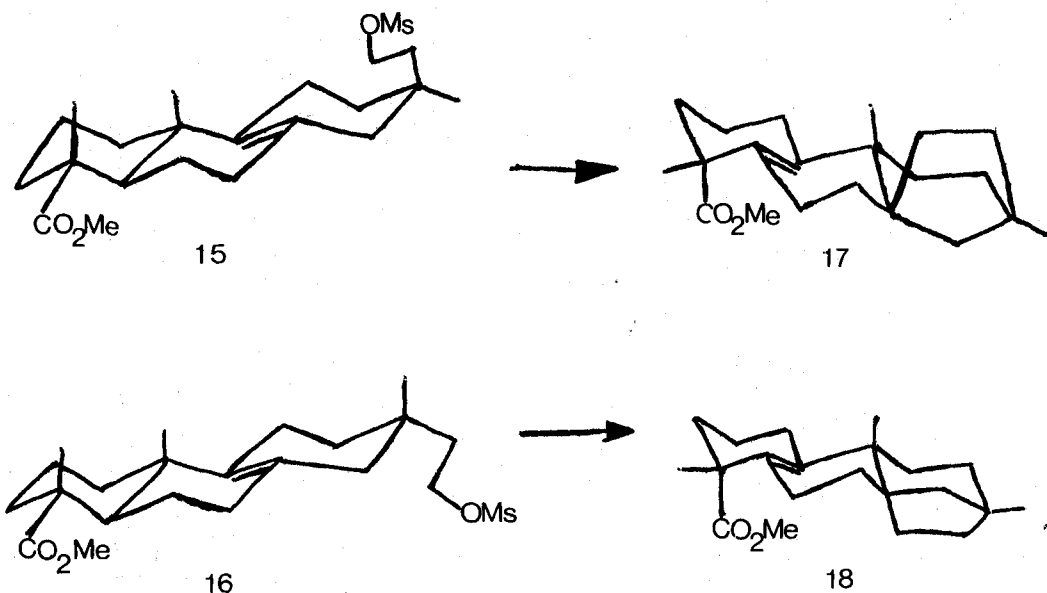
This rearrangement has been imitated in the laboratory by the treatment¹⁹ of dihydropimaric acid (11; R=Et, R¹=CH₃) and dihydroisopimaric acid (11; R=CH₃, R¹=Et) with cold concentrated sulphuric acid to give the corresponding 5 β - γ -lactones (12).



Further cyclisation of the carbonium ion species (7) and (8) in vivo is considered to give the corresponding tetracyclic ions (13) and (14).

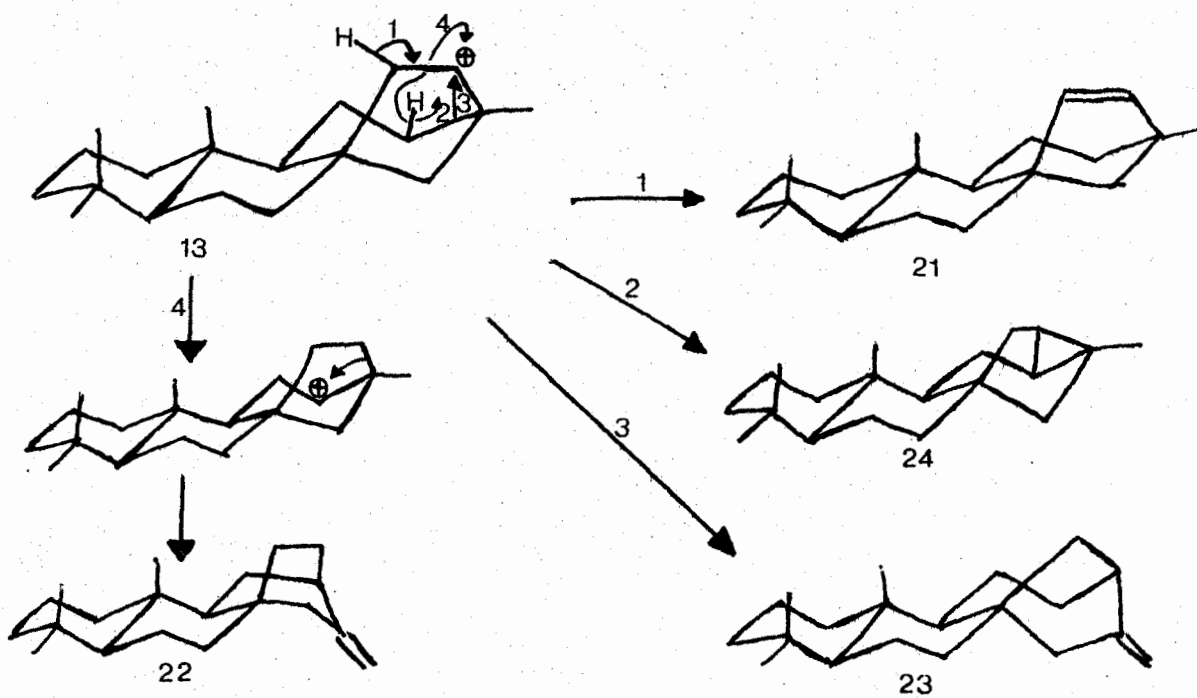
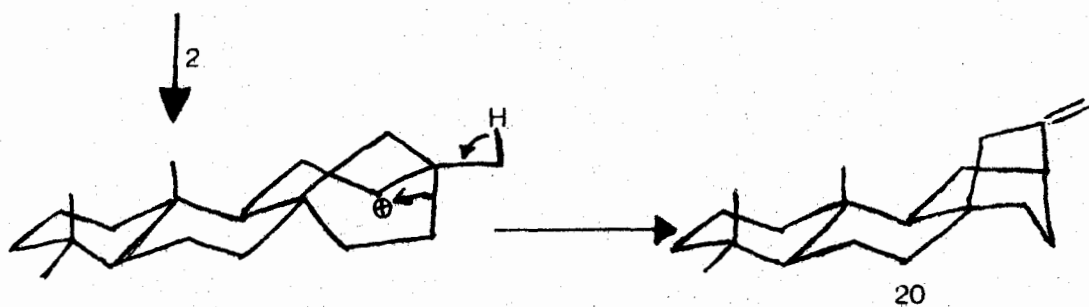
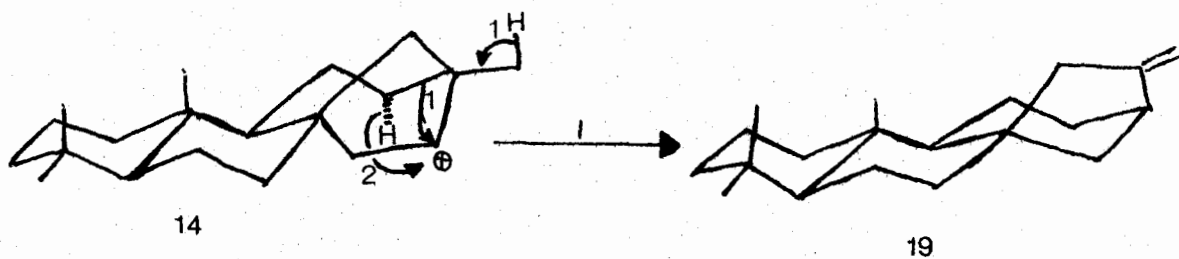


Strictly speaking this cyclisation has not, to date, been imitated in the laboratory. The tricycyclic to tetracycyclic transformation has been performed for each series however, with an electron shift in the opposite direction, and this was accompanied by a backbone rearrangement. Thus the solvolysis of pimarene mesylate (15)²⁰ and isopimarene mesylate (16)²¹ gave the rearranged hibaene (17) and isohibaene (18) skeletons respectively.



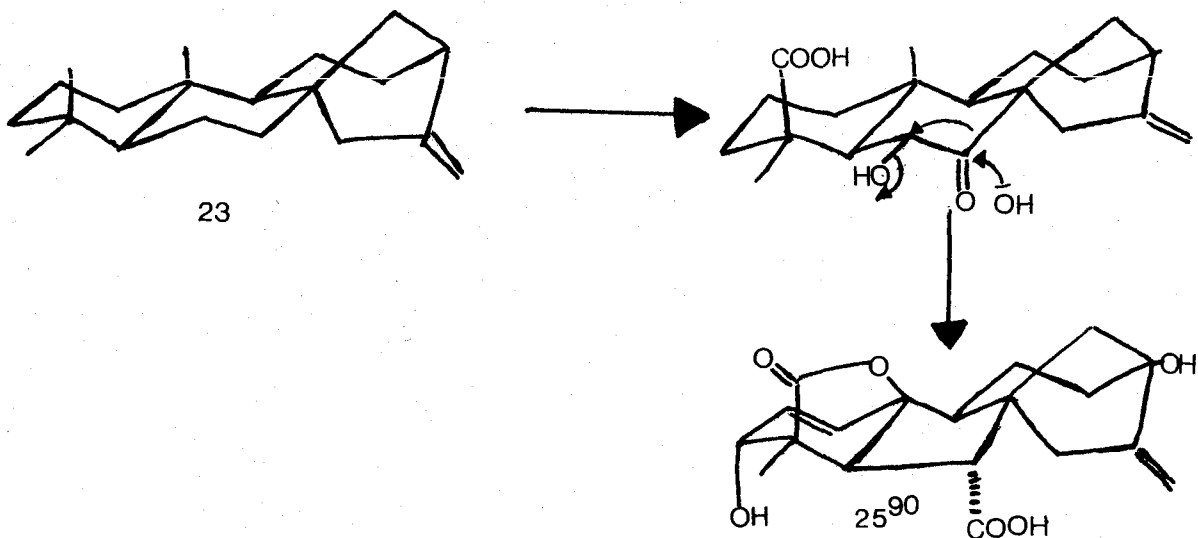
Whereas phyllocladene (19) and neo-atisene (20) are the only known naturally occurring diterpenes with skeletons derivable from (14), the isolation of diterpenes possessing the hibaene (21), atisene (22), kaurene(23) and trachylobane (24) skeletons (derivable from 13) indicate

the variety of pathways available to this type of intermediate.

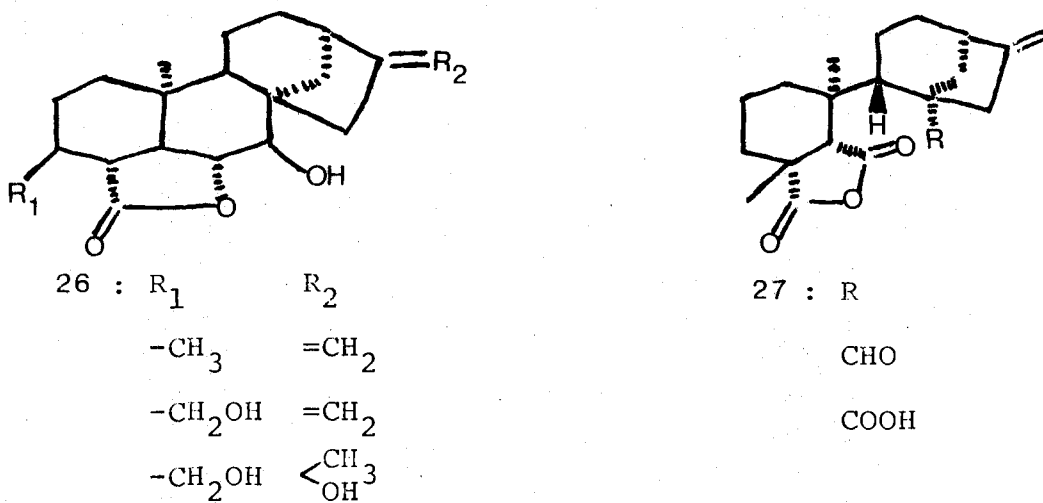


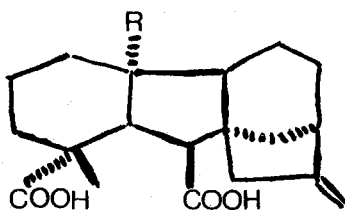
There are many reports of the cationic in vitro inter-
conversion of these skeletons ²²⁻³².

The biologically important gibberellic acid ⁹⁰ (25)
is envisioned as being formed via contraction of ring B
in the kaurene skeleton ⁸⁹ (23).



This sequence is supported by the isolation of the re-
lated intermediate compounds (26) ³³, (27) ³⁴, (28) ³⁴.



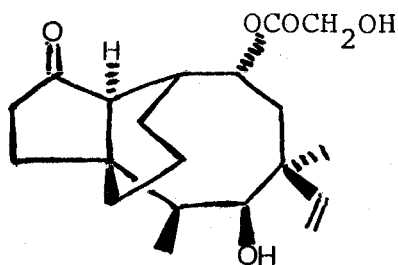


28 : R

CH₃

COOH

Strong support is lent to the above described biosynthetic pathways by the ¹⁴C labelling studies which have been performed in investigating the pathways which lead to pleuromutilin (29)³⁵⁻³⁸, rosenonolactone (9)^{35, 36, 39,40} and gibberellic acid (25)^{39,40}.

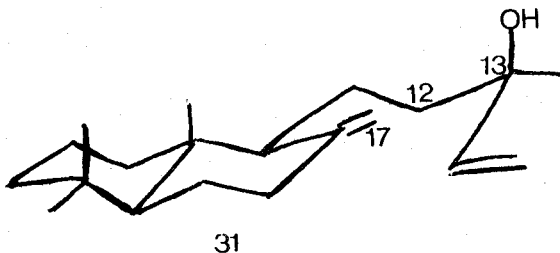


29

The overall scheme considered above for the biogenesis of the more extensively cyclised and rearranged diterpenes is illustrated by the biogenetic derivation of the label in gibberellic acid from acetate -1 ¹⁴C and ¹⁴C-2 mevalonate as shown in Figure 1. (p. 13)

Inspection of the overall scheme shows that the biogenesis of the diterpenes can proceed via intermediate carbonium ions. The transformations may occur by elimination to intermediate olefins and re-protonation but in the plant this is less likely than a process involving transfer of the cationic centre simply by π or σ electron migration. Many of the processes have, indeed, been copied in the laboratory by generating a cationic centre in an appropriate skeleton. At the beginning of this research there had, however, been very few in vitro studies on the bicarbocyclic \rightarrow tricarbicyclic \rightarrow tetracarbicyclic processes and it was the purpose of this work to investigate in the laboratory, reactions which may imitate these transformations.

A convenient starting material was manool (31), since it was commercially available and would, on acid treatment provide the required bicyclic C-13 carbonium ion.



The purpose then was to use conditions sufficiently vigorous to consume the manool but not so vigorous as to cause extensive rearrangement of the primary products. The primary products were to be isolated, identified and reacted further and this process repeated as often as possible. In this way, it was proposed to study the readiness with which each encountered carbonium ion species underwent transformations resembling those proposed for the in vivo processes involved in the biosynthesis of the more extensively cyclised diterpenes.

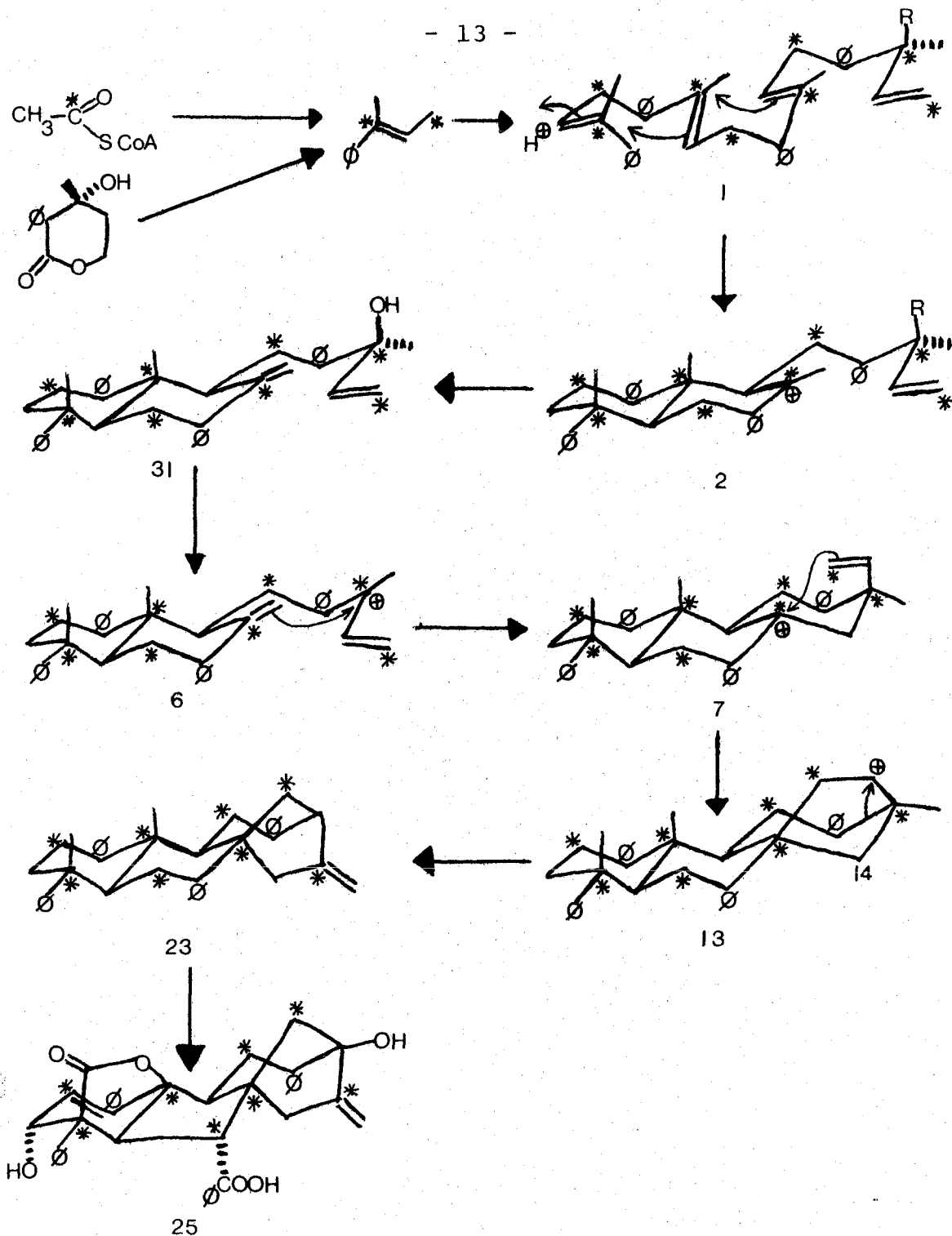
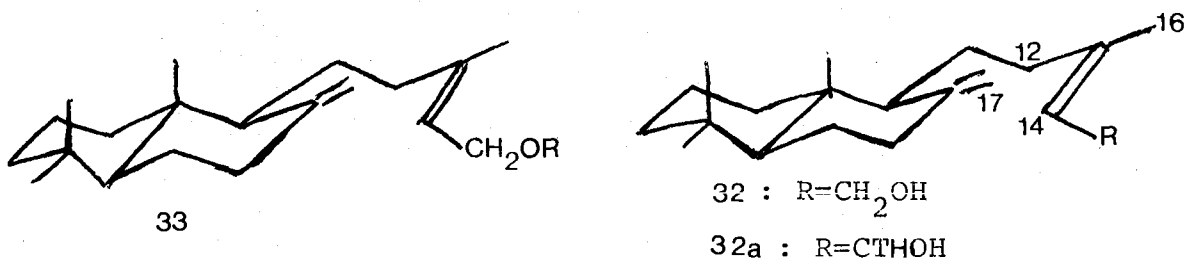


Figure 1: Biogenesis of Gibberellic Acid⁸⁹

CHAPTER 2

RESULTS

Reaction of both manool (31) and Δ^{13} -manool (32) in refluxing acetic acid gave hydrocarbon mixtures with identical vapour phase chromatograms (see Figures 2, p.28 and 3, p.29).



The results are summarised in Table 1 (p.24). Each component was identified by its IR, NMR and Mass Spectrum and where possible by comparison with authentic samples.

After appropriate work-up and extensive chromatographic separation each of the hydrocarbon products was subjected to the original reaction conditions and all except trans-biformene (34) were shown to be completely stable, as determined by vapour phase chromatographic analysis on column A or column B. After 1 hour reaction, 70% of the trans-biformene was consumed. No additional peaks were, however, observed and the trans-biformene was considered to have polymerised.

The determination of product distribution in the hydrocarbon mixture was performed by VPC on columns A and B. It was therefore necessary to calibrate the response of each of the components to be determined. This

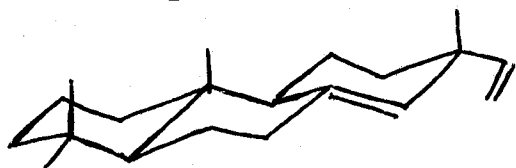
was done by defining a "specific response" (SR) of compound X with respect to the internal reference compound octadecane (OD)

$$SR = \frac{Wt.X}{\text{response X}} \cdot \frac{Wt.OD}{\text{response OD}}$$

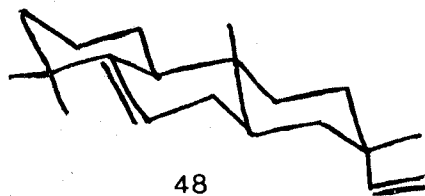
From this the actual weight and hence the yield of a component X in a mixture could be determined if the specific response of that component had been determined and if the weight of octadecane was known, simply by measuring on the VPC trace the ratio of the areas of the peaks corresponding to X and OD.

$$\therefore Wt.X = SR \times \frac{\text{response X}}{\text{response OD}} \times \frac{Wt.OD}{1}$$

To this end the specific response of each isolated hydrocarbon was determined and the results are summarised in Table 2. (p.25)



41



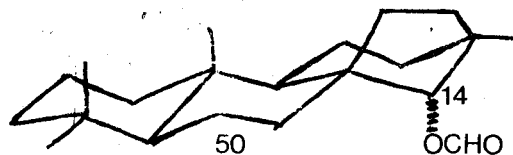
48

Due to decomposition of the samples it was not possible to determine the specific response for sandaracopimaradiene(41) or rimuene(48). In addition to the above described compounds there was a further component(49) which appeared in several chromatograms but which eluded

all attempts at isolation and hence this component could not be calibrated. The yields of these three compounds are therefore uncorrected and are marked with an asterisk*.

The experimental error was estimated at $\pm 10\%$ of each value determined, except for yields of less than 1% which were rounded off to the nearest tenth of a percent.

Reaction of manool (31) and Δ^{13} -manool (32) with refluxing formic acid gave hydrocarbon mixtures with identical chromatograms (see Figures 4, p.30 and 5, p.31), just as they did on reaction with acetic acid. The results are summarised in Table 3. (p.27)



The interconversions of the hydrocarbons in the above reaction were studied by subjecting them individually to the reaction conditions for various lengths of time. The results of this treatment for the tricyclic compounds (40, 42, 44) possessing a C-13 α vinyl group are summarised in Tables 4 (p.54), 5 (p.55), and 6 (p.56) and the results for the epimeric compounds (37, 38, 39, 43) possessing a C-13 β vinyl group are summarised in Tables 7 (p.57), 8 (p.58), 9 (p.59) and 10 (p.60). 8-Epi5(10), 15-rosadiene (46) was shown to be stable under the reaction conditions and 5(10), 12-abietarosadiene (47), although

40% reacted after 1 hour, did not give products detectable by VPC and IR spectroscopy of the reaction mixture showed no formate absorption. Hence the formation of this compound was irreversible and it was considered to have polymerised. 8,13-Burnabadiene (45) on refluxing with 97% formic acid for 1 hour was converted quantitatively into hiban-14 α -yl-formate (50) which was also isolated from the reaction mixture.

Additional information concerning the pathway by which hiban-14 α -yl-formate (50) was formed from the bicyclic skeleton was provided by treatment with formic acid of Δ 13-manool (32) deuterated at C-12, C-14 and C-16. This compound was prepared according to the scheme outlined in Figure 6 (p.32). Thus, reduction of the allylic ester (33; R=Ac) with LiAlH_4 and subsequent oxidation with chromic anhydride and pyridine gave the aldehyde (51). Attempts at further oxidation using Jones reagent or Tollen's reagent or by prolonged reaction with chromic anhydride and pyridine proved unsuccessful. Oxidation according to the method of Corey⁴¹, gave enantio-methyl copalate (52). Base-catalysed deuterium exchange followed by LiAlH_4 reduction gave Δ 13-manool deuterated at C-12, C-14 and C-16 (54). The extent of deuteration at C-

14 was ascertained to be 50% by NMR comparison of the intensity of the pair of signals (τ 5.20, 5.53) due to the C-17 hydrogens with the triplet (τ 4.74) due to that attached to C-14. After chromatography of the reaction mixture, hiban-14 α -yl-formate (50) was isolated in low yield. Integration of the NMR signal due to the formate hydrogen (τ 1.80) and comparison with that due to the 14 β -hydrogen (τ 5.55) in this hiban-14 α -yl-formate revealed that all of the deuterium originally at C-14 in the starting material was located at C-14 in this product. Furthermore, the isotope distribution around the parent peak in the mass spectrum of both of these compounds was the same, indicating that the deuterium at C-12 and C-16 in the deuterated Δ 13-manool (54) were not lost in the reaction.

An attempt was made to maintain asymmetry at C-13 in the ring closure between C-17 and C-13 of manool by decreasing the ease of the step which generates a carbonium ion centre at C-13. It was hoped that asymmetric capture of C-13 could be detected by an increase in the yields of products derived from the pimarane skeleton (C-13 β -vinyl), formed by a backside displacement, at the expense of products derived from the isopimarane

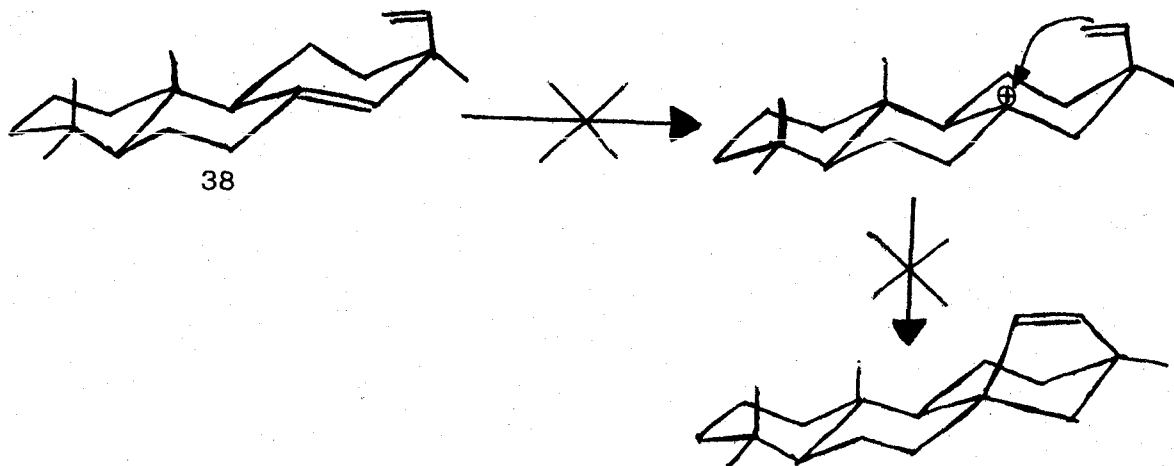
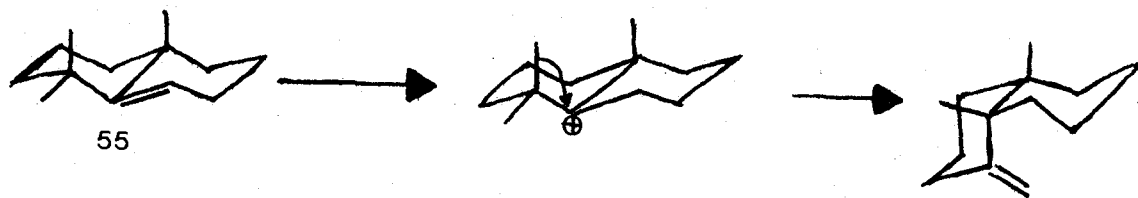
skeleton (C-13 α -vinyl). The latter compounds can only be formed after complete ionisation and rotation about the C-12 - C-13 bond. To this end the p-nitrobenzoate of manool was prepared and its solvolysis attempted in a variety of solvents. Unfortunately, the compound was so stable that no ionisation occurred and pure starting material was recovered on each occasion.

The compound to which the structure 5(10),12-abiatarosadiene (47) was subsequently assigned was hydrogenated and the product compared by VPC with a sample of authentic hibane prepared from hiban-14 α -yl-formate. The two compounds were not identical and it was therefore concluded that this compound, whose structure had not yet been determined, did not possess the hibane carbon skeleton.

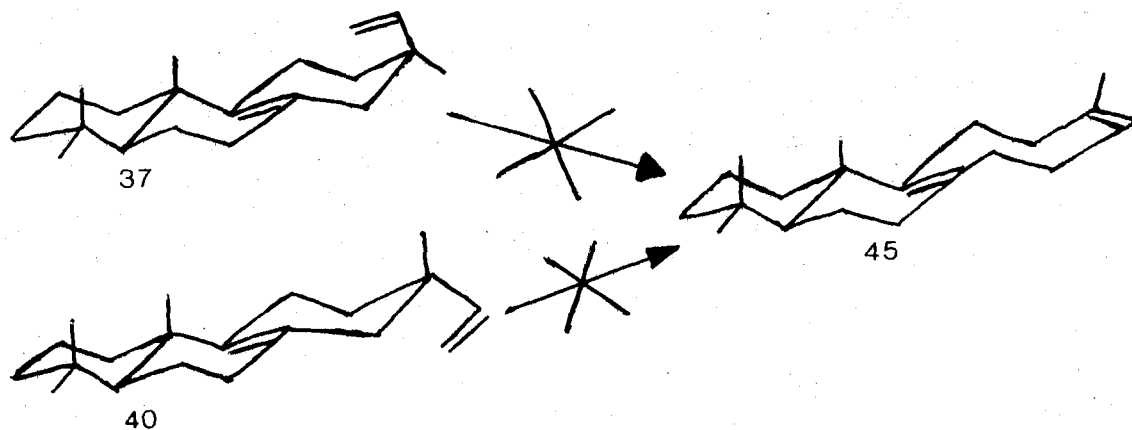
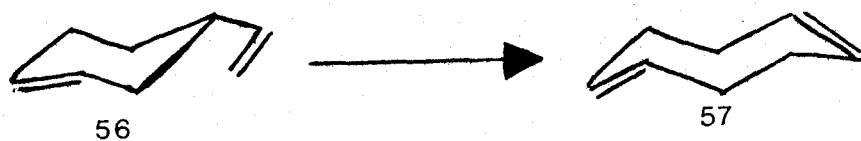
In order to substantiate structure (45) for 8,13-burnabadiene several attempts were made to synthesise it.

Photolysis of pimaradiene(38) in tert-butanol with xylene as sensitiser, the same conditions as have been previously used⁴² to induce cationic rearrangements via photolytically generated carbonium centres in cyclohexane systems eg. (55)⁴³, failed to give any reaction.

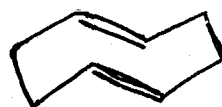
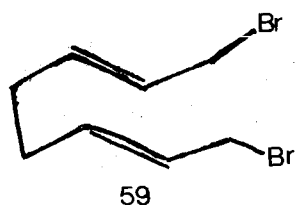
Exposure of 8,15-pimaradiene(37) and 8,15-iso-pimaradiene(40) to $(\text{OCN})_2 \text{PdCl}_2$ ⁴⁴ under the same conditions as have been reported⁴⁵ for the isomerisation of 4-vinyl-



cyclohexene (56) to 1,5-cyclooctadiene (57) did not produce any 8,13-burnabadiene. In each case the pure starting material was recovered.



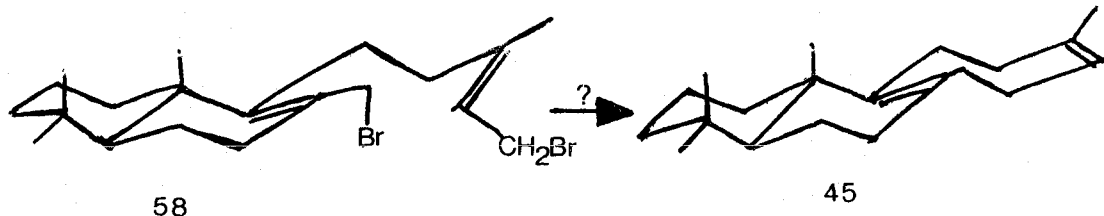
The third procedure which was attempted involved as the last step the cyclisation of the diallyl bromide (58) using $\text{Ni}(\text{CO})_4$ or $\text{P}(\text{C}_6\text{H}_5)_3\text{Ni}(\text{CO})_3$ ⁴⁶ according to the procedure previously reported⁴⁷ for the cyclisation in 5-20% yield of the diallyl bromide (59) to 1,5-cyclooctadiene (60).



60

$\text{Ni}(\text{CO})_4$ 5% yield

$\text{P}(\text{C}_6\text{H}_5)_3\text{Ni}(\text{CO})_3$ 20% yield



The bicyclic precursor (58) was to be synthesised according to the procedure outlined in Figure 7 (p.33), utilising enantio-methyl copalate (52) as starting material.

The bromination step⁴⁸ to give (61) and the subsequent dehydrobromination⁴⁹ to (62) proceeded successfully but an attempted purification of (62) by column chromatography gave only polymerised products. This synthesis was, there-

fore, discontinued at this stage.

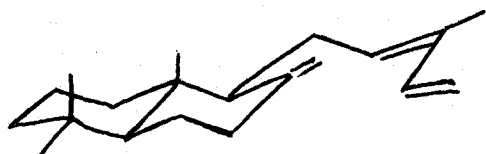
TABLE I

Product Distribution from Reaction of Manool with Acetic

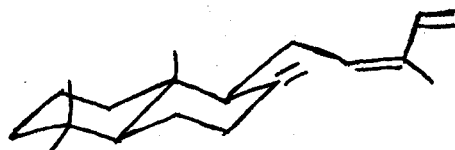
	<u>Acid</u>	
<u>Compound</u>		<u>Yield %</u>
Manool	(31)	36
Δ 13-Manool Acetate (33; R=Ac)		30
<u>Trans</u> -biformene	(34)	15
<u>Cis</u> -biformene	(35)	5.4
Sclarene	(36)	6.2
8,15-Pimaradiene	(37)	3.4
Pimaradiene	(38)	0.5
7,15-Pimaradiene	(39)	1.1
8,15-Isopimaradiene	(40)	2.9
Sandaracopimaradiene	(41)	0.4*
Isopimaradiene	(42)	1.2
5(10),15-Rosadiene	(43)	0.2
13- <u>Epi</u> -5(10),15-rosadiene (44)		0.4
8,13-Burnabadiene	(45)	1.4
	Total	104.1

TABLE 2

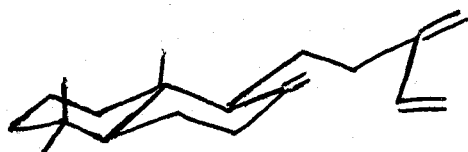
Specific Response of Hydrocarbon Products



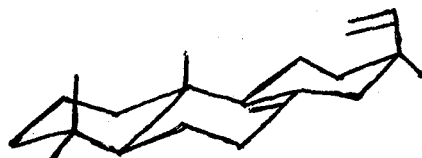
Trans-biformene (34) : 4.2



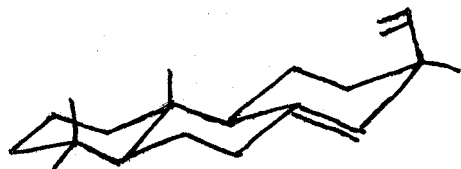
Cis-biformene (35) : 5.3



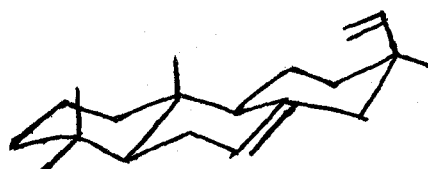
Sclarene (36) : 1.8



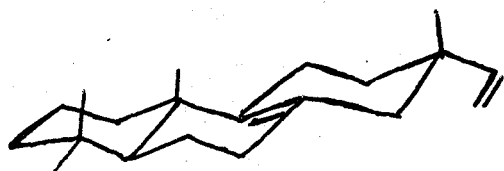
8,15-Pimaradiene (37) : 1.3



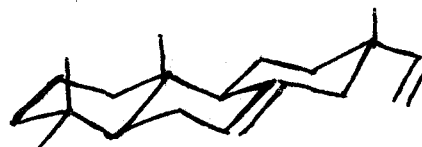
Pimaradiene (38) : 1.3



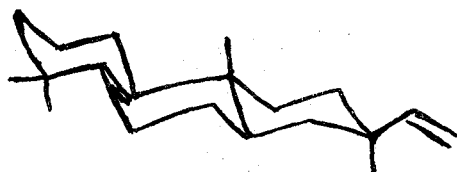
7,15-Pimaradiene (39) : 1.4



8,15-Isopimaradiene (40) : 1.1



Isopimaradiene (42) : 1.1

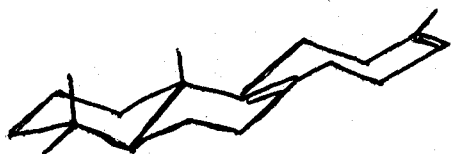


5(10),15-Rosadiene (43) : 1.1

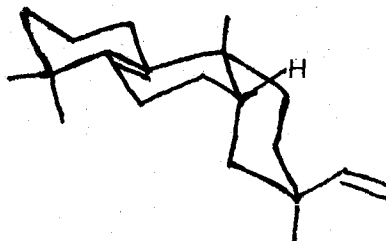


13-Epi-5(10),15-rosadiene (44) : 1.5

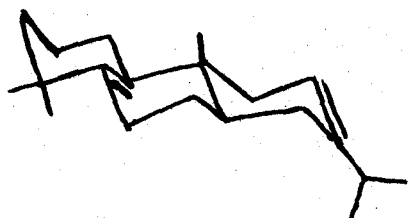
TABLE 2, Cont.



8,13-Burnabadiene (45):2.0



8-Epi-5(10),15-
Rosadiene (46):1.2

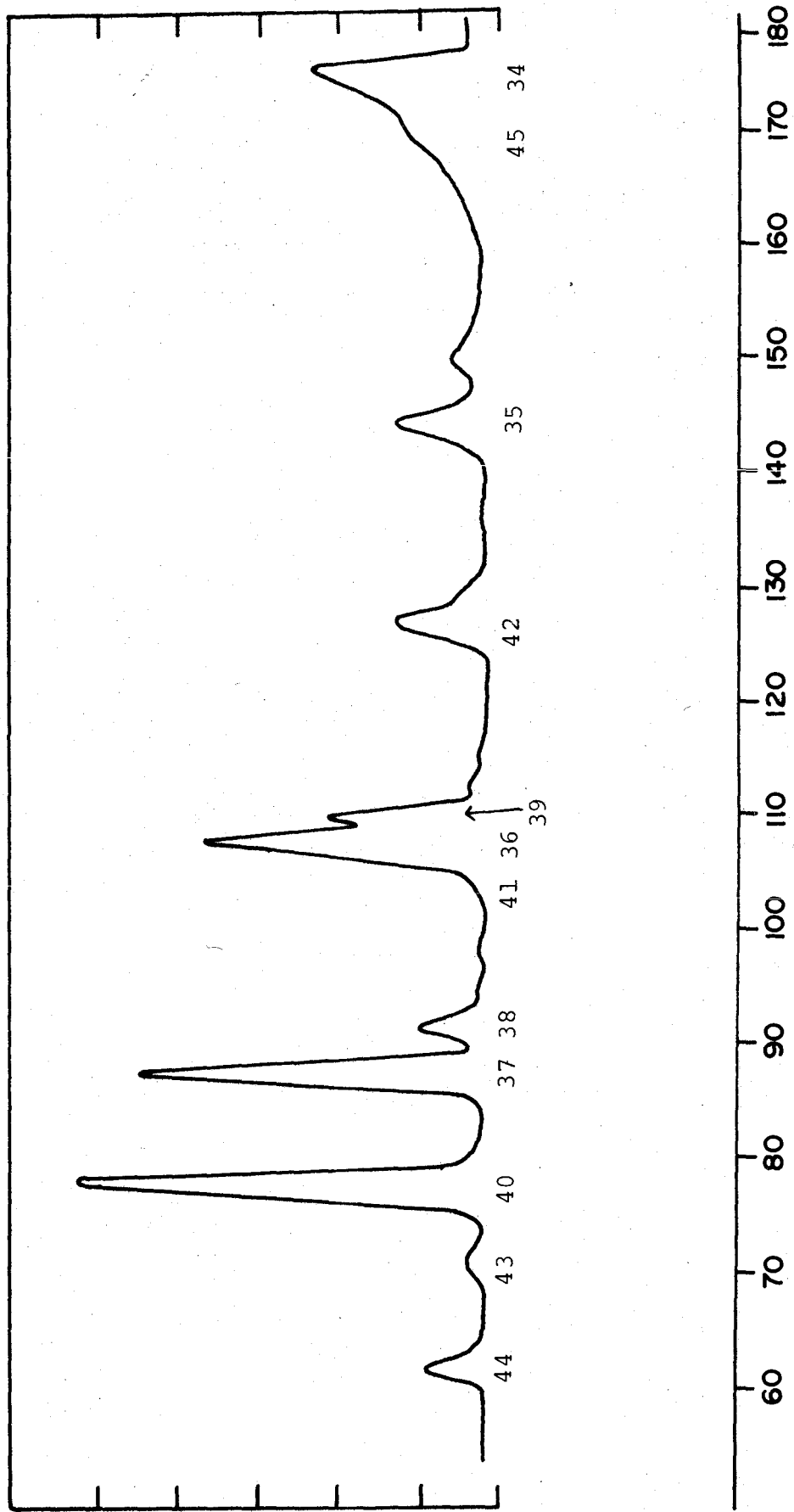


5(10),12-Abietarosadiene (47):1.3

TABLE 3

Product Distribution from Reaction of Manool with Formic
Acid

<u>Compound</u>	<u>Yield %</u>
Δ 13-Manool formate (33; R=CHO)	11
8,15-Pimaradiene (37)	24
Pimaradiene (38)	1.5
7,15-Pimaradiene (39)	1.8
8,15-Isopimaradiene (40)	19
Sandaracopimaradiene (41)	1.1*
Isopimaradiene (42)	1.7
5(10),15-Rosadiene (43)	5.0
13- <u>Epi</u> -5(10),15-rosadiene (44)	10
Rimuene (48)	1.8*
8- <u>Epi</u> -5(10),15-rosadiene (46)	1.2
5(10),12-Abietarosadiene (47)	1.1
Unknown (49)	1.0*
8,13-Burnabadiene (45)	2.0
Hiban-14 α -yl formate (50)	8.0
Total	90.3



RETENTION TIME IN MINUTES

Figure 2: VPC on column A; hydrocarbon fraction from 1 hour reaction of manool with acetic acid. 28

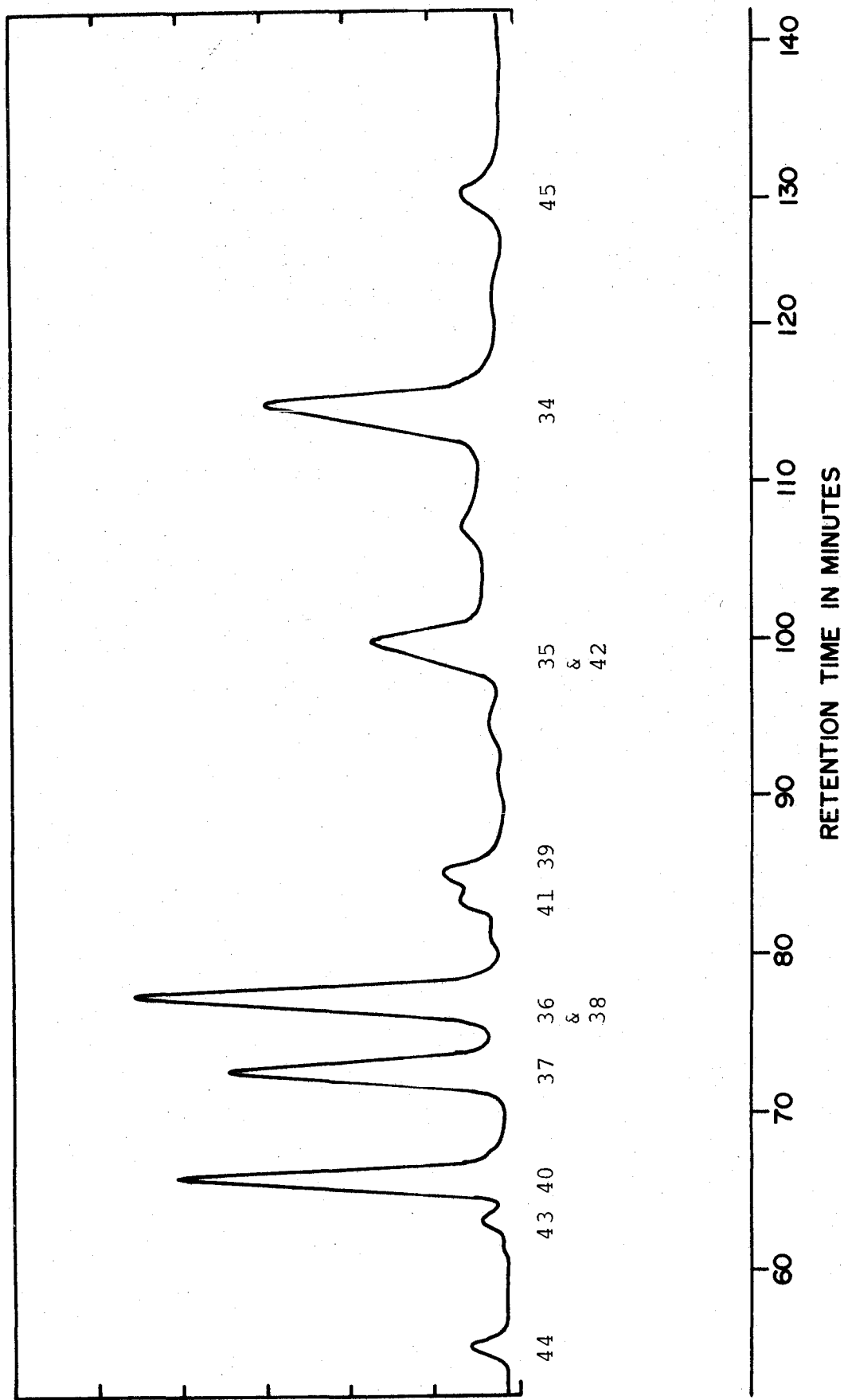


Figure 3: VPC on column B of hydrocarbon fraction from 1 hour reaction of manool with acetic acid.

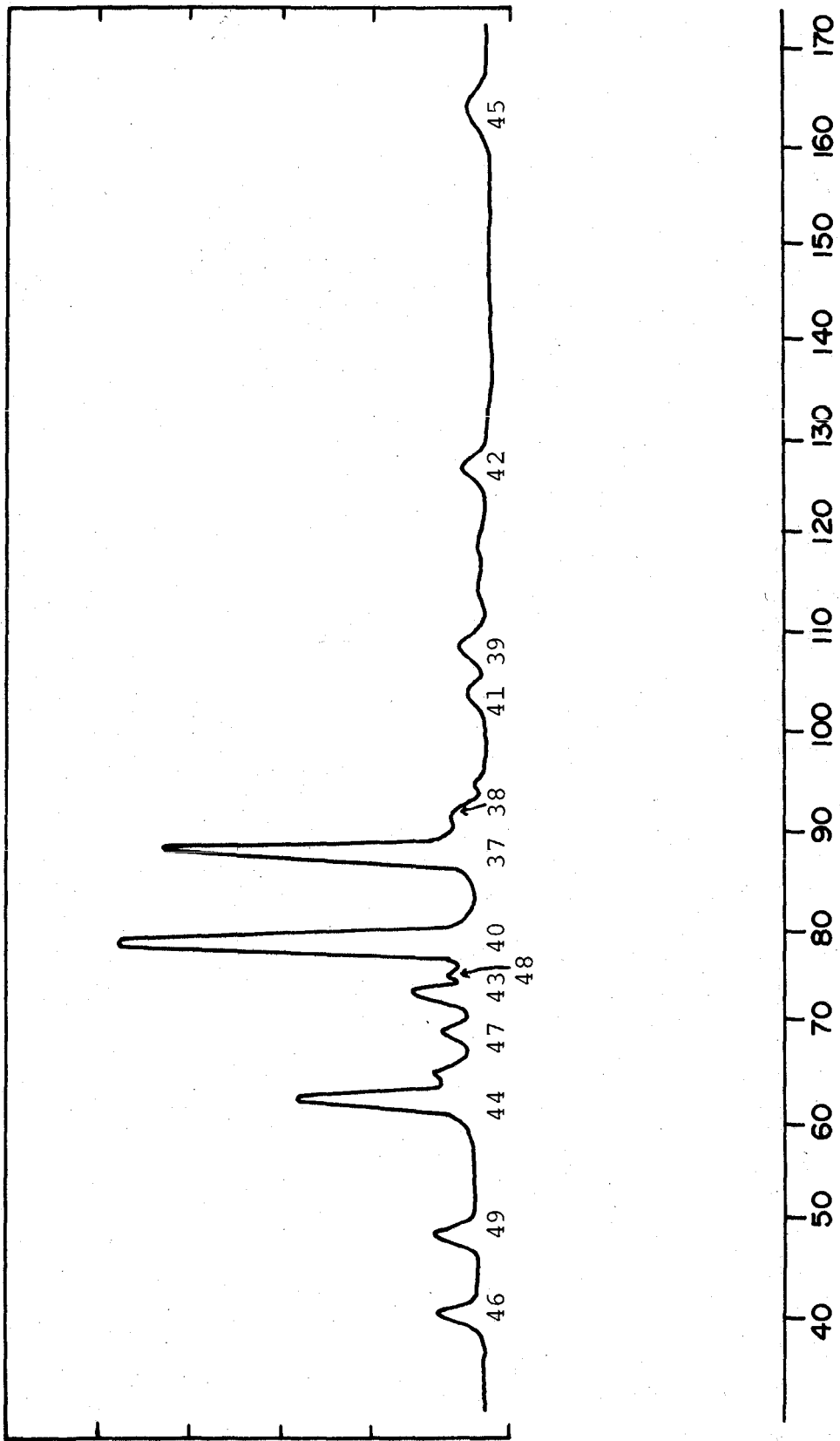


Figure 4: VPC on column A of hydrocarbon fraction from 1 hour reaction of manool with formic acid.

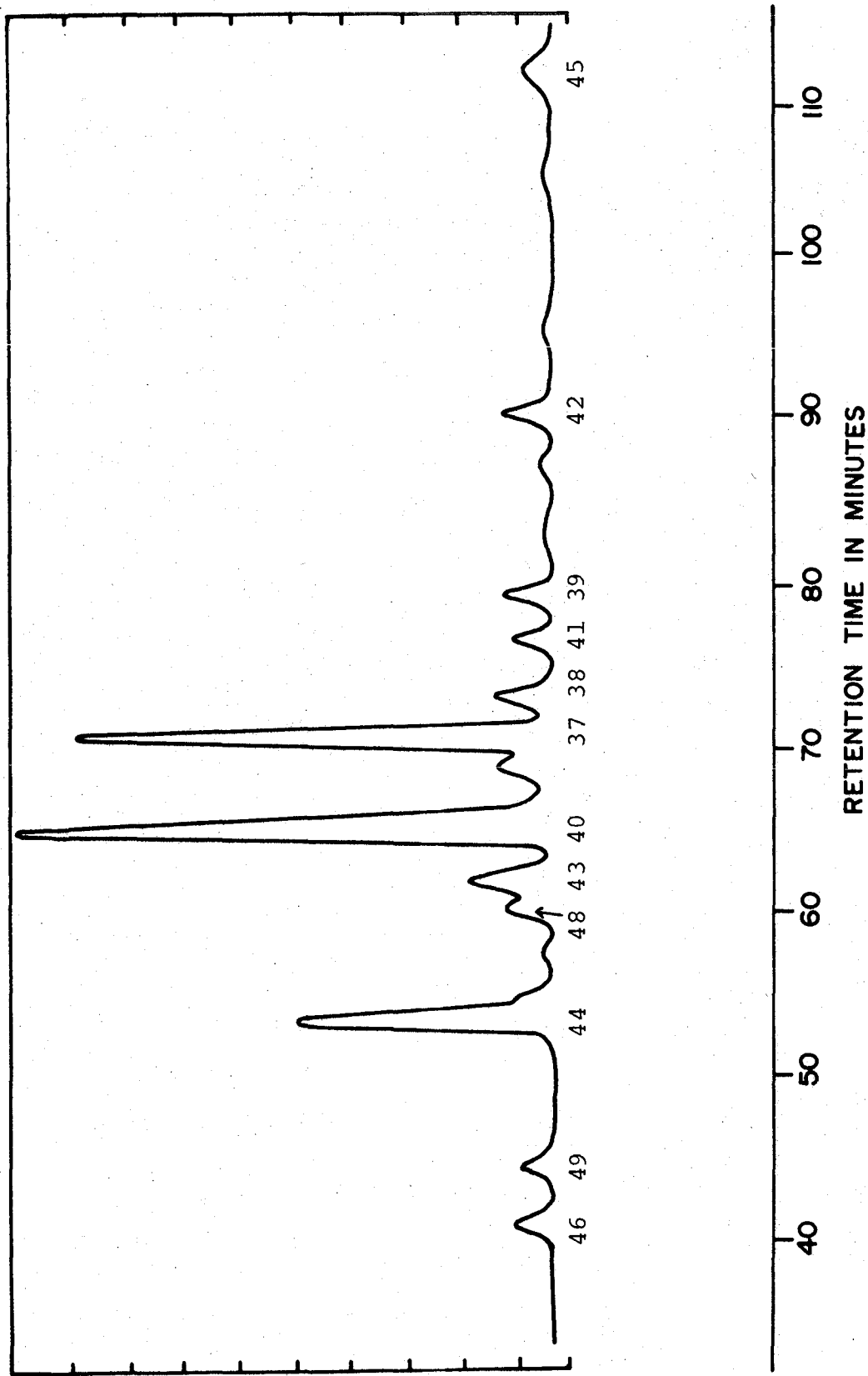


Figure 5: VPC on column B of hydrocarbon fraction from 1 hour reaction of manool with formic acid

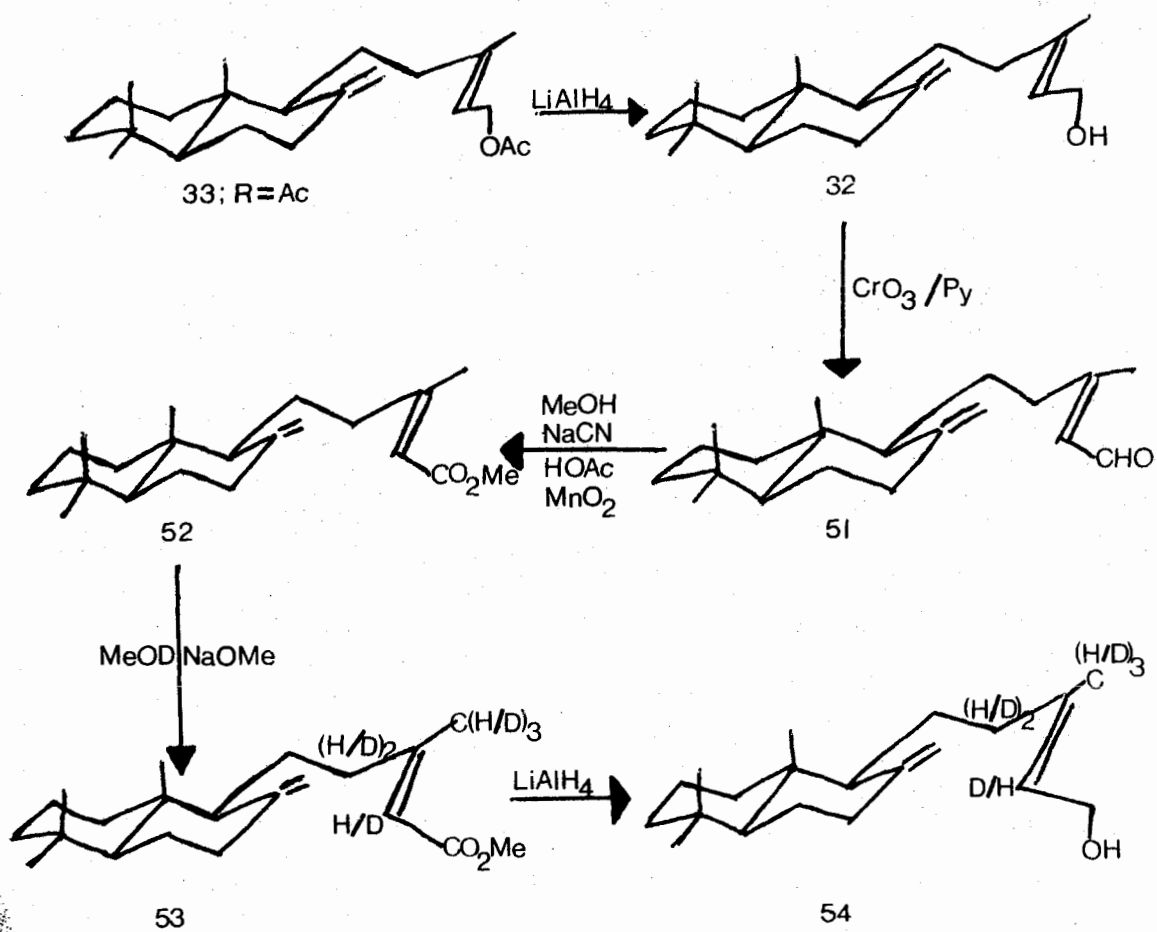


Figure 6: Synthesis of Deuterated Δ^{13} -Manool

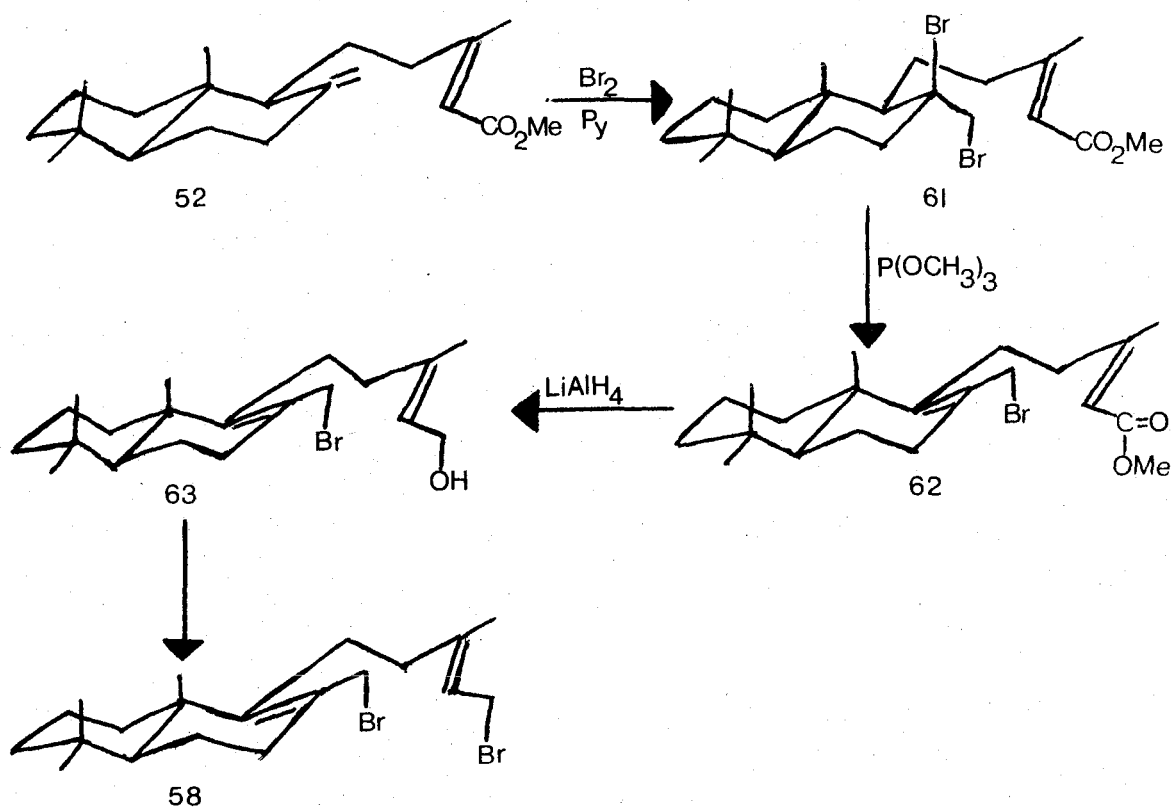


Figure 7: Attempted Synthesis of Diallyl Bromide Precursor (58)

CHAPTER 3

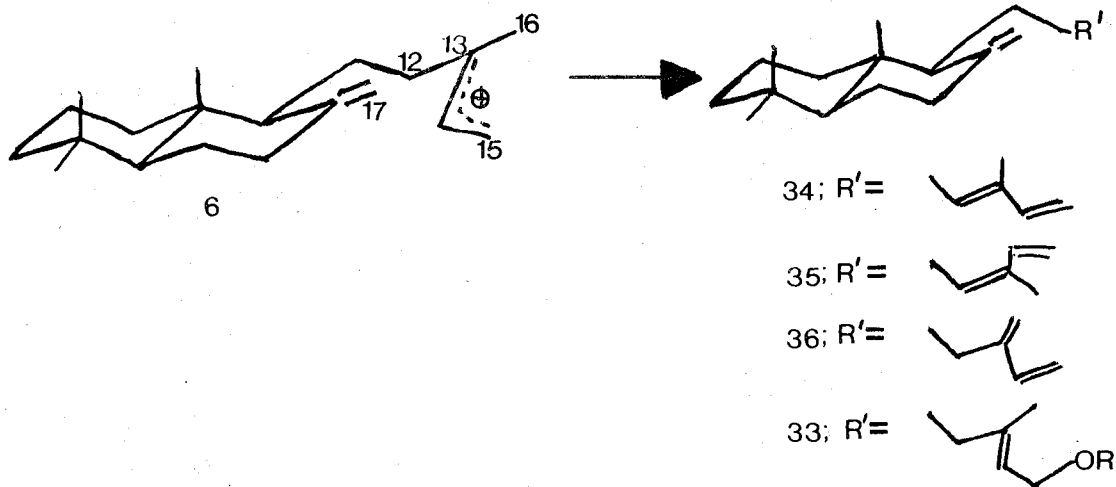
DISCUSSION OF RESULTS

That the bicyclic species undergoing cyclisation is best represented by (6) is indicated by the following results.

- a) The identical product distribution from both manool(31) and Δ^{13} -manool(32)
- b) Comparable yields of the two C-13 epimeric tricyclic series of compounds
- c) The isolation of Δ^{13} -manool formate and acetate (33; R=CHO, COCH₃)
- d) The isolation of the cyclised product 8,13-burnabadiene(45)

The first three observations are supported by the preliminary reports^{50, 51, 52} of other workers which were published while this work was in progress.

The lower pKa of formic acid compared with acetic acid resulted in a greater consumption of manool and the formation in higher yields of more extensively cyclised and rearranged products(to be discussed) in this reaction medium.



Neutralisation at C-15 by the appropriate gegenion led to the corresponding ester (33; R= -CHO, -COCH₃). In acetic acid deprotonation to trans-biformene(34), cis-biformene(35) or sclarene(36) was the predominant alternative fate of (6). These products have been isolated from natural sources and their formation under these reaction conditions has also recently been reported.⁵³

Reactions in 97% formic acid, however, failed to result in the detection of any of these three products, and the compounds which were formed instead were derived from (6) via alternative pathways involving cyclisation to C-17. This observation can be explained in two ways. The labdatrienes, if formed, would be expected to be re-protonated much more readily in formic acid than in acetic acid, in a process possibly resulting in the regeneration of (6), which could then react in a non-reversible fashion via the alternative cyclisation pathways. This would explain the failure to detect any of the bicyclic trienes in the reaction mixture. This explanation was eliminated, however, by the complete consumption of cis-biformene(35) and trans-biformene(34) and sclarene(36), when subjected individually to the reaction conditions, to give mixtures whose vapour phase chromatograms possessed peaks at retention times

shorter than those of the earliest peaks observed in the chromatogram for the manool reaction. It was, therefore, concluded that, once the bicyclic ion (6) was formed, it underwent cyclisation at a rate which precluded deprotonation by formate ion to a labdatriene.

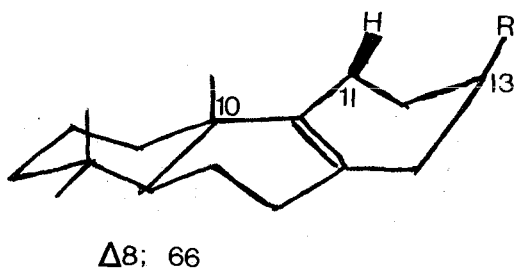
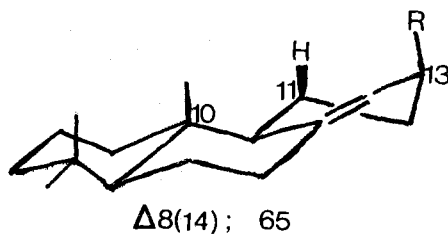
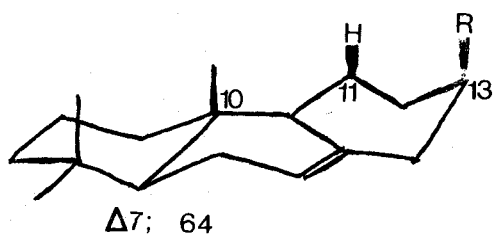
The stability of the labdatrienes to refluxing acetic acid showed that in this reaction, the cyclised products resulted directly from reaction of (6) without the participation of bicyclic olefin intermediates. Hence acetate ion is sufficiently basic to allow proton elimination to compete favourably with cyclisation.

In both acetic acid and formic acid cyclisation of (6) between C-13 and C-17 gave the tricyclic pimaradienes (37,38,39) and isopimaradienes (40,41,42) as well as the corresponding backbone rearranged products (43,44).

Analogous cyclisations in model systems are well known^{54, 55}. In addition, reports^{50, 51, 52} of this ring closure were published concurrent with our work.

Many compounds possessing structures derivable from the regular or backbone rearranged tricyclic skeleton have been isolated from natural sources, and evidence that in the in vivo process the bicyclic ion (6) is a precursor of these products is provided by the incorporation⁵⁶ of 15-T- Δ 13-manool (32a) into rosenonolactone (9) by Tricothecium roseum.

The preponderance of the Δ^8 isomers may be rationalised in terms of the greater stability of the tetra-substituted double bond with respect to the trisubstituted double bond of the other two isomers. An investigation of molecular models, however, reveals that this is not the only factor to be considered. The most stable conformations (64,65,66) of the three isomers involved are shown below.



Although the bonds associated with rings B and C in the Δ^7 and $\Delta^{8(14)}$ compounds are all staggered, there is 1,3 diaxial interaction of the C-11 β -H with the C-10 methyl and the C-13 β -substituent. The Δ^8 isomer, however, possesses a reduced 1,3 diaxial interaction between these

three groups. The presence of these additional interactions adds further explanation to the higher yields of the $\Delta 8$ isomers compared to the $\Delta 7$ and $\Delta 8(14)$ isomers. Consistent with this is the observation that the $\Delta 7$, $\Delta 8(14)$ and $\Delta 8$ isomers on treatment with formic acid for a short time underwent a facile interconversion in an equilibrium process giving mixtures consisting of the above isomers in the approximate ratio 1:1:25. The less stable isomers were consumed more rapidly than the $\Delta 8$ isomers.

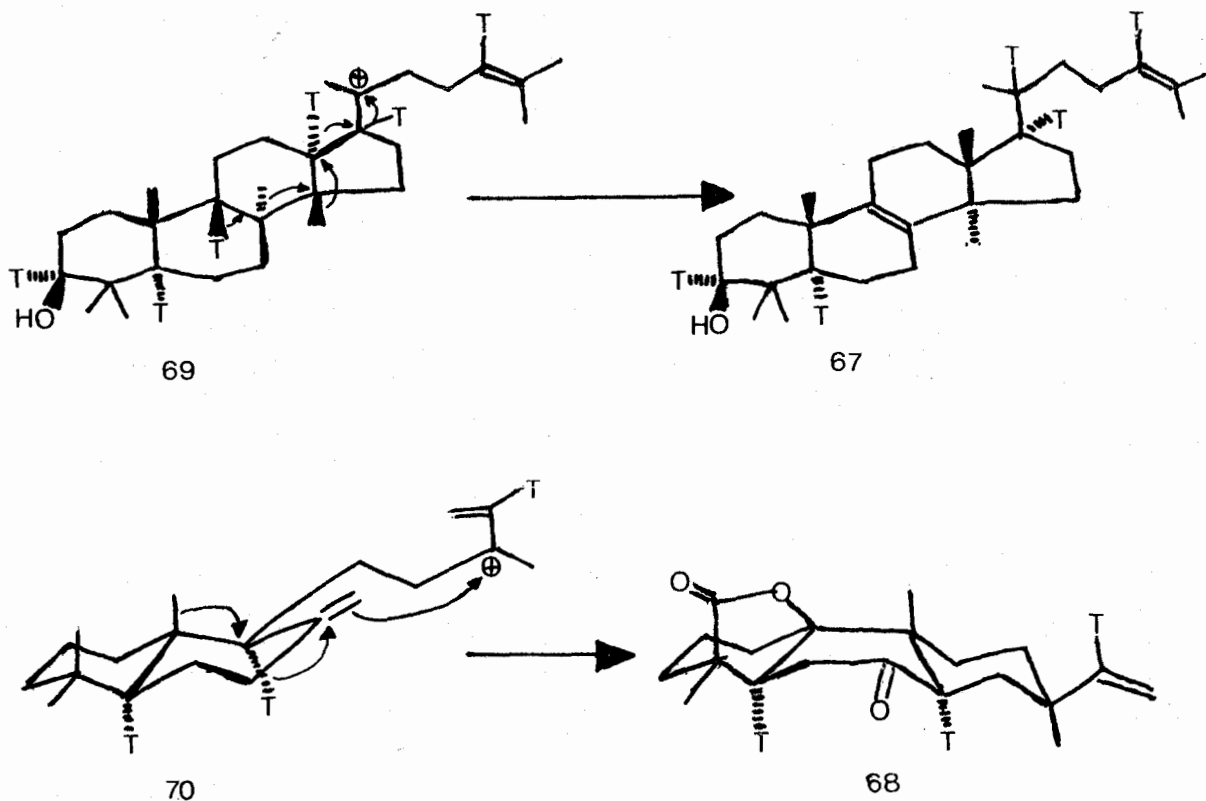
The fact that each of the tricyclic products(37-42) was stable to refluxing acetic acid raises one interesting point which has not previously been observed. The backbone rearranged products(43) and (44) formed on reaction with acetic acid must have come directly from the initially cyclised carbonium ions (7) and (8) without the participation of olefinic intermediates. By analogy with the argument above concerning the fate of the bicyclic ion(6), the increase in the yields of the backbone rearranged products upon reaction in formic acid is attributable, at least in part, to an increase in the competitiveness of backbone rearrangement of the initially formed tricyclic ion (7) or (8) since proton elimination is retarded in this reaction. The extent of the contribution from re-protonated olefins, however, cannot be determined

from the available data, but the fact that it did participate to some extent was shown by the further reactions of the primary products of cyclisation to give backbone rearranged compounds when reacted individually in formic acid.

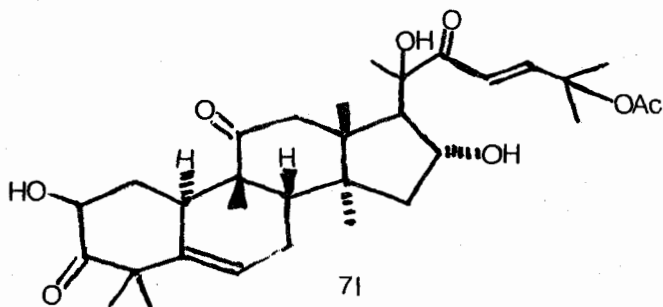
These observations are especially interesting in the light of the many reports of both in vivo and in vitro backbone rearrangements which proceed without the participation of olefinic intermediates.

The earliest experiments^{57,58} designed to investigate the mechanism of in vivo backbone rearrangements were the enzymatic cyclisation and subsequent rearrangement of squalene to lanosterol(67) in the presence of D₂O. No measurable amount of deuterium was incorporated. More recently the incorporation of 4R-[4T : 2-¹⁴C] mevalonolactone into lanosterol (67)⁵⁹ and rosenonolactone(68)^{60,61} showed that the in vivo backbone rearrangements involved in the biogenesis of these compounds from their polycyclic progenitors (69), (70) proceeded with retention of label as shown. These results are consistent with a pathway which does not involve olefinic intermediates.

It has also been shown⁶² that the biosynthesis of the backbone rearranged cucurbitacin B (71) does not involve

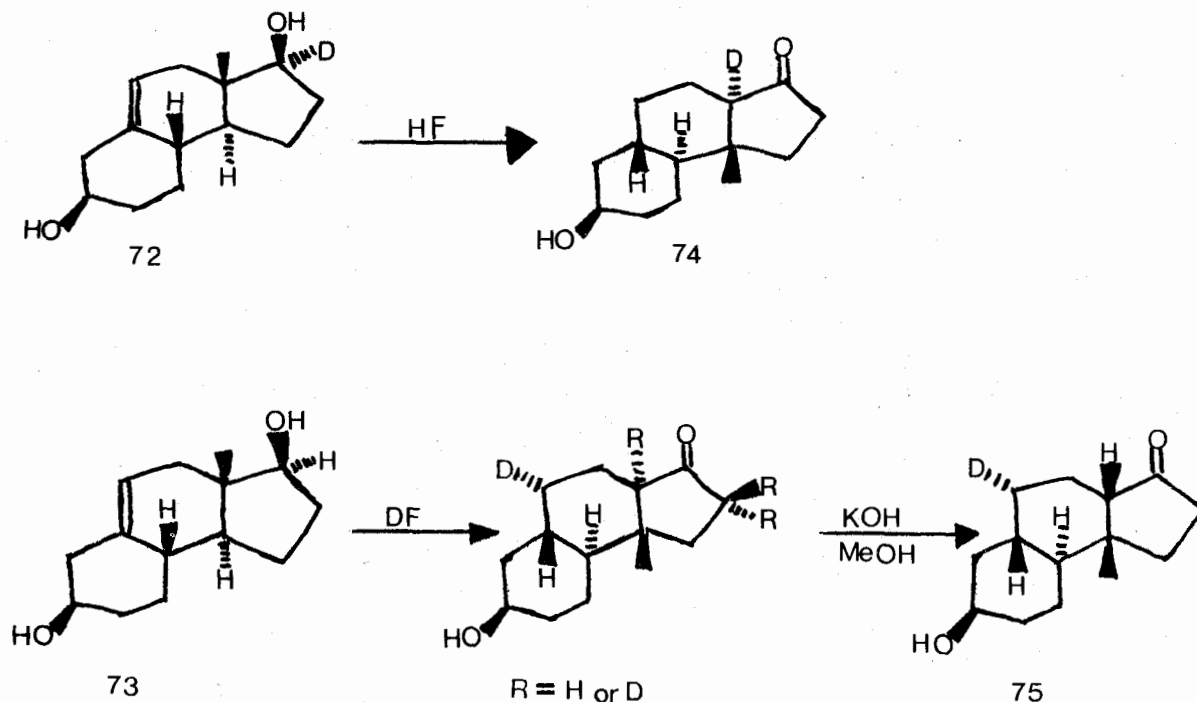


the olefinic intermediate lanosterol(67).



Also of interest is the acid catalysed⁶³ backbone rearrangement of the two diols(72), (73) in anhydrous HF and DF respectively. The presence of only one deuterium atom in each of the corresponding products (74), (75) eliminates the possibility of deprotonation-reproton-

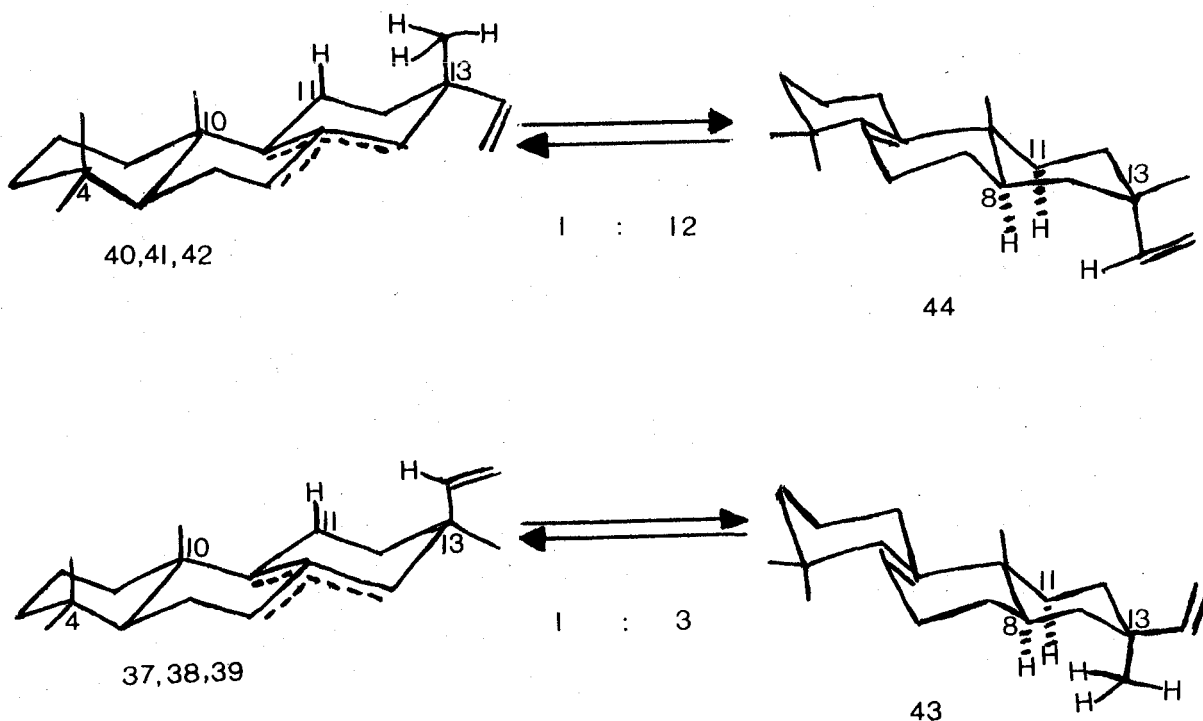
ation reactions of the intermediate carbonium ions.



Prolonged formic acid treatment of the pimaradienes (37,38,39) or isopimaradienes (40,41,42) resulted in equilibrium mixtures in which the ratios of yields of regular: backbone rearranged skeletons were approximately 1:3 and 1:12 respectively. The same equilibrium mixtures were obtained upon starting with 5(10),15-rosadiene(43) and 13-epi-5(10),15-rosadiene(44). A rationale for the preponderance of the backbone rearranged products may be adduced by examination of the 1,3-diaxial interactions in each isomer. Thus in the backbone rearranged products 1,3-diaxial interaction between the methyl groups at C-4

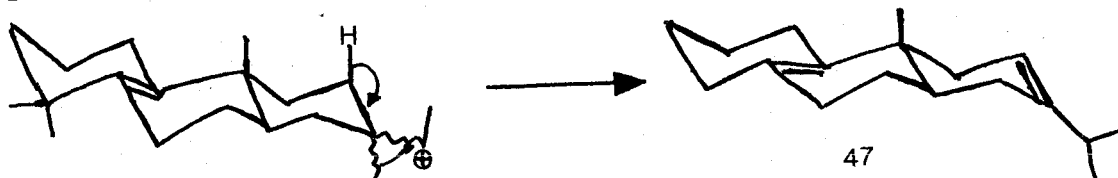
and C-10 is relieved.

It is interesting to note that the backbone re-arranged product was more favoured in the C-13 α -vinyl series than in the C-13 β -vinyl series. This can be seen by examination of molecular models which reveal that 1,3-diaxial interaction in the backbone rearranged skeletons between the C-13 α group and the α protons at C-8 and C-11 will be greater when the axial α group at C-13 is methyl rather than vinyl. Similarly the loss of steric interaction between the C-13 β group and the C-11 β -proton is greater for rearrangement in the isopimarane series (40,41,42). These effects indicate that loss of



steric interaction on formation of (44) is greater than on formation of (43) in agreement with the observed product ratios.

Inspection of the results in Tables 4-10 (pp.54-60) reveals that 5(10),12-abieta-rosadiene(47) was formed much faster from 5(10),15-rosadiene(43) than from the pimara-dienes (37, 38, 39), or 13-epi-5(10),15-rosadiene(44) and it is in this compound that the migrating C-13 methyl group is in the least stable configuration. This indicates that formation of (47) proceeded by initial backbone rearrangement followed by C-13 - C-15 methyl migration and deprotonation.



It might be expected that backbone rearrangement would precede methyl shift since protonation to give a tertiary carbonium ion usually proceeds faster than protonation to give a secondary carbonium ion, the requisite intermediates for the two transformations.

The tetracyclic hiban-14 α -yl formate was also formed on reaction of manool with formic acid. There have been isolated from natural sources many compounds possessing carbon skeletons which can readily be derived from this

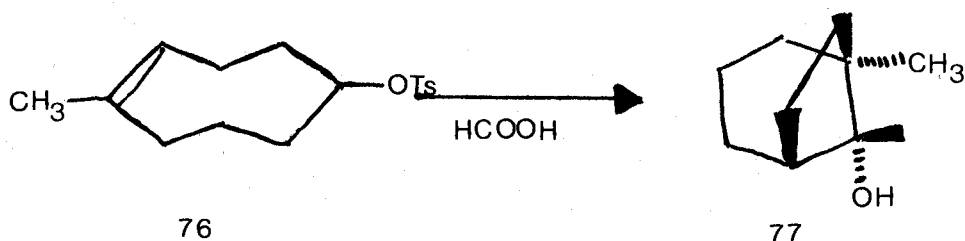
structure.

Two pathways (Figure 8, p.61) leading to this product are mechanistically attractive.

Cyclisation of (6) between C-17 and the tertiary C-13 cationic centre (pathway a) leads to the cation (7) which is the precursor of the previously discussed pimaradienes (37,38,39). Further ring closure between C-16 of the vinyl group and the cationic centre at C-8 followed by hydride shift and neutralisation as shown might result in the observed product (50). This is the pathway by which the tetracyclic and pentacyclic diterpenoids have long been considered to be synthesised in vivo⁶⁴, and by which this compound was initially considered to be formed in the laboratory cyclisation⁵⁰.

A more mechanistically interesting route, however, has been proposed⁵² in which the initial cyclisation of the bicyclic cation (6) occurs between C-17 and the primary C-15 cationic centre (pathway b). Further ring closure by migration of the π electrons in the Δ^{13} bond followed by Wagner-Meerwein shift and neutralisation as shown could conceivably lead to the observed product (50). While the failure of the pimaradienes(37,38,39) to cyclise to hiban-14 α -yl formate on treatment with formic

acid appeared to exclude pathway (a), the solvolysis⁵² of the sulphonate (76) to yield after LiAlH_4 treatment the alcohol (77) was a good indication that pathway (b) might be the correct one.



Inspection of the two proposed pathways reveals that C-14 of the bicyclic precursor (6) becomes in (50) C-16 via pathway (a) and C-14 via pathway (b). Accordingly, deuterated Δ^{13} -manool(54) was synthesised and converted to deuterated hiban-14 α -yl formate. NMR spectroscopy as discussed earlier revealed that all of the deuterium originally at C-14 in the starting material was located at C-14 in this product. This result excludes pathway (a) and confirms that pathway (b) is the route by which the tetracyclic skeleton is formed in vitro. Labelling experiments by other workers^{65,66} have also demonstrated this.

Mass Spectroscopy, as discussed earlier revealed that the deuterium at C-12 and C-16 in the starting material were not lost in the reaction. This observation eliminates biformene or sclarene intermediates in this

transformation, in agreement with the conclusion drawn from the results of formic acid treatment of these labdatrienes discussed earlier.

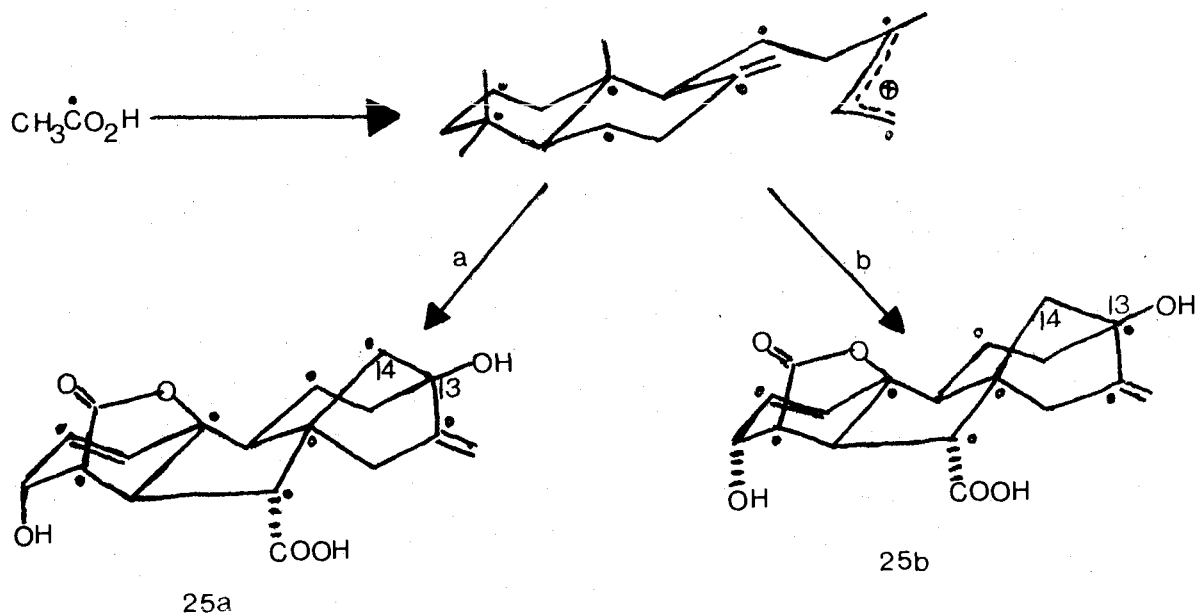
A compound to which the 8,13-burnabadiene structure (45) was assigned was isolated from the reaction of manool in acetic acid and was shown by vapour phase chromatography to be a product of the formic acid reaction also. Reaction of this compound in refluxing formic acid resulted in its quantitative conversion to hiban-14 α -yl formate(50).

Hence the reaction of manool with acetic acid and with formic acid may be formulated as in Figures 9 (p.62) and 10 (p. 63) respectively.

The exclusion of pathway (a) in the in vitro process necessitates examination of the experiments on which the evidence for this in vivo pathway is based, especially since attempts^{67,68} to incorporate labelled pimaradiene into gibberellic acid (25) were unsuccessful. That this latter result could have been due merely to a transportation problem, however, is indicated by the absence of reports of the natural occurrence of compounds reasonably derivable from a C-14 cationic hibane whereas derivatives of a C-16 cationic hibane are plentiful.

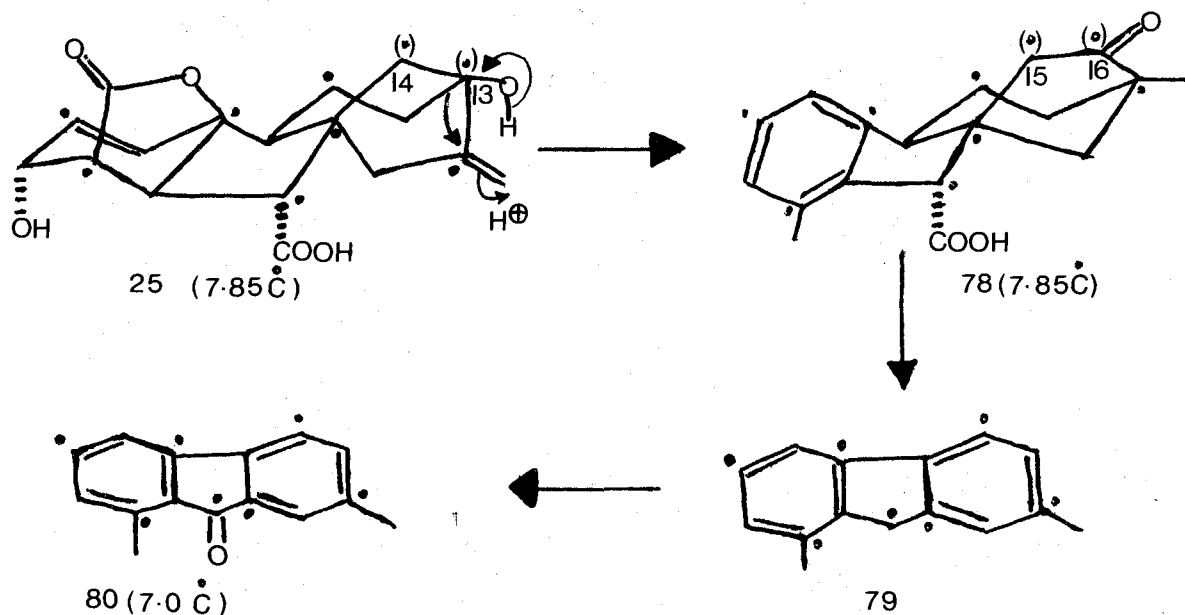
In addition, a close examination of the previously reported³⁹ biosynthesis of gibberellic acid in Gibberella

fujikuroi from $\text{CH}_3^{14}\text{CO}_2\text{Na}$ reveals that the degradative technique used was incapable of distinguishing between the two pathways. Incorporation of $\text{CH}_3^{14}\text{CO}_2\text{Na}$ into gibberellic acid would lead to labelling pattern (25a) via pathway (a) and labelling pattern (25b) via pathway (b).



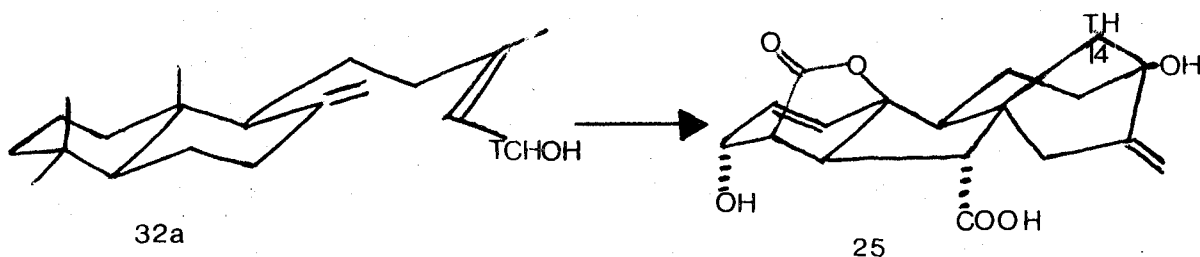
It can be seen that to distinguish between the two pathways the location of the label at C-13 or C-14 of gibberellic acid must be ascertained.

The reported³⁹ degradation involved acid treatment of gibberellic acid (25) to give gibberic acid (78) which on dehydrogenation with selenium and subsequent oxidation gave gibberenone (80). This procedure involved loss of C-16 and C-15 in gibberic acid (C-13 and C-14 respectively in 25) in one step so that although it was shown that one



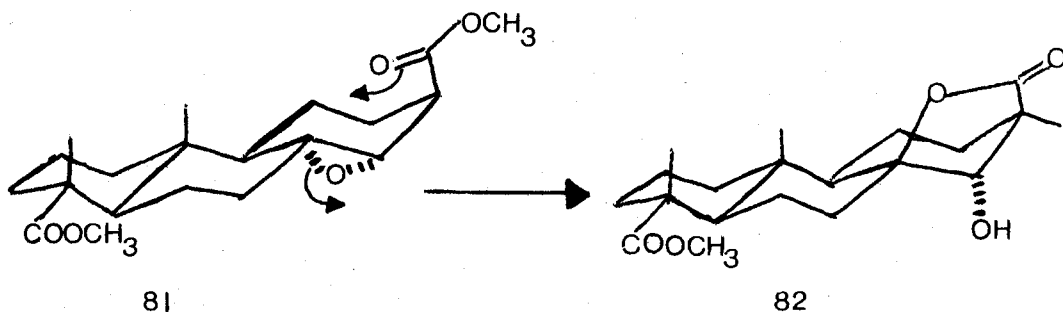
of them was active, which one it was could not be ascertained. Hence this work did not distinguish between pathway (a) and pathway (b).

Simultaneous with this work incorporation⁶⁸ of 15-T- Δ 13-Manool(32a) into gibberellic acid by *Gibberella fujikuroi* and subsequent degradation located the label specifically at C-14 in the gibberellic acid. This conclusively establishes the pimarane route (a) as the pathway by which gibberellic acid is biosynthesised.



It is intriguing that the tetracyclic hibane skeleton is formed from the bicyclic labdane skeleton by a route in the plant which is quite different from the pathway to the same skeleton followed by a cation generated in the laboratory.

The difficult step in the in vitro reaction is the ring closure between the vinyl C-16 and the cationic centre at C-8. This is not surprising since the vinyl group would be expected to have a preferred orientation away from the rest of the molecule and hence the ring closure would be sufficiently slow to allow the other observed processes (backbone rearrangement and elimination) to occur. In support of this idea is the recently reported⁶⁹ ring closure of the α -epoxide(81) to the hydroxy- γ - lactone (82).



In this molecule the bulk of the $-OCH_3$ group preferentially orientates the source of the π electrons over the developing carbonium site and thereby facilitates cyclisation.

The novel compound 8,13-burnabadiene possesses a hitherto unknown skeleton. It results from an alternative mode of cyclisation which is in competition with the in vitro cyclisation of the bicyclic precursor (6) to the carbon skeleton of the well-known pimarane diterpenoids. In evaluating the possibility of encountering this structure in a natural system, the following information should be considered.

a) Bicyclic precursors have been incorporated into rosenonolactone⁵⁶ and gibberellic acid⁶⁸ whereas attempts to incorporate tricyclic precursors have failed.

b) Both bicyclic and tetracyclic diterpenes have been isolated from Gibberella fujikuroi but there are no reports of the isolation of tricyclic pimaradiene metabolites from this source⁷⁰.

c) Although there are numerous examples of bicyclic diterpenes from both the 10 α and the 10 β series, the tricyclic compounds possess predominantly the 10 β configuration whereas most of the tetracyclic diterpenoids are members of the 10 α series.

Hence it would appear that most plants investigated to date possess the ability to transform the bicyclic skeleton possessing a 10 α stereochemistry to the 10 α

tetracyclic structure with very little production of 10α tricyclic compounds. This might suggest an in vivo process in which the closures of the C and D rings of the 10α tetracyclic diterpenes are directed by a single or closely associated set of enzymes. Pathways leading to 8,13-burnabadiene in the 10α series apparently do not form part of this natural process.

The observation that the 10β tricyclic compounds are generally isolated from tree trunk resins seems to suggest that the many systems synthesising these 10β skeletons are able to release them prior to their conversion to tetracyclic derivatives. Accordingly one might encounter less resistance to in vivo incorporation of labelled 10β pimarenes into 10β tetracyclics than noted above in the 10α series.

An alternative explanation for the predominance of 10β tricyclic pimarenes in trunk resins is that many higher plants lack the ability to cyclise the 10β bicyclic progenitor. This raises the possibility that these tricyclics may arise from a non-enzymatic cyclisation which in the laboratory has indeed been shown to give moderate yields of these compounds. Following this line of reasoning, one might expect that if compounds based on the 8,13-burnabadiene skeleton were to be encountered in Nature,

they are more likely to occur with the 10β stereochemistry.

Perusal of the literature reveals no apparent evidence for the production of 10β -tetracyclic diterpenes via the cyclooctenyl route. Thus all naturally occurring hibanes possess oxidation or unsaturation at C-16 (expected via the pimarene route) and not at C-14 (expected via the cyclooctenyl route).

TABLE 4

Product Distribution (%) from Reaction of 8,15-isopimaradiene with Formic Acid

Product	0	Reaction Time						
		2½min	7½min	12½min	30min	1hr	3 hr	5 hr
8,15-Isopimaradiene (40)	100	87	68	51	30	17	7	5
Sandaracopimaradiene (41)		0.4*	1.8*	2.2*	0.6*			
Isopimaradiene (42)		0.9	3.0	3.5	1.2			
13-Epi-5(10),15-rosadiene(44)		2.5	14.2	31	46	43	44	37
Rimuene (48)			0.8*	1.7*	2.5*	5.9*	6.6*	5.5*
5(10),12-Abietarosadiene (47)						0.8	3.4	5.2
Unknown (49)			1.3*	5.0*	8.9*	8.8*	11*	12*
Totals	100	90.8	89.1	94.4	89.2	75.5	72.0	64.7

TABLE 5

Product Distribution (%) from Reaction of Isopimaradiene with Formic Acid

<u>Product</u>	<u>Reaction Time</u>			
	0	5 min	10 min	20 min
8,15-Isopimaradiene (40)		34	51	17
Sandaracopimaradiene (41)		2.2*	2.4*	
Isopimaradiene (42)	100	40	11	
13-Epi-5(10),15-rosadiene (44)		21	40	64
Rimene (48)		1.1*	3.4*	8.6*
Unknown (49)		2.6*	5.6*	12 *
Totals	100	100.9	113.4	101.6

TABLE 6

Product Distribution (%) from Reaction of 13-Epi-5(10), 15-rosadiene with Formic

Product	Acid	<u>Reaction Time</u>						
		0	10min	30min	1hr	2hr	4hr	7hr
8,15-Isopimaradiene (40)					2.3	3.2	3.6	3.2
13-Epi-5(10), 15-rosadiene (44)	100	69	50	48	47	39	37	
Rimuene (48)		3.5*	5.4*	8.6*	11.2*	7.6*	7.6*	
5(10), 12-Abietaradiene (47)				1.4	1.8	4.0	6.7	
Unknown (49)					2.1*	5.3*	8.5*	
Totals	100	72.5	55.4	60.3	65.3	59.5	63.0	

TABLE 7

Product Distribution (%) from Reaction of 8,15-Pimaradiene with Formic Acid

<u>Product</u>	<u>Reaction Time</u>					
	0	10min	30min	1hr	2hr	5hr
8,15-Pimaradiene (37)	100	83	73	47	29	6
7,15-Pimaradiene (39)			3.6	2.2	1.5	
5(10), 15-Rosadiene (43)		3.1	11	13	19	21
5(10), 12-Abietarosadiene (47)						3.2
8-Epi-5(10), 15-rosadiene (46)		1.1	5.6	8.2	17	26
Totals	100	87.2	93.2	70.4	66.5	56.2

TABLE 8

Product Distribution (%) from Reaction of Pimaradiene with Formic Acid

<u>Product</u>	<u>Reaction Time</u>				
	0	5min	10min	20min	1hr
8,15-Pimaradiene (37)		32	52	54	21
Pimaradiene (38)	100	53	27	9	1.4
7,15-Pimaradiene (39)		3.2	4.1	3.2	1.5
5(10),15-Rosadiene (43)		3.5	5.4	13	23
5(10),12-Abietarosadiene (47)					1.3
8-Epi-5(10),15-rosadiene (46)		0.6	2.1	7.4	16
Totals	100	92.3	90.6	86.6	64.2

TABLE 9

Product Distribution (%) from Reaction of 7,15-Pimaradiene with Formic Acid

<u>Product</u>	<u>Reaction Time</u>					
	0	10min	15min	30min	1hr	2hr
8,15-Pimaradiene (37)		56	78	70	47	23
7,15-Pimaradiene (39)	100	15	5.6	4.4	1.7	
5(10),15-Rosadiene (43)		3.4	6.4	12	22	24
5(10),12-Abietarosadiene (47)						0.5
8-Epi-5(10),15-rosadiene (46)		1.1	2.8	7.0	18	24
Totals	100	75.5	92.8	93.4	88.7	71.5

TABLE 10
 Product Distribution (%) from Reaction of 5(10), 15-Rosadiene with Formic Acid

<u>Product</u>	<u>Reaction Time</u>					
	0	15min	30min	1hr	2hr	5hr
8,15-Pimaradiene (37)		15	20	16	14	10
5(10), 15-Rosadiene (43)	100	69	60	48	37	25
5(10), 12-Abietarosadiene (47)			1.9	3.0	8.5	14
8-Epi-5(10), 15-rosadiene (46)			3.6	4.2	13	21
Totals	100	84	85.5	72.0	72.5	70

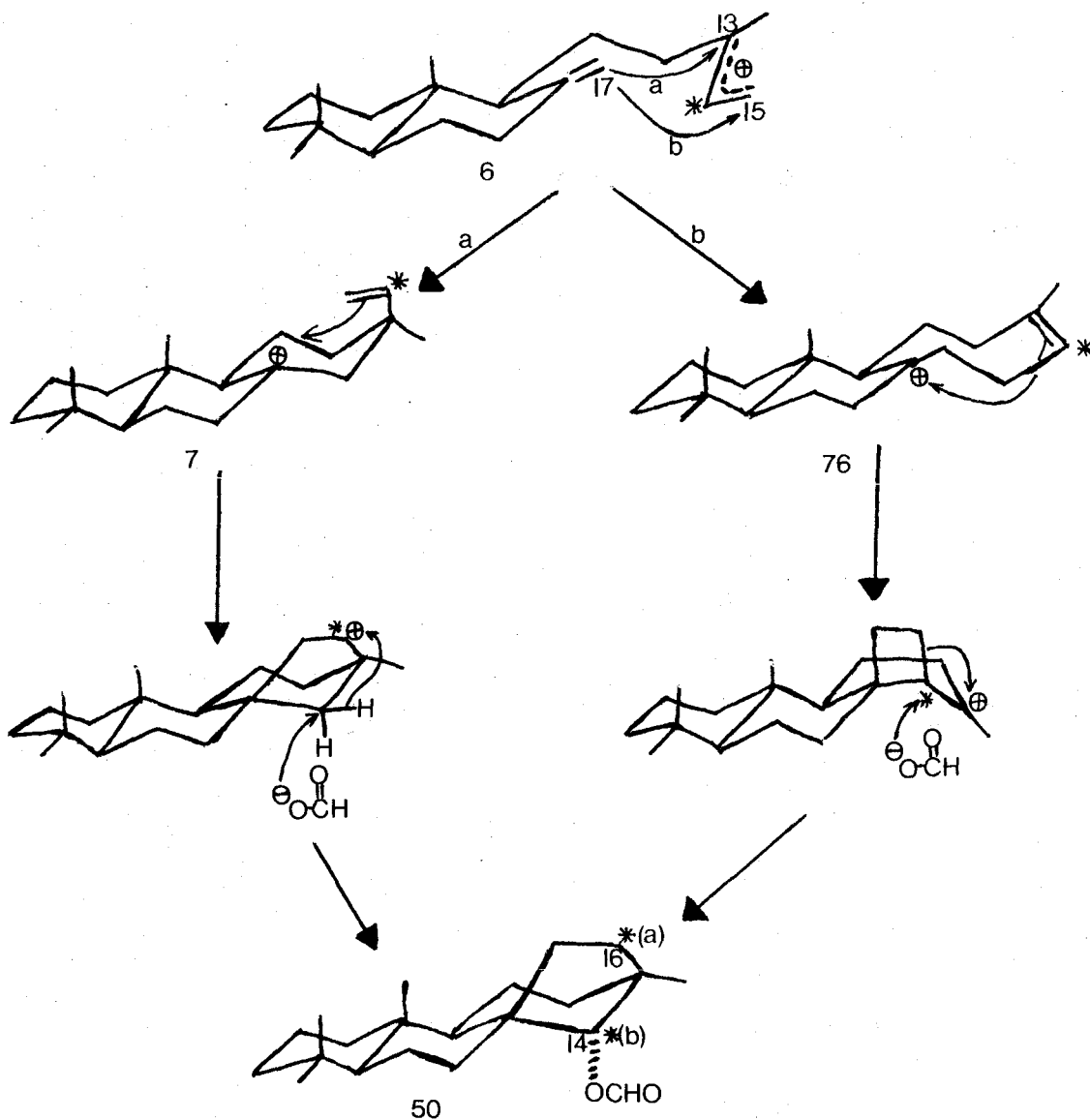


Figure 8: Possible Routes to Hiban-14 α -yl Formate

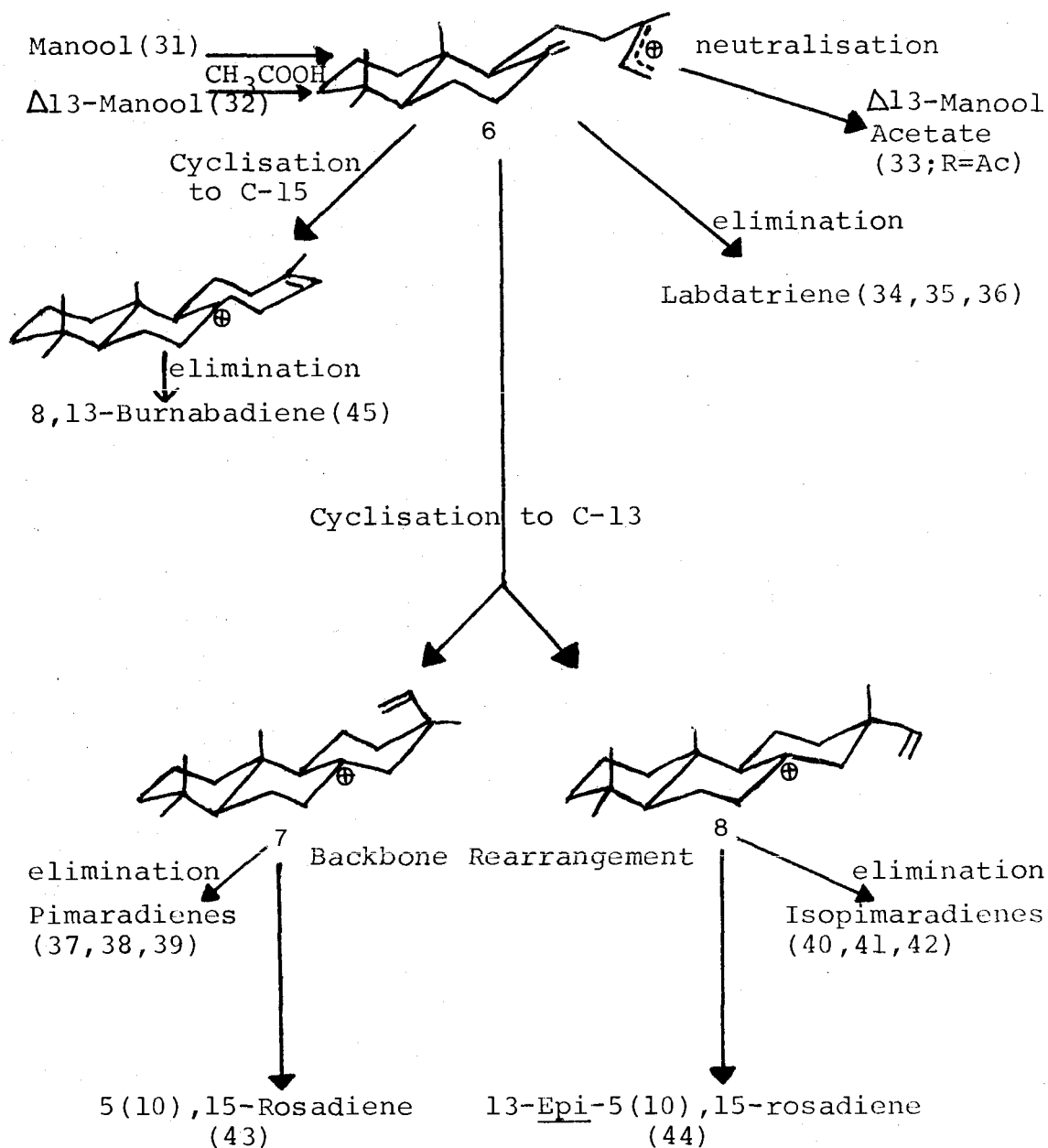


Figure 9: Reaction Scheme for Manool and Acetic Acid

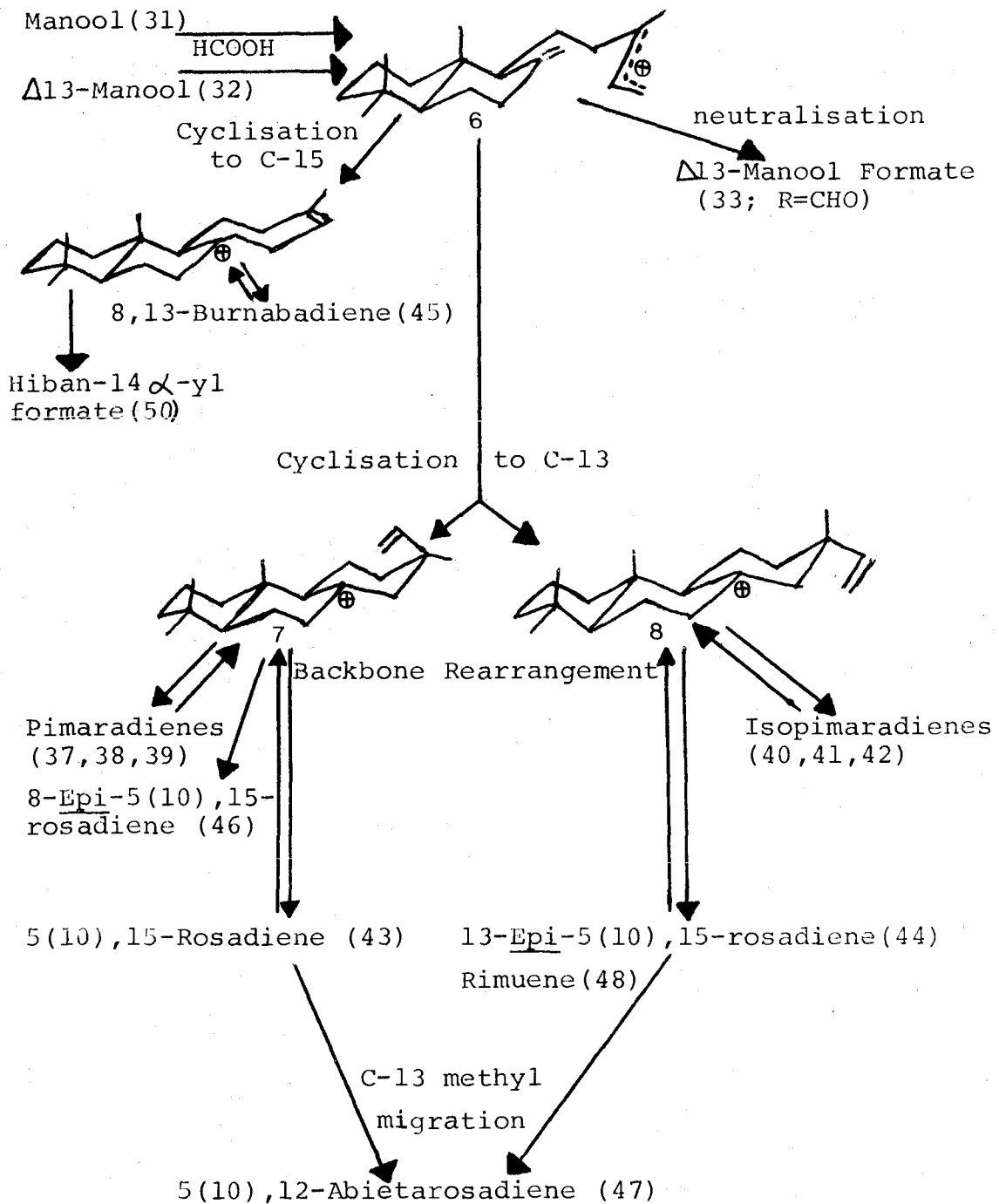


Figure 10: Reaction Scheme for Manool and Formic Acid

CHAPTER 4

DISCUSSION OF STRUCTURAL ASSIGNMENTS

Identification of the products of the acid catalyzed reactions of manool was performed by comparison of IR, NMR and MS data with previously published results and where possible by comparison with authentic samples. This, however, could not be done in the case of 7,15-pimaradiene(39), 8,13-burnabadiene(45), or 8-Burnabene(89) since these compounds had not been previously reported. In addition, no reports could be found describing compounds with the same spectral properties as the products to which the 5(10),12-abietarosadiene(47) and 8-epi-5(10),15-rosadiene(46) structures were assigned. A more detailed evaluation of the spectra of these compounds was therefore required.

NMR of Tricyclic Compounds

The chemical shifts of the CH₃ groups in the NMR spectra of the pimaradienes(37⁵⁰, 38⁵⁰) the isopimaradienes (40⁵⁰, 41⁵⁰, 42⁷¹) 5(10),15-rosadiene(43)⁷², 13-epi-5(10),15-rosadiene(44)⁵¹ and rimuene⁷¹ have been published. The NMR data of the compounds isolated in this work and to which the above structures were assigned were in precise agreement with these published results. 7,15-Pimaradiene (39) was also isolated and the chemical shifts of the CH₃ groups in this compound, together with the corresponding data for the aforementioned structures are summarised in

Table II (p.81). The full NMR spectra of all of these compounds except for sandaracopimaradiene are shown in Figures 11 - 18 (pp. 82-89).

Some very interesting correlations can be made from an examination of the data in Table II. Moving the endocyclic double bond from the $\Delta 8(14)$ position to either the $\Delta 8(9)$ or the $\Delta 7(8)$ position causes an upfield shift of the C-13 methyl group in both epimeric series. Furthermore, moving this double bond from the $\Delta 8(9)$ position to the $\Delta 7(8)$ or the $\Delta 8(14)$ position results in an upfield shift of the C-10 methyl. On the other hand, the more remote methyl groups at C-4 are relatively unaffected by any of these changes.

The structures of five of this set of six tricyclic isomers (37, 38, 40, 41, 42) are well established. The C-13 configuration of the hitherto unreported member (39) was readily established from its acid catalysed interconversion with (37) and (38) and the width of the peak at $\tau 4.57$ in its NMR spectrum (Figure 13, p.84) was consistent with a $\Delta 7(8)$ double bond. The above analysis of the changes in chemical shift of the CH_3 groups of the isopimaradienes (40, 41, 42) as the endocyclic double bond was moved from one position to another allowed an analogy to be drawn

with the pimaradienes (37, 38, 39) and the NMR data of 7,15-pimaradiene was consistent with this correlation.

It can therefore be seen that not only do the spectra of all of these tricyclic dienes agree with published data but the six pimaradienes (37, 38, 39) and isopimaradienes (40, 41, 42) form a self consistent set wherein the changes in spectral characteristics caused by structural modification can be easily rationalised.

Mass Spectra of Tricyclic Pimaranes and Isopimaranes and Their Backbone Rearranged Products

Examination of the mass spectra of the tricyclic products (Figures 19-27, pp. 90-98) reveals that compounds which are epimeric at C-13 but whose structures are otherwise identical have mass spectra which are distinguishable only by minor intensity differences. This indicates that the primary cleavage in the fragmentation of these compounds occurs in either the A or B ring to give intermediates in which the asymmetry of the C ring is lost.

The assignments previously reported⁷³ for 8,15-isopimaradiene, sandaracopimaradiene and isopimaradiene appear to be based on this factor but the authors did not point out that epimeric isomers exhibit nearly identical fragmentation patterns. In addition the peaks in

the mass spectra of the backbone rearranged products may be assigned by analogy with the assignments for the regular Pimarane and isopimarane skeletons.

8,15-Pimaradiene (37) and 8,15-Isopimaradiene (40)

The mass spectra of (37) (Figure 19, p.90) and (40) (Figure 22, p.93) obtained by us were identical with that previously reported⁷³ for 8,15-isopimaradiene. The assignments made⁷³ for the ions m/e 272, 257, 187, 175, 161 are shown in Figure 28 (p.99). In support of this scheme is the appearance of a metastable ion in the mass spectrum of each of these compounds at m/e 101 corresponding to the m/e 257 \rightarrow 161 fragmentation.

Pimaradiene (38) and Sandaracopimaradiene (41)

The mass spectra of (38) (Figure 20, p.91) and (41) (Figure 23, p.94) obtained by us were identical with those previously reported for pimaradiene⁷¹ and sandaracopimaradiene⁷³. The assignments made⁷³ for the ions m/e 272, 137, 136 are shown in Figure 29 (p.100). In addition to the previously reported⁷³ assignments the ion at m/e 257 may be caused by loss of the C-13 methyl to give the allylic ion (84).

7,15-Pimaradiene (39) and Isopimaradiene (42)

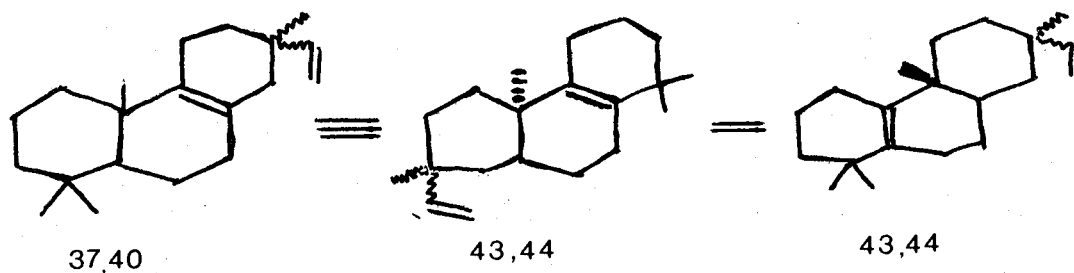
The mass spectrum of (42) (Figure 24, p.95) obtained by us was identical with that previously reported^{71, 73}

for isopimaradiene. The assignments made⁷³ for the ions m/e 272, 148, 133, 124, 109 are shown in Figure 30 (p.101). In addition to the previously reported⁷³ assignments the ion at m/e 257 may be caused by loss of the C-13 methyl to give the allylic ion (85). In support of this scheme the mass spectra of both isopimaradiene and of the compound to which the 7,15-pimaradiene structure (Figure 21, p.92) was assigned showed metastable ions at m/e 119.5 and 96 corresponding to the m/e 148 \rightarrow 133 and 124 \rightarrow 109 fragmentations respectively. The only significant difference between the mass spectra of these two compounds is that the ion at m/e 148 for 7,15-pimaradiene is more intense than for isopimaradiene. If, however, the ionisation voltage was increased the intensity of this ion decreased in relation to its daughter at m/e 133. This observation was also reported⁷³ for isopimaradiene. Therefore the mass spectral data are in complete agreement with the assignment of the 7,15-pimaradiene structure to this compound.

5(10), 15-Rosadiene (43) and 13-Epi-5(10), 15-rosadiene (44)

The mass spectra of these compounds (Figure 25, p.96; 26, p.97 respectively) were identical. Inspection of their gross structure reveals that for the purpose of proposing a cracking pattern they may be regarded as 10 α analogs of

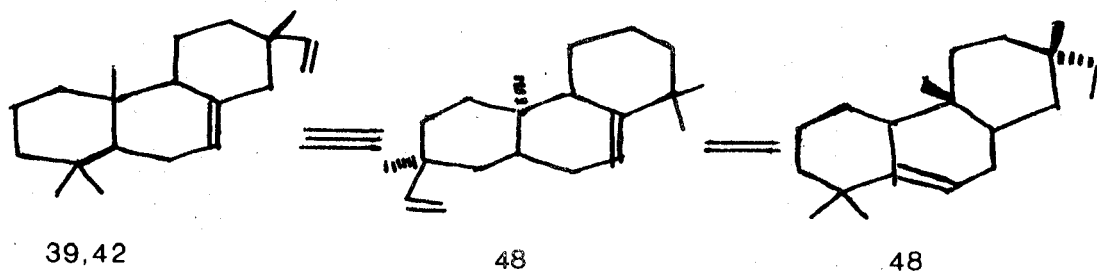
8,15-pimaradiene and 8,15-isopimaradiene.



A sequence of rearrangements analogous to those proposed for the fragmentation of (37) and (40) is shown in Figure 31 (p.102) and this predicts ions at m/e 257, 163, 149, 175. Ions of these masses are in fact observed and additional support is given by the appearance of a metastable ion at m/e 119 corresponding to the m/e 257 \rightarrow 175 fragmentation.

Rimuene (48)

The mass spectrum of (48) (Figure 27, p.98) obtained by us was identical with that previously reported^{71, 73}. The large peak at m/e 257 has been assigned⁷³ to loss of methyl from either C-4 or C-13. A close look at the structure of this compound reveals that if it is considered a 10α analog of 7,15-pimaradiene and isopimaradiene a fragmentation scheme analogous to that proposed for (39) and (42)



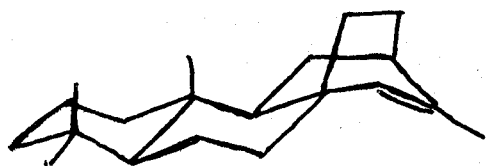
as shown in Figure 32 (p.103) predicts ions at m/e 257, 136, 121. Ions of these masses are in fact observed and a metastable ion appears at m/e 107.5 corresponding to the m/e 136 \rightarrow 121 transformation.

The Structure of 8,13-Burnabadiene (45)

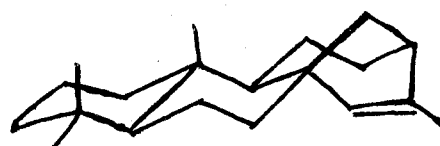
Mass spectral analysis (Figure 33, p.104) revealed that this compound possessed M.Wt. 272. Compound (45) must therefore either be bicyclic with three double bonds, tricyclic with two double bonds or tetracyclic with one double bond. The UV spectrum of (45) was transparent above 200 $m\mu$ showing that if (45) possessed two or more double bonds they were not conjugated.

Its NMR spectrum (Figure 34, p.105) possessed three sharp singlets (3H each) at τ 9.15, 9.18, 9.28 which were assigned to three methyl groups attached to fully substituted carbon atoms. In addition a sharp doublet $J=2.0$ cps at τ 8.45 (3H) was assigned to a vinyl methyl group coupled with either a cis or a trans vinyl hydrogen. In support of this latter assignment was the appearance at τ 4.60 of a broad singlet (1H). Integration over the range τ 7.50-8.25 accounted for ten hydrogens, indicating the presence of ten allylic hydrogens. The absence of the vinyl ABX pattern which was present in the NMR spectra of the tricyclic compounds discussed earlier suggested that

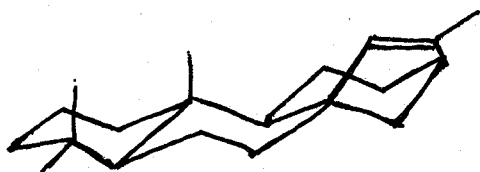
this compound might be tetracyclic. Reasonable possibilities were isoatisene(86), isokaurene(87) and isophylloc-ladene(88).



86



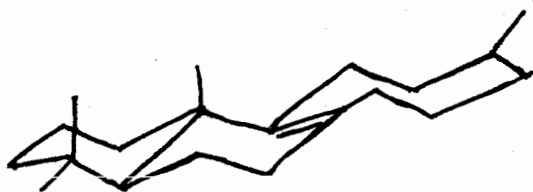
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88

Each of these structures possesses three methyl groups attached to fully substituted carbons, a vinyl methyl group cis to a vinyl hydrogen and Mwt. 272. They do not, however, possess ten allylic hydrogens and the vinyl singlet in the NMR of our compound was much broader than would be expected for a hydrogen which was coupled only with a cis methyl group. A skeleton possessing the $RCH_2-CH=CR^1CH_3$ moiety would account more successfully for the observed spectrum. Indeed, comparison of the spectral data of the compound with that reported for (86)^{74,75}, (87)^{75,76,77} and (88)⁷⁶ revealed that this compound was not identical with any of these structures.

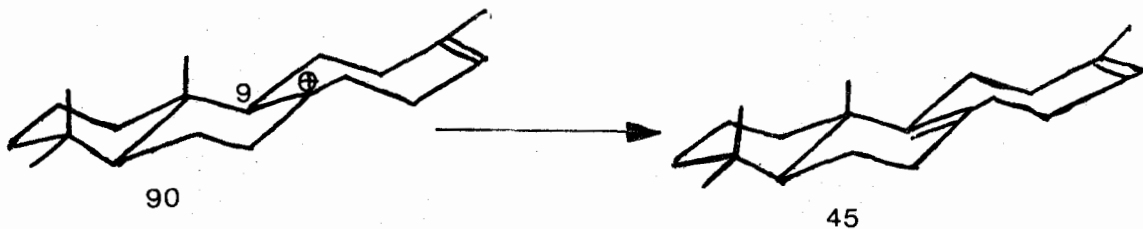
The presence of at least two double bonds was proven by partial hydrogenation. Thus one mole of hydrogen was absorbed giving a product (89) Mwt.274 which still gave a positive tetranitromethane test.



89

That the positive tetranitromethane test was not caused by unreduced starting material was proven by the absence of an ion at m/e 272 in the mass spectrum (Figure 35, p.106) of (89). In the NMR spectrum of the reduction product (Figure 36, p.107), the vinyl hydrogen had disappeared, the vinyl CH_3 doublet had moved upfield and only six allylic hydrogens remained.

It had been shown by labelling studies (discussed earlier) that the hibane-14 α -yl formate (50) isolated from the reaction of manool with formic acid was formed via the tricyclic cation (90), and proton loss from C-9 in tri-



cyclic cations of the type (7), (8) was considered the origin of the 8,15-pimaradiene(37) and 8,15-isopimaradiene (40) isolated from the acid catalysed reactions of manool. Hence by analogy proton loss from C-9 in(90) to give(45) seemed a reasonably attractive possibility. Indeed, structure (45) fits all of the above mentioned spectral data since it possesses:

- a) three methyl groups attached to fully substituted carbons
- b) ten allylic hydrogens
- c) unsaturation featuring the $\text{RCH}_3\text{C}=\text{CHCH}_2\text{R}^1$ moiety
- d) no vinyl group
- e) Mwt. 272

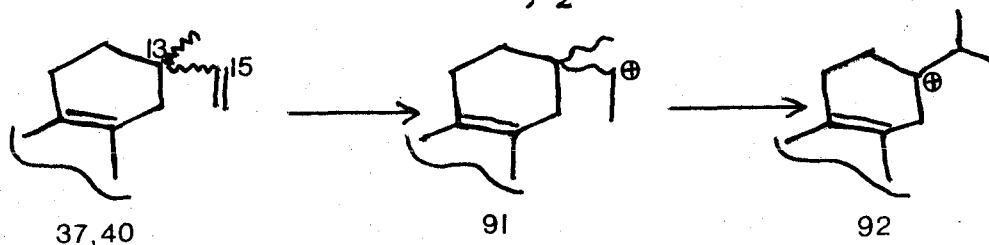
Completing the course of thought concerning this compound one would expect that upon treatment with acid that if protonation occurred at C-9 to regenerate the cation(90) then hiban-14 α -yl formate should be one of the products. Indeed, although the compound was stable in refluxing acetic acid, treatment with refluxing formic acid resulted in its quantitative conversion to hiban-14 α -yl formate.

The location of the Δ 8(9) double bond appears clear from the absence of vinyl hydrogens in the NMR spectrum of (89), however, it could be argued that the Δ 13(14) double bond could perhaps be at the Δ 12(13) position. This can

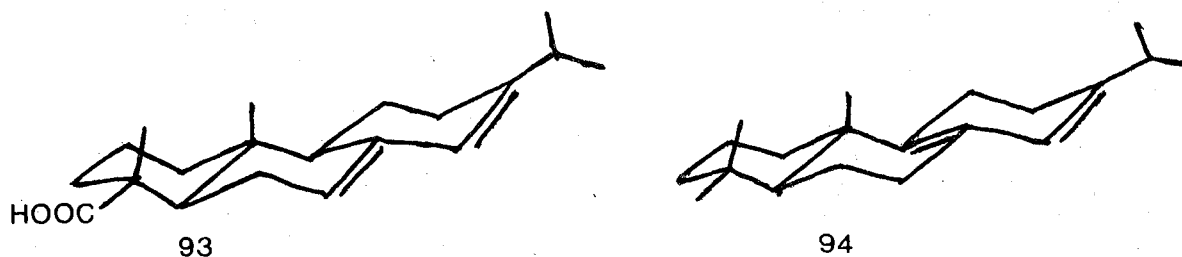
be ruled out since the deuterium labelling studies established that deprotonation-reprotonation processes do not occur at C-12 or C-14 during the conversion of manool to hiban-14 α -yl formate via (90).

The Structure of 5(10),12-Abietarosadiene(47)

The formation of this compound from both the pimarane (37) and the isopimarane(40) skeletons is immediately suggestive of a migration of the C-13 methyl to C-15(91 \rightarrow 92) in a fashion analogous to the reported¹ transformation of either pimaric (11; R=vinyl, R¹=CH₃) or isopimaric (11; R=CH₃, R¹=Vinyl) acid to abietic acid(93) and the abietic lactone (12; R, R¹= -OH, -CH(CH₃)₂).

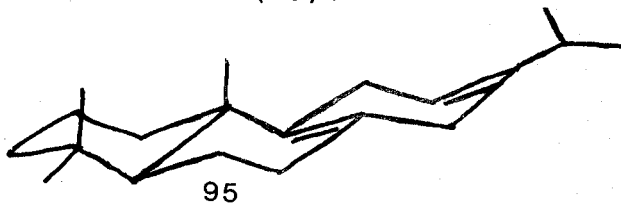


Therefore a reasonable first guess of the structure of this compound would be (94).



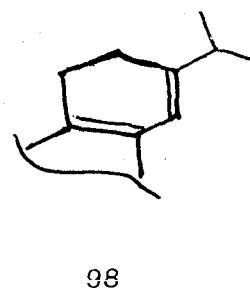
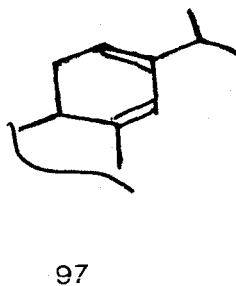
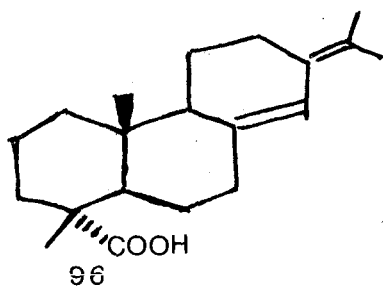
Indeed, the mass spectrum (Figure 37, p. 108) showed that the compound had MWt. 272 and the NMR spectrum (Figure 38, p.109) showed the presence of a broad singlet at τ 4.67 corresponding to a vinyl proton. In addition, the appearance of four sharp singlets at τ 8.97, 9.02, 9.07, 9.22 with a total integration corresponding to fifteen hydrogens indicated five methyl groups. The largest peak at τ 9.07 was assigned to a methyl group attached to a fully substituted carbon superimposed on the upfield peak of the doublet $J=6.5$ cps corresponding to the two methyl groups of the $-\text{CH}(\text{CH}_3)_2$ at C-13. The peak at τ 8.97 constituted the downfield partner of this doublet.

The structure (94), however, would predict a sharp singlet for the vinyl proton whereas the peak at τ 4.67 was fairly broad. In addition, the UV spectrum was transparent above 200 μ showing that the compound did not possess conjugated double bonds. An alternative assignment might therefore be (95).

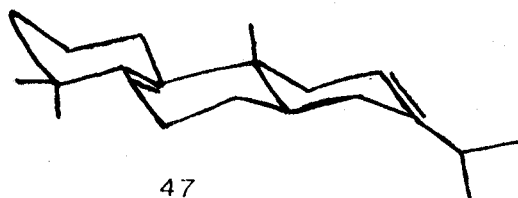


The stability of this compound, however, to acid treatment compared to the reported² facile interconversions of abietic (93), neoabietic (96), levopimaric(97) and

palustric (98) acids suggests that this assignment is probably incorrect.



The facile backbone rearrangement which we observed in both the pimarane (37) and isopimarane (40) skeletons as well as the reported¹ formation of the abietic lactone (12; $R, R^1 = -OH, -CH(CH_3)_2$) on treatment of pimaric (11; $R = \text{vinyl}, R^1 = -CH_3$) or isopimaric (11; $R = -CH_3, R^1 = \text{vinyl}$) acid with cold sulphuric acid suggests that this compound might possess, in addition to a rearranged side-chain, a rearranged backbone. Therefore the structure (47) is proposed for this compound.



This structure exhibits all of the features required by the foregoing discussion. It is not clear, however, why this structure would be so reluctant to undergo reversal of the backbone rearrangement which we have found

to occur with 5(10),15-rosadiene(43) and 13-epi-5(10,15-rosadiene(44), since an examination of molecular models reveals that the changes in steric interaction occurring in this process are the same in both types of systems

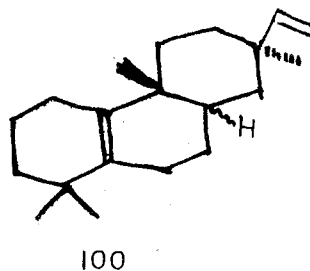
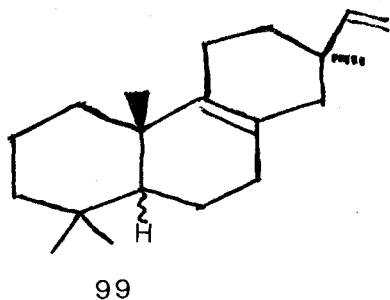
In conclusion, although the structure of this compound is by no means secure, we feel that from the information available structure (47) is the most likely.

The Structure of 8-Epi-5(10),15-Rosadiene(46)

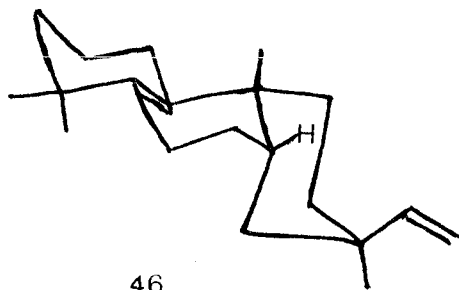
The NMR spectrum (Figure 39, p.110) of this compound showed the ABX pattern of the vinyl group as the only absorption for vinylic hydrogens, and the absence of any absorption corresponding to a vinylic methyl group. The appearance of three sharp singlets at τ 9.01, 9.05, 9.10 with a total integration corresponding to twelve hydrogens was assigned to four methyl groups attached to fully substituted carbons. These data suggested that the compound was tricyclic and possessed both a vinyl group and an additional double bond which was fully substituted by groups other than methyl.

Its exclusive formation from the pimarane(37) series showed that the vinyl group was in the C-13 β position. This data, together with a Mwt. 272 from its mass spectrum (Figure 40, p.111) suggested structures of the type (99) and (100).

The mass spectrum was nearly identical with that of



5(10),15-rosadiene(43), (and substantially different from that of 8,15-pimaradiene) which suggested that these compounds contained similar carbon skeletons and might be epimeric. (It was shown earlier that compounds which were epimeric at C-13 but which were otherwise identical possessed nearly identical mass spectra). Therefore structure (46) was proposed for this compound.



Inspection of the above structure predicted that the compound would be much more stable to acid reaction than the epimeric 5(10),15-rosadiene(43), and indeed it was completely stable in refluxing formic acid. Analysis of molecular models of (46) and the epimeric 5(10),15-

rosadiene revealed that structure (46) possessed fewer 1,3-diaxial interactions than its epimer (See Figure 41, p.112). This difference successfully explains the stability of the compound in refluxing formic acid.

In conclusion, although the structure of this compound is not firmly established, the information available suggests that structure (46) is the most likely.

TABLE 11

Chemical Shift of CH₃ Groups in Tricyclic Diterpenes

Compound	Chemical Shift () of CH ₃ Groups			NMR Figure
	C-4	C-10	C-13	
8,15-Pimaradiene (37)	9.12, 9.15	9.04	9.07	11
Pimaradiene (38)	9.13, 9.15	9.27	9.02	12
7,15-Pimaradiene (39)	9.13, 9.20	9.09	9.04	13
8,15-Isopimaradiene (40)	9.12, 9.15	9.04	9.04	14
Sandaracopimaradiene (41)	9.13, 9.14	9.20	8.96	
Isopimaradiene (42)	9.13, 9.13	9.13	9.08	15
5(10),15-Rosadiene (43)	8.96, 9.03, 9.03, 9.15	9.03, 9.15	(positions not assigned)	16
13-Epi-5(10),15-Rosadiene (44)	9.07, 9.07, 9.07, 9.16	9.07, 9.16	(positions not assigned)	17
Rimuene (48)	8.95, 9.00, 9.05, 9.33	9.05, 9.33	(positions not assigned)	18

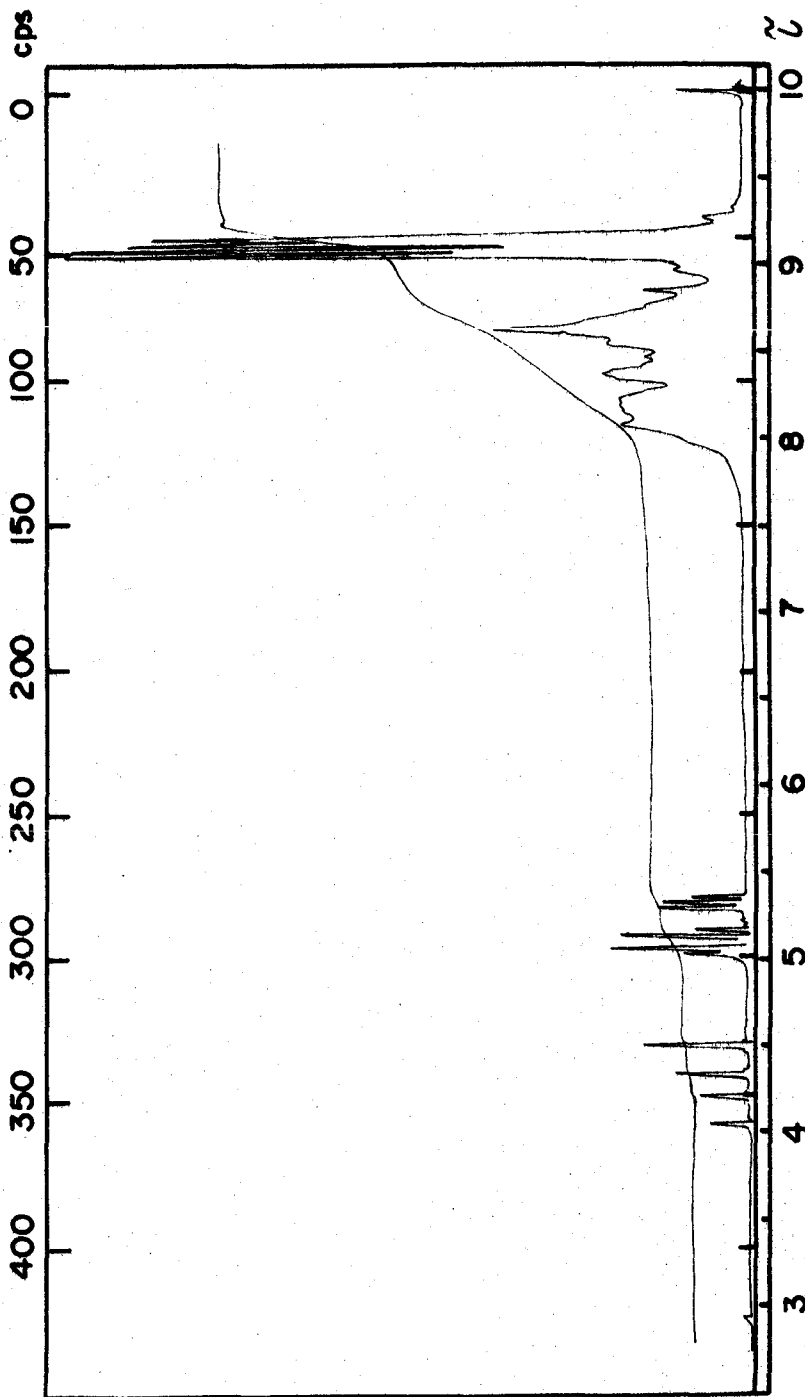


Figure 11: NMR OF 8,15-PIMARADIENE (37)

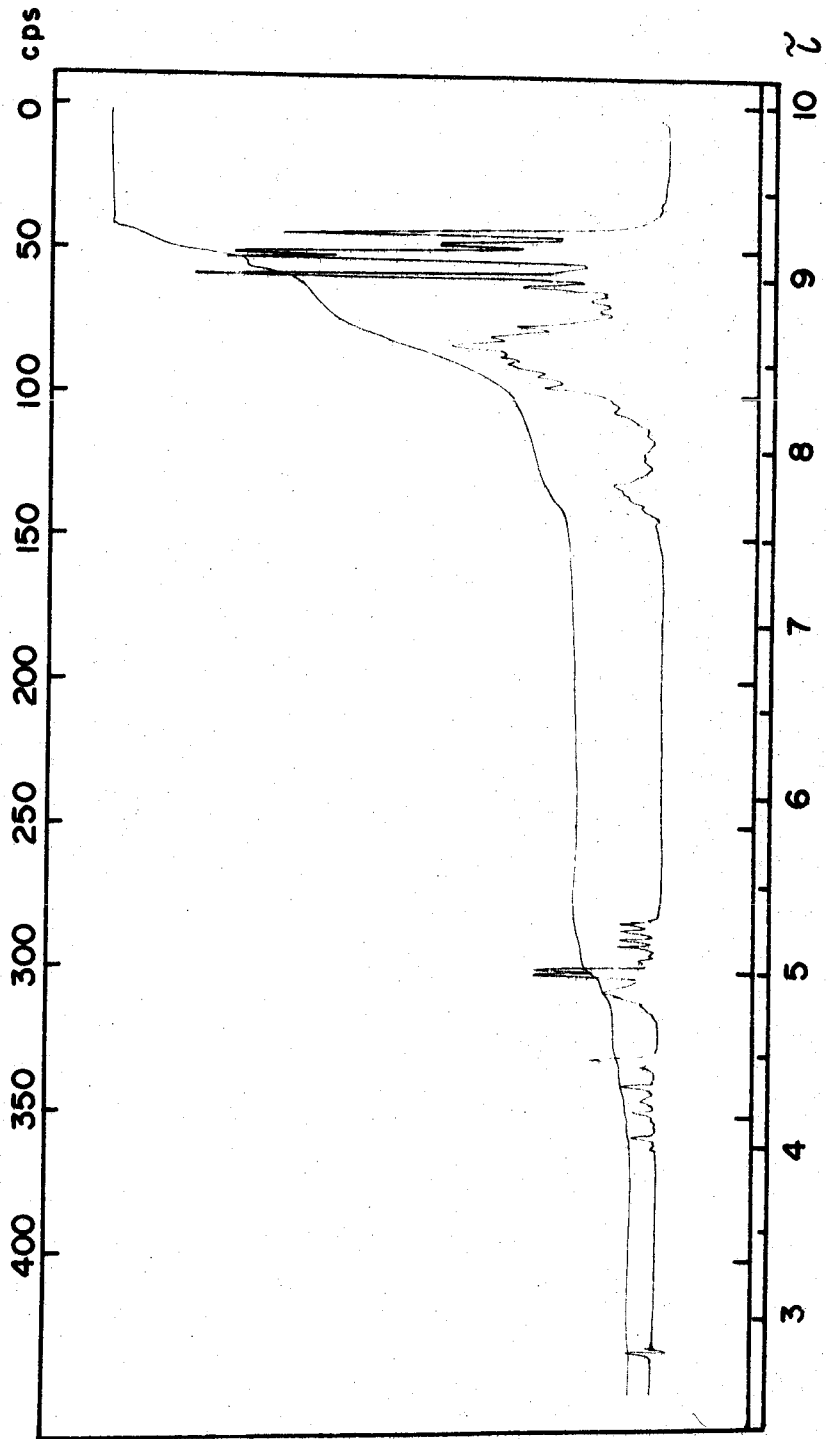


Figure 12: NMR OF PIMARADIENE (38)

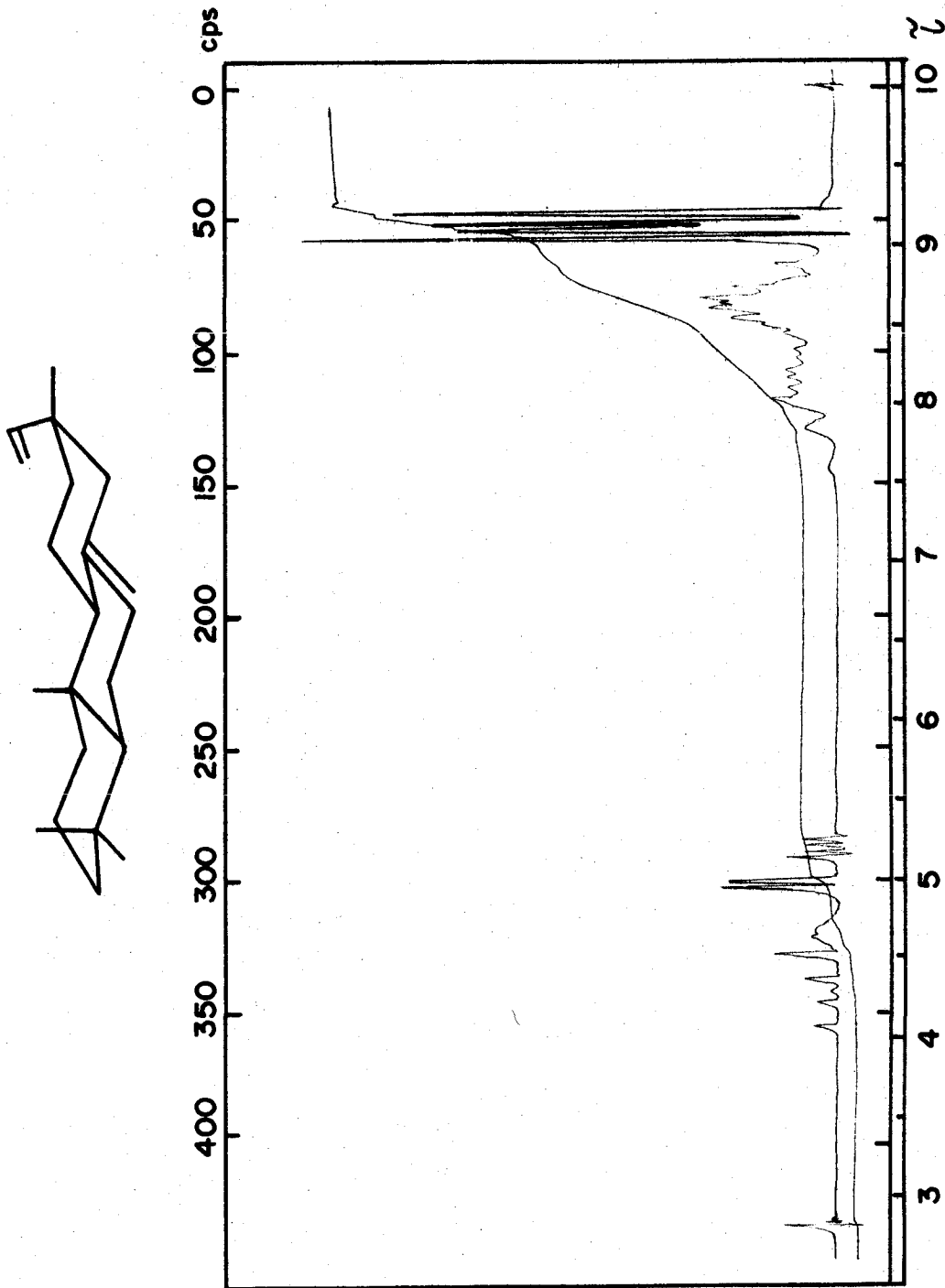


Figure 13: NMR OF 7,15-PIMARADIENE (39)

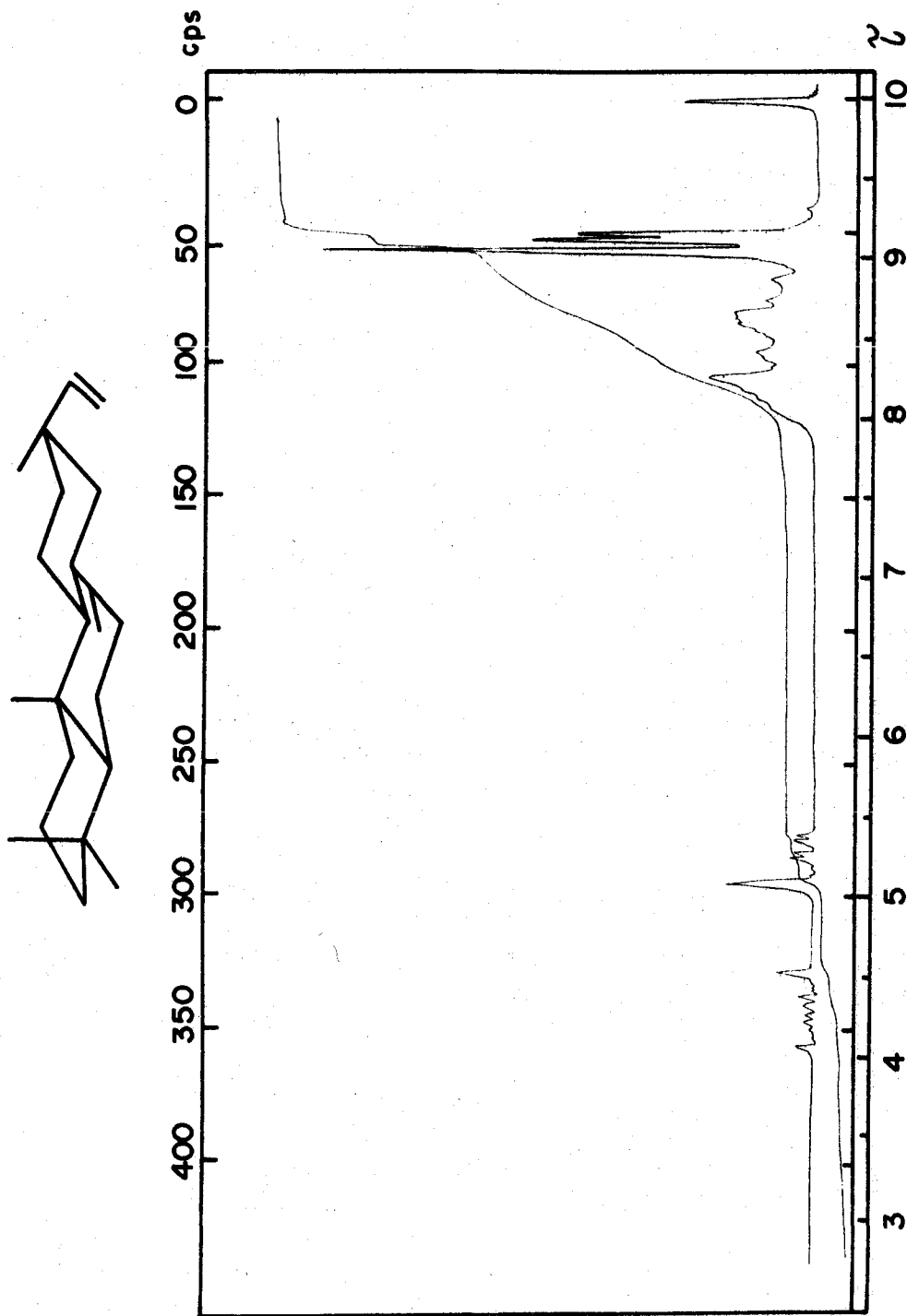


Figure 14: NMR OF 8,15-ISOPIMARADIENE (40)

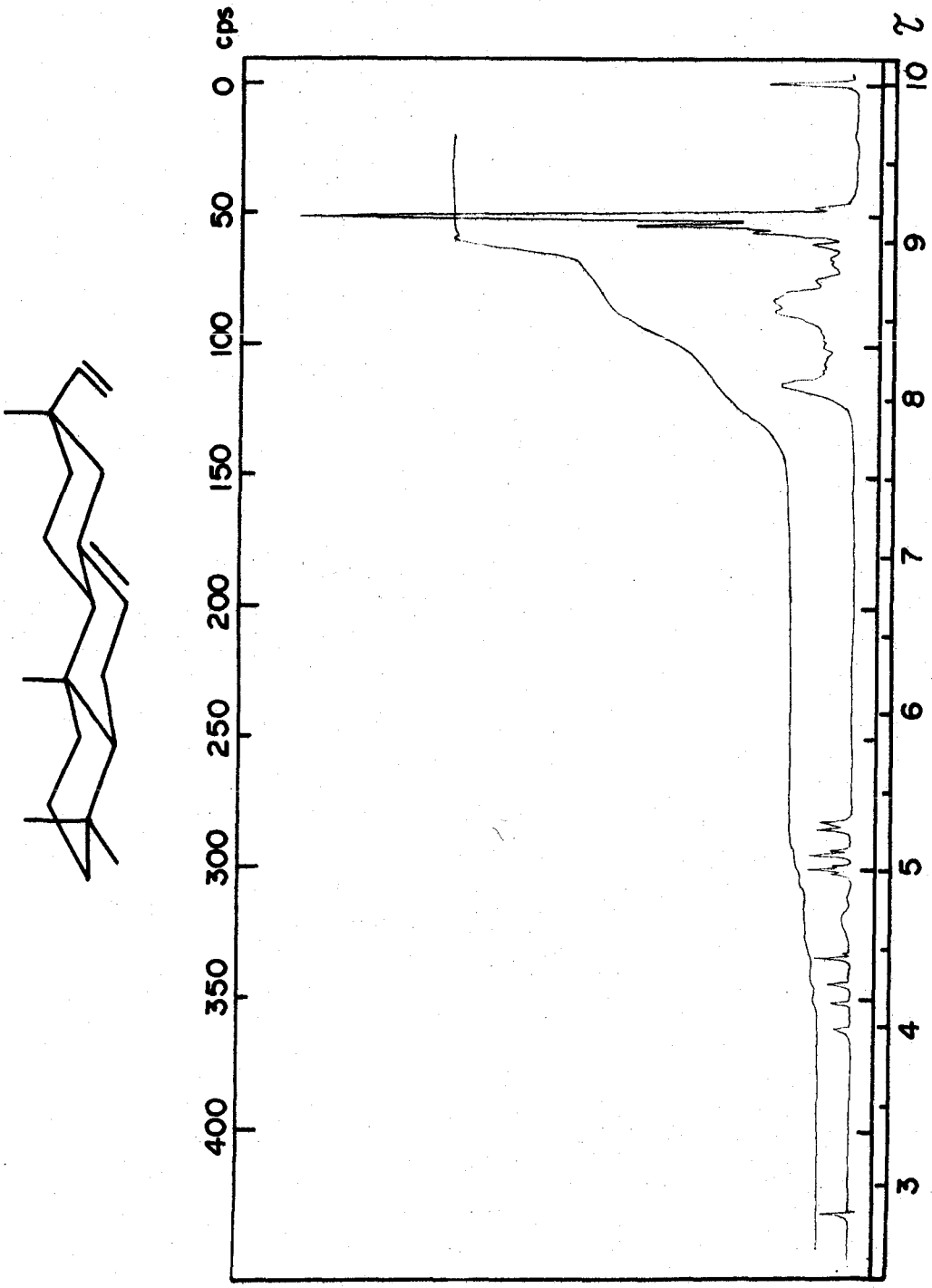


Figure 15: NMR OF ISOPIMARADIENE (42)

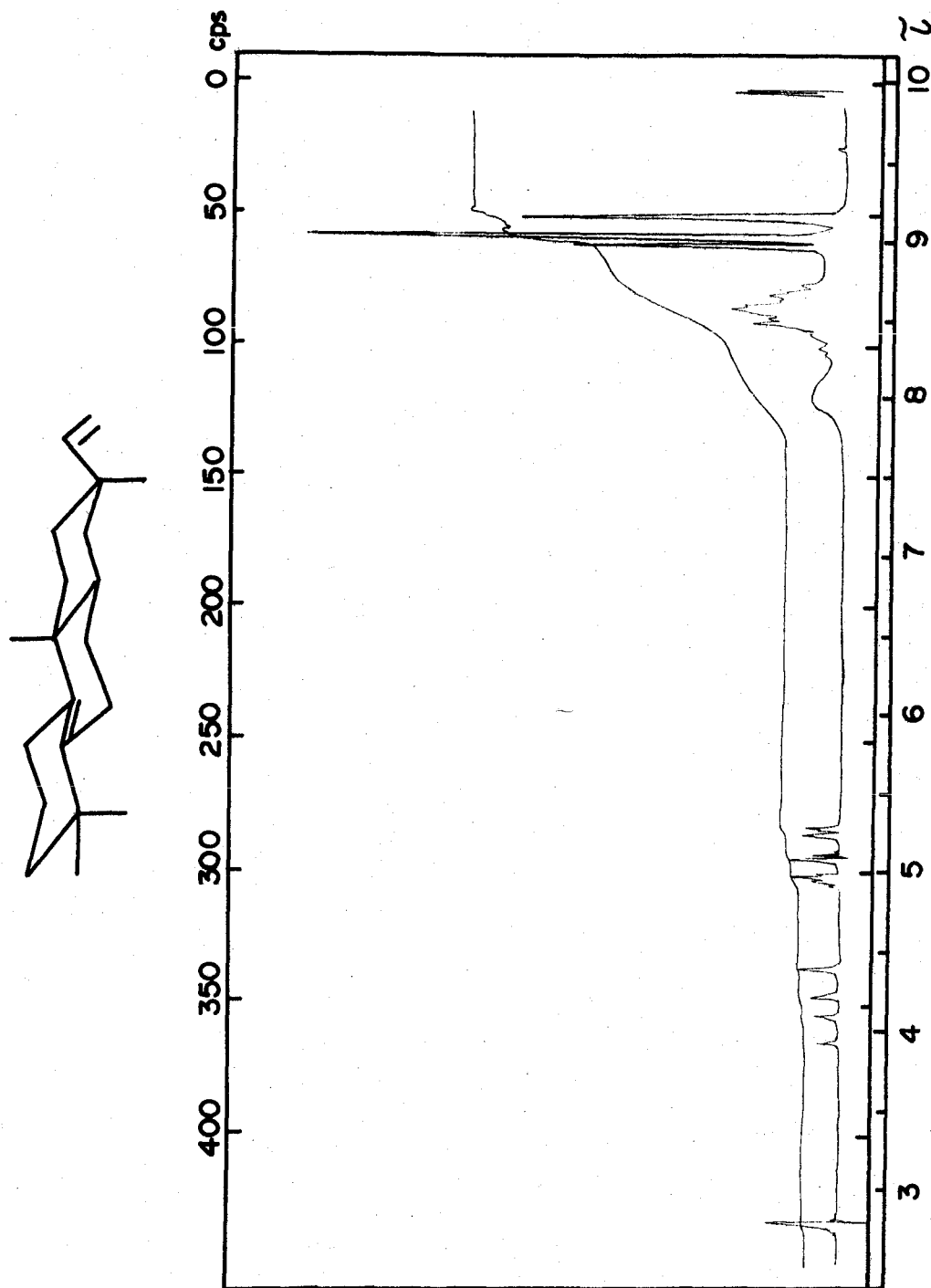


Figure 16: NMR OF 5(10),15-ROSADIENE (43)

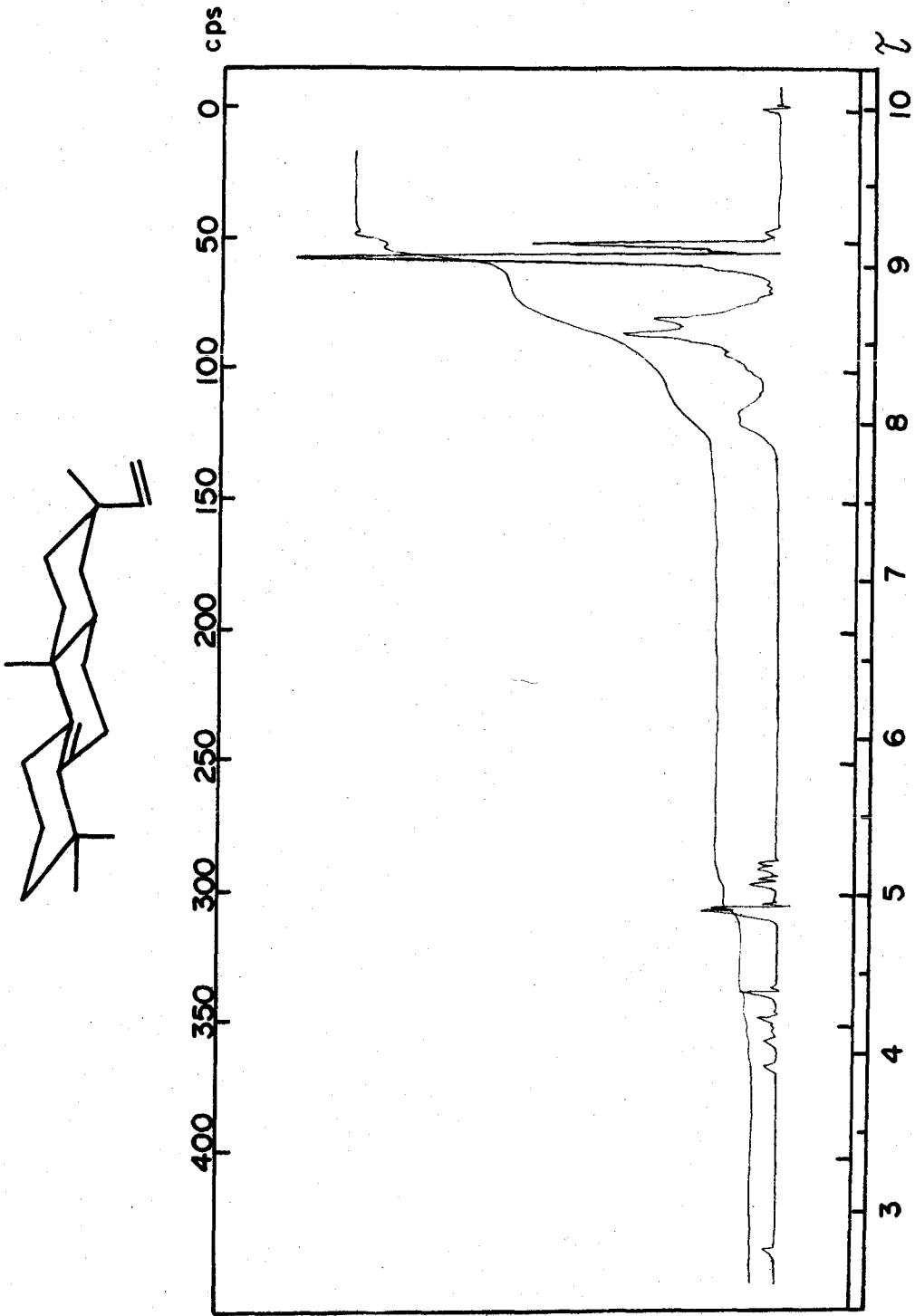


Figure 17: NMR OF 13-EPI-5(10), 15-ROSADIENE (44)

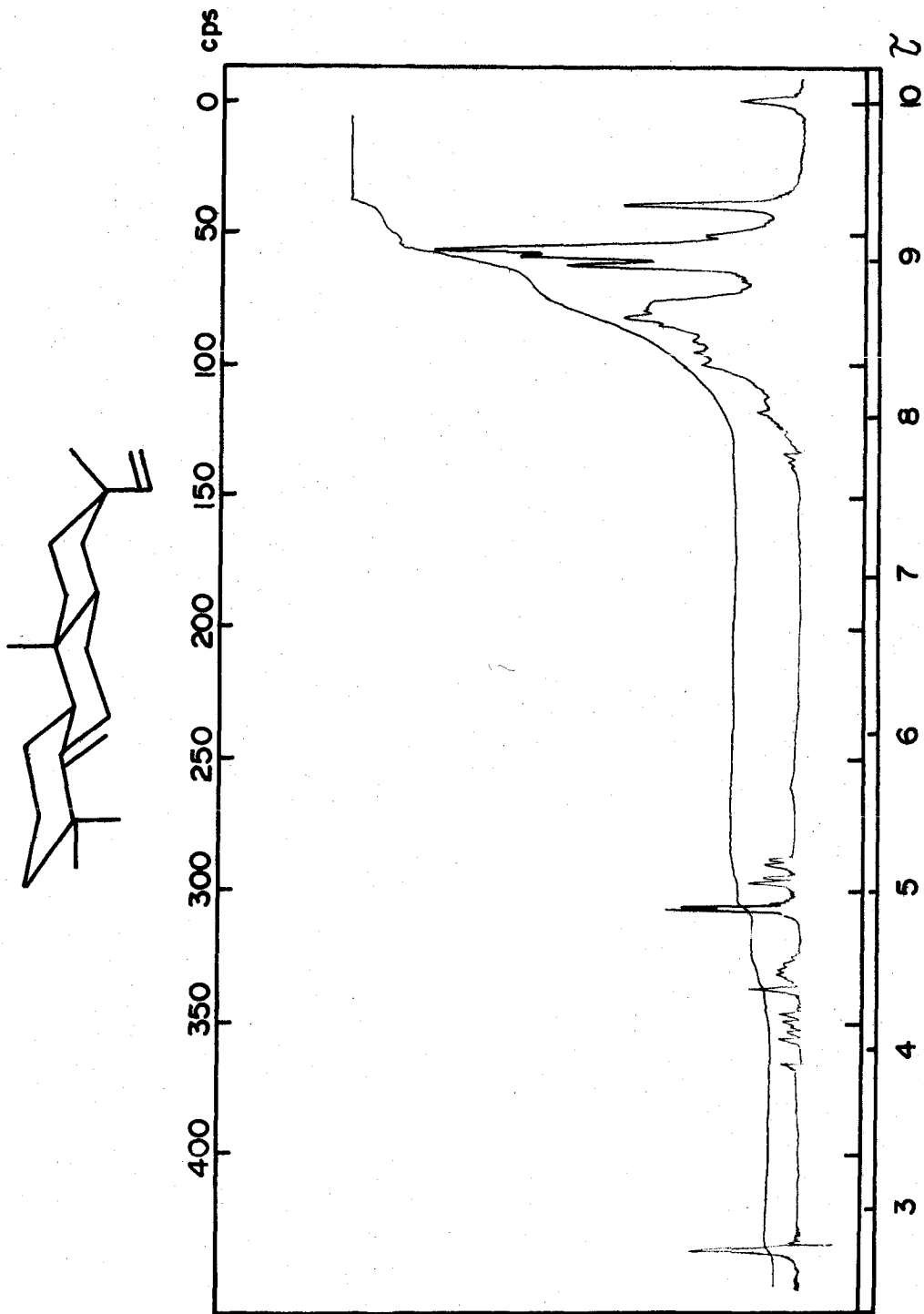


Figure 18: NMR OF RIMUENE (48)

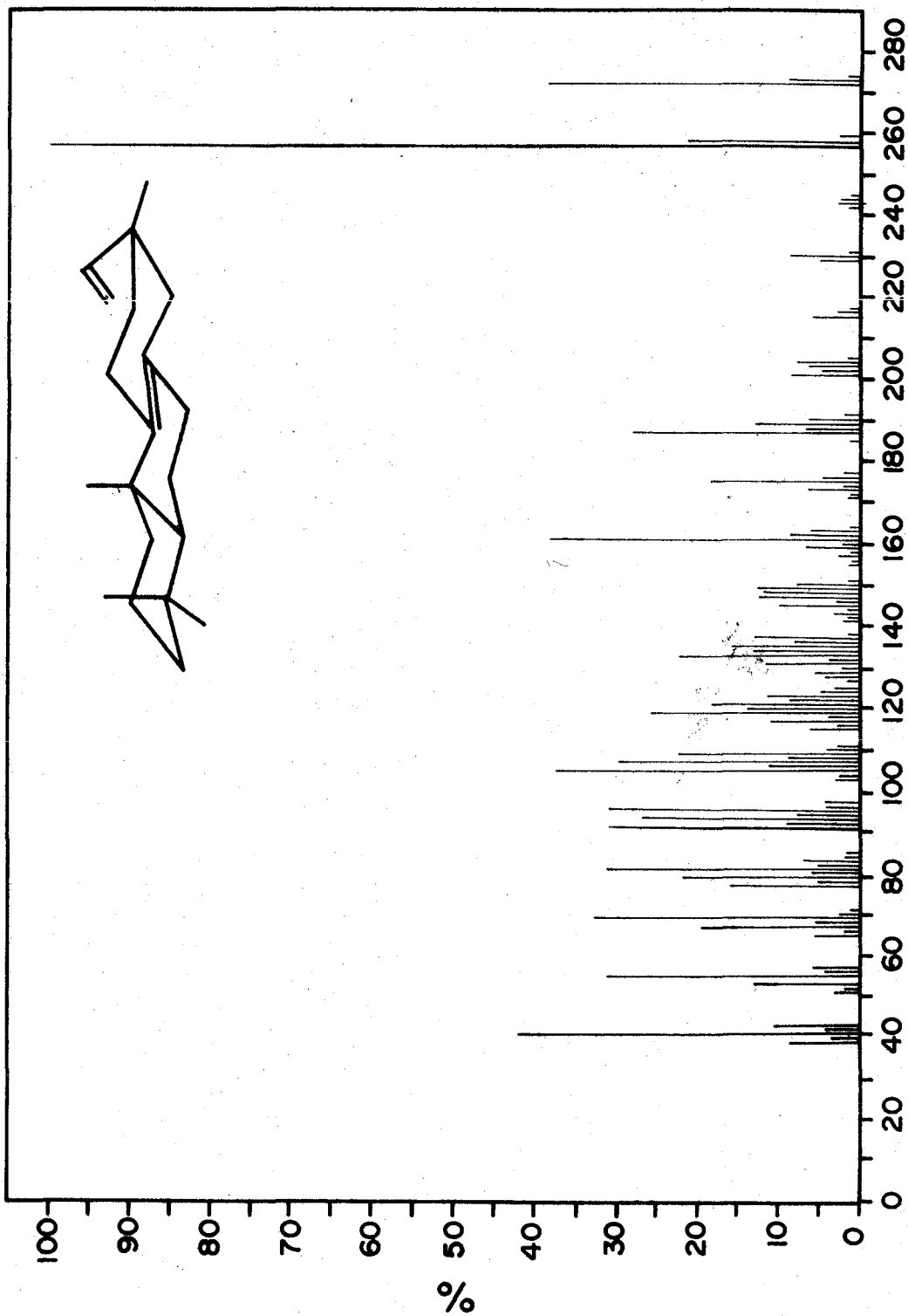


Figure 19: MASS SPECTRUM OF 8,15-PIMARADIENE (37)

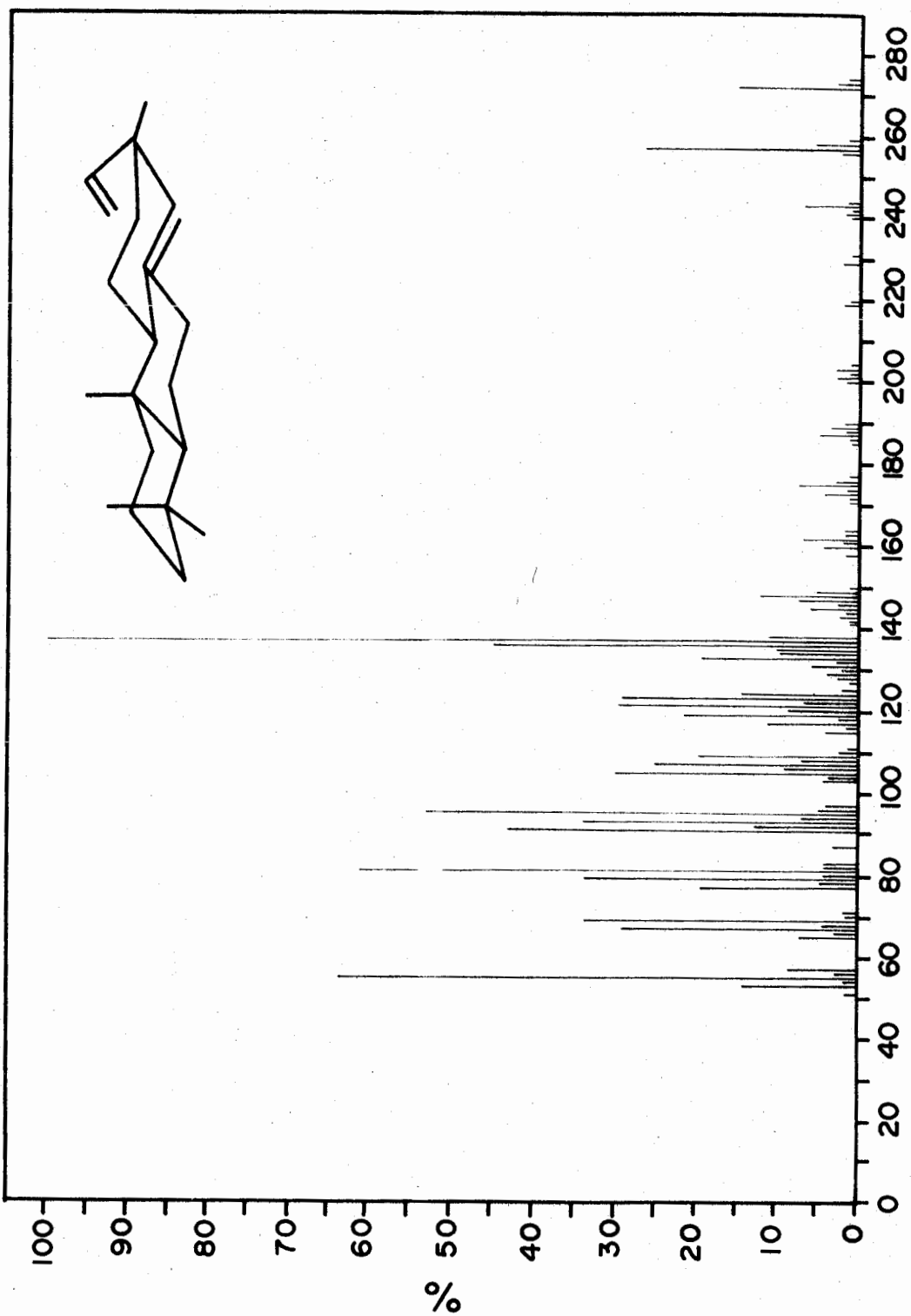


Figure 20: MASS SPECTRUM OF PIMARADIENE (38)

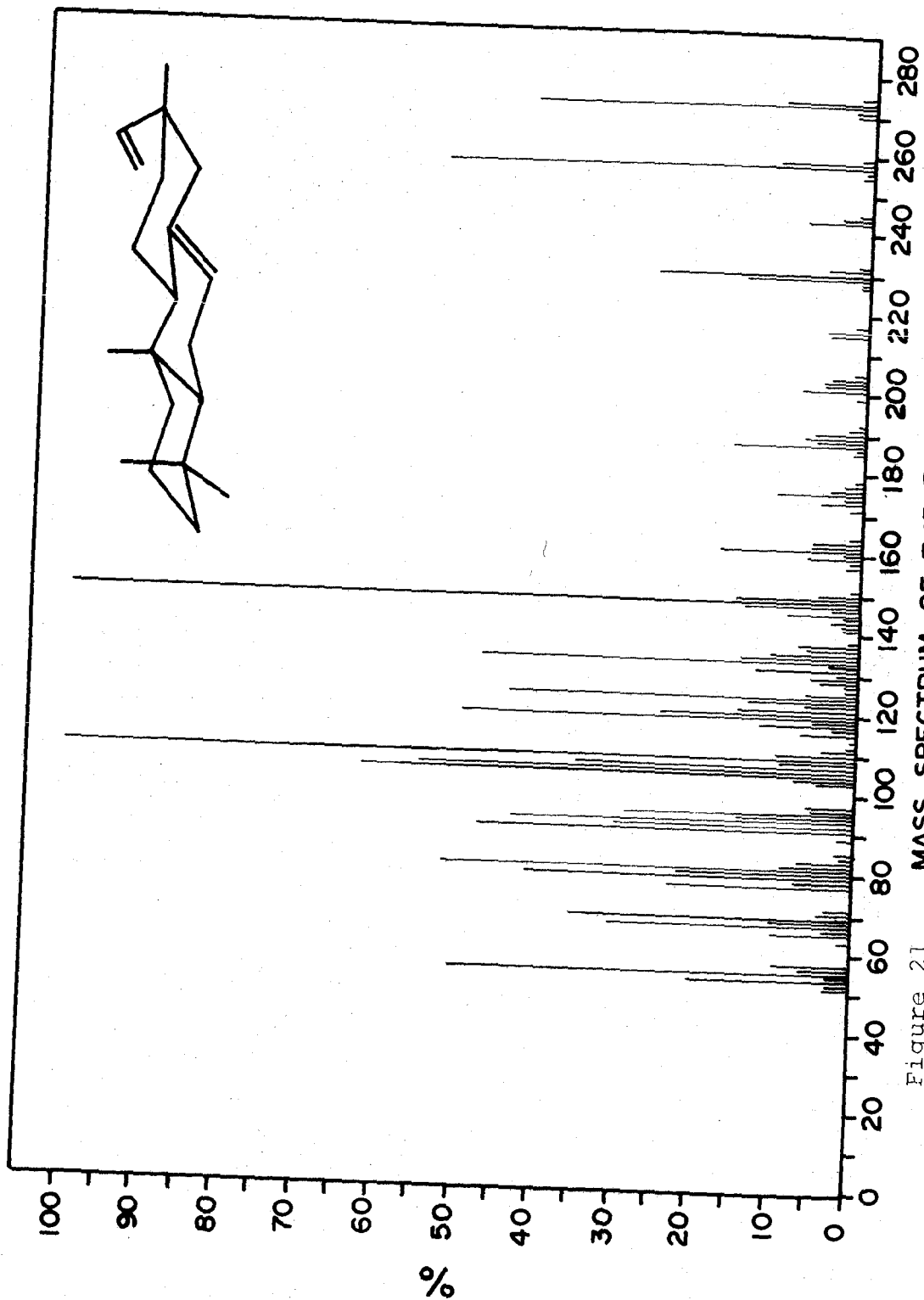


Figure 21 MASS SPECTRUM OF 7,15-PIMARADIENE (39)

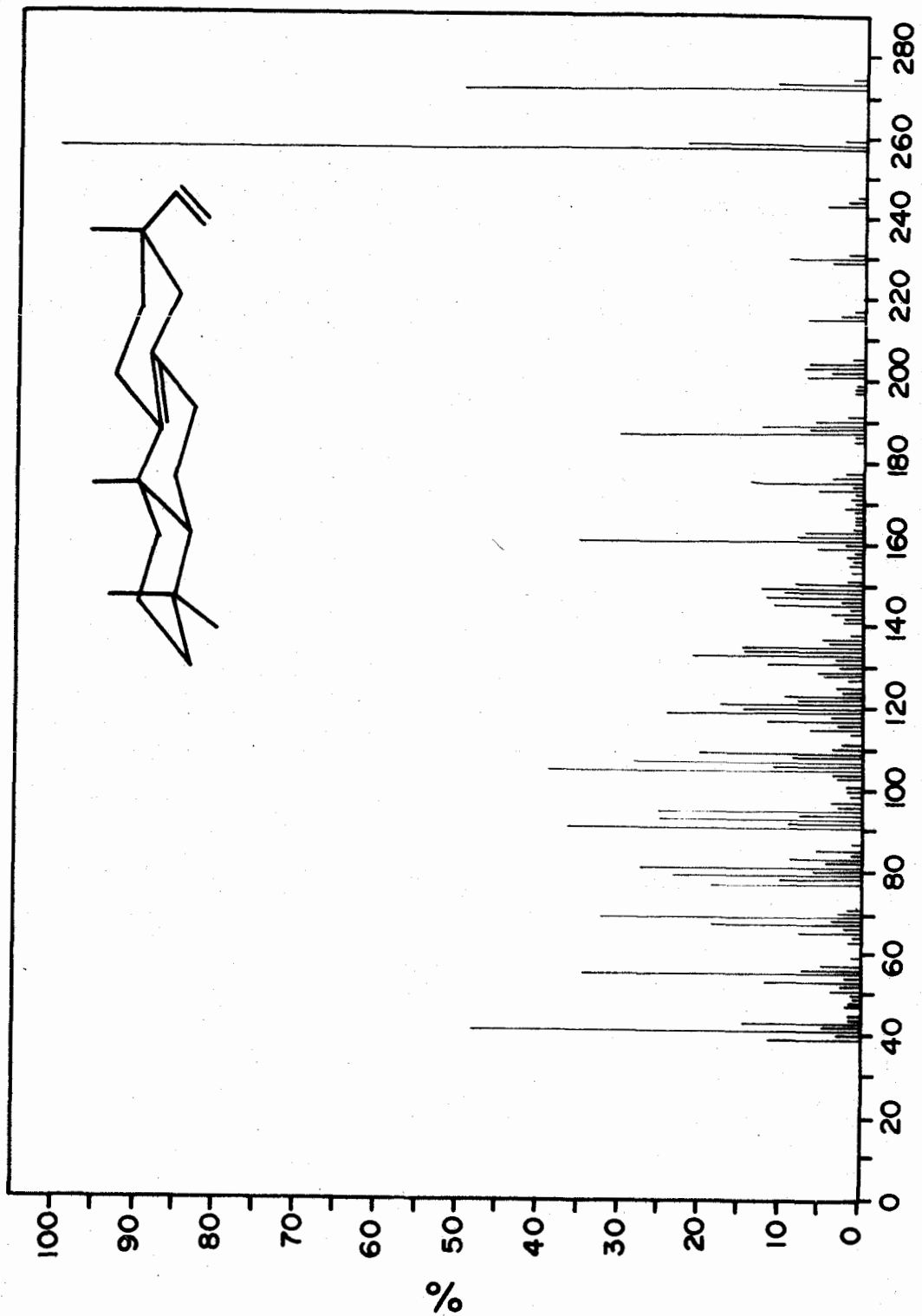


Figure 22: MASS SPECTRUM OF 8,15-ISOPIMARADIENE (40)

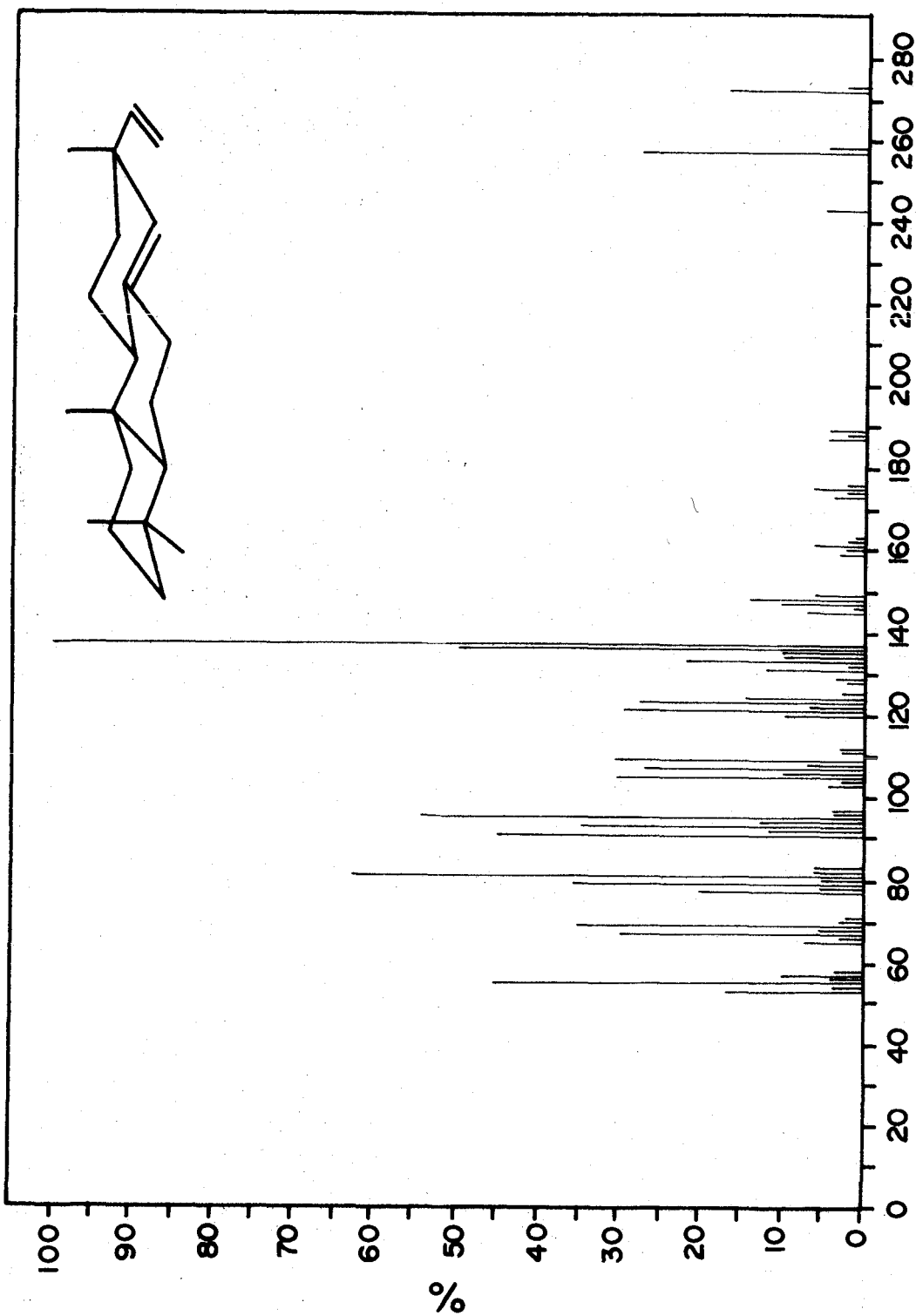


Figure 23: MASS SPECTRUM OF SANDARACOPIMARADIENE (41)

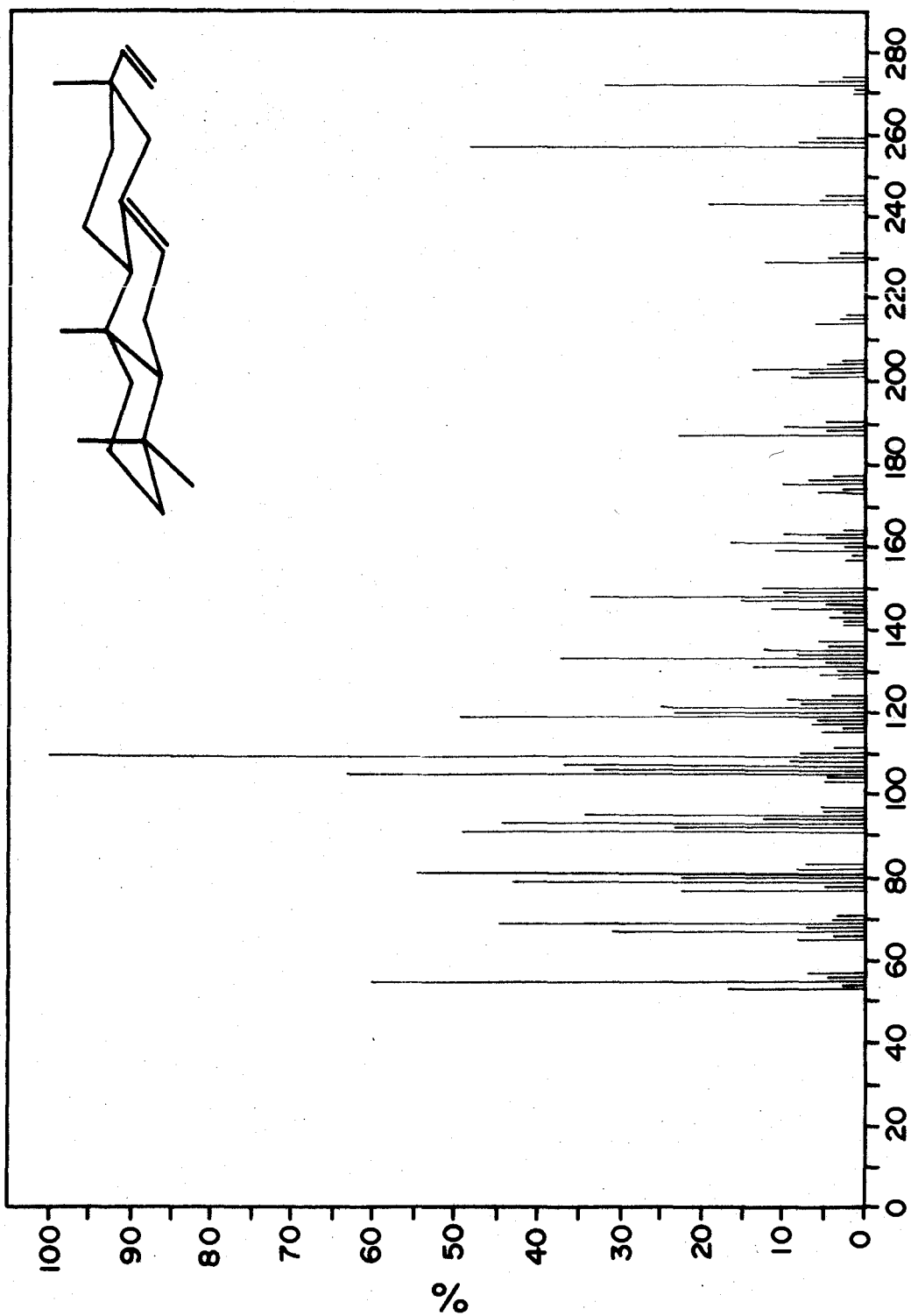


Figure 24: MASS SPECTRUM OF ISOPIMARADIENE (42)

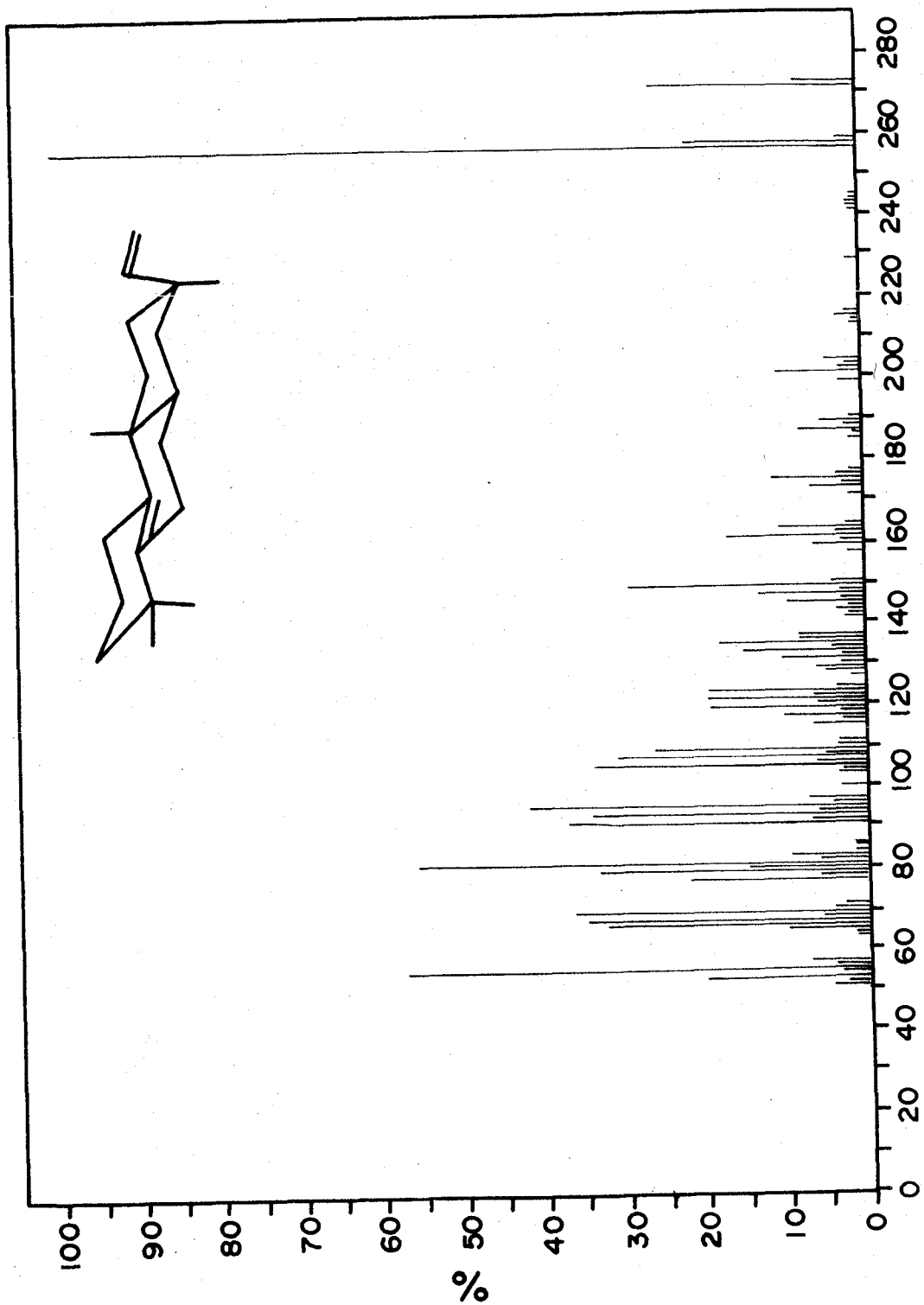


Figure 25: MASS SPECTRUM OF 5(10), 15-ROSADIENE (43)

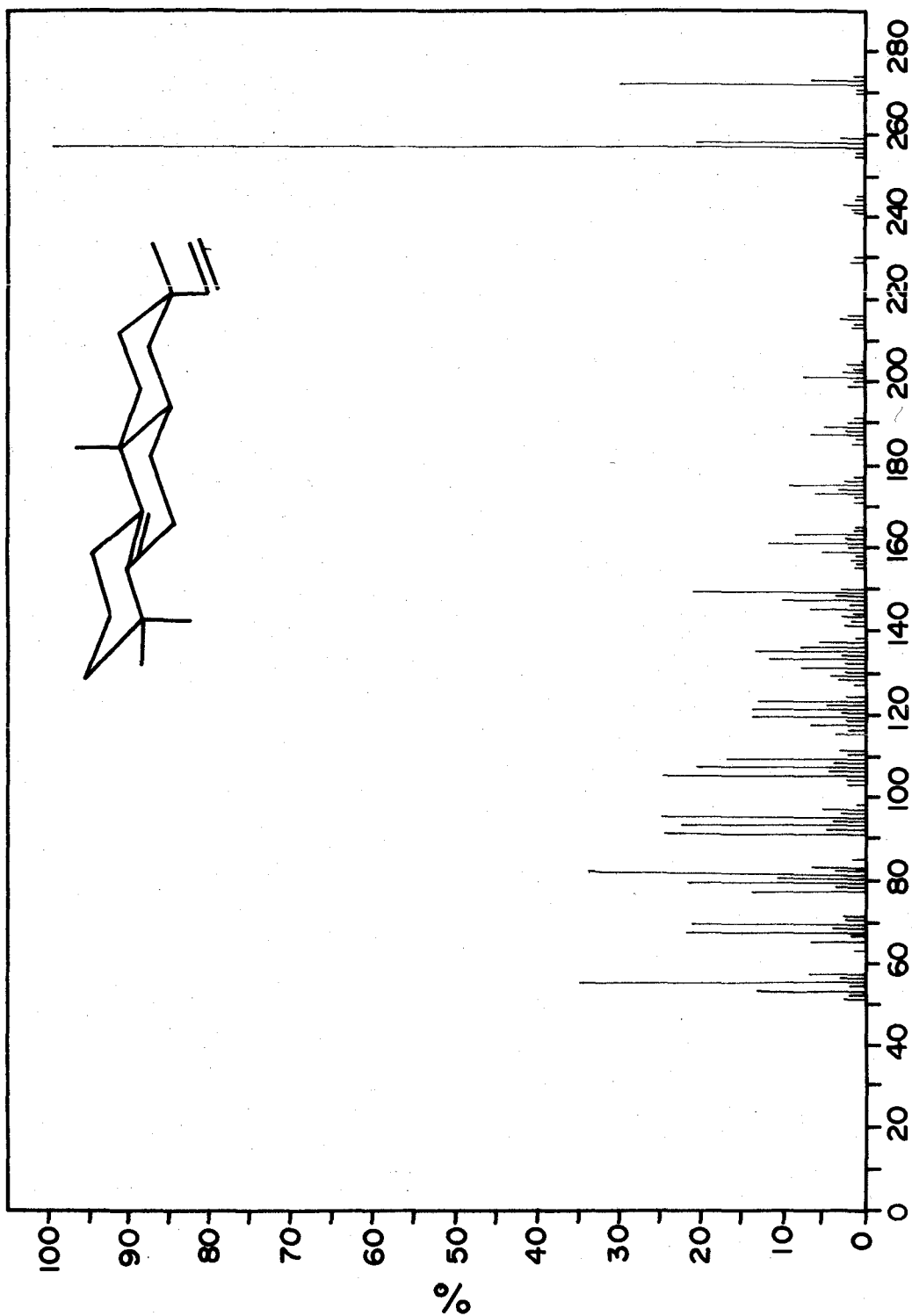


Figure 26: MASS SPECTRUM OF 13-EPI-5(10), 15-ROSADIENE (44)

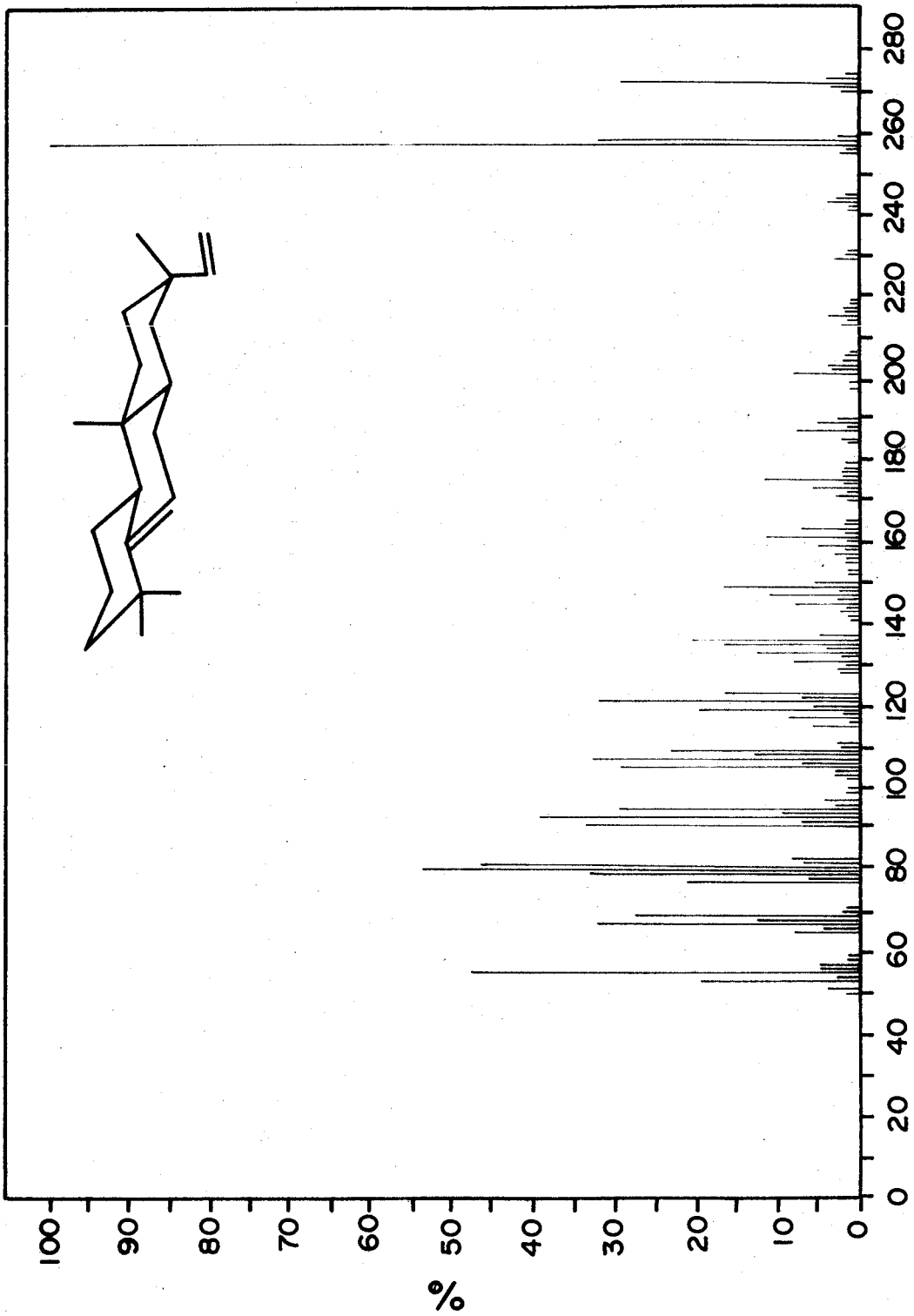


Figure 27: MASS SPECTRUM OF RIMUENE (48)

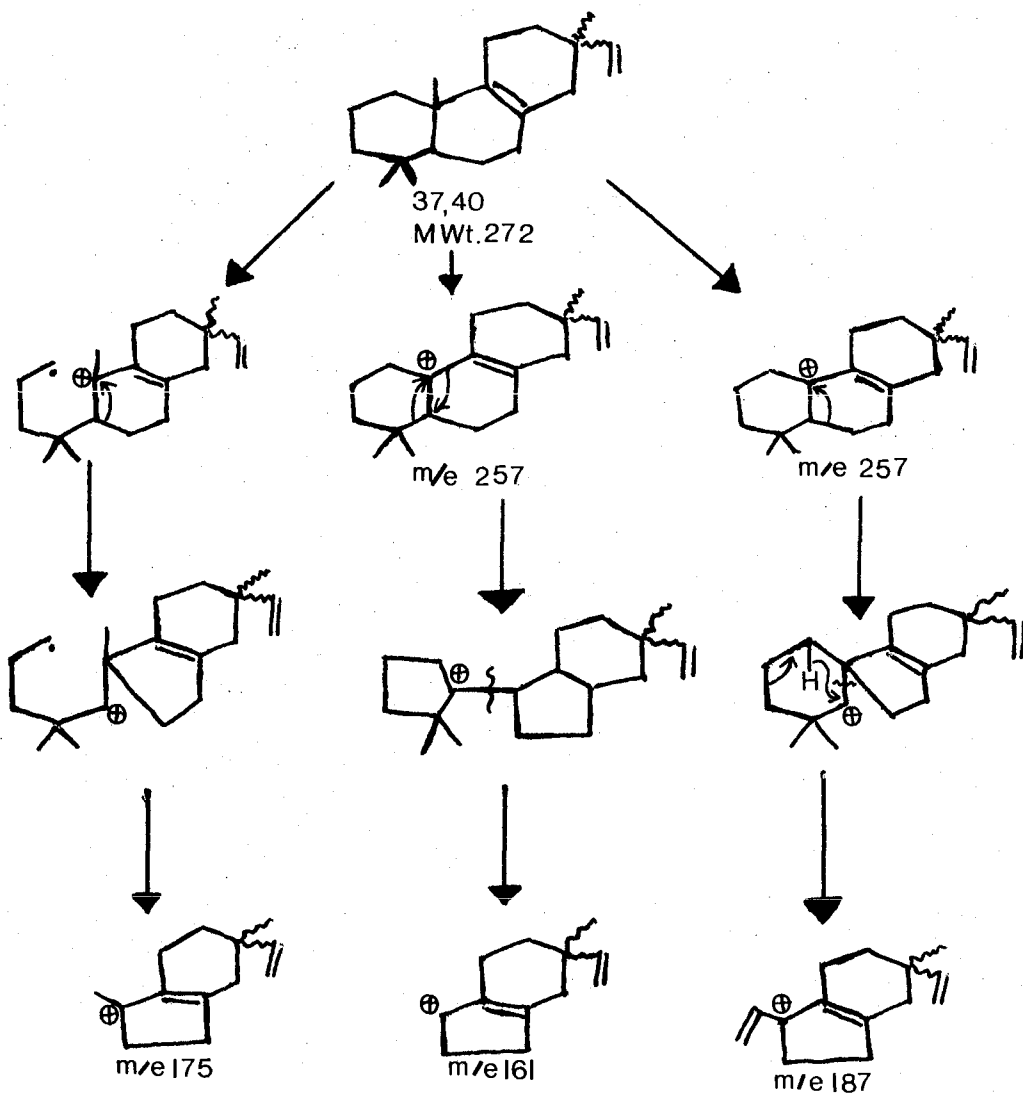


Figure 28: Fragmentation Pattern of 8,15-Pimaradiene(37) and 8,15-Isopimaradiene(40)

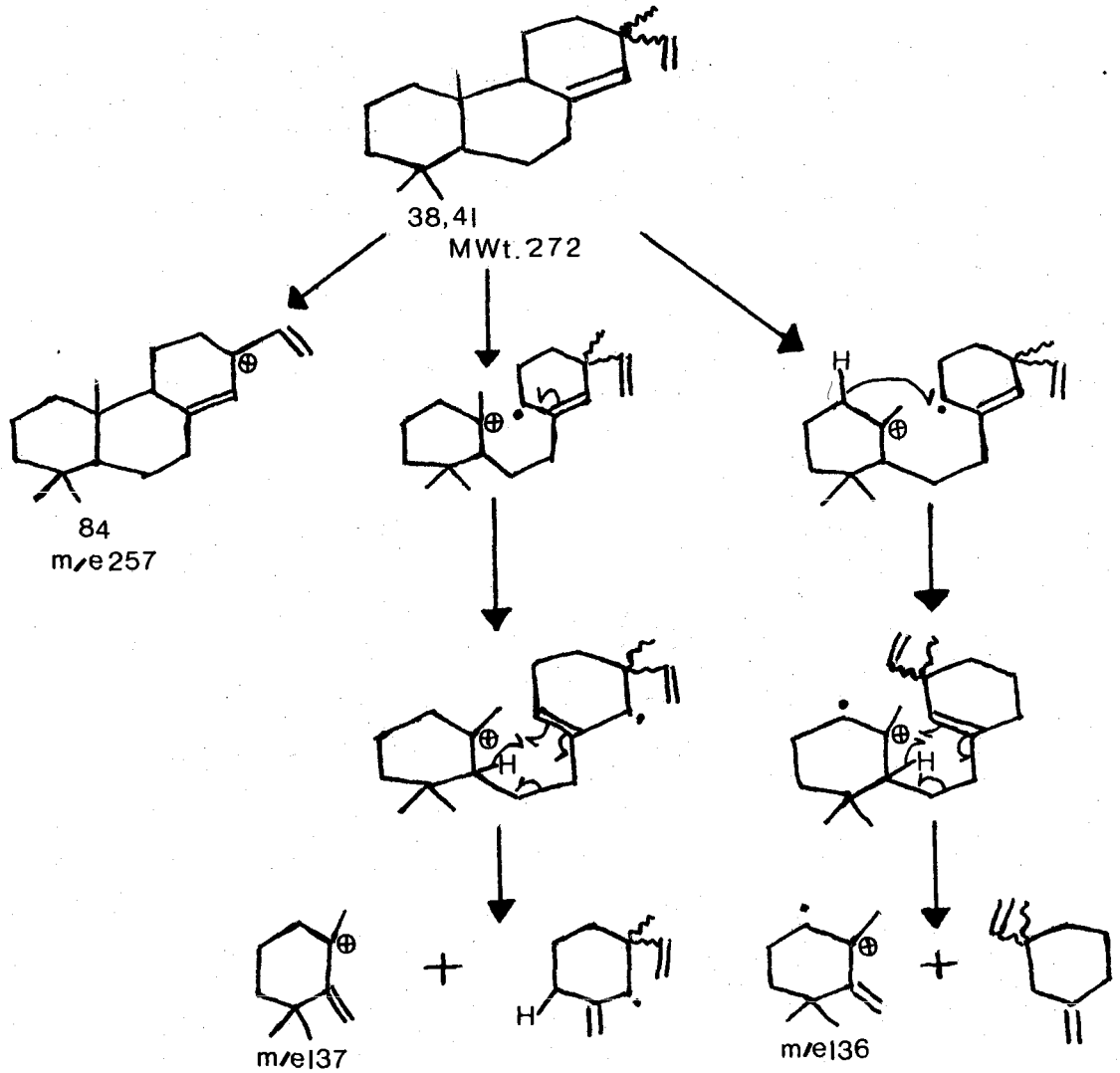


Figure 29: Fragmentation Pattern of Pimaradiene (38) and Sandaracopimaradiene (41)

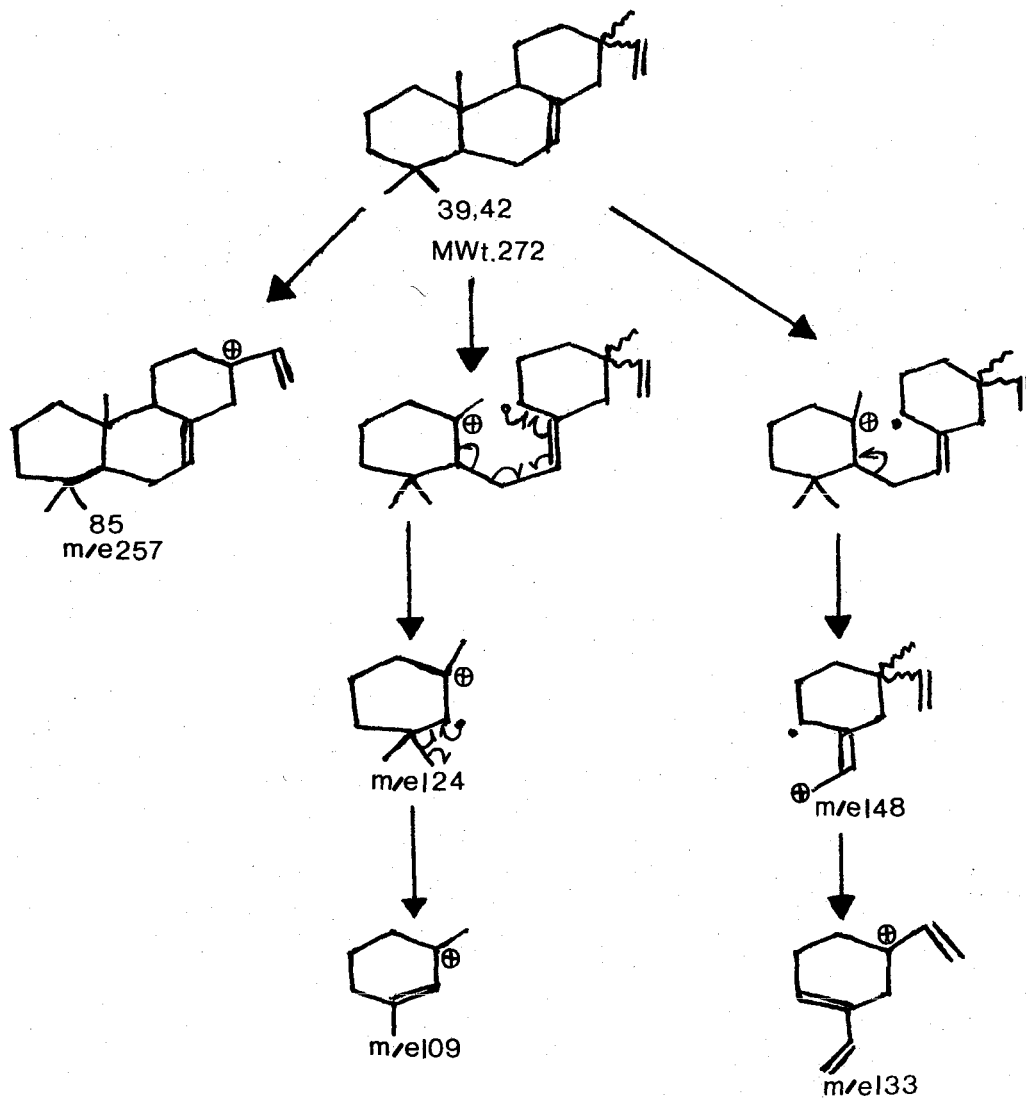


Figure 30: Fragmentation Pattern of 7,15-Pimaradiene(39) and Isopimaradiene(42)

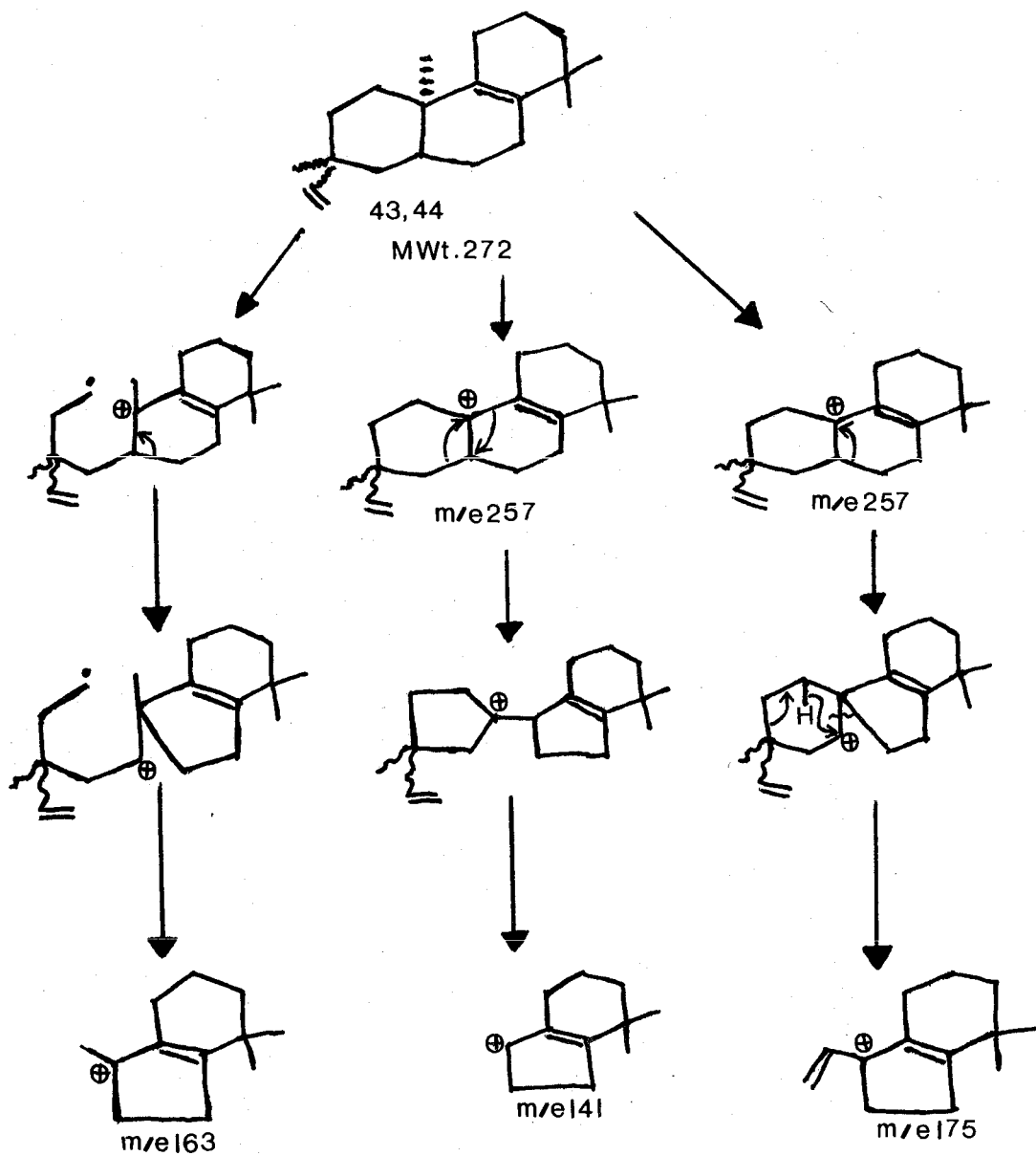


Figure 31: Fragmentation Pattern of 5(10),15-Rosadiene (43) and 13-Epi-5(10),15-rosadiene(44)

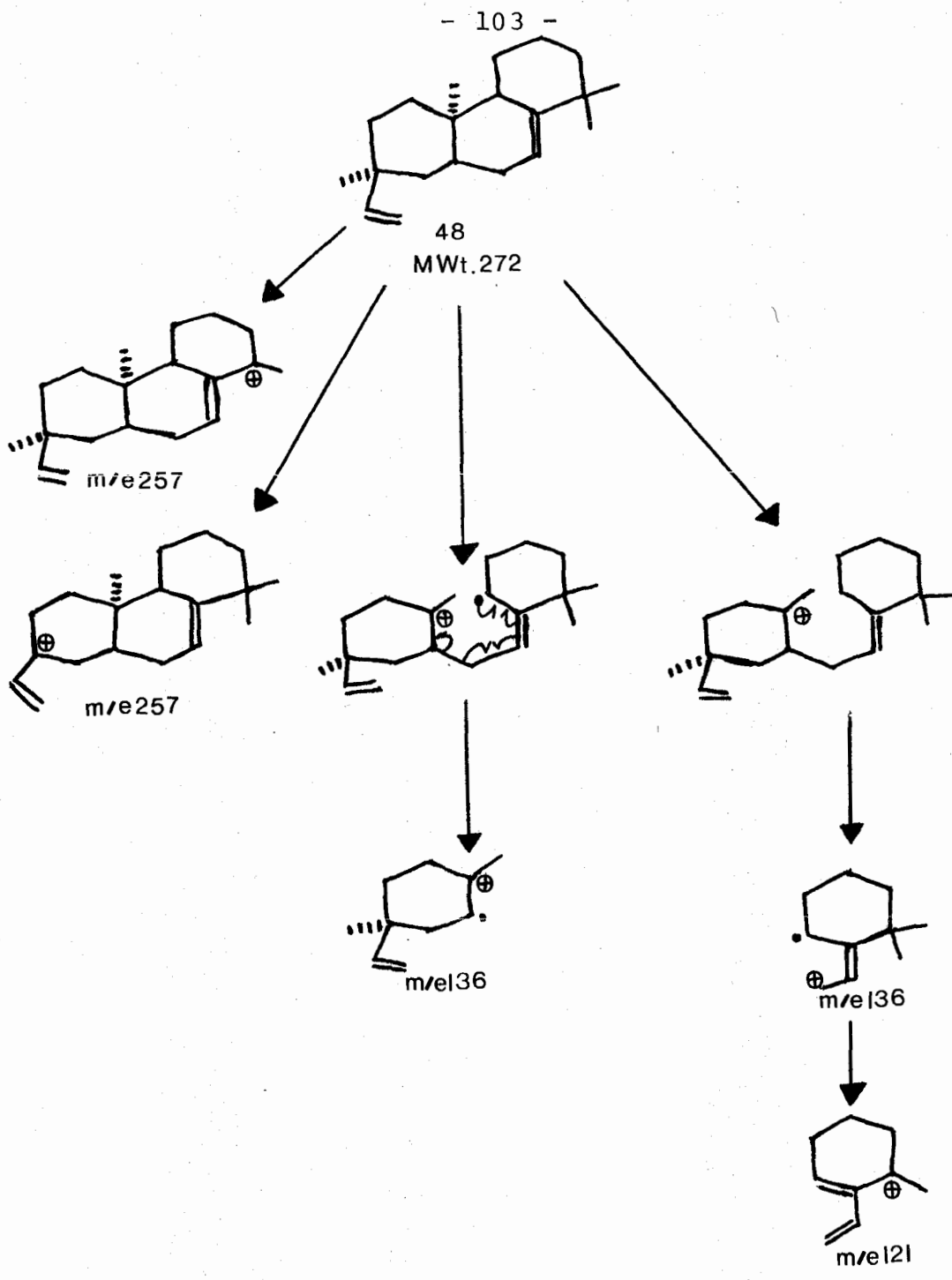


Figure 32: Fragmentation Pattern of Rimuene(48)

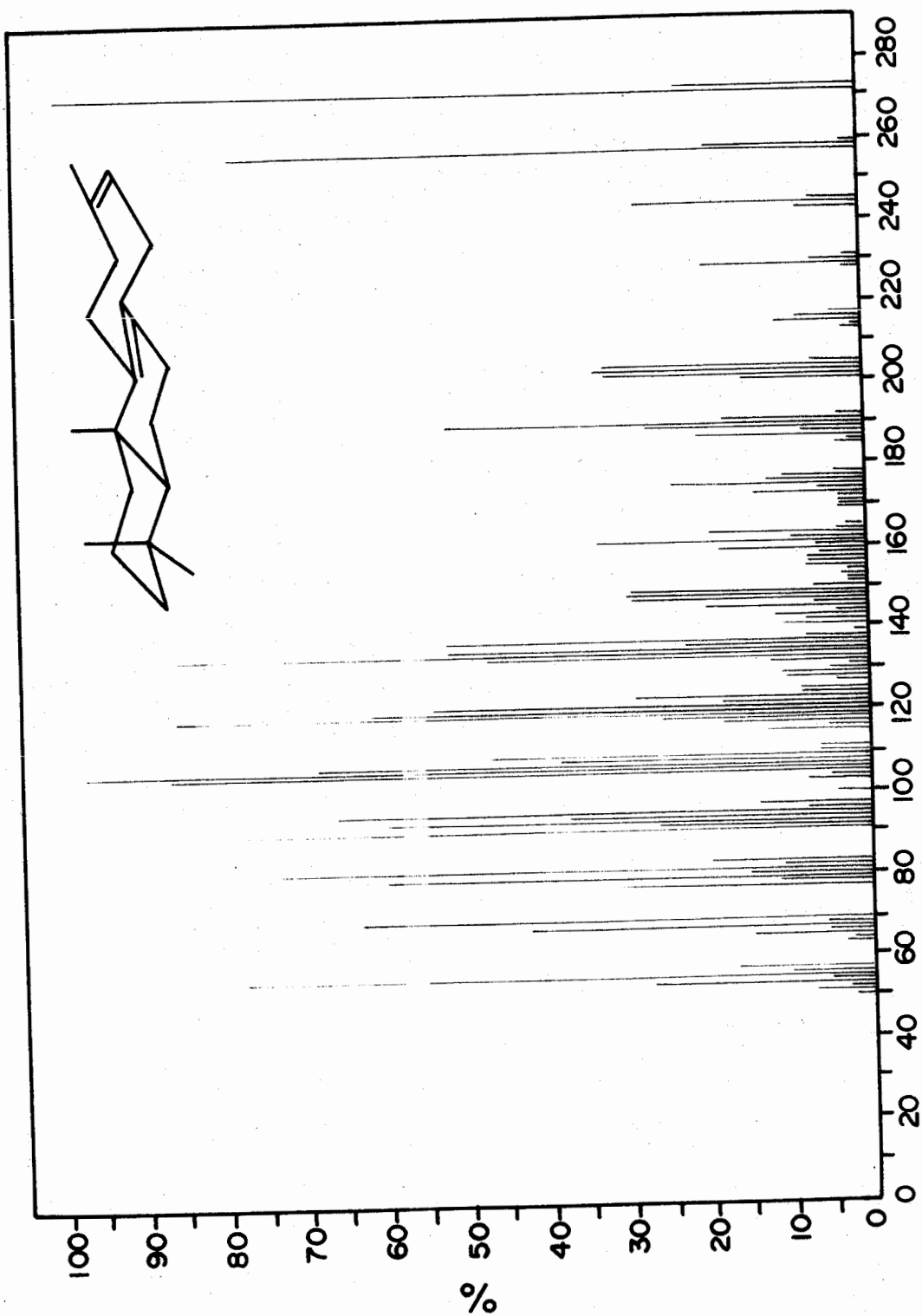


Figure 33: MASS SPECTRUM OF 8,13-BURNABADIENE (45)

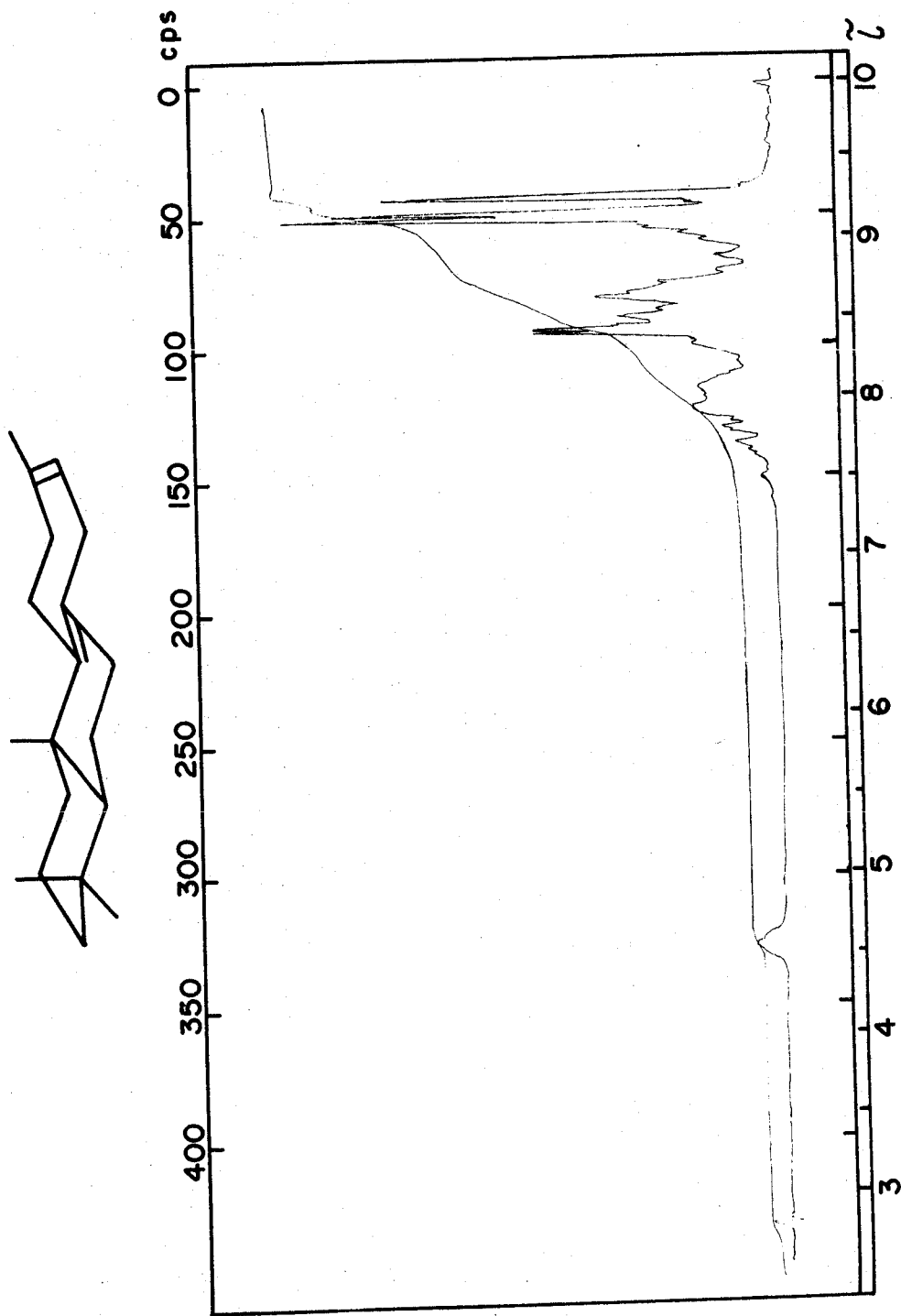


Figure 34: NMR OF 8,13 - BURNABADIENE (45)

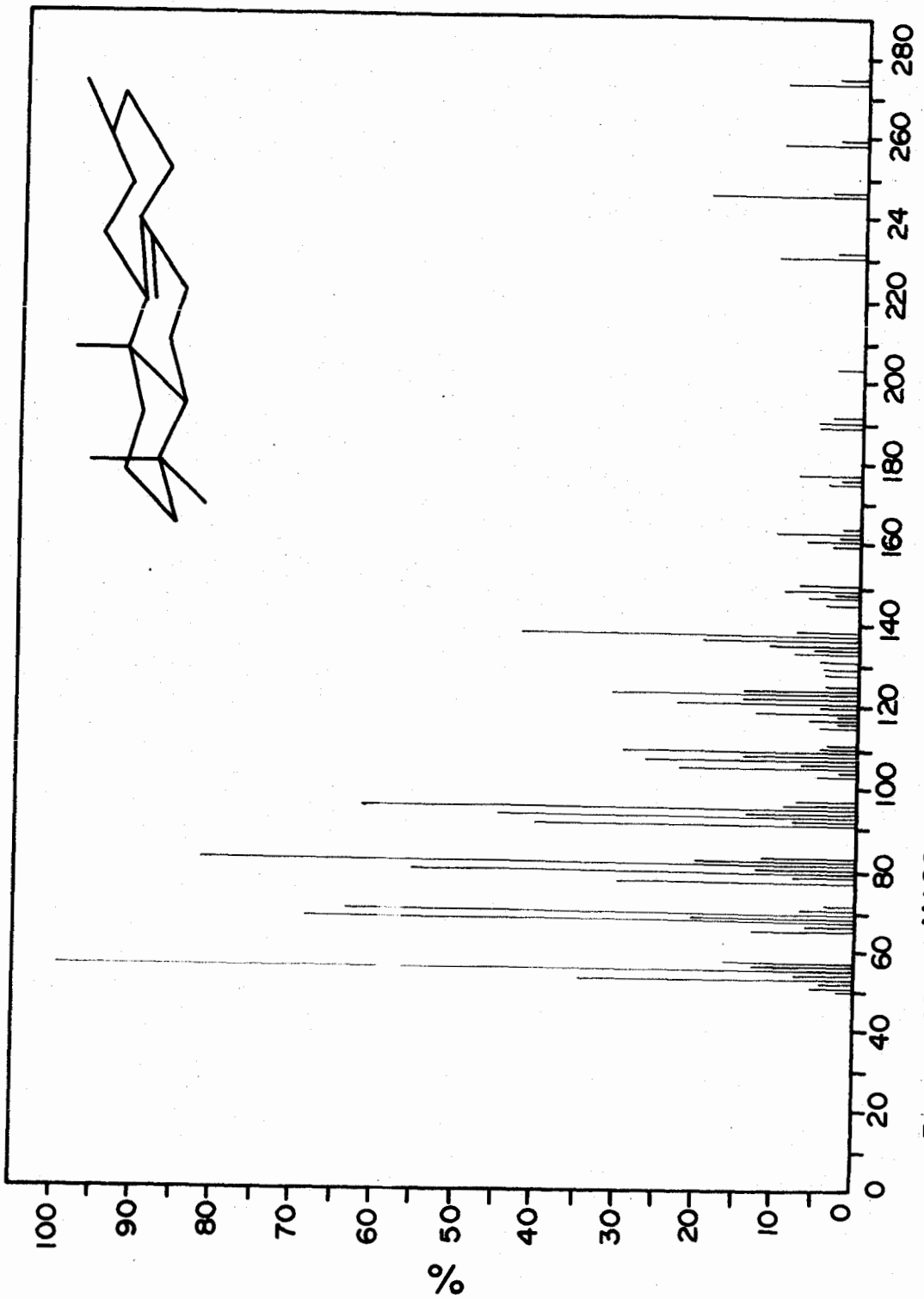


Figure 35: MASS SPECTRUM OF 8-BURNABENE (89)

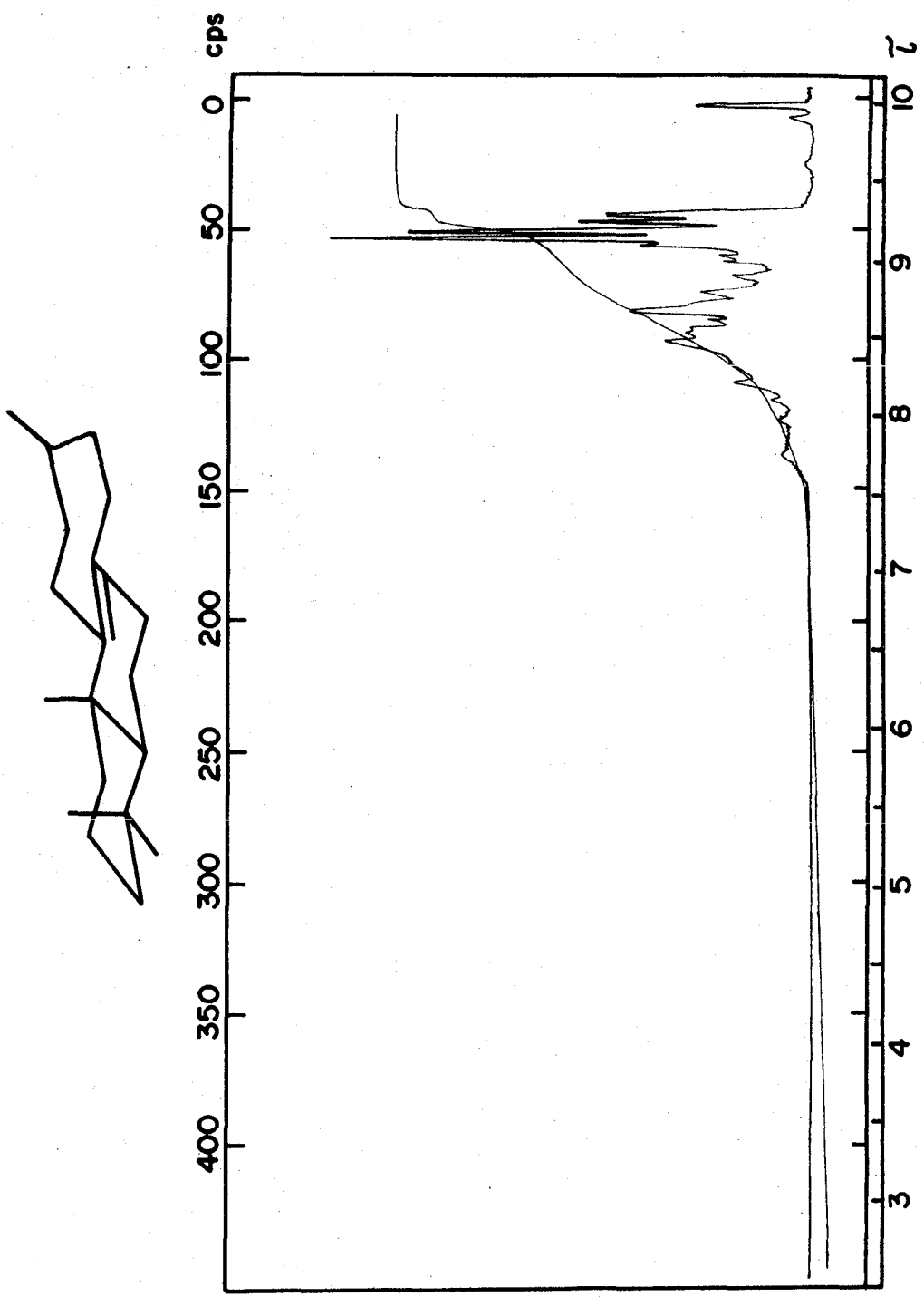


Figure 36: NMR OF 8 - BURNABENE (89)

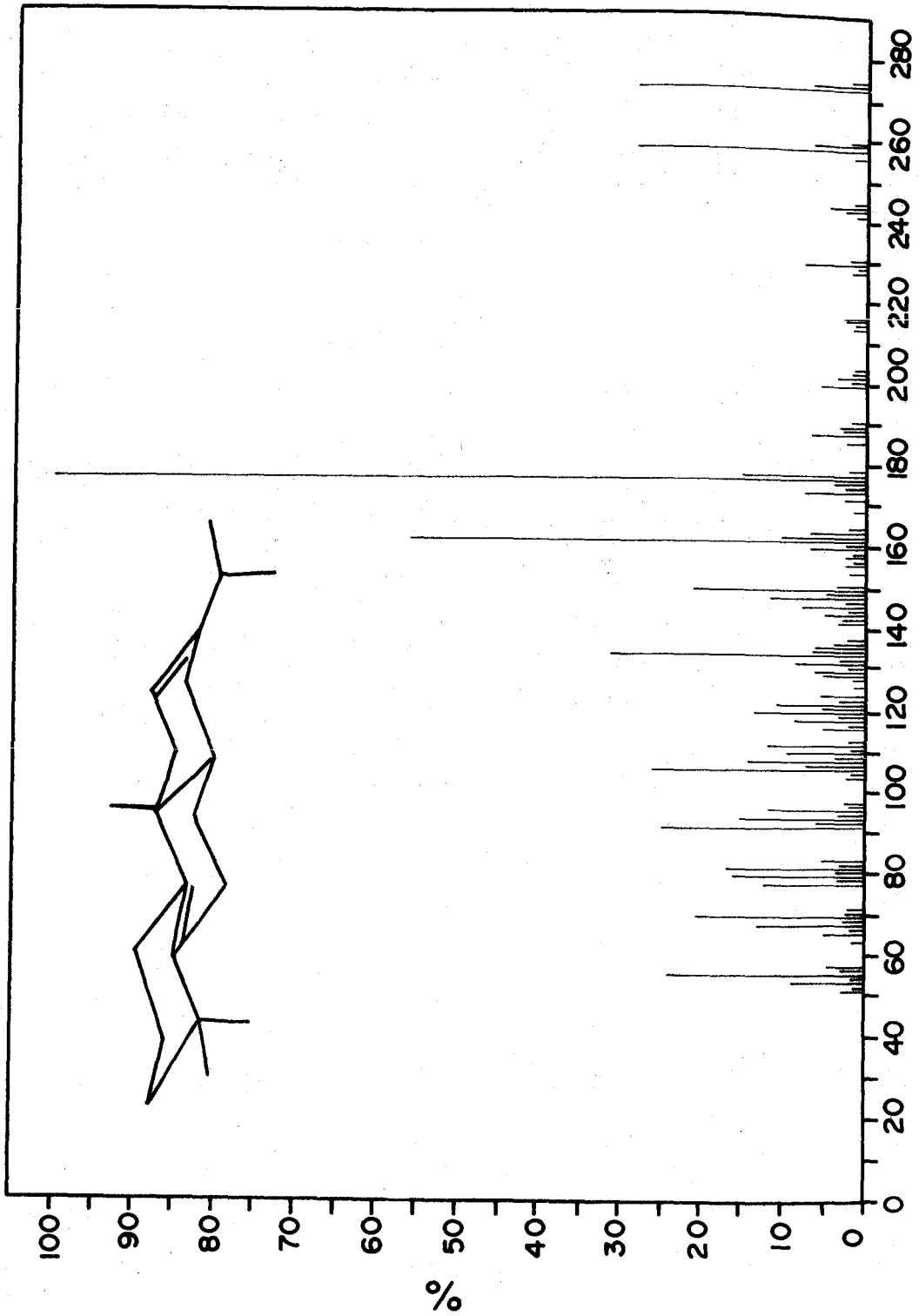


Figure 37: MASS SPECTRUM OF 5(10),12-ABIETAROSADIENE (47)

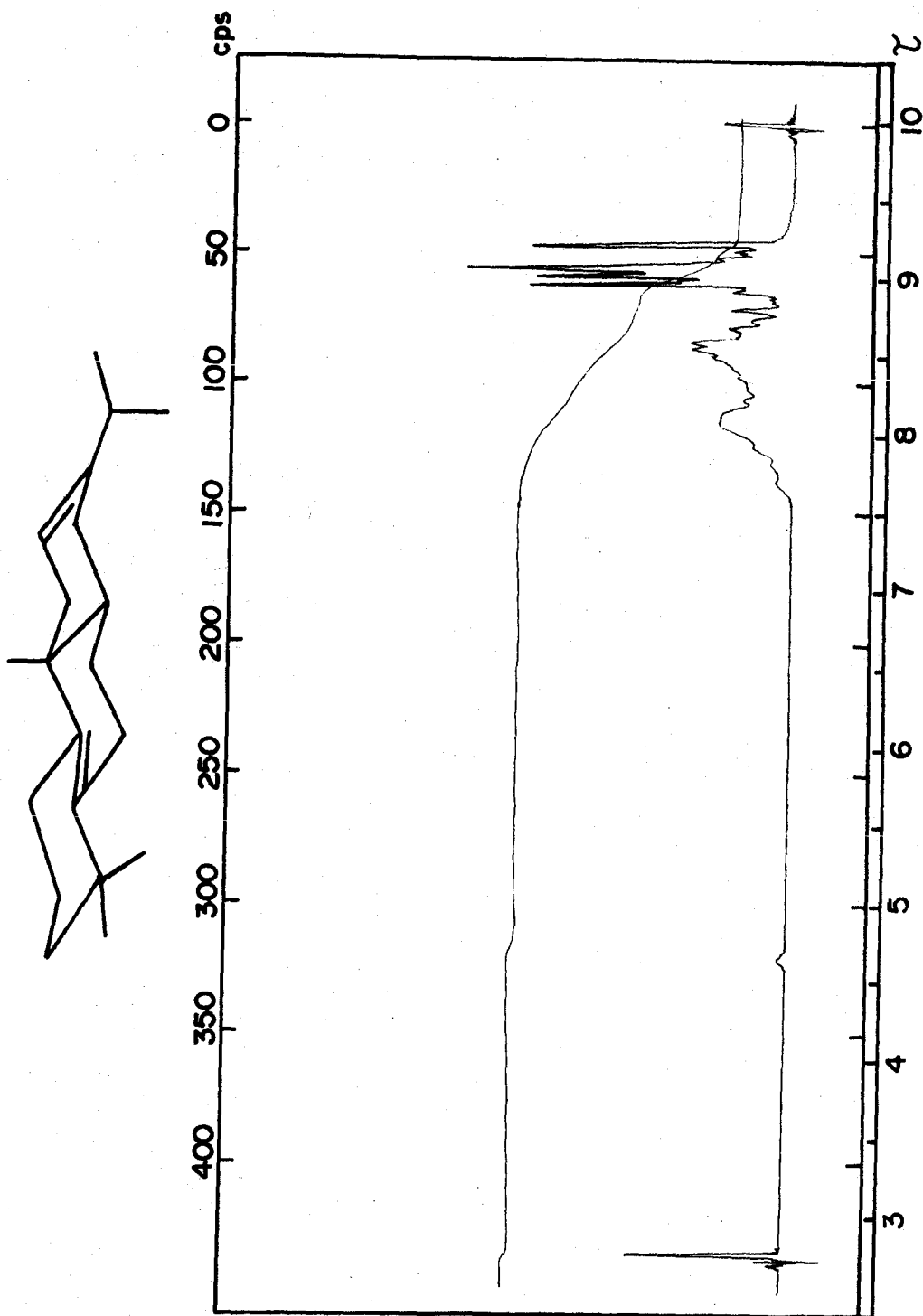


Figure 38: NMR OF 5(10),12-ABIETAROSADIENE (47)

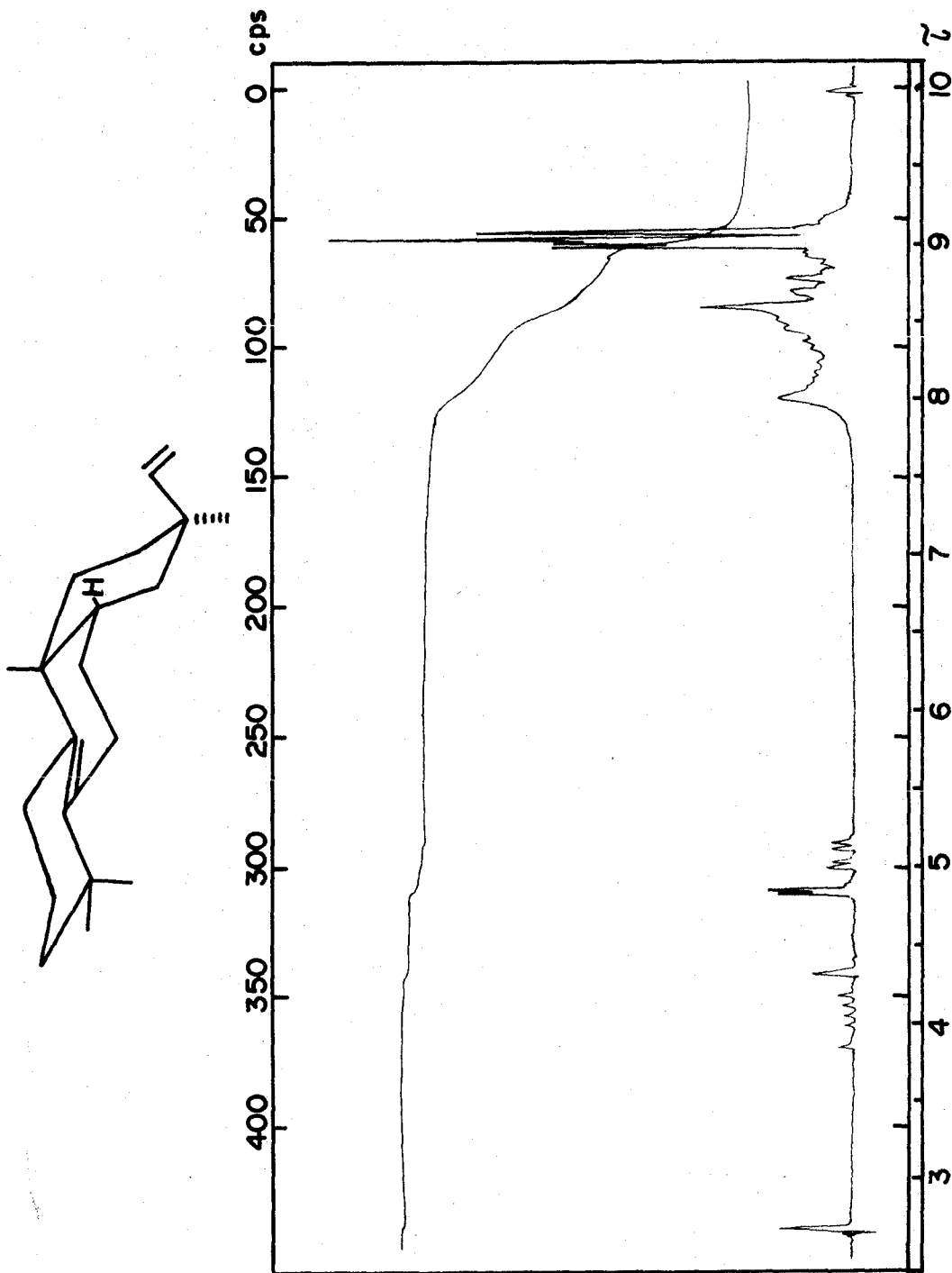


Figure 39: NMR OF 8-EPI-5(10),15-ROSADIENE (46)

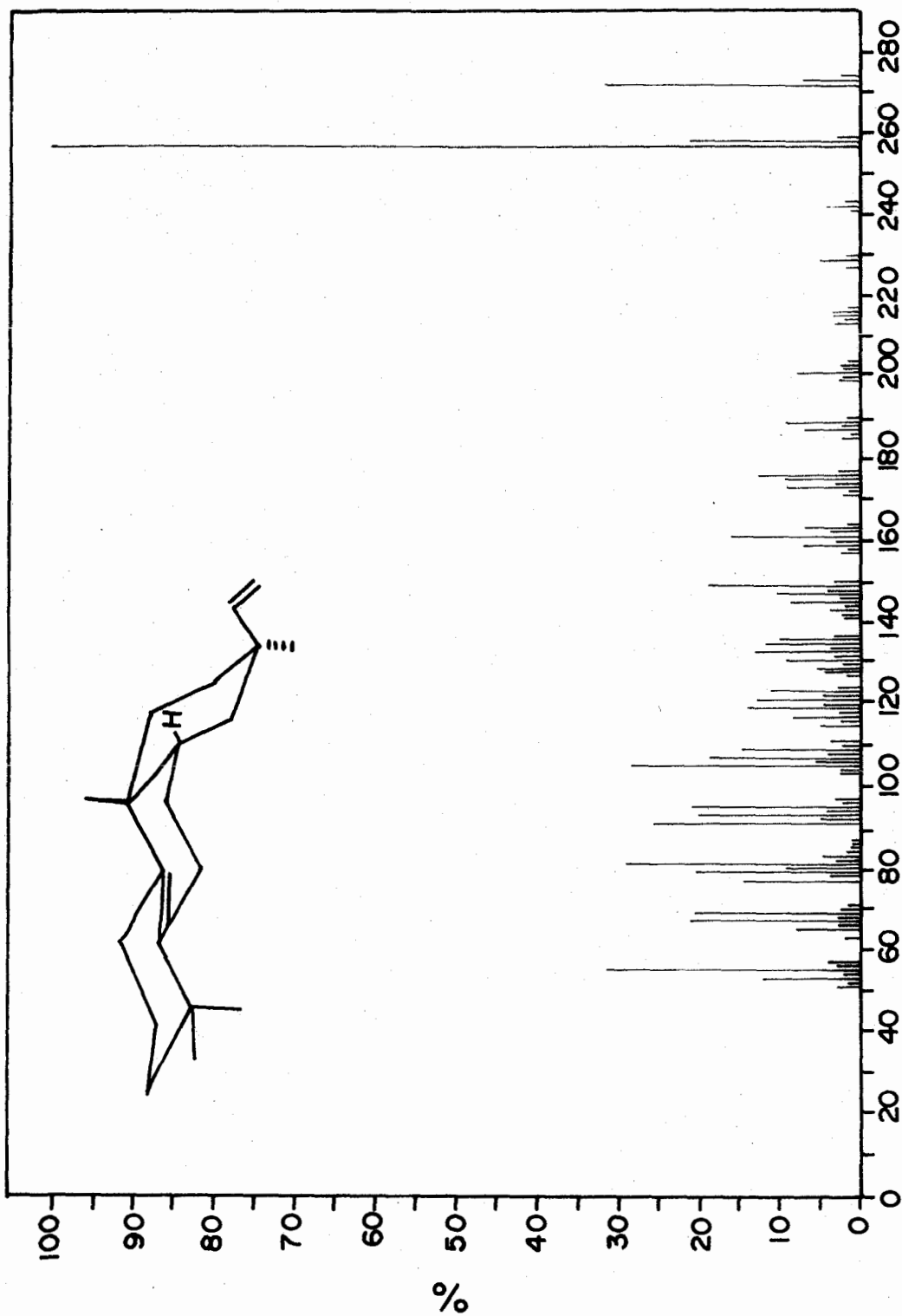


Figure 40: MASS SPECTRUM OF 8-EPI-5(10),15-ROSADIENE (46)

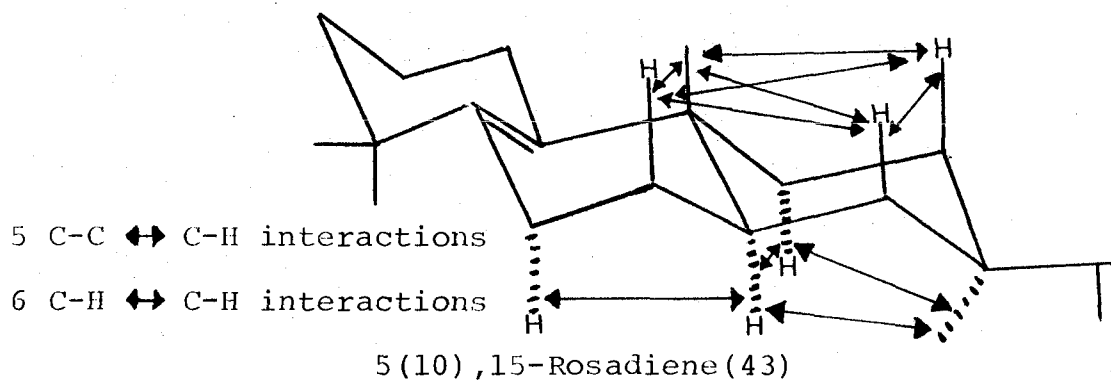
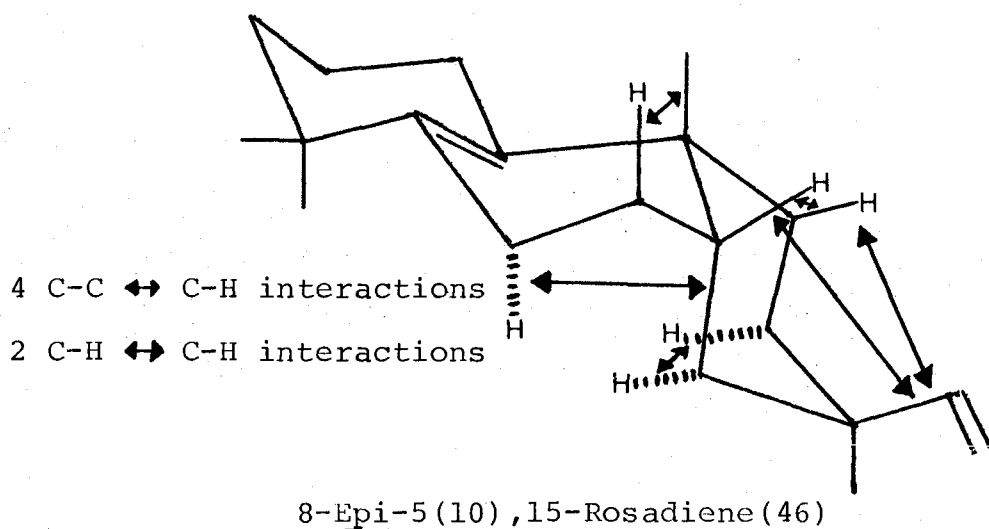


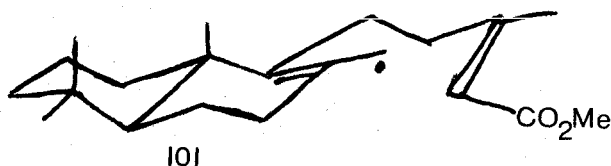
Figure 41: Comparison of 1,3-diaxial interactions in rings Band C of 5(10),15-Rosadiene and 8-Epi-5(10),15-Rosadiene

CHAPTER 5

RECOMMENDATIONS FOR FURTHER RESEARCH

Synthesis of 8,13-Burnabadiene(45)

The bromo ester (62) was prepared as an intermediate compound in an attempted synthesis of 8,13-burnabadiene. Its decomposition upon attempted purification by column chromatography probably proceeded by loss of Br to give the allyl radical (101) which then polymerised. In fact the



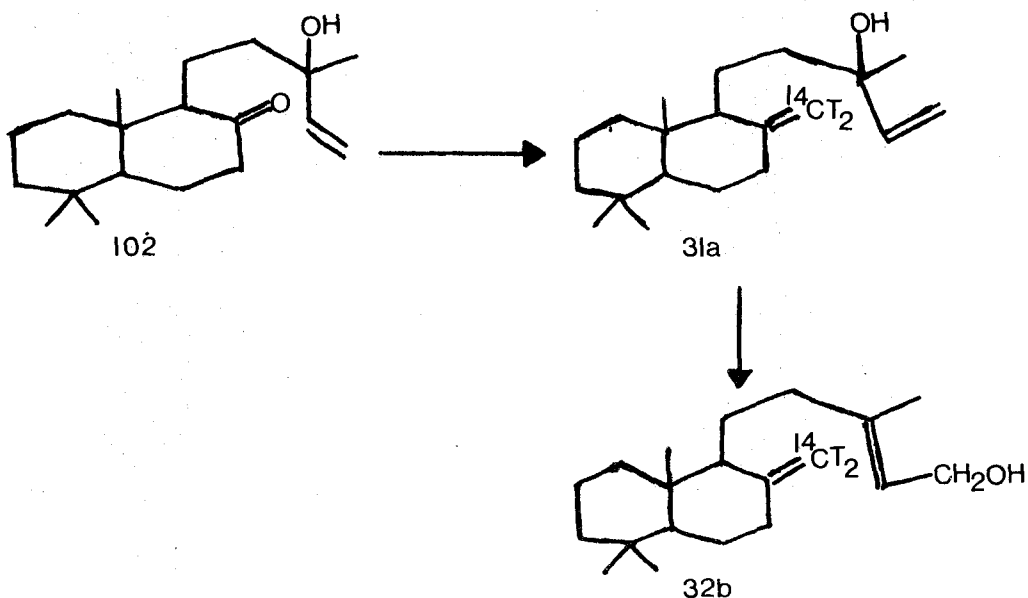
reported⁸⁷ synthesis of allyl bromides recommends that these compounds be protected from light, but in this case, the precaution was not taken. If the previously outlined procedure were to be repeated, protecting from light the compounds (62,63,58) which possess an allyl bromide function a successful synthesis of 8,13-burnabadiene would likely result.

Determination of the Intermediacy of Pimaradiene(38) in Gibberellic Acid Biosynthesis

It has been shown⁸⁸ by the incorporation of 4-(R)-[4-³H,2-¹⁴C] mevalonate and [2-³H₂, 2-¹⁴C] mevalonate into gibberellic acid that 8,15-pimaradiene(37) and 7,15-pimaradiene(39) are not involved in the biosynthesis of this compound. The absence of reports of a successful incorporation of pimaradiene into gibberellic acid indi-

cates that either transportation difficulties were encountered or that the tricyclic ion(7) undergoes ring closure to the tetracyclic carbonium ion(13) without the participation of any intermediate olefins.

These two processes could be distinguished by incorporation of $[17\text{-}^3\text{H}_2, 17\text{-}^{14}\text{C}] \Delta^{13}\text{-manool}(32\text{b})$ ⁸⁹ into gibberellic acid using previously reported procedures.⁶⁸ A decrease in T:¹⁴C ratio on going from starting material to gibberellic acid would indicate that indeed pimaradiene is an intermediate in this biosynthetic process, since C-17 in the labdene precursor(32) becomes C-14 in the pimaradiene intermediate(7). Formation of pimaradiene would therefore necessarily result in loss of tritium. If no change in T:¹⁴C ratio were observed then all olefinic intermediates derivable directly from (7) would be finally eliminated.



A synthesis of (32b) could be achieved by Wittig reactions upon the previously reported⁶⁵ ketone(102) introducing $^{14}\text{CH}_2$ and CT_2 separately with subsequent mixing of the radioactive precursors to give (31a). Isomerisation as previously described would give the desired product.

Determination of the Intermediacy of Hibaene in Gibberellic Acid Biosynthesis

The reported⁶⁸ incorporation of $[\text{15-}^3\text{H}] \Delta\text{13-manool}$ ⁸⁹ into gibberellic acid proved that the biosynthetic route proceeded via the tetracyclic carbonium ion(13). This experiment, however, was not able to distinguish between a pathway proceeding by the rearrangement of (13) to the kaurene skeleton(23) directly and one involving the intermediacy of the olefin hibaene(21) derived by deprotonation of (13).

These two pathways could be distinguished by a mixed labelling experiment with $[\text{15-}^3\text{H}, \text{17-}^{14}\text{C}] \Delta\text{13-manool}$ ⁸⁹. Since C-15 in the labdene precursor (32) becomes C-16 in the tetracyclic ion(13) a decrease in T: ^{14}C ratio on going from starting material to gibberellic acid would show that some elimination to hibaene and subsequent reprotonation did occur. If no change in T: ^{14}C ratio was observed then

this process would be precluded.

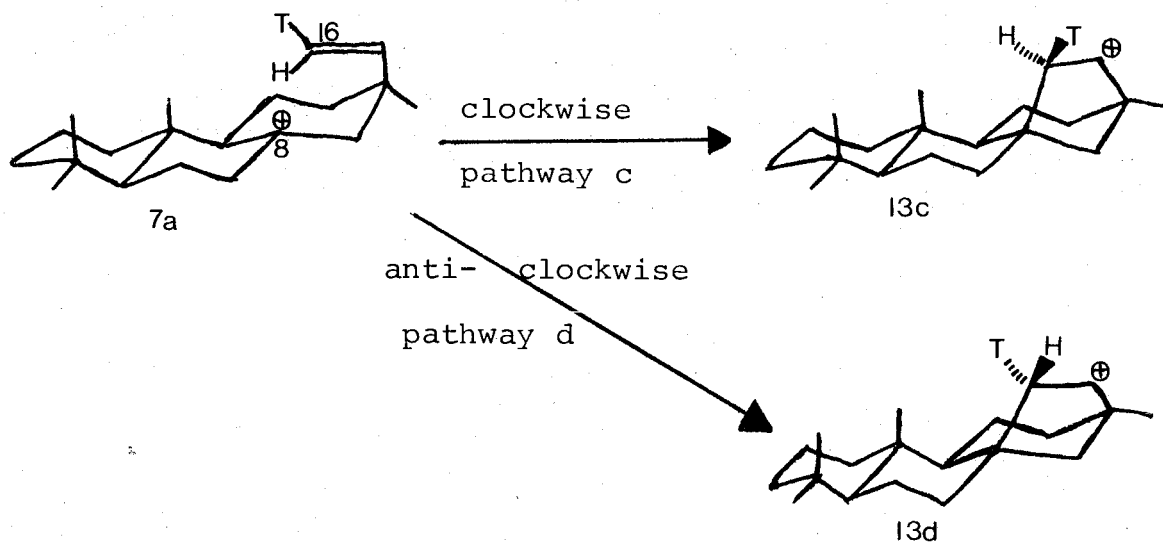
The placement of the 15-³H has been previously reported⁶⁸ and the placement of the 17-¹⁴C was described above.

The Mode of Closure of ring D in the Biogenesis of
Gibberellic Acid

In considering the stereochemistry involved during the formation of the C-D ring system of gibberellic acid, the stereochemistry at C-15 is especially interesting. This carbon arises from C-5 of mevalonate.

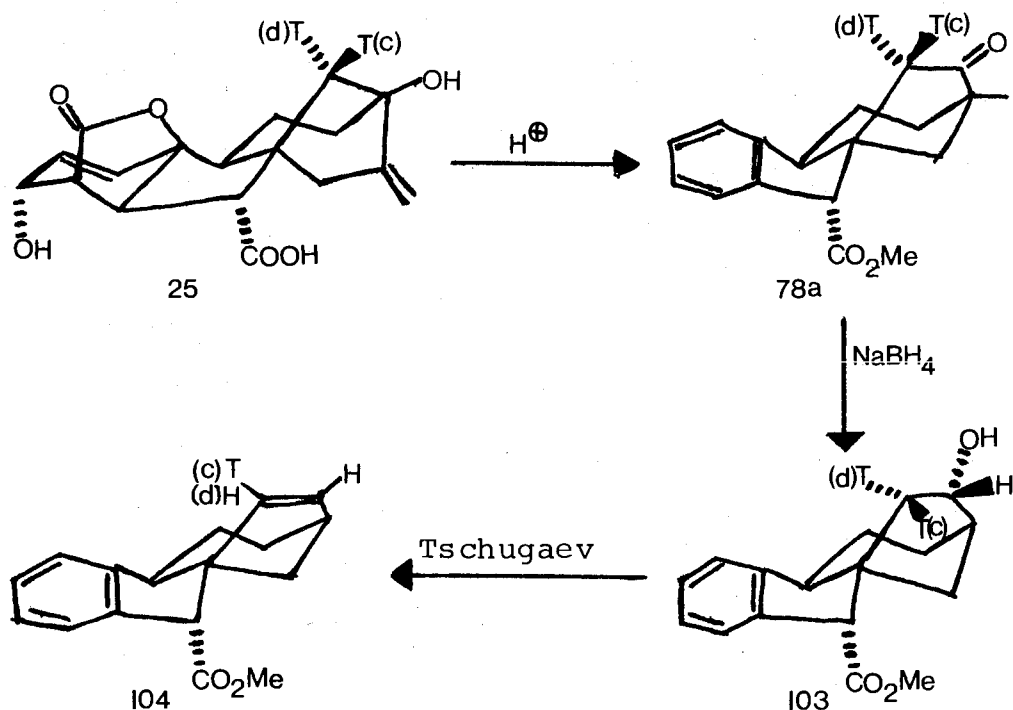
Formally, two transformations involving stereochemistry occur at this centre during this biogenetic process. One is the isomerisation of the $\Delta^{13(14)}$ double bond in the bicyclic progenitor(32)⁸⁹ to the $\Delta^{15(16)}$ position in the pimarane skeleton(7) which must occur during the formation of ring C. The other is ring closure between C-8 and C-16 in the formation of ring D in (13). Since the stereochemistry involved in the double bond isomerisation cannot be settled without knowledge of the stereochemistry involved in the formation of the D ring, this latter point should receive the initial attention.

The vinyl group involved in the closure of ring D in the formation of (13) must rotate in order for cyclisation to occur between C-8 and C-16.⁸⁹



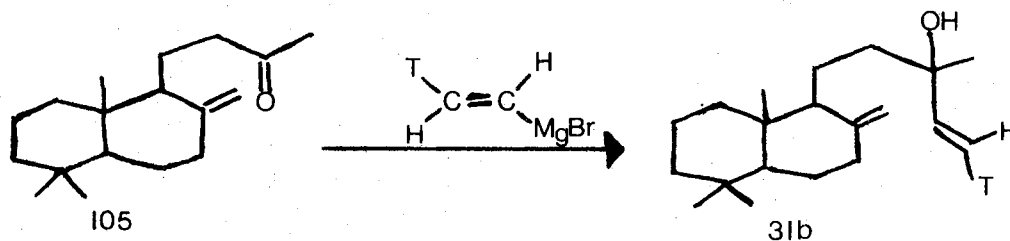
If C-16 in (7a) was labelled as shown, clockwise rotation (pathway c) would result in the labelling pattern (13c) and anti-clockwise rotation (pathway d) would result in labelling pattern (13d). Both processes appear equally feasible. If the results of the experiments suggested earlier showed that hibaene was not involved and that the kaurane skeleton was formed directly from (13) then (13c) and (13d) would give gibberellic acid with labelling pattern (25c) and (25d) respectively.

Methylation followed by acid treatment would give the corresponding methyl gibberate (78a) which upon reduction



would afford the alcohol (103). Tschugaev elimination would then give the olefinic compound (104). If tritium was not lost in the transformation (103-104) then this would show that the vinyl group in (7) rotated in a clockwise fashion upon closure of ring D. If tritium was lost in this transformation then an anti-clockwise rotation would be indicated.

The precursor of (7a) which would be fed to Gibberella fujikuroi to test the stereochemistry of the CD ring closure described above could be synthesised as shown below⁸⁹.



Oxidation of manool with potassium permanganate has been shown^{91,92} to give the ketone (105). A Grignard reaction as shown would result in the formation of a mixture of manools (31b) diastereoisomeric at C-13 with the tritium trans- in the vinyl position as shown. The Grignard reagent would be prepared by addition of magnesium to the adduct of acetylene and TBr. Addition of hydrogen halides to simple acetylenes has been shown⁹³ to be stereospecific-trans.

Although the incorporation of the diastereoisomeric mixture would most likely result in the incorporation of one diastereoisomer, conversion of each diastereoisomer is possible since the separation of this diastereoisomeric set is readily effected by chromatography on $\text{SiO}_2\text{-AgNO}_3$ ⁹⁴.

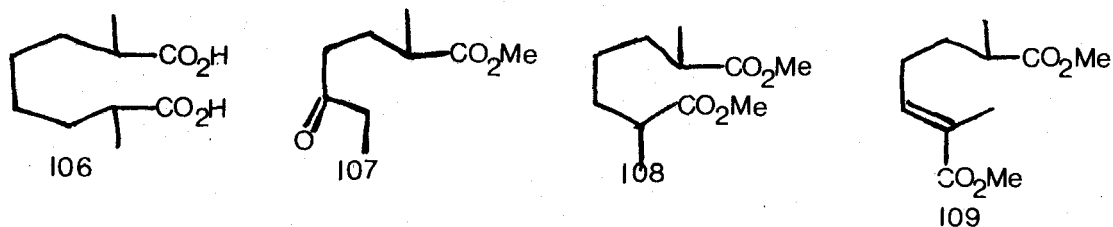
With the above stereochemical point settled, the only remaining question of stereochemistry arising in the trans-
 ition of a mevalonate C-5 to a gibberellin C-14 could be solved by the incorporation of 5-(R)- $[5\text{-}^3\text{H}, 2\text{-}^{14}\text{C}]$
 mevalonate and the corresponding(S) mevalonate into gibbe-

rellic acid. Degradation in the manner described above to determine the stereochemistry of the hydrogens at C-14 in gibberellic acid thus derived would then yield the absolute stereochemistry of the $\Delta_{13(14)}$ to $\Delta_{15(16)}$ isomerisation which must occur during the formation of the CD ring system of gibberellic acid.

The Metabolism of Gibberellic Acid

Of more general interest would be an investigation of the metabolism of the gibberellins. Gibberellic acid is well known for its growth regulating properties and as such has potential use for control of the development of plants, especially those used as food sources. It is therefore considered important to determine the effects of ingestion of gibberellins and their metabolites. A perusal of the literature reveals that little work has been done on diterpene metabolism.

Biogenetic degradation of dehydroabietic acid⁹⁵ and its methyl ester⁹⁶ has been shown to result in hydroxylation of rings A and B leading eventually to their cleavage to give a mixture of low molecular weight carboxylic acids (106-109)



These results indicate that the organism uses the diterpene skeleton as a source of carbon. Similar studies on the gibberellins might be expected to give a similar result, and an examination of the toxicity of the resulting degradation products would be worthwhile.

CHAPTER 6
EXPERIMENTAL

Purification of Manool(31)

Manool(20g) as supplied by Koch-Light was purified by chromatography on silica gel impregnated with 10% AgNO_3 (500g). Unidentified impurities were eluted by petroleum ether through 60% benzene in petroleum ether and pure manool (13.5g) was eluted by benzene.

Reaction of Manool with Acetic Acid

A solution of Manool(12.5g) in glacial acetic acid (125 ml) was heated under reflux for 1 hour. The reaction mixture was poured into water (400 ml), neutralised with NaHCO_3 and extracted several times with ether. The ether extracts were combined, washed with water, dried over anhyd. MgSO_4 and evaporated to give a brown oil (11.3g) which was chromatographed on act. 1 neutral alumina (300g): petroleum ether eluted a mixture of hydrocarbons (3.50g); 10% benzene in petroleum ether eluted Δ^{13} -manool acetate(33; R=Ac) (3.23g) whose spectra were in agreement with published data⁷⁸; 60% benzene in petroleum ether eluted unreacted manool(2.76g).

The mixture of hydrocarbons was chromatographed on silica gel impregnated with 10% AgNO_3 (200g): 10% benzene in petroleum ether eluted 8,13-burnabadiene(45) (109 mg; for spectral data see Ch. 4) and mixture A

(840mg); 15% benzene through 20% benzene in petroleum ether eluted mixture B (1.263g); benzene eluted cis-biformene (35) (532mg); 3% chloroform through 20% chl. in benzene eluted trans-biformene (34) (517mg). The spectra of the two biformenes were in agreement with literature spectra^{53,78,79}. In a subsequent repeat of this experiment using larger amounts of materials, 40% benzene in petroleum ether eluted 7,15-pimaradiene (39) (255mg; for spectral data see Ch. 4).

Mixture A was re-chromatographed on silica gel impregnated with 10% AgNO₃ (25g): petroleum ether eluted 8,13-burnabadiene (45) (70mg) and 8,15-pimaradiene, (37) (500mg; for spectral data see Ch. 4) identical on columns A and B with an authentic sample.⁵⁰ In the repeat experiment, 4% benzene in petroleum ether eluted mixture C (630mg) which was separated by prep. VPC on column C at 183°C into 8,15-pimaradiene (188mg) and mixture D (106mg). Mixture D was purified by prep. VPC on column D at 150°C to give isopimaradiene (42) (50mg; for spectral data see Ch. 4)

Mixture B was separated by prep. VPC on column E at 130°C to give 8,15-isopimaradiene (40) (125mg; for spectral data see Ch. 4) m.p. 50-1°C (lit.⁵⁰ m.p. 51-2.5°C), identical on columns A and B with an authentic

sample⁵⁰, and sclarene, whose spectral data were in agreement with those published⁷⁹.

Diels-Alder Adduct of Sclarene with Tetracyanoethylene

A solution of sclarene (36) (100mg; 0.37 m. moles) and TCNE (47mg; 0.37 m. moles) in EtOAc (10ml) was refluxed for 24 hours, when the solution was evaporated and the solid product recrystallised from ethanol-water to give the adduct as white needles (80mg) m.p. 138-9°C (lit.⁷⁹ m.p. 115°C) whose spectral properties were identical with previously published findings⁷⁹. TLC on silica gel impregnated with 10% AgNO₃ and eluting with benzene indicated a single component R_f. = 0.6. The white needles were sublimed at 120°C / .25mm. to give an analytical sample (12mg) m.p. 139-9.5°C. Found: C, 77.52; H, 7.93; residue, 2.20. C₂₆H₃₂N₄ requires: C, 77.96; H, 8.05.

Reaction of Manool with Formic Acid

A solution of manool (14g) in 97% formic acid (140ml) was refluxed for 1 hour, when the solution was poured into water (450ml), neutralised with K₂CO₃ and extracted several times with ether. The ether extracts were combined, washed with water, dried over anhyd. MgSO₄ and evaporated to give a brown oil (11.7g).

A portion of the oil (10.7g) was chromatographed on silica gel (500g): petroleum ether eluted a mixture of hydrocarbons (7.26g); chlf. eluted mixture A (2.287g) ν_{\max} 1730s, 1170s cm^{-1} no O-H.

The mixture of hydrocarbons was chromatographed on silica gel impregnated with 10% AgNO_3 (350g): petroleum ether through 10% benzene in petroleum ether eluted unidentified mixtures (799 mg); 10% benzene in petroleum ether eluted mixture B (1.316g); 10% benzene in petroleum ether through 40% benzene in petroleum ether eluted a mixture of 8,15-pimaradiene (37) and 8,15-isopimaradiene (40) (1:1, 4.623g); chlf. eluted 7,15-pimaradiene (39) (100mg).

A solution of mixture A in dry ether (100ml) was added dropwise with stirring to LiAlH_4 (0.5g) in dry ether (200ml) during 30 min. and the reaction mixture refluxed for 30 min. Excess reagent was destroyed with water, the solution filtered, dried over anhyd. MgSO_4 and evaporated to give a yellow oil (1.79g) ν_{\max} 3450 cm^{-1} , no c=o. The yellow oil was chromatographed on silica gel (50g): 20% benzene in petroleum ether eluted hiban-14 α -ol (400mg) m.p. 110.5-12 $^{\circ}\text{C}$ (lit.⁵⁰ m.p. 114-5 $^{\circ}\text{C}$) whose physical and spectral properties were identical with those published⁵⁰; 60% benzene in petroleum ether

eluted the known Δ 13-manool^{51,78} as an impure oil (527mg).

Mixture B was separated by prep. VPC on column C at 186°C into 13-epi-5(10),15-rosadiene (44) (211mg; for spectral data see Ch. 4), 8,13-burnabadiene (45) (112mg), 8-epi-5(10),15-rosadiene (46) (35mg; for spectral data see Ch. 4) and rimuene (48) (25mg; for spectral data see Ch. 4).

Hiban-14 α -yl Acetate

Hiban-14 α -ol(100mg) was dissolved in pyridine (3 drops), acetic anhydride (5 drops) added and the reaction mixture allowed to stand at 100°C for 28 hours. The mixture was then allowed to stand over P₂O₅ and NaOH in a vacuum dessicator for 20 hours, whereupon hiban-14 α -yl acetate crystallised (100mg) m.p. 84-5°C (lit.⁵⁰ m.p. 85-6°C). The spectra and physical properties were identical with those published.⁵⁰

Hiban-14 α -yl Formate. From Hiban-14 α -ol

Hiban-14 α -ol (100mg) was dissolved in HCl (4mg of 38% soln.) and formic acid (270mg of 97% solution). The reaction mixture was allowed to remain in a sealed tube at 90°C for 18 hours whereupon it was added to water and extracted several times with ether. The ether extracts were combined, washed with NaHCO₃ solution and water, dried over anhyd. MgSO₄ and evaporated to give a colourless oil

which showed only one spot on TLC and a single peak on VPC column F at 190°C v_{\max} 1730s, 1175s cm^{-1} . NMR spectrum (τ): 1.80 (sharp singlet, 1H, formyl hydrogen), 5.55 (sharp singlet, 1H, H-C-O-), 9.07, 9.13, 9.17, 9.20 (sharp singlets, 4CH₃ groups). m/e 318 (P⁺)

Hiban-14 α -yl Formate. From 8,13-Burnabadiene

8,13-Burnabadiene (45) (50mg) was refluxed with formic acid (1ml) for 1 hour. The reaction mixture was neutralised with 10% Na₂CO₃ solution and extracted several times with ether. The ether extracts were combined, washed with water, dried over anhyd. MgSO₄ and evaporated to give hiban-14 α -yl formate (50mg) identical in all respects with the sample prepared above.

Hydrogenation of 8,13-Burnabadiene

A solution of 8,13-burnabadiene (48mg) in EtOAc (3ml) was shaken with Pt on charcoal (30mg) under 70 p.s.i.g. H₂ for 4 days. The catalyst was removed by centrifugation and the supernatant evaporated to give 8-burnabene (89) as a white solid (46mg) m.p. 60-62°C. This product gave a positive tetranitromethane test and a mass spectrum which possessed no peak at m/e =272. (For spectral data see Ch. 4).

Formic Acid Treatment of 8,15-pimaradiene (37)

A solution of 8,15-pimaradiene (880mg) in 97% formic

acid (10ml) was refluxed for 6 hours. The reaction mixture was poured into water (100ml), neutralised with Na_2CO_3 and extracted several times with ether. The ether extracts were combined, washed with water, dried over anhyd. MgSO_4 and evaporated to give a brown oil (806mg), a portion of which (680mg) was chromatographed on silica gel impregnated with 10% AgNO_3 (40g): 3% benzene in petroleum ether eluted 5(10),12-abietarosadiene(47) (27mg; for spectral data see Ch. 4) and 8-epi-5(10),15-rosadiene (46) (74mg; for spectral data see Ch. 4); 5% benzene in petroleum ether eluted a mixture(108mg) which was purified by prep. VPC on column D at 150°C to give 5(10),15-rosadiene (43)^{51,72} (34mg; for spectral data see Ch. 4)

Hiban-14-one

Chromic oxide (.37g; 3.7m moles) was added with stirring to ice-cold anhyd. pyridine(12ml) during 10 min. A solution of hiban-14 α -ol (372mg; 1.33 m. moles) in anhyd. pyridine (3ml) was added in one portion whereupon the reaction darkened. The reaction mixture was stirred at 0°C for 30 min. and at room temperature for 8 hours then poured into water (60ml) and extracted several times with ether. The ether extracts were combined and evaporated under vacuum until only a few ml. of material remained. This residue was dissolved in ether and washed

quickly with 2% NaHSO₄ solution until the washings remained acidic. The ether solution was then washed with water, dried over anhyd. MgSO₄ and evaporated to give hiban-14-one (348mg) whose spectra and physical properties were identical with those published⁵⁰.

Hibane

Sodium (400mg) was dissolved in diethylene glycol (15ml) under dry He. Hydrazine (95%, 2.0ml) and hiban-14-one (157mg) were added, the mixture refluxed at 180°C for 2 hours, the temperature raised to 217°C by distillation and reflux continued for 15 hours. The mixture and distillate were poured into water (100ml) and extracted several times with ether. The ether extracts were combined and evaporated and the residue dissolved in petroleum ether, washed with water, dried over anhyd. MgSO₄ and evaporated to give hibane (129mg) whose properties and spectra were identical with published data⁵⁰.

Hydrogenation of 5(10),12-Abietarosadiene(47)

Impure 5(10),12-abietarosadiene(47) (6mg) was purified by prep. VPC on column D at 130°C. The pure compound was hydrogenated in EtOAc (2ml) over Pt on charcoal (20mg) at 78 p.s.i.g. H₂ with shaking for 5 d. The catalyst was removed by centrifugation and

the reaction mixture evaporated to give a clear oil (1.9mg) whose VPC on column B showed only one peak. This had a retention time different from the starting material. Both starting material and product had retention times different from that of hibane described above.

Methyl Sandaracopimarate

A dry ether solution of diazomethane⁸⁰ (3ml; 3.9m. moles) was added with cooling and stirring to a solution of sandaracopimaric acid* (130mg; 0.43m. moles) in dry ether (2ml) and the solution allowed to stand overnight. The reaction mixture was evaporated to give methyl sandaracopimarate (103mg) m.p. 63-4°C (lit.⁸¹ m.p. 64-5.5°C) whose spectra were identical with those previously reported^{50,80,81}.

Sandaracopimarol

A solution of methyl sandaracopimarate (90mg; 0.29 m moles) and LiAlH_4 (23mg; 0.6 m moles) in dry ether (10ml) was refluxed overnight. The excess reagent was destroyed with water, the solution filtered, dried over anhyd. MgSO_4 and evaporated to give sandaracopimarol as a clear

*A sample of sandaracopimaric acid was generously supplied by Dr. J. W. ApSimon

oil (84mg) whose spectra are identical with those published^{50,81}.

Sandaracopimaral

Chromic oxide (84 mg; .84 m. moles) was added with stirring to ice-cooled anhyd. pyridine (3ml) during 10 minutes. A solution of sandaracopimarol (84mg; .29 m. moles) in anhyd. pyridine (1ml) was added in one portion. The reaction mixture was stirred at 0^o C for ½ hour and at r.t. for 2 hours, then poured into water (20ml) and extracted several times with ether. The ether extracts were combined, washed with water and evaporated until only a very small amount of material remained. The residue was re-dissolved in ether, washed quickly with 2% NaHSO₄ solution, water, 2% K₂CO₃ solution and again with water, then dried over anhyd. MgSO₄ and evaporated to give sandaracopimaral (67mg) whose spectra and properties were identical with previously reported data⁸¹.

Sandaracopimaral semicarbazone

To semicarbazide hydrochloride solution⁸³ (0.12ml; 0.234 m moles) was added sandaracopimaral (67mg; 0.234 m moles) in methanol (1ml) and 1d. of pyridine. The reaction mixture was heated gently with vigorous stirring. After a few minutes a white precipitate appeared.

Heating was discontinued and stirring continued for 1 hour, then the reaction mixture allowed to stand at r.t. for a further hour. The reaction mixture was filtered and the residue washed with methanol to give sandaracopimaral semicarbazone as a white solid (35mg) m.p. 212-4°C (lit.⁸¹ m.p. 218-20°C).

Sandaracopimaradiene

A mixture of sandaracopimaral semicarbazone (35mg) and diethylene glycol (2ml) was heated to 100°C with stirring under dry He. Solid KOH (0.45g) was added and the temp. raised to 205°C. The reaction mixture was maintained at this temp. with stirring under dry He for 3 hours after which it was poured into water (30 ml) and extracted several times with ether. The ether extractions were combined, washed with water, dried over anhyd. MgSO₄ and evaporated to give sandaracopimaradiene (41) (6mg; for spectral data see Ch. 4).

Methyl Pimarate

An ether solution of diazomethane⁸⁰ (30ml; 0.039 moles) was added with cooling and stirring to a solution of pimaric acid* (1.017g; 3.3 m moles) in dry ether

*Pimaric acid is commercially available from Koch-Light.

(20ml). The solution was allowed to stand overnight and then evaporated to give methyl pimarate (1.021g) m.p. 65-6°C (lit.⁸¹ m.p. 69°C) possessing previously reported spectral properties^{50,81,82}.

Pimarol

A solution of methyl pimarate(1.066g) in dry ether (70ml) was added drop-wise with stirring to LiAlH_4 (0.4g) in dry ether (100ml) and the reaction mixture refluxed overnight. Excess reagent was destroyed with water, the solution filtered, dried over anhyd. MgSO_4 and evaporated to give pimarol (869mg) whose spectral properties were identical with previously published findings⁵⁰.

Pimaral

Chromic oxide (870mg; 8.7 m moles) was added with stirring to ice-cold anhyd. pyridine(30ml) during 10 minutes. A solution of pimarol (869mg; 3.0 m moles) in anhyd. pyridine(10ml) was added in one portion and the reaction mixture stirred at 0°C for ½ hour then at r.t. for 2 hours. The solution was poured into water (200ml) and extracted several times with ether. The ether extracts were combined, washed with water and evaporated until only a few ml. of material remained.

The residue was redissolved in ether, washed quickly with 2% NaHSO₄ solution, water, 2% K₂CO₃ solution and again with water, then dried over anhyd. MgSO₄ and evaporated to give pimaral (626mg) whose spectral characteristics were identical with previously published data^{50,81}.

Pimaral Semicarbazone

To semicarbazide hydrochloride solution⁸³ (1.1ml; 2.2m moles) was added a solution of pimaral (626mg; 2.2 m moles) in methanol (8ml) and pyridine (8 drops). The reaction mixture was heated gently with vigorous stirring. After a few minutes a white precipitate appeared. Heating was discontinued and stirring continued for 1 hour. The reaction mixture was allowed to stand for 1 hour further at r. t. and then filtered to give pimaral semicarbazone (391mg) m.p. 214-7°C (lit.⁸¹ m.p. 213-6°C) which exhibited the same spectral characteristics as previously reported⁵⁰.

Pimaradiene

A mixture of pimaral semicarbazone (391mg) and diethylene glycol (14ml) was heated under dry He to 100°C solid KOH (4.6g) was added and the temperature gradually raised to 205°C where it was maintained under

dry He for 3 hours. The reaction mixture was poured into water (150ml) and extracted several times with ether. The ether extracts were combined, washed with water, dried over anhyd. MgSO_4 and evaporated to give pimaradiene (38) (185mg; for spectral data see ch. 4).

Δ 13-Manool(32)

A solution of Δ 13-manool acetate (33; R=Ac) (4.4g) in dry ether(150ml) was added slowly with stirring to LiAlH_4 (0.9g) in dry ether(300ml) and the reaction refluxed overnight. Excess reagent was destroyed with water and the reaction mixture filtered, dried over anhyd. MgSO_4 and evaporated to give the previously reported^{51,78} Δ 13-manool(32) (3.868g).

Enantio-copaldehyde(51)

Chromic oxide (3.9g; 3.9×10^{-2} moles) was added with stirring to ice-cold anhyd. pyridine(100ml) during 10 min. A solution of Δ 13-manool(3.868g) in anhyd. pyridine(20ml) was added in one portion whereupon the reaction mixture darkened. Stirring at 0°C was continued for $\frac{1}{2}$ hour and at r.t. for 15 hours. The reaction mixture was poured into water(1000ml) and extracted several times with ether. The ether extracts were combined and evaporated until only a few ml. of material remained. The residue was dissolved in ether,

washed with 2% NaHSO₄ solution, water, 2% K₂CO₃ solution and again with water, then dried over anhyd. MgSO₄ and evaporated to give a mixture of cis and trans enantio-copaldehyde(51) (2.995g) ν_{\max} 1678cm⁻¹. NMR spectrum (τ): 0.13(triplet J=8cps, 1H, is actually two superimposed doublets at 0.07 and 0.22, aldehyde proton, cis-trans mixture), 4.20(doublet, J=8cps, 1H, C-14 vinyl hydrogen), 5.18 (singlet, 1H, C-17 vinyl hydrogen), 5.55 (singlet, 1H, C-17 vinyl hydrogen), 7.87 (doublet, J=1cps, 1.5H approx., vinyl CH₃ of trans-isomer), 8.05 (doublet, J=1cps, 1.5H approx., vinyl CH₃ of cis-isomer), 9.14, 9.21, 9.32 (3 sharp singlets, 3CH₃ groups).

Enantio-methyl copalate(52)

A solution of enantio-copaldehyde(51) (4.46g) NaCN (4.13g), acetic acid(1,52g) and freshly prepared⁸⁴ MnO₂ in methanol(90ml) was stirred at r.t. for 12 hours. MnO₂ was removed by centrifugation and the solution evaporated to give an amber solid which was dissolved in water and ether. The aqueous layer was extracted several times with ether and the ether extracts combined, washed with 10% Na₂CO₃ solution, and then water, dried over anhyd. MgSO₄ and evaporated to give a mixture of cis and trans enantio-methyl copalate(52) previously des-

cribed⁸⁵ (3.868g) ν_{\max} 1720 cm^{-1} . NMR spectrum (τ):
4.23(singlet, 1H, C-14 vinyl hydrogen), 5.07(singlet,
1H, C-17 vinyl hydrogen), 5.40(singlet, 1H, C-17 vinyl
hydrogen), 6.28 (singlet, 3H, ester CH_3), 7.81(doublet
 $J=1\text{cps}$, 1.5H approx., vinyl CH_3 of trans-isomer), 8.08
(doublet $J=1\text{cps}$, 1.5H approx, vinyl CH_3 of cis-isomer),
9.12, 9.18, 9.31(3 sharp singlets, 3 CH_3 groups).

Deuterium Exchange of Enantio-methyl Copalate

A solution of enantio-methyl copalate(2.533g) and sodium methoxide(3.7g) in CH_3OD (250ml) was stirred at r.t. for 2½ hours and then the reaction mixture evaporated to dryness at 40°C. The residue was dissolved in D_2O and dry ether. The D_2O layer was extracted several times with dry ether, the ether layers combined, washed with D_2O , dried over anhyd. MgSO_4 and evaporated to give partially deuterated enantio-methyl copalate(53)(1.477g). Integration of C-14 vinyl hydrogen vs. ester CH_3 and C-17 hydrogens indicated 50% deuteration at C-14 and the two doublets corresponding to vinyl CH_3 were very small.

Deuterated Δ^{13} -Manool(54)

A solution of deuterated enantio-methyl copalate (1.477g) in dry ether(54ml) was added dropwise with stirring to LiAlH_4 (0.4g) in dry ether(100ml) and the reaction

refluxed overnight. Excess reagent was destroyed with water, the solution filtered, dried over anhyd. MgSO_4 and evaporated to give deuterated Δ^{13} -manool. The NMR spectrum again indicated 50% deuteration at C-14 and the singlet at τ 8.37 corresponding to vinyl CH_3 was buried in the background. The doublet $J=7\text{cps}$ centred at τ 5.95 corresponding to the two C-15 protons had collapsed to a singlet. In the mass spectrum the isotopic distribution around the parent peak was the same as that for the deuterated enantio-methyl copalate(53).

Formic Acid Treatment of Deuterated Δ^{13} -Manool

A solution of deuterated Δ^{13} -manool(1.3g) in formic acid(13ml) was refluxed for 1 hour when the solution was poured into water (50ml) neutralised with K_2CO_3 and extracted several times with ether. The ether extracts were combined, washed with water, dried over anhyd. MgSO_4 and evaporated to give a yellow oil (1.23g) which was chromatographed on silica gel (70g): petroleum ether eluted a mixture of hydrocarbons (542mg); 10% benzene in petroleum ether eluted a mixture of hiban-14 α -yl formate and unidentified impurity A(254mg); A (76mg) was also eluted by this solvent. The above mixture was re-chromatographed on silica gel(12.5g) to

give hiban-14 α -yl formate contaminated with A and VPC on column F at 170°C showed that the tetracyclic ester was 72% pure. Mass spectral analysis showed that the isotopic distribution around the parent peak was unchanged. Integration of the NMR spectrum of this hiban-14 α -yl formate showed that the peak due to the 14 β hydrogen was 50% of that due to the formate hydrogen. The NMR spectrum of the impurity A possessed no absorption in these two regions.

Studies of Acid Catalysed Reactions of Hydrocarbons

To a known weight (10-70mg) of pure hydrocarbon was added a known weight of octadecane, a sample of the mixture passed through VPC column A or B and the area of the peak for each component measured. To this mixture was added the appropriate acid(2ml) and the mixture heated under reflux for the appropriate length of time. The solution was then poured into water(20ml), neutralised with Na₂CO₃ and extracted several times with ether. The ether extracts were combined, washed with water, dried over anhyd. MgSO₄ and evaporated. A sample of the residue was passed through one of the above VPC columns, and the peak area measured for each component. The components were identified by peak

enhancement from mixed injection. The procedure was repeated as required.

Manoolp -nitrobenzoate

A solution of manool(1.45g; 5 m moles) and p - nitrobenzoyl chloride(1.86g; 10 m moles) in dry pyridine (100ml) was stirred at r.t. for 6 d., after which time the colour of the reaction mixture had changed from yellow to red. TLC on silica gel impregnated with 10% AgNO₃ eluting with benzene showed complete consumption of manool and the presence of a single new product at higher Rf. The reaction mixture was evaporated and the residue filtered through act. III neutral alumina(60g). Benzene eluted a pale yellow solid which was recrystallised from benzene to give manoolp -nitrobenzoate(1.2g) m.p. 149-50°C ν_{\max} 1700cm⁻¹. NMR spectrum(τ): 3.93(quartet $J_{\text{cis}}=10\text{cps}$, $J_{\text{trans}}=18\text{cps}$, 1H, C-14 vinylhydrogen), 4.78 (doublet, $J=18\text{cps}$, 1H, C-15 vinyl hydrogen), 4.82(doublet, $J=10\text{cps}$, 1H, C-15 vinyl hydrogen), 5.20(singlet, 1H, C-17 vinyl hydrogen), 5.52(singlet, 1H, C-17 vinyl hydrogen), 8.30(singlet, 3H, C-13 methyl), 9.13, 9.20, 9.33(3 sharp singlets, 3CH₃ groups). m/e 439 (P⁺).

Attempted Solvolysis of Manool p -nitrobenzoate

A solution of manool p-nitrobenzoate(1g) in 80%

acetone-water(10ml) was refluxed for 1 hour. After removal of the solvent, unreacted manool p-nitrobenzoate was recovered. The same result was obtained upon using chl_f., 90% ethanol-water or 80% DMF-water as solvent.

Bromination of Enantio-methyl copalate(52)

To a solution of enantio-methyl copalate(52) (2.786g; 8.77 m moles) and pyridine(0.70g; 8.77 m moles) in dry methylene chloride(50ml) at 0°C was added dropwise with stirring, a solution of bromine (1.40g; 8.77 m moles) in dry methylene chloride(25ml) during 8 hours. The reaction mixture was allowed to stir at r.t. overnight, and evaporated to give a yellow oil and a yellow gum. The residue was washed several times with ether and the ether fractions combined, washed with 2% NaHSO₄ solution and then with water, dried over anhyd. MgSO₄, and evaporated to give 9β,17-dibromo-enantio-methyl copalate(61) as a yellow oil (2.149g). TLC on silica gel eluting with benzene showed two major spots at the same R_f. as the two spots corresponding to cis and trans-enantio-methyl copalate. IR spectrum: peak at $\nu_{\max} 900\text{cm}^{-1}$ (C=CH₂) disappeared. NMR spectrum(τ): 5.07, 5.40 (C=CH₂) disappeared, 9.31(C-10CH₃) shifted to 8.93*; 6.11, 6.17

* See footnote p. 144

(2 singlets, 2H, $-\text{CH}_2\text{Br}$).

Dehydrobromination of 9 β -17-Dibromo-enantio-methyl copalate(61)

A solution of (61) (2.149g; 4.50 m moles) in xylene (10 ml) was added dropwise to a vigorously boiling solution of $\text{P}(\text{OCH}_3)_3$ (558mg; 4.50 m moles) in xylene (10ml). After refluxing for 90 min. the reaction mixture was evaporated to dryness to give a yellow oil (1.979g). TLC on silica gel eluting with benzene showed one major spot. NMR spectrum (τ): 8.93(C-10 CH_3) shifted to 9.13*. Chromatography of the reaction mixture on silica gel (100g) resulted in a number of unidentifiable mixtures and the column gradually turned orange. Only a small fraction(338mg) had the same Rf. on silica gel TLC eluting with benzene as the major spot in the reaction mixture and NMR analysis showed it to be an unidentifiable mixture.

*The 0.38ppm downfield shift of the C-10 methyl group on introduction of a C-8 β -Br in the labdene skeleton compares favourably with the previously reported⁸⁶ 0.27 ppm downfield shift on introduction of a C-8 β -OH. The upfield shift of only 0.20ppm (rather than 0.38ppm) on dehydrobromination is justified by the proximity of the newly introduced $\Delta^8(9)$ double bond.

Reaction⁴⁵ of 8,15-Pimaradiene and 8,15-Isopimaradiene
with $(\text{OCN})_2\text{PdCl}_2$ ⁴⁴

To solutions of 8,15-pimaradiene (37mg; 0.136 m moles) and 8,15-isopimaradiene (37mg; 0.136 m moles) in dry benzene (1ml) was added $(\text{OCN})_2\text{PdCl}_2$ (52mg; 0.136 m moles). The reaction mixtures were examined by VPC on column G after stirring at r.t. for 20 hours and after refluxing for 98 hours. In each case the chromatogram showed the presence of starting material without the appearance of any 8,13-burnabadiene.

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APPENDIX

Description of Equipment and Services

- IR spectra : Perkin Elmer 457 Grating Infrared Spectrophotometer. Samples were run between sodium chloride discs. Oils were run neat and solids as nujol muls.
- NMR spectra : Varian A-56/60A analytical NMR Spectrophotometer. CDCl_3 was used as solvent with TMS as internal standard.
- UV spectra : Cary 14 Recording Spectrophotometer. The solvent was absolute ethanol.
- OR : Perkin-Elmer P22 Spectropolarimeter. The solvent was hexane.
- MS : Hitachi Perkin-Elmer RMU-6E Mass Spectrometer. Inlet temperature: 80°C Ionisation Voltage: 80ev.
- VPC : Analytical, Varian Series 1200 Gas Chromatograph equipped with flame ionisation detector. Preparative, Varian Autoprep 700 equipped with cathrometer detector.
- Column A : 150' x .02" wall coated DEGS.
- Column B : 150' x .02" wall coated Ucon oil LB-550-X (Perkin-Elmer liquid phase letter designation R)

Column C : 20' x 3/8" 30% DEGS
Column D : 5' x 1/4" 20% DEGS
Column E : 6' x 1/2" 20% XF 1150
Column F : 5' x 1/8" 5% DEGS
Column G : 6' x 1/8" 20% DEGS

Elemental Analysis: Alfred Bernhardt, Microanalytisches
Laboratorium, Mulheim, Germany.