STRUCTURE AND STEREOCHEMISTRY OF THE DITERPENES OF JAMAICA-GROWN *HYMENAEA COURBARIL* SEED POD RESIN

by

SIOW-FONG KHOO

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Name: Siow-Fong Khoo

Degree: Master of Science

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Examining Committee:

Dr. A.C. Oehlschlager
Senior Supervisor

Dr. A.M. Unrau
Examining Committee

Dr. C.H.W. Jones
Examining Committee

Dr. J. Rahe
Department of Biological Sciences

Date Approved: 25 Sept., 1972

ABSTRACT

The isolation and structure determination of four new bicarbocyclic diterpenes (**76**, **85**, **86** and **93**) from Jamaica-grown *Hymenaea courbaril* seed pod resin is described. In addition, cyclosativene, caryophyllene, α-himachalene, selina 4(14),7(11)-diene were identified as major components of the sesquiterpene fraction while β-bourbonene, calarene, selina 4(14),7-diene, humulene, δ-cadinene, α-calcorene and α-muurolene were identified as minor components. The diterpene composition of this resin is more highly rearranged than those isolated from the trunk resin of the same tree.

To

My Parents and Che-Young

ACKNOWLEDGEMENTS

The author wishes to express her sincere thanks to Dr. A.C. Oehlschlager for his guidance and advice during the course of her work. Acknowledgement is also made to:

Dr. N. Anderson for samples of sesquiterpenes (humulene, α-copaene, γ-cadinene, α-muurolene, γ-muurolene and β-cadinene) and for the GLC analysis of a chromatographed *Hymenaea courbaril* fraction.

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ABBREVIATIONS

ATP	Adenosine triphosphate
CD	Circular dichroism
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
Eq	Equatorial
$Eu(fod)_3$	[tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octane dione)Eu(III)]
FPP	Farnesyl pyrophosphate
GLC	Gas liquid chromatography
IPP	Isopentyl pyrophosphate
IR	Infrared
M.S.	Mass spectrum
NBS	N-Bromosuccinimide
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
Pet. ether	Petroleum ether
Quat	Quaternary
Tert	Tertiary
TLC	Thin layer chromatography
UV	Ultra violet

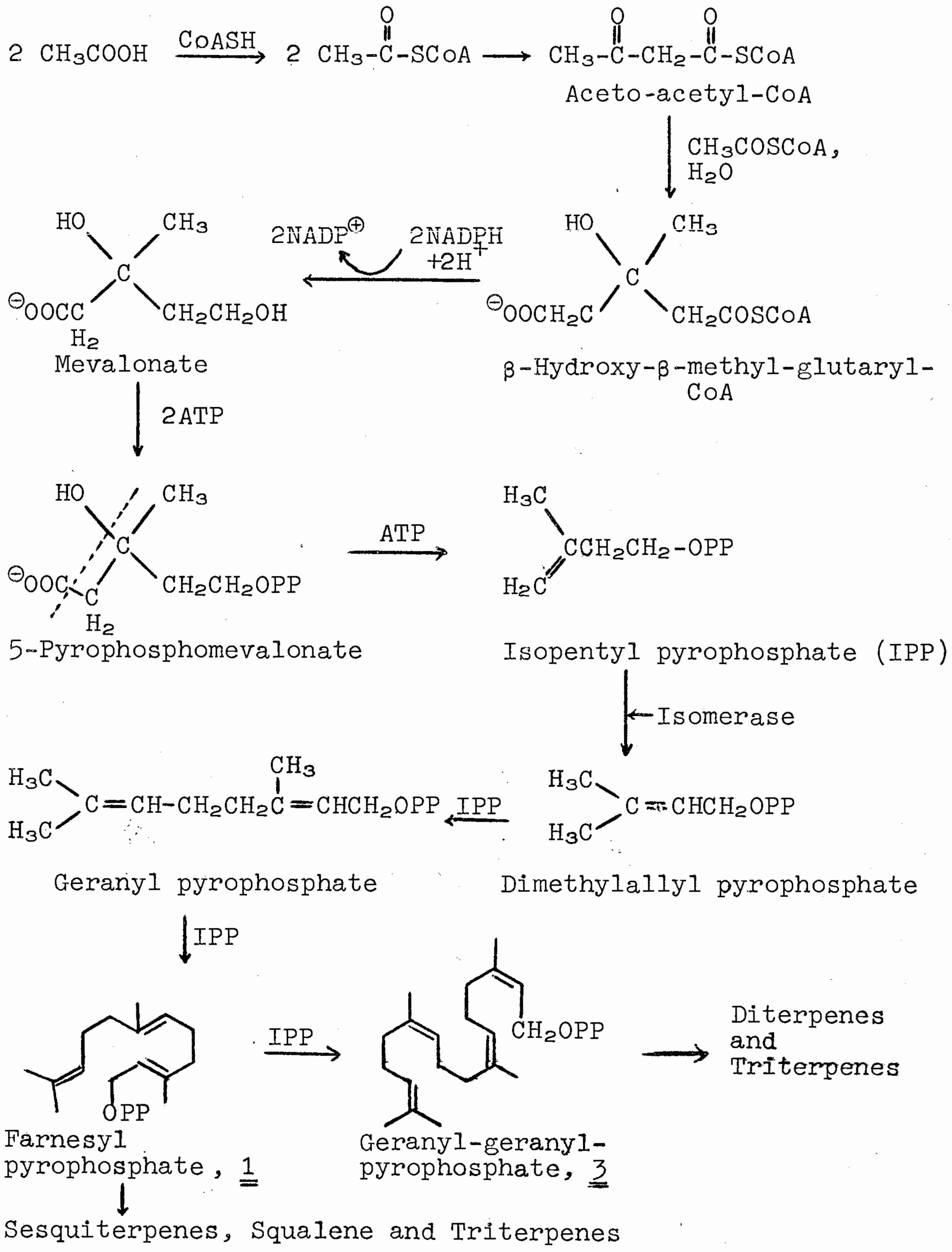
Chapter 1

Introduction

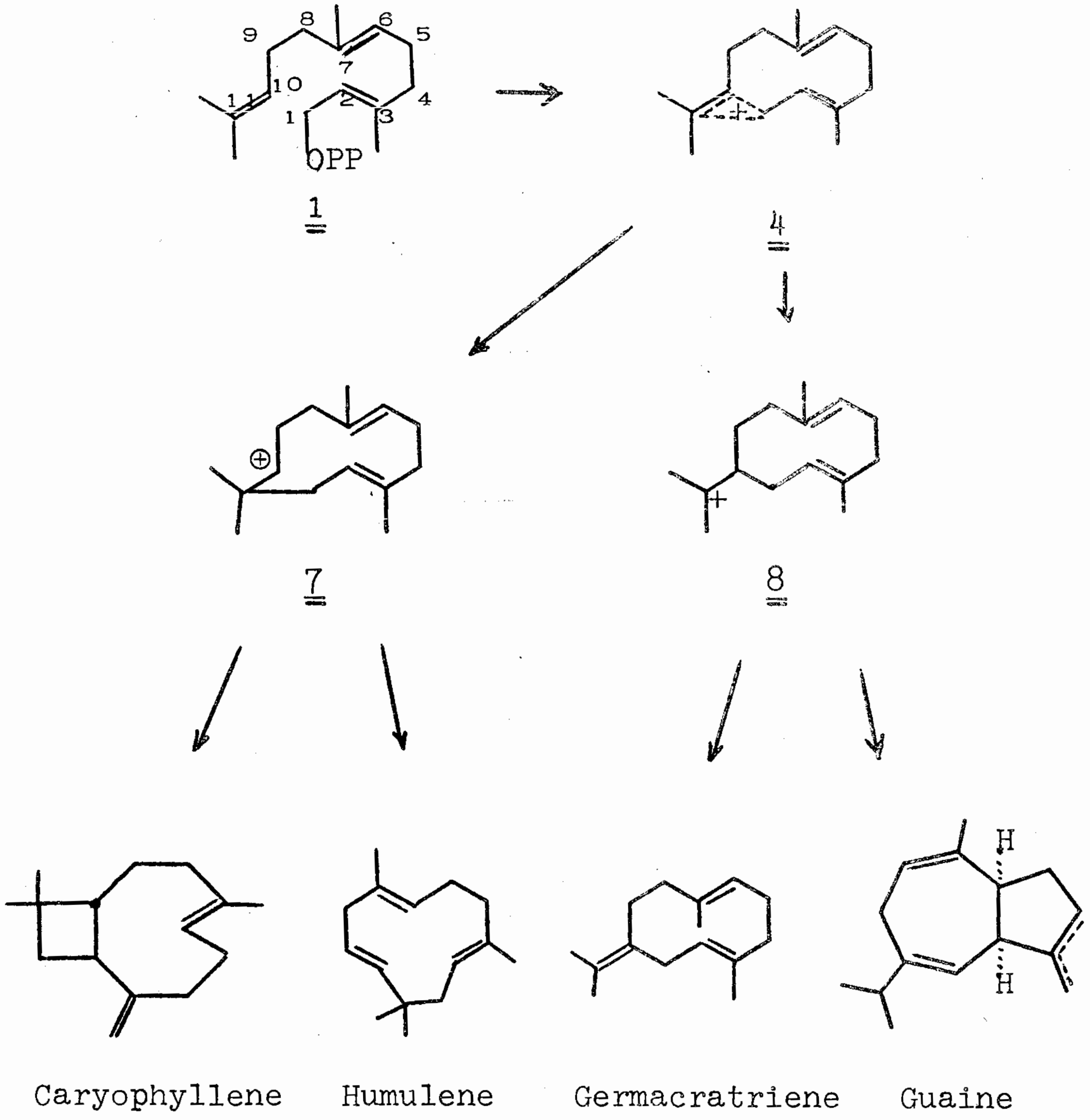
The polyisoprenoids (terpenes) are widely distributed in nature. Their biogenesis involves the conversion of acetate to mevalonate and thence via isopentenyl pyrophosphate to acyclic 1,5-polyenes. Cyclizations and rearrangements of these acyclic polyenes provide reasonable routes to most of the carbocyclic skeletons encountered in nature. The acyclic intermediate leading to the sesquiterpenes, the C_{15} polyisoprenoids, is farnesyl pyrophosphate (FPP), **1**, (Scheme 1)[1]. Cyclization of FPP is considered to proceed from a variety of conformations and configurations (Scheme 2)[2]. Cyclization of the *all-trans* FPP, **1**, or the *trans-cis* FPP, **2**, to either the central or terminal double bonds leads to the cations **7** - **12** through the intermediacy of the nonclassical cations **4** - **6**[2]. Further rearrangement and cyclization of cations **7** - **12** leads to the various classes of sesquiterpenes such as the caryophyllenes, humulene, germacratriene, guaines, cadinenes, muurolenes and himachalenes.

The biogenesis of diterpenes is considered to arise from geranyl-geranyl pyrophosphate **3** (Scheme 3)[3]. Cyclization of **3** is considered to proceed to the biological equivalent of the bicyclic C_8 carbonium ion

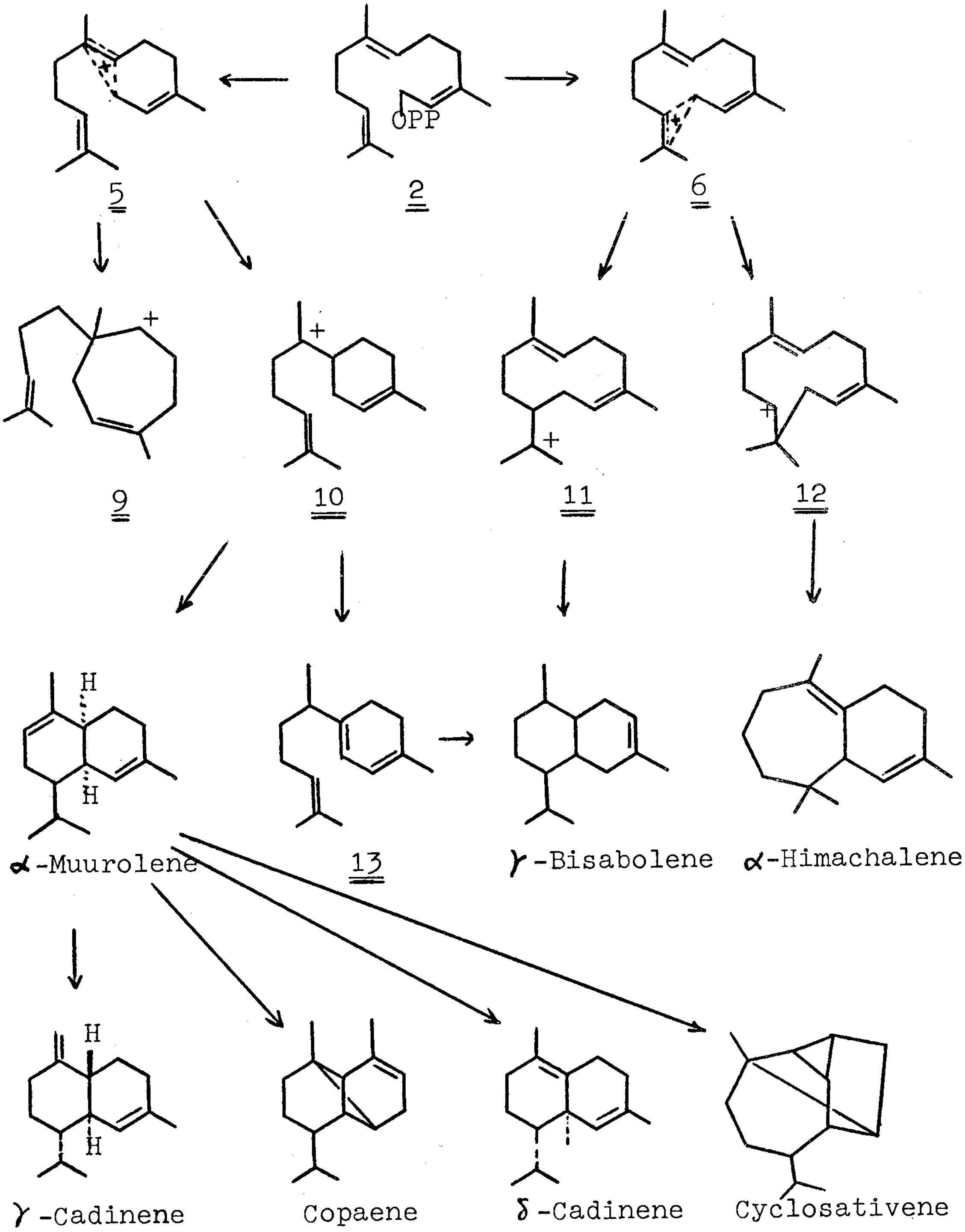
Scheme 1: Biogenesis of Farnesyl and Geranyl-geranyl Pyrophosphate



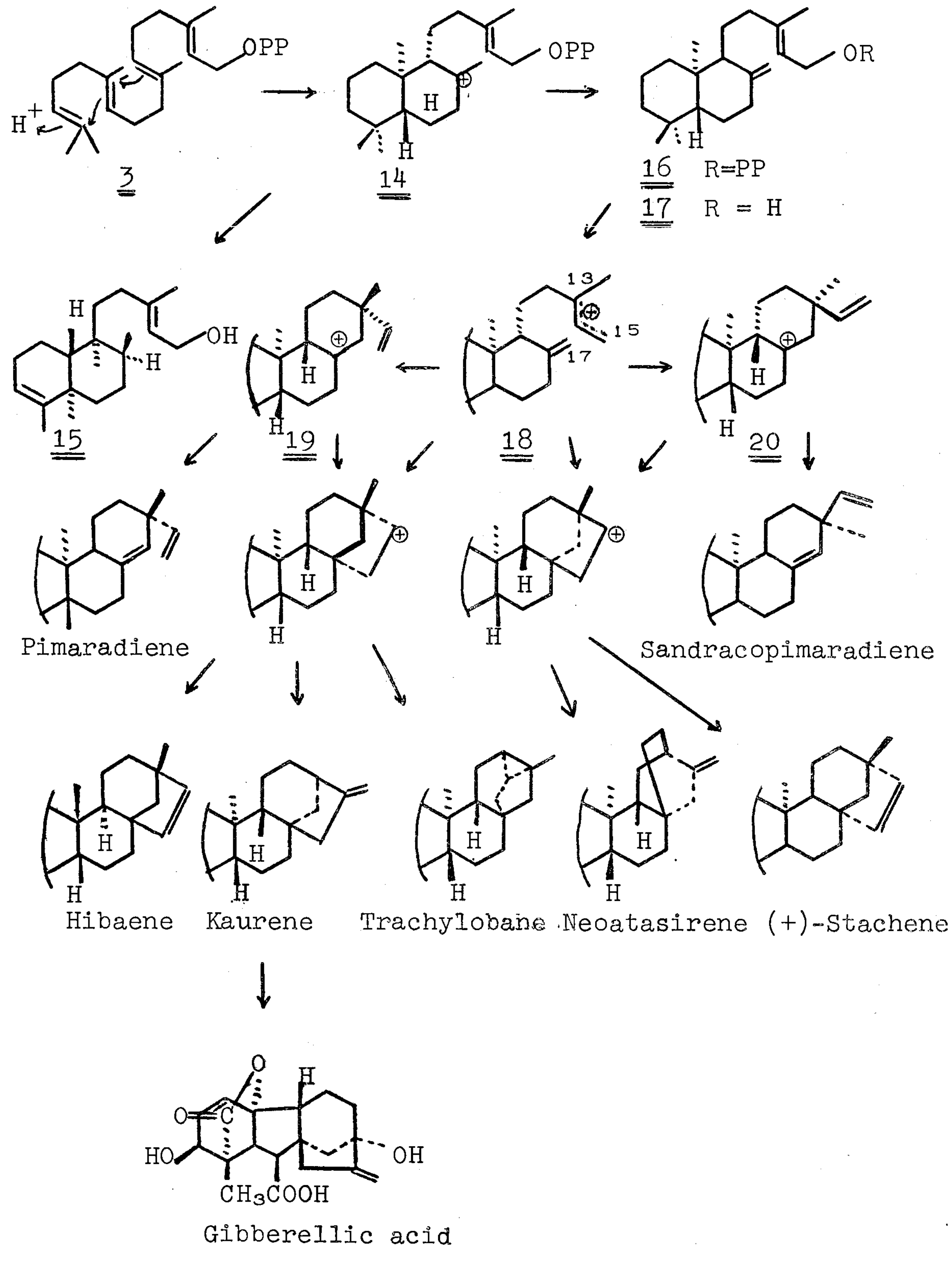
Scheme 2: Biosynthetic Routes to Sesquiterpenes



Scheme 2: Biosynthetic Routes to Sesquiterpenes



Scheme 3: Biosynthetic Routes to Diterpenes



(14, Scheme 3) and thence via hydration, neutralization and rearrangement to the labdenes (e.g. 17) and rearranged labdenes (e.g. 15)[3,4]. This course of events is supported by labelling experiments[4], laboratory cyclization of geranyl-geraniol and enzymatic conversion of 3 to 16 by Gibberella fujikuroi[5]. Further cyclization of 16 provides the tricyclic skeletons of pimaradiene and sandracopimaradiene type, presumably via 18, 19, and 20. These intermediates may, in turn, cyclize to the tetracarbocyclic skeletons of the kaurenes and gibberellins. West has recently shown that kaurene, trachylobane and enantio-sandracopimaradiene are produced from 16 in castor bean seedlings[5a-5c].

Since obvious precursor-product relationships exist in the bioelaboration of the cyclic diterpenoids it would be of interest to determine if plants have developed the ability to produce diterpenes of varying degrees of cyclization in different organs. If this was observed one could begin to consider possible reasons for such differentiation in synthesis of these secondary metabolites. Evidence bearing on this question is difficult to interpret because diterpene content has often been obtained on whole plants. Furthermore most plants investigated to date (even the most primitive, e.g. fungi) possess the ability to produce gibberellins. Since these growth regulating substances are derived from kaurene which is one of the most highly cyclized diterpene skeletons, it is difficult to draw conclusions based on isolation of diterpenes of lesser degrees of sophistication.

A further disadvantage in earlier studies arises from the fact that investigations of diterpene content of plants has not been systematic. Investigations are often on isolated species, justified by an economic (Rutaceae, Dipterocarpaceae, Amherstieae) or pharmaceutical (Euphorbiaceae) interest in a plant. Sometimes even the local abundance (Leguminosae) of a plant is sufficient reason for an investigation.

Ecological and physiological studies of seventeen species of Hymenaea grown in Central and South America have recently been taken up by Dr. J.H. Langenheim and coworkers who are interested in its capacity to secrete trunk resin and possible ecological and evolutionary trends indicated by changes in resin composition[6]. Furthermore, pods of several related species are available for comparison of diterpene composition with that in trunk.

We have investigated the seed pod resin of *Hymenaea courbaril* for both diterpenes and sesquiterpene content to determine if the skeletons of the compounds of these classes of terpenes isolated from the pod are different from those isolated from the trunk resin.

To provide a basis of comparison with other studies we chose to investigate the seed pod resin of *Hymenaea courbaril*. This tree is an *Amherstieae* member of the *Leguminosae* family.

The family *Leguminosae* is one of the largest and most important families from an economic point of view. The seeds of plants in this family are used widely for food. Many of the tropical trees of this family are rich in gums and resins while dyes are obtained from others.

The family is divided into three subfamilies, Caesalpinioideae, Mimosoideae and Faboideae. The Caesalpinioideae can be subdivided into several tribes, the latest revision resulting in the recognition of seven tribes (Table 1)[7].

Investigations of Caesalpinioideae have generally been isolated. Thus in the Dimorphandreae only the genus Erythrophleum has been extensively studied. Alkaloids based on the cassaic acid residue (21, R = H) are widespread in this genus and structures have been isolated which possess oxidation in positions 3, 6, 7, 11, and 12. For example, the alkaloid Erythrophleguine, (22), and cassminic acid (23) have been isolated from the bark of Erythrophleum guineense (G. Don)[8,9].

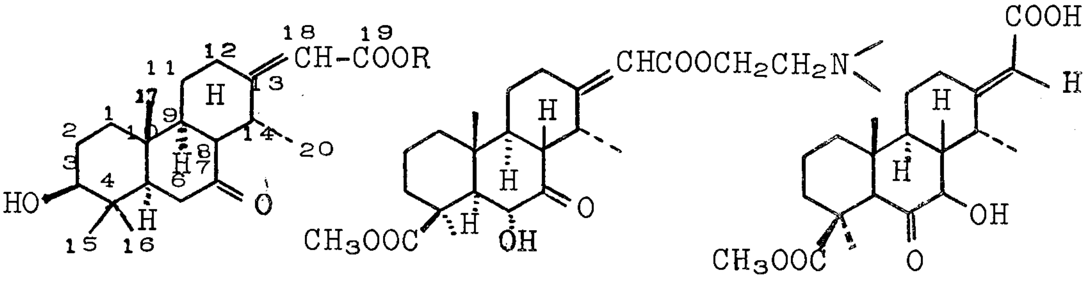


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TABLE 1

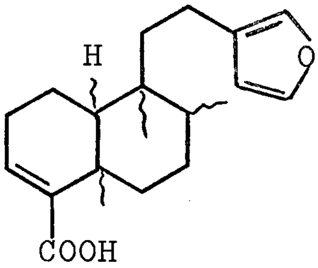
Phylogenetic Relationships within Caesalpinioideae Subfamily

Tribe	Genus	Species	Species investigated for terpenes
Dimorphandreae	Erythrophleum	E. guineense	+
	Dimorphandra	D. gigantea	-
	Mora	M. oleifera	-
	Burkea	B. africana	-
Caesalpinieae	Gleditsia		-
	Gymnocladia		-
	Caesalpinia	C. echinata	-
		C. digyna	-
		C. sappan	-
		C. coriaria	-
		C. pulcherrima	+
		C. bonducella	+
	Haematoxylon	H. brasiletto	-
		H. campechianum	-
	Delonix	D. regia	-
	Mezoneuron	M. Welwitschianum	-
Bauhinieae	Cercis	C. siliquastrum	-
		C. canadensis	-
	Bandeiraea or Griffonia	G. tenniflora	-
	Bauhinia	B. platycalyx	-
		B. siqueiraei	-
Amphimanteae	Amphimas	A. pterocarpoides	-
Cassieae	Ceratonia siliqua		-
	Koompassia		-
	Dialium		-
	Distemonanthus	D. benthamianus	-
	Cassia	C. aphylla	-
		C. angustifolia	-
		C. senna	-
		C. fistula	-
		C. occidentalis	-
		C. auriculata	-

Tribe	Genus	Species	Species investigated for terpenes
Amherstieae	Cynometra	C. sessiliflora	-
	Peltogyne	P. paniculata	-
		P. pubescens	-
	Tachigalia	T. formicarum	-
	Tessmannia		-
	Saraca	S. indica	-
	Copaefera	C. reticulata	-
		C. lansdorfii	-
		C. multijuga	+
		C. officinalis	+
	Gibourtia	G. demeusii	-
		G. copallifera	-
	Colophospherum	C. mopane	-
	Detarium	D. macrocarpum	-
	Trachylobium	T. verrucosum	+
	Brachylobium		-
	Cryptosepalum		-
	Baikiaea	B. insignis	-
	Afzelia	A. africana	-
		A. bijuga	-
	Daniellia	D. oliveri	+
		D. ogea	+
	Eperua	E. falcata	+
	Julbernardia		-
	Humboldtia	H. laurifolia	-
	Tamarindus	T. indica	-
	Amherstia	A. nobilis	-
	Heterostemon	H. mimosoides	-
	Hymenaea	H. courbaril	+
		H. altissima	+
		H. stilbocarpa	+
		H. martiana	+
		H. velutina	+
		H. eriogyne	-
		H. stigonocarpa	+
		H. oblongifolia	+
		H. palustris	-
		H. parvifolia	+
		H. reticulata	+
		H. intermedia	+
		H. adenotricha	-
		H. davisii	-
		H. multiflora	-
		H. rubriflora	-
		H. torrei	-
Swartzieae	Exostyles		-
	zollernia		-
	Mildbraediodendron		-
	cordyla		-

The tribe Amherstieae has been the subject of several investigations and is homogeneous from a chemical point of view. Fresh oleoresin of many copal producing Amherstieae have been shown to contain bicyclic diterpenes possessing the labdane or rearranged labdane skeleton. Thus far, more than two dozen of them have been characterized.

The resin from the heartwood of Copaefera officinalis contains (+)-hardwickic acid (24)[10] but the oleoresin of Hardwickia pinnata contains (-)-hardwickic acid (25) as well as kolavic acid (26), kolavenic acid (27) and kolavenol (28)[11,12].



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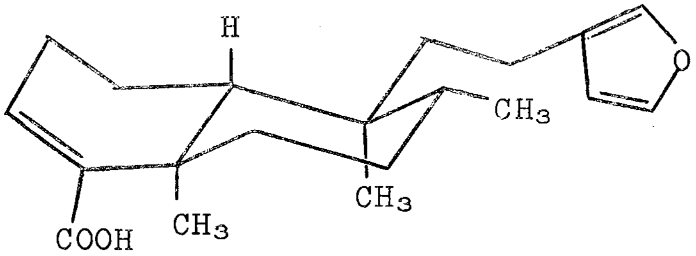

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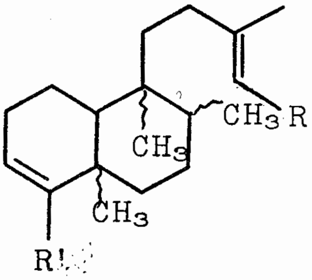
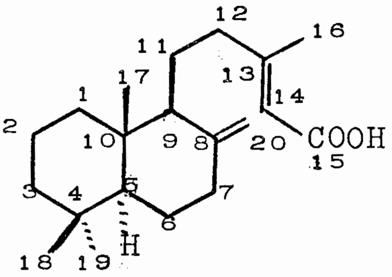


26 R = R' = COOH

27 R = COOH, R' = CH_3

28 R = CH_2OH, R' = CH_3

The oleoresin of Copaifera multijuga contains copaiferic acid (29), (+)-7-hydroxy hardwickic acid (30), copaiferolic acid (31), methyl-11-acetoxylabda-8(20)-13-dien-15-oate (32) and (+)-hardwickic acid (24)[13 - 15].



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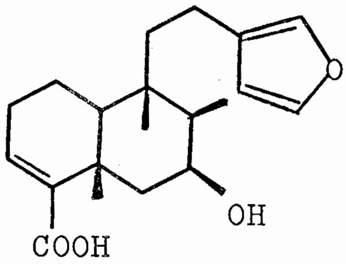

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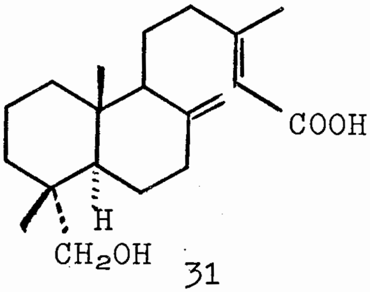

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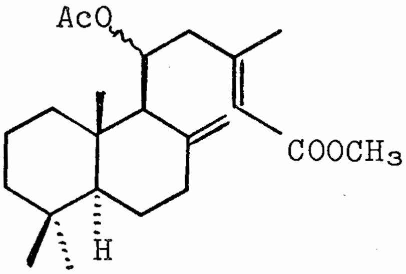

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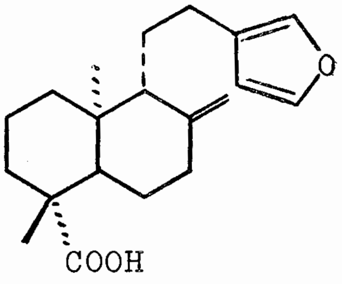
The trunk resin of Daniellic oliveri contains daniellic (33)[16] and oliveric acids (34)[17]. While both possess axial carboxyl groups at C_4, the closely related *Daniellia ogea* contains the C_4 equitorially oxidized ozic acid (35)[18].

The oleoresin of *Eperua falcata* contains eperuic (36) and isoeperuic ($\Delta^{8,9}$-eperuic acid) acids[19,20], while *Prioria copaifera* contains cativic acid (37)[21] which is antipodal, isomeric and epimeric at C_{13} with eperuic acid (36).



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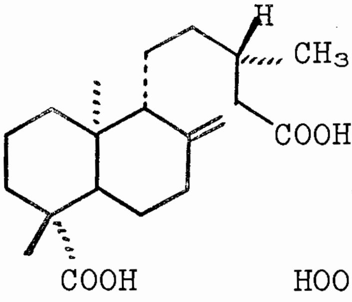

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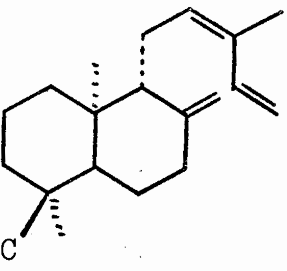

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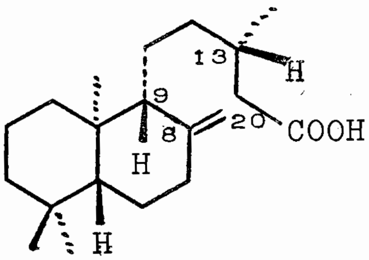

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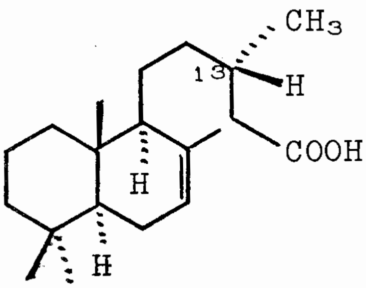

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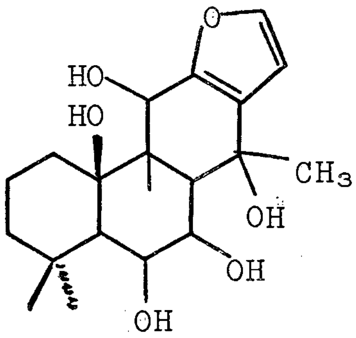
The seeds of *Caesalpinia bonducella* (tribe *Caesalpiniea*) contain γ-caesalpin(38) which possesses the rearranged cassane skeleton[22], and α- (39), β- (40), δ- (41)and ε-caesalpin(42) which possess normal voucapane skeleton with tertiary hydroxyl group at C_{14}[23,24]. The bark of *Caesalpinia pulcherrima*,however, contains χ-caesalpin(43) which has a voucapane skeleton[24].



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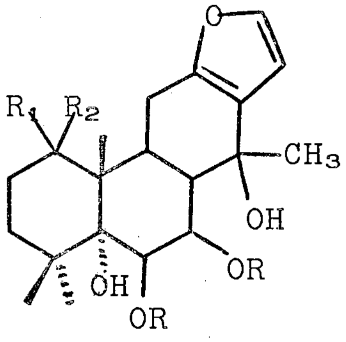
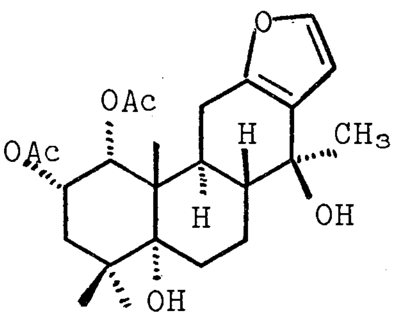


39 R = Ac $\left.\begin{matrix}R_1\\R_2\end{matrix}\right\} = O$

40 R = H $\left.\begin{matrix}R_1\\R_2\end{matrix}\right\} = O$

41 R = H $\left.\begin{matrix}R_1\\R_2\end{matrix}\right\} = H,OH$



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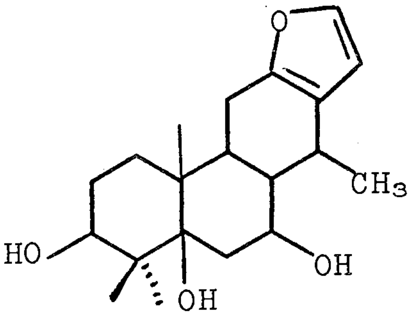

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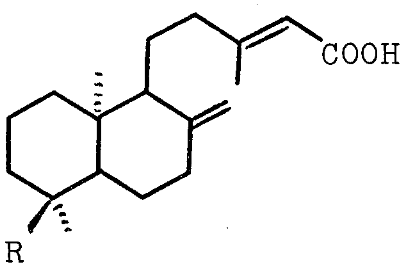
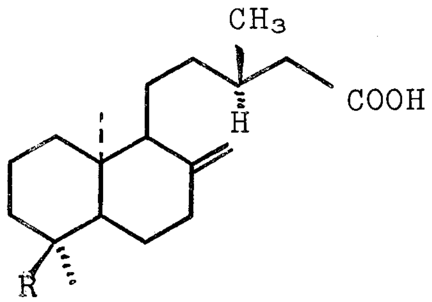
The trunk resin of Trachylobium verrucosum contains bicyclic diterpenes 44 - 54[25] whereas the resin extracted from the seed pods contains principally tetra- and penta-cyclic diterpenes 55 - 61 possessing the (-)-kaurene (62) and (-)-trachylobane (63) skeletons[26] although one bicyclic diterpene, 64, was also isolated from this source[27].



44 R = CH_3

45 R = CH_2OAc

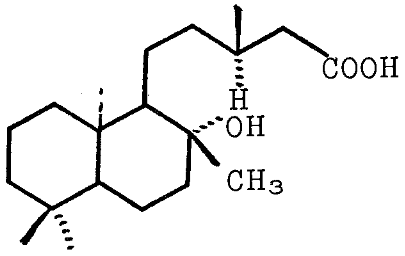


46 R = CH_3

47 R = COOH

48 R = CH_2OH

49 R = CH_2OCH_3



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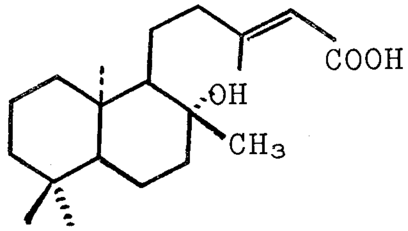

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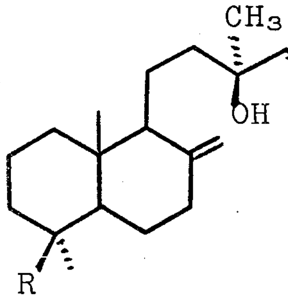
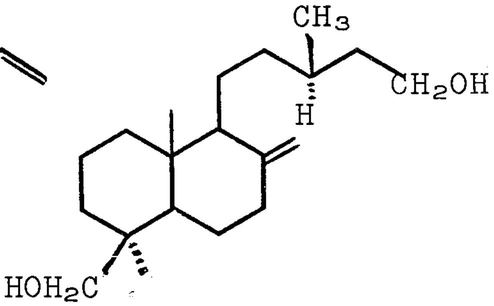


52 R = CH_3

53 R = CH_2OH



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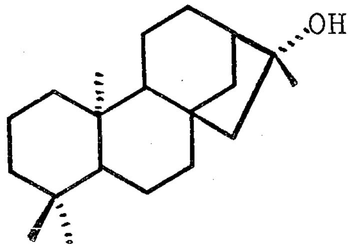

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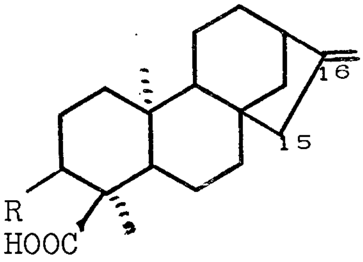
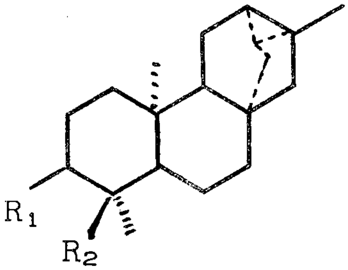


56 R = H

57 R = OAc

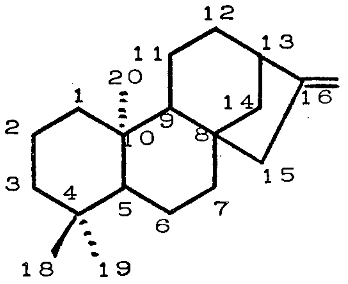
58 $\Delta^{15,16}$ isomer, R = H



59 R = H, R_1 = CH_2OH

60 R_1 = H, R_2 = COOH

61 R_1 = OAc, R_2 = COOH



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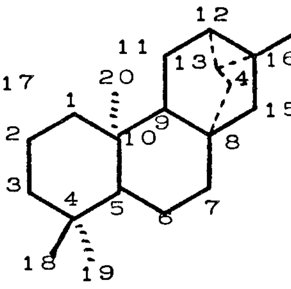

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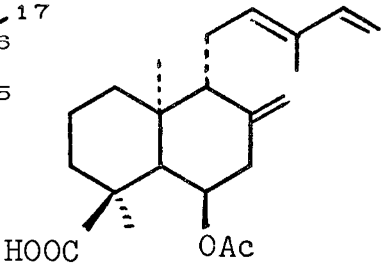

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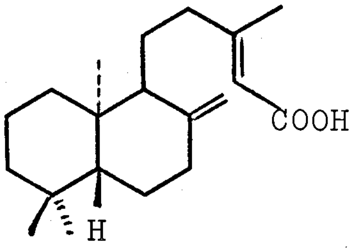
Such examples are rather important since they show that biogenetically related diterpenes isolated from different organs of the same plant may possess different skeletons and that compounds isolated from the trunk resin of each species are more elementary than those of the pod resin[28]. Whether these observations are isolated cases or examples of a general phenomenon can be determined by investigation of different organs of a larger number of species.

We chose to extend these investigations to Hymenaea courbaril seed pod resin since the trunk resin of this tree has previously been shown to contain labdane diterpenes. Moreover, several Hymenaea trunk resins are under investigation in the laboratory of Prof. J.H. Langenheim and pods of several of these species are available for comparisons with Hymenaea courbaril pod resin components.

The trunk oleoresin of H. courbaril, grown in Northern Brazil, has been reported to contain copalic acid (65) as well as its $\Delta^{7,8}$ (66) and $\Delta^{8,9}$ (67) isomers[29] as major constituents.



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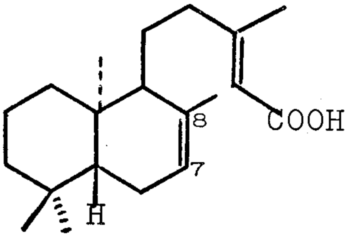

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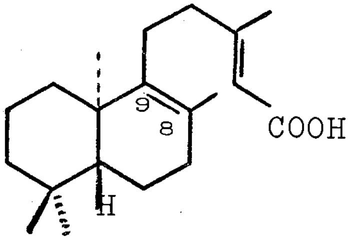
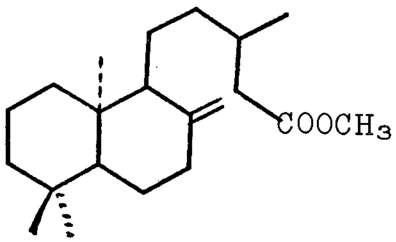


67

Literature covering work on *Hymenaea* has been reviewed[30]. To date about seventeen species of *Hymenaea* (Table 1) have been described. Investigation of the diterpenoid resin acids of ten species of *Hymenaea* (including *H. courbaril*) indicates that the total amount of resin (per gram fresh weight) and the quantitative proportions of the resin constituent varied significantly among the species studied and even among *Hymenaea courbaril* at different localities. From the trunk resins of *H. courbaril* in different localities, several compounds (68 - 71) were isolated and characterized as indicated below:



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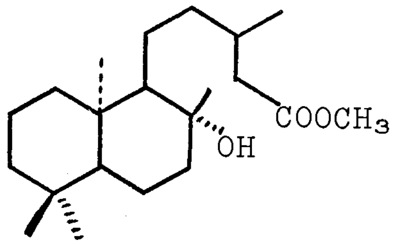

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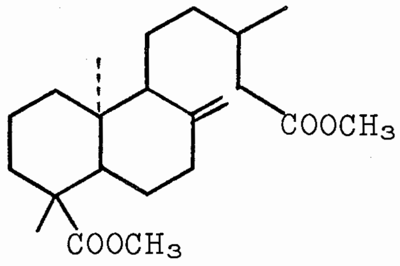
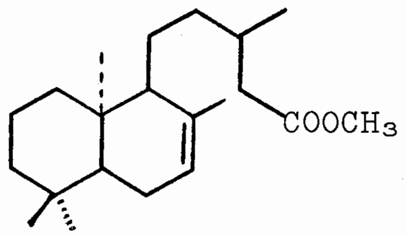
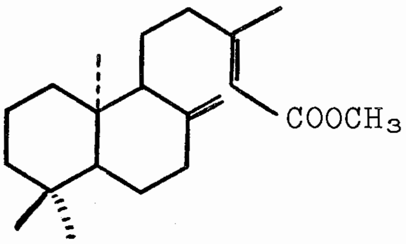


70



71

Investigation on the trunk resins of *H. altissima* resulted in the isolation of 70 - 73. Compounds 68 - 73 were found in variable amount in the trunk resins of *H. martiana*, *H. stilbocarpa*, *H. velutina*, and *H. stigonocarpa*[30].



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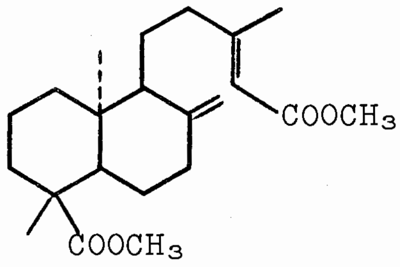
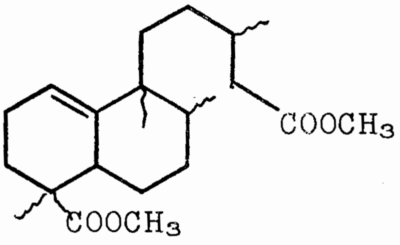
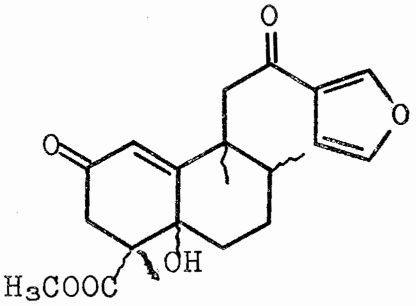


73

The resin extracted from the seed pods of *H. courbaril* grown in Africa was studied by Oehlschlager and Ourisson and preliminary results indicated that this resin contained bicarbocyclic diterpenes (e.g. 74) which possess rearranged labdene skeletons different from any of those previously reported[31].



74



75

Diterpenes possessing the rearranged skeleton of 74 have recently been reported to occur in the roots of *Adenochlaena siamensis* Ridl. (*Euphorbiaceae*)[32]. The roots of this plant, which give a drug extract applied for stomach disorders in Thailand, yield as the major component of the methylene chloride extract, 75.

Chapter 2

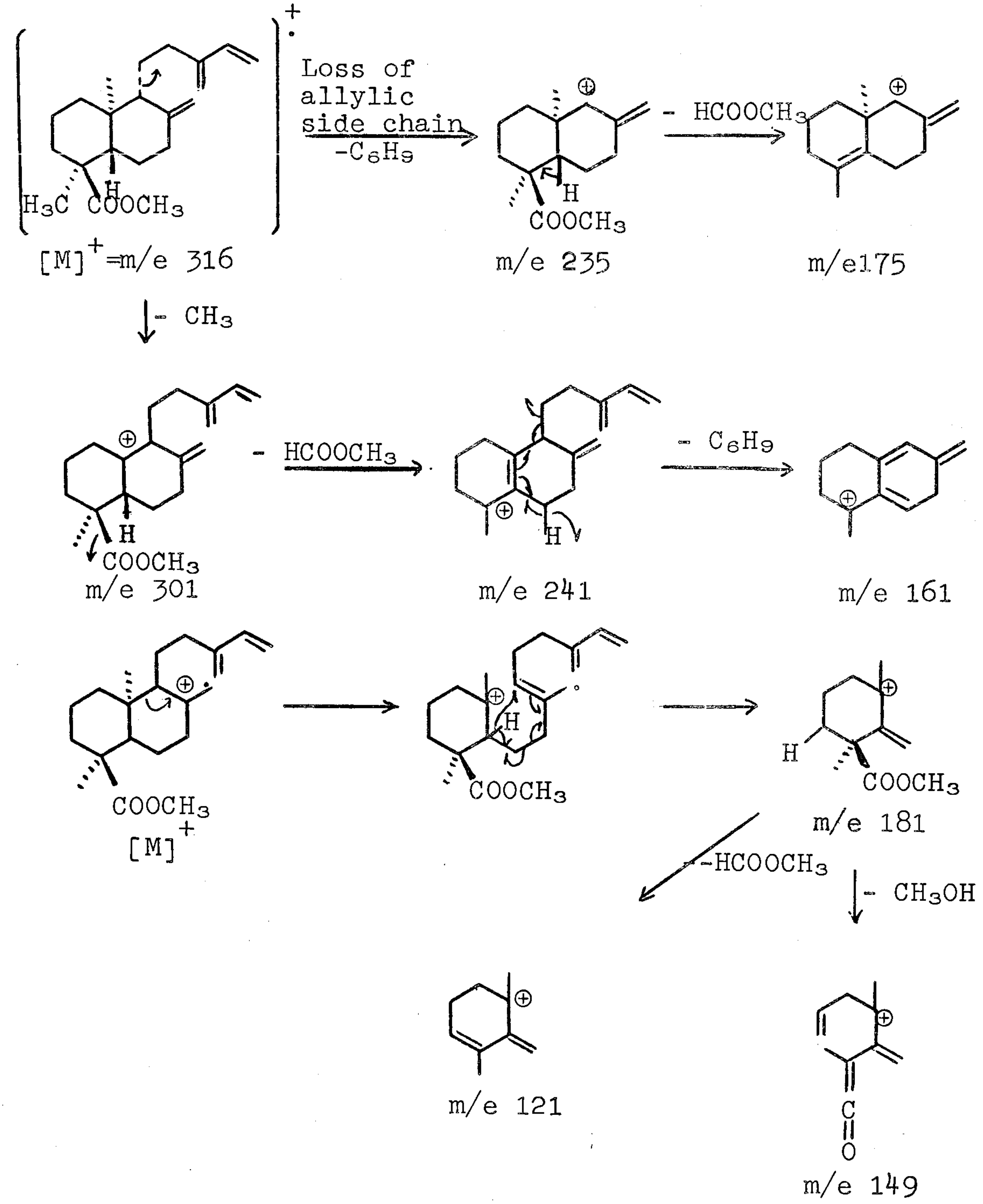
Results and Discussion of Structural Assignments

The present work involves the isolation and identification of the resin extracted from the seed pods of Jamaica-grown H. courbaril. The resin, obtained by benzene-chloroform extraction of the pulverized pods, was initially separated into its acidic and neutral components by treatment with lithium carbonate solution. The acidic constituents were subsequently methylated and both fractions separated by column, thin layer and gas chromatography yielding diterpenes 76, 85, 86, 93 and several sesquiterpene hydrocarbons.

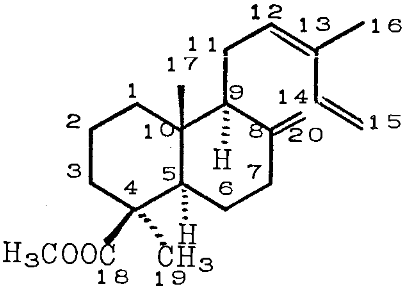
Structure of 76:

Infrared investigation of the neutral portion revealed a considrable carboxylic content; thus this fraction was also methylated with diazomethane prior to chromatography which yielded primarily 76. The mass spectrum of 76 revealed a molecular weight corresponding to $C_{21}H_{32}O_2$ and suggesting a bicarbocyclic diterpene ester. The mass spectral fragmentation pattern of 76 shown in Scheme 4 resembles closely those observed by Enzell and Ryhage[33] for bicarbocyclic diterpene esters such as methyl communate (77)[34]. Thus most diterpenes possessing the normal labdane skeleton with unsaturation at C_8-C_{20} and a carbomethoxy

Scheme 4: Mass Spectral Fragmentation Pattern of 76

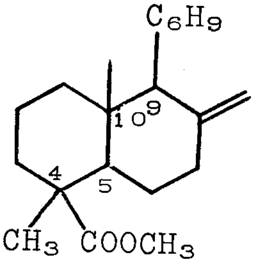


function at C_4 undergo the fragmentations leading to a prominent mass peak m/e at 121. Loss of the side chain usually gives rise to another prominent peak for this moiety. In the present case this corresponds to m/e 81 or C_6H_9.



77

The similarity of the mass spectral cracking pattern of 76 with that of 77 and related bicarbocyclic diterpenes indicated that 76 possessed a normal labdane skeleton, i.e.



The infrared spectrum of 76 possessed absorptions at 1645 and 890 cm^{-1} attributable to an exocyclic methylene group[17,25]. Absorptions at 1726 cm^{-1} and 1245 cm^{-1} attributable to an ester group were also present. Relying on the mass spectral fragmentation pattern of 76 the carbomethoxy function was located at C_4. The strong absorption at 1245 cm^{-1} in the infrared spectrum was assignable to this function only if an equatorial stereochemistry was assumed[18,35]. Finally, absorptions at both 1645 and 1595 cm^{-1} were indicative of the presence of a conjugated diene[36]. The band at 1595 cm^{-1} distinguishes the conjugated system from a single olefinic linkage which would absorb only weakly in the 1660-1640 cm^{-1} region.

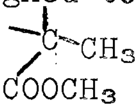
The NMR ($CDCl_3$) spectrum of 76 showed the presence of two methyl groups at quaternary positions (δ0.70, δ1.14). The lowfield signal was assigned to a methyl group α to a carbomethoxyl function ( $\overset{|}{\underset{COOCH_3}{-C-}}CH_3$) whereas the highfield signal was assigned to quaternary methyl which was not flanked by any neighboring electronegative functions. In addition, a three-hydrogen singlet appearing at δ3.63 was assigned to an ester methoxyl. From the chemical shifts of the C_4 and C_{10} methyl resonances of 78[25] and the dimethyl ester of oliveric acid 79 [17], the carbomethoxy group of 76 is further indicated to be at C_4 in an equatorial position (Table 2). Two one-hydrogen signals at δ4.56 and δ4.83 were assigned to the resonance of a $>C=CH_2$ group, confirming the presence of the exocyclic methylene group [17,25]. The two one-hydrogen doublets centered at $\delta 5.17$ ($J = 17.5$ Hz) and $\delta 5.0$ ($J = 12$ Hz) were attributable to the gem hydrogens of $\mathrm{{>}C(H){=}CH_2}$ group while the quartet present at $\delta 6.35$ ($J = 17.5$ Hz) was assigned to the resonance of the vinyl proton on a $\mathrm{{>}C(H){=}CH_2}$ group.

TABLE 2

Chemical Shifts of Methyl Groups in 76, 78 and Dimethyl Oliverate (79)

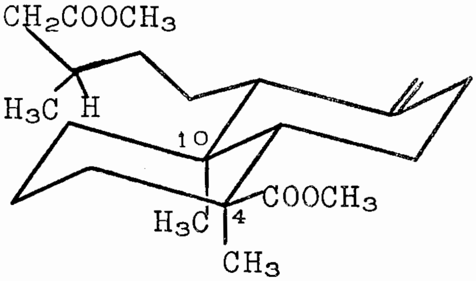
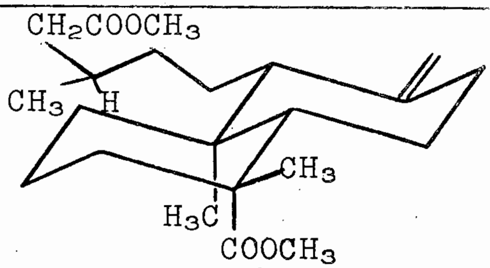
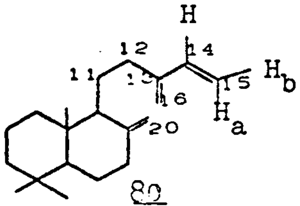
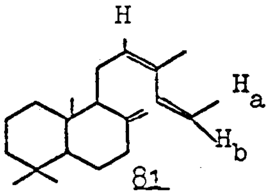
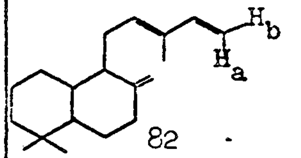
Compounds	C-4-Methyl	C-10-Methyl
76	1.14	0.70
 78	1.13	0.70
 79	1.15	0.48

TABLE 3

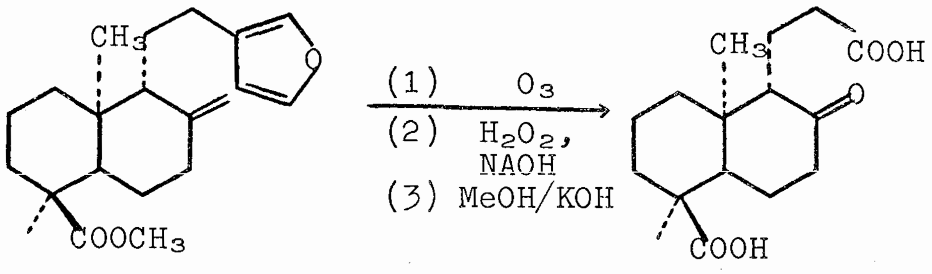
Comparison of Vinyl Hydrogen Resonances
of cis- and trans-biformene, sclarene and 76

Compounds	C_{12} Hydrogen	C_{14} Hydrogen	C_{15} Hydrogen	C_{16} Hydrogen	C_{20} Hydrogen	C_{13} Methyl
 80	—	δ 6.28 (AB quartet, J=17.5 Hz)	H_a: δ 5.01 (D, J=17.5 Hz) H_b: δ 4.92 (D, J=10.5 Hz)	δ 4.88 (2H)	δ 4.52, (1H,m) δ 4.78 (1H,m)	—
 81	δ 5.19 (T, J=7 Hz 7 Hz)	δ 6.72 (AB quartet, J=17.5 Hz)	H_a: δ 5.07 (D, J=18 Hz) H_b: δ 4.99 (D, J=10 Hz)	—	δ 4.45, (1H,m) δ 4.78 (1H,m)	δ 1.73 (D, J=1.2 Hz)
 82	δ 5.33 (T, J=6 Hz 6 Hz)	δ 6.24 (AB quartet, J=17.5 Hz)	H_a: δ 4.93 (D, J=17 Hz) H_b: δ 4.78 (D, J=10 Hz)	—	δ 4.44 (1H,m) δ 4.76 (1H,m)	δ 1.71 (S)
76	—	δ 6.35 (AB quartet, J= 17.5 Hz)	H_a: δ 5.17 (D, J=17.5 Hz) H_b: δ 5.0 (D, J=10.5 Hz)	δ 4.96 (2H)	δ 4.56, (1H,m) δ 4.83 (1H,m)	—

Direct comparison of the olefinic region of the NMR spectrum of 76 with those of sclarene (80)[37], cis- (81), and trans-biformene (82)[37], (Table 3), revealed that sclarene and 76 possessed nearly superimposable patterns whereas the pattern of 76 and those of biformenes differed significantly in this region. It follows that 76 possesses a side chain identical to that in sclarene, i.e.

C_6H_9 =

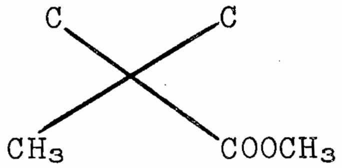
The stereochemistry and absolute configuration of 76 was arrived at by comparison with the methyl ester of polyalthic acid (83) whose structure and configuration have been established. Ozonolysis of polyalthic acid followed by oxidation with alkaline hydrogen peroxide has been reported to yield a keto-dicarboxylic acid, 84[38], which possesses a melting point and optical rotation identical to that of the keto-dicarboxylic acid, 84, derived from ozonolysis of 76.



83 84

Structure of esters 85 and 86

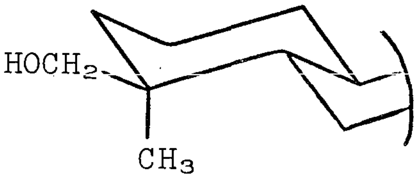
Chromatography of the acidic portion yielded upon elution with 50/50 petroleum ether/ether , a liquid from which two components 85 and 86 were separated by preparative GLC. The major component 85 was found to have a molecular weight corresponding to $C_{22}H_{36}O_4$. The infrared spectrum of 85 possessed significant absorptions indicating the presence of ester functions[35](1725, 1250, 1190, 1160 and 1110cm^{-1}) and an olefinic linkage (2940,1650 cm^{-1}). The n.m.r. spectrum indicated the presence of one vinyl hydrogen (δ5.37), two carbomethoxyl groups (δ3.63), two quaternary methyl groups (δ 1.11, 0.88) and two tertiary methyl groups (δ 0.95, d, J = 6.5 Hz; δ 0.70, d, J = 7.0 Hz). The chemical shift of the low field quaternary methyl (δ 1.11) compares closely with signals in the n.m.r. spectrum of bicarbocyclic diterpenes possessing C_4 carbomethoxyl groups suggesting the presence of partial structure a.

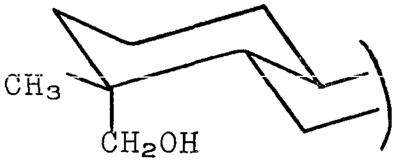


a

The presence of this moiety was further indicated by the shift of the low field quaternary methyl (δ1.11) to δ0.84 and by appearance of hydroxymethylene function as a sharp AB quartet centered at δ 3.34 (δ_A 3.18, δ_B 3.45, J_{AB} = 10.5 Hz) in the n.m.r. spectrum of the diol, 87, produced upon LAH reduction of 85 [35].

The chemical shifts of the hydroxy methylene hydrogens were those expected of an equatorial but not an axial hydroxymethylene function attached to a fused six-membered ring[39,40].

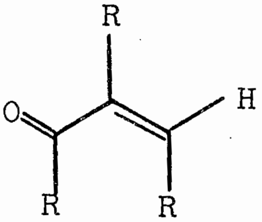




The n.m.r. spectrum of the diol 87 also revealed a triplet at δ 3.60 ($J = 6.8$ Hz) due to hydroxymethylene hydrogens produced upon reduction of the second ester function. This ester is therefore attached to a methylene group ($-CH_2-COOCH_3$).

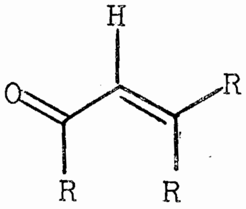
The n.m.r. spectrum of the diacetate 88 prepared by acetylation of the diol 87 with acetic anhydride revealed the appearance of an AB quartet centered at δ 3.85 ($\delta_A = 3.74$, $\delta_B = 3.95$, $J_{AB} = 10.5$ Hz) again in the rather narrowly defined region expected of an equatorial acetoxymethylene function attached to a decalin system [39,40].

Treatment of 85 with sodium chromate in acetic acid gave an α,β-unsaturated ketone 89 λ_{max} 237 mμ ($\varepsilon = 3.4 \times 10^4$) indicating either partial structures b_1 or b_2 associated with an acyclic or cyclohexenyl system.



b$_1$

$\lambda_{\text{max}}^{\text{cald}}$[36] 237 mμ

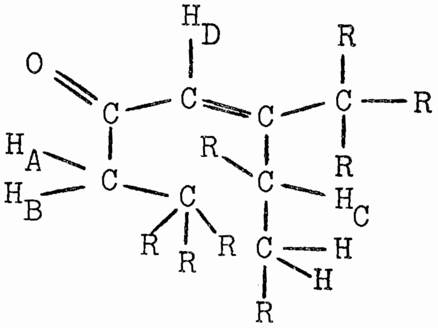


b$_2$

$\lambda_{\text{max}}^{\text{cald}}$[36] 239 mμ

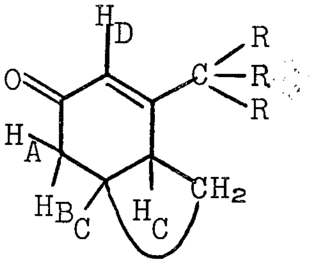
A decision in favor of b$_2$ was made by comparison of the change of chemical shift experienced by the vinyl hydrogen upon oxidation (Δδ 0.42) with those experienced by the α (Δδ 0.32) and β (Δδ 1.38) hydrogens upon oxidation of cyclohexene to 2-cyclohexenone[41].

Partial structure b$_2$ was extended to c upon the observation of base exchange of a two hydrogen AB quartet (δ_A 2.24, δ_B 2.72, J = 16 Hz), an exchangeable one hydrogen quartet (δ_C 3.04, J = 5 Hz and 11.5 Hz) and the vinyl hydrogen (δ_D 5.79).

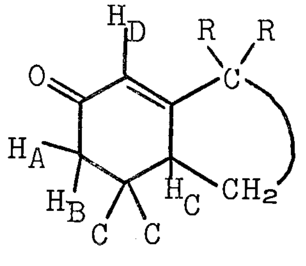


c

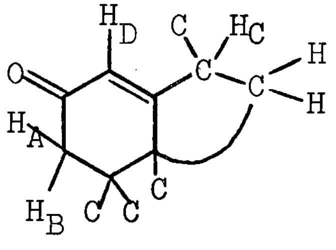
Since the exchangeable AB quartet possessed the characteristics of a geminal pair adjacent to a keto function of a cyclohexenone upon benzene solvent induced shift experiments (Fig. 1) [42] ($\Delta\delta_A$ $CDCl_3$-C_6H_6 = 0, $\Delta\delta_B$ $CDCl_3$-C_6H_6 = +0.25), structure c was modified to d_1 - d_4. These structures also take account of the observation that H_C is exchangeable and coupled to two adjacent hydrogens (not A or B) in a nonequivalent fashion. This latter observation places these hydrogens in a conformationally rigid carbon frame.



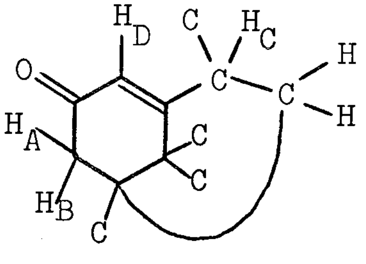
d_1



d_2



d_3



d_4

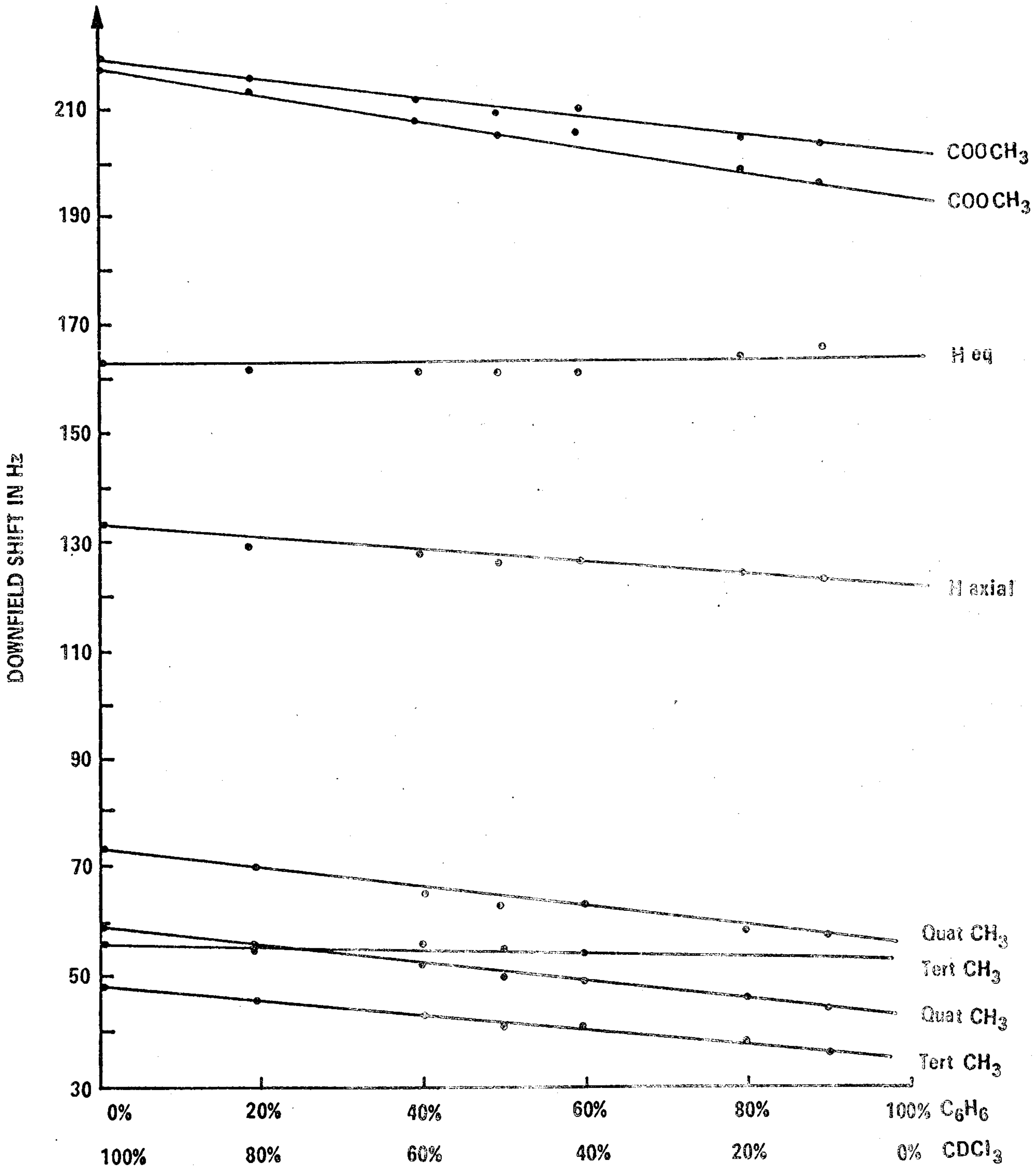
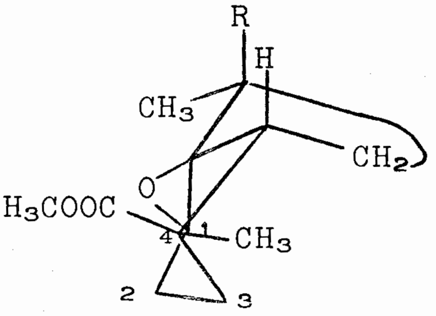


Figure 1: PLOT OF CHEMICAL SHIFT OF PROTONS AND CH$_3$ GROUPS IN 89
VS % COMPONENT OF C$_6$H$_6$ AND CDCl$_3$

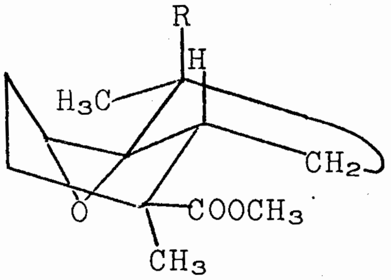
Evidence for the spatial relationship between the trisubstituted cyclohexenyl double bond of **85** and its two quaternary methyls was deduced from examination of the n.m.r. spectra of the isomeric epoxides (**90** and **91**) obtained from reaction of **85** with p-nitroperbenzoic acid. The n.m.r. spectrum of the less polar epoxide, **90**, revealed the epoxidic hydrogen as a triplet at δ2.92 (J = 2.5 Hz). Significantly the high field quaternary methyl (δ0.88) of **85** moved to δ0.65 in this epoxide whereas the other methyl resonance remained relatively unchanged. The n.m.r. spectrum of **91** revealed the epoxidic hydrogen as a doublet at δ2.94 (J = 4.0 Hz) and both the quaternary methyls (δ0.88 and δ1.11) at higher field (δ0.70 and δ1.01). These observations place the quaternary methyl not associated with the carbomethoxyl-bearing carbon in the shielding cone of the epoxide functions in **90** and **91**, while the quaternary methyl associated with the carbomethoxy group is placed in the shielding cone of the epoxide function of **91**.

The partial structure most consistent with this spatial relationship is d_2 wherein the quaternary methyl not associated with the carbomethoxyl function is in a *peri* relationship to the epoxide ring as in **e**[43].



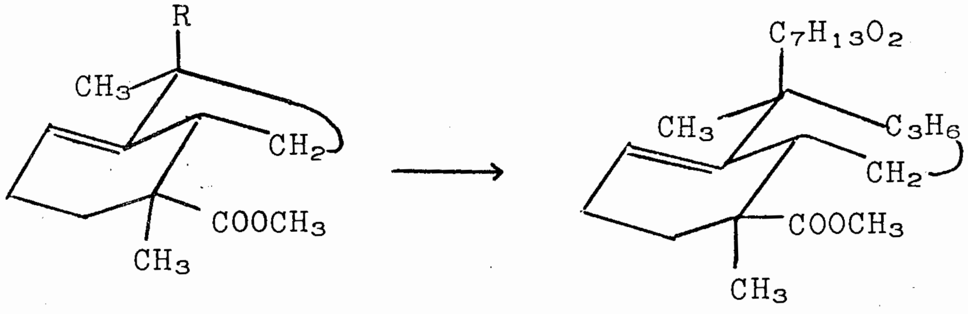
90

e



91

Additional evidence for a *peri* relationship between the high field quaternary methyl and the unsaturation of 85 was obtained by NOE.[44] Thus irradiation at the frequency of the high field quaternary methyl of 89 (derived from 85 by allylic oxidation) resulted in a 35 ± 10% increase in the integrated intensity of the vinyl hydrogen signal (relative to both internal chloroform and the carbomethoxyl resonances). This is expected only if the quaternary methyl has close steric proximity to the vinyl hydrogen. The above implied extention of the partial structure of 85 to f.



f

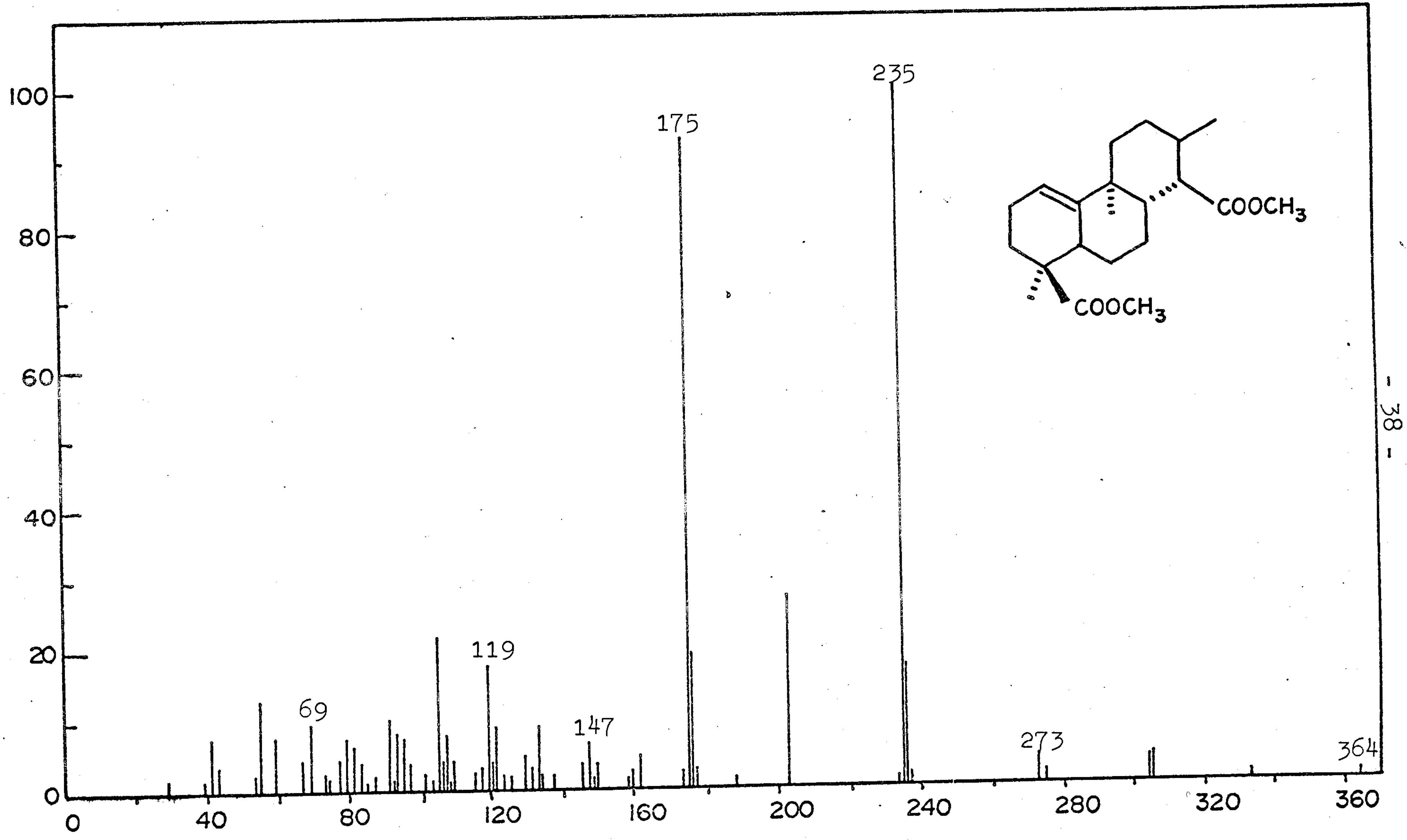


Figure 2: Mass Spectrum of 85

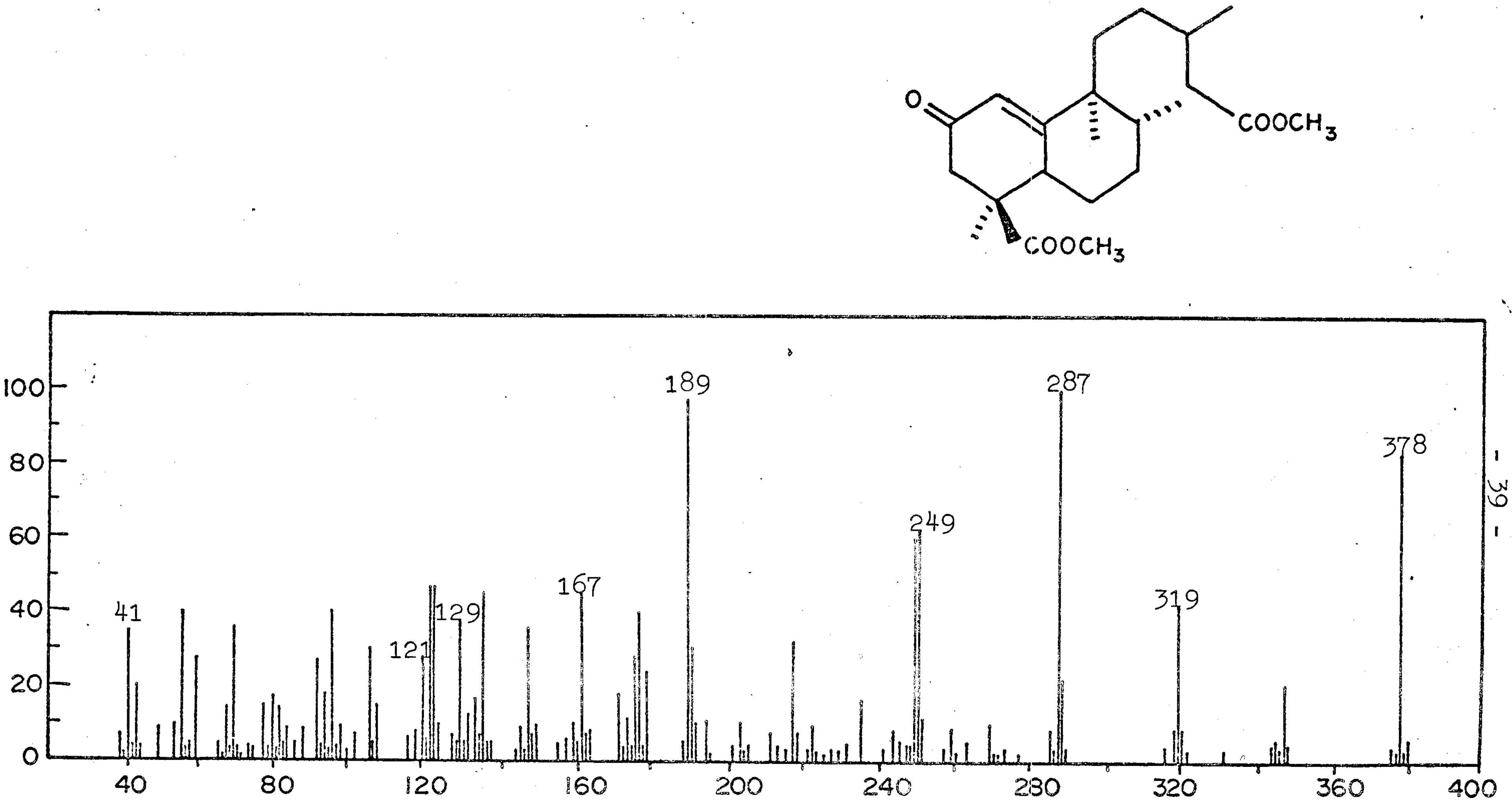


Figure 3: Mass Spectrum of 89

Structure f accounts for $C_{12}H_{17}O_2$ of $C_{22}H_{36}O_4$ in 85. The mass spectrum of 85 (Fig. 2) reveals a base peak at m/e 235 which corresponds to loss of $C_7H_{13}O_2$[‡] (m/e 129). The next most prominent peak is at m/e 175 and is produced by loss of $HCOOCH_3$ (m/e = 60) from the m/e 235 ion as evidenced by a metastable ion at 130. Considering the most probable point of fragmentation of partial structure f to be the allylic center containing the quaternary methyl, if rupture occurred eliminating the ion of m/e 129, the formula of the remaining fragment would be $C_{15}H_{23}O_2$ of which $C_{12}H_{17}O_2$ is deduced. The remaining undetermined portion of the m/e 235 fragment is C_3H_6. Of the possible structural arrangements for this fragment the $-CH_2\text{-}\overset{|}{C}HCH_3$ formulation is compatible with the presence of one of the two tertiary methyl doublet resonances in the n.m.r. spectrum of 85. Scrutiny of the mass spectrum of 89 (Fig. 3) reveals a major ion (m/e 249) also produced by loss of an m = 129 fragment. The m/e 249 ion subsequently eliminates $HCOOCH_3$ to yield a fragment m/e 189 containing the oxygenation additional to 89. The ions at m/e 161, 122, 121 and m/e 41 are considered to originate from m/e 189 via retro-Diels

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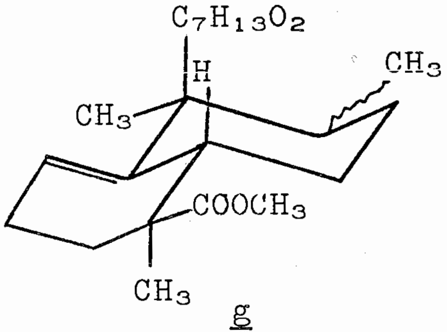
[‡] This composition was supported by the observation of a fragment at m/e 69, i.e. 129 - $HCOOCH_3$.

Alder reactions (Scheme 5) and require the tertiary methyl of the C_3H_6 fragment to be adjacent to the quaternary methyl. Structure f may thus be extended to g.

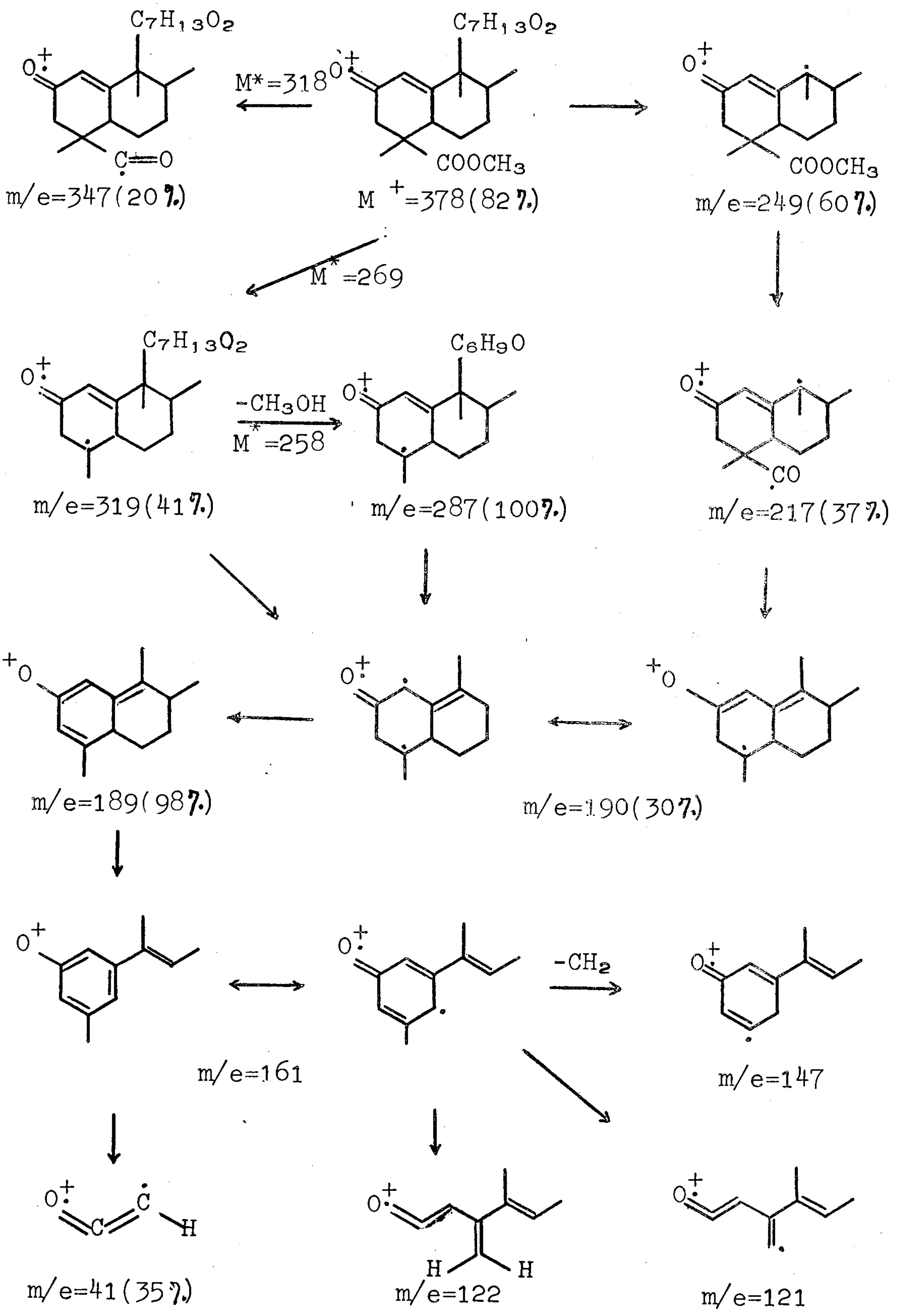


g

The above assignments were corroborated by mass spectral analysis of the diethylene ketal derivative of 89 (Fig. 4). In this derivative, 89a, ionization occurred primarily at the ketal function followed by loss of dioxane then loss of the side chain and retro-Diels Alder reactions to give m/e 121 as the dominant ion in the spectrum.

Considerable but unfruitful effort was invested in attempting to extend the conjugation of ring A of 89 to ring B (Appendix). It was presumed that definitive evidence as to the location of the ring B secondary methyl group could be obtained if this could be achieved.

Scheme 5: Fragmentation Pattern of 89



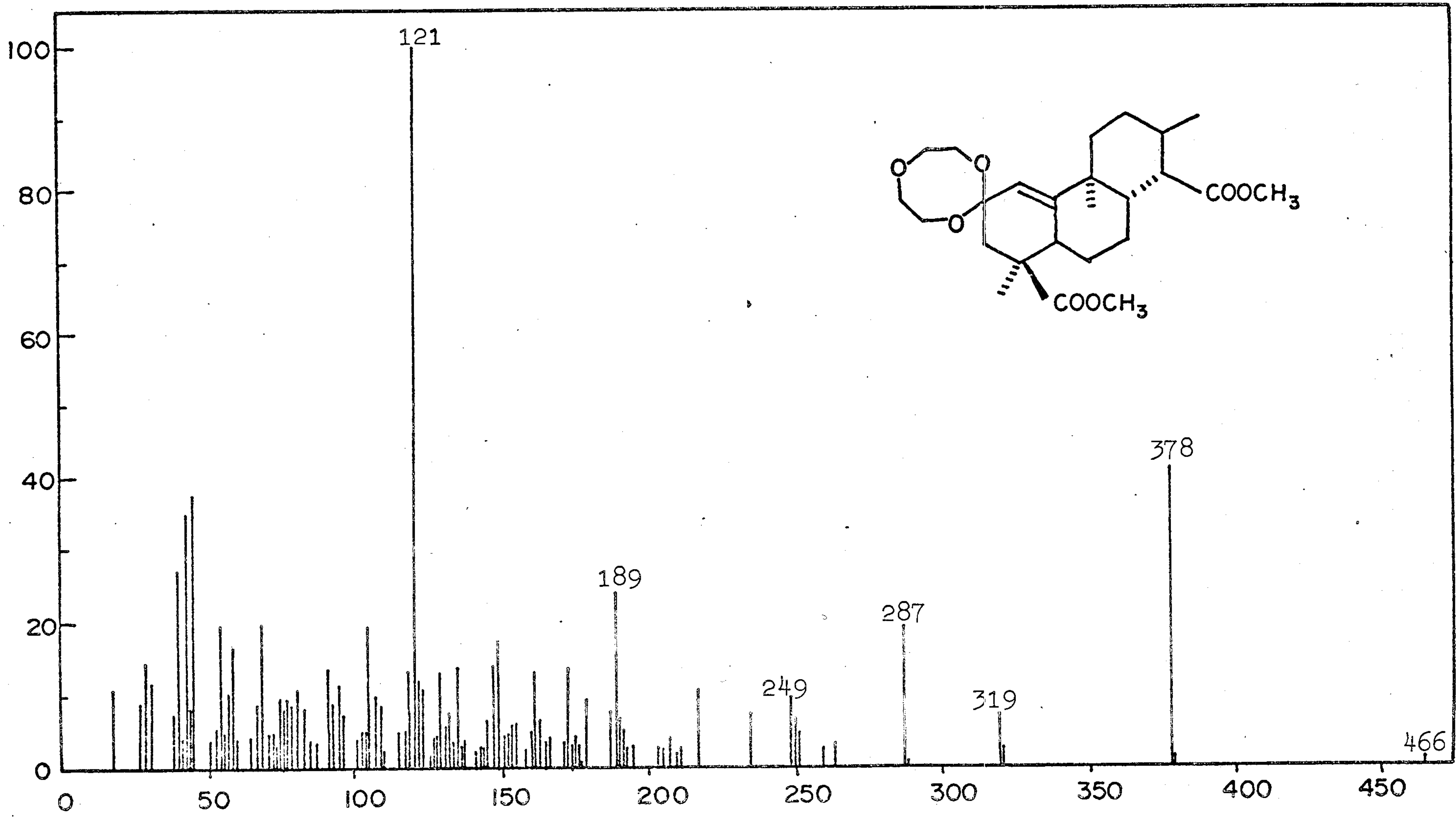
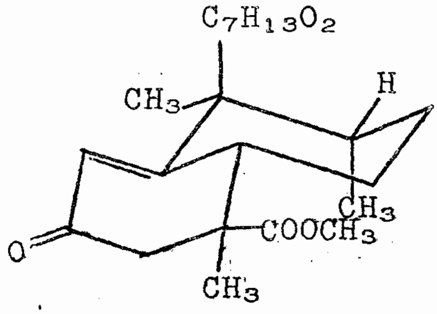


Figure 4: Mass Spectrum of 89a

The stereochemistry of this tertiary methyl was deduced from benzene solvent induced n.m.r. shifts of 89 and by Eu(fod)$_3$ induced shifts[45] on epoxides 90 and 91. In the former experiment (Fig. 1) it was found that both quaternary methyls and one of the two tertiary methyl resonances shifted by nearly identical amounts to higher field as the solvent was changed from chloroform to benzene. Although the quaternary methyls have previously been located relatively near the conjugated carbonyl, in order for there to be a distinct upfield shift of a tertiary methyl resonance in this experiment, the tertiary methyl group must either be sterically near this group or be axial in the proposed decalin system. Since the mass spectral cracking pattern placed a tertiary methyl at C$_8$, if it is axial it must be α as given in partial structure h.



h

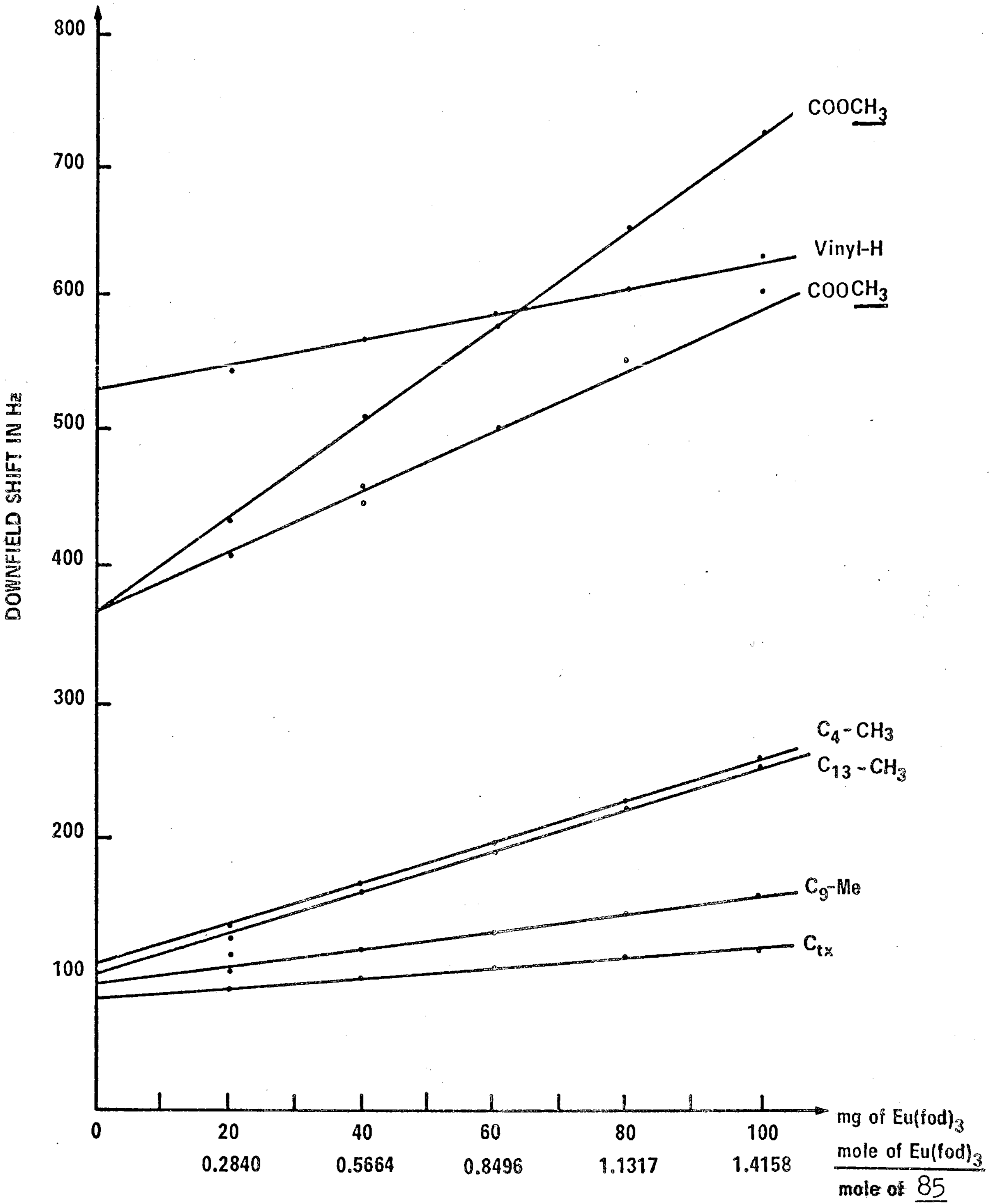


Figure 5: VARIATION OF INDUCED SHIFT WITH MOLAR RATIO $(EU(FOD)_3)$ / (SUBSTRATE) OF 85 IN CCl_4 SOLUTION

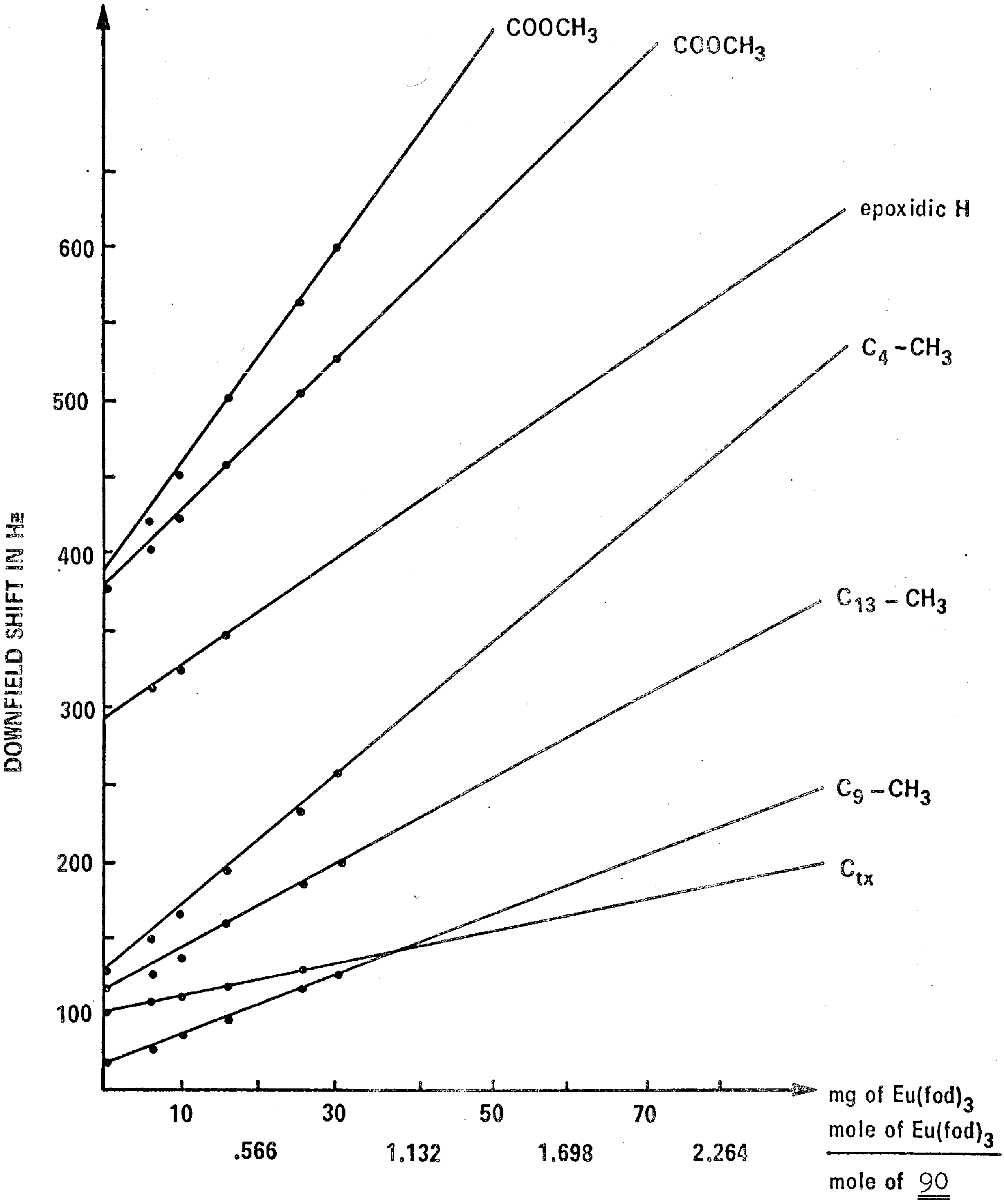


Figure 6: VARIATION OF INDUCED SHIFT WITH MOLAR RATIO (EU(FOD)$_3$) / (SUBSTRATE) OF 90 IN CCl$_4$ SOLUTION

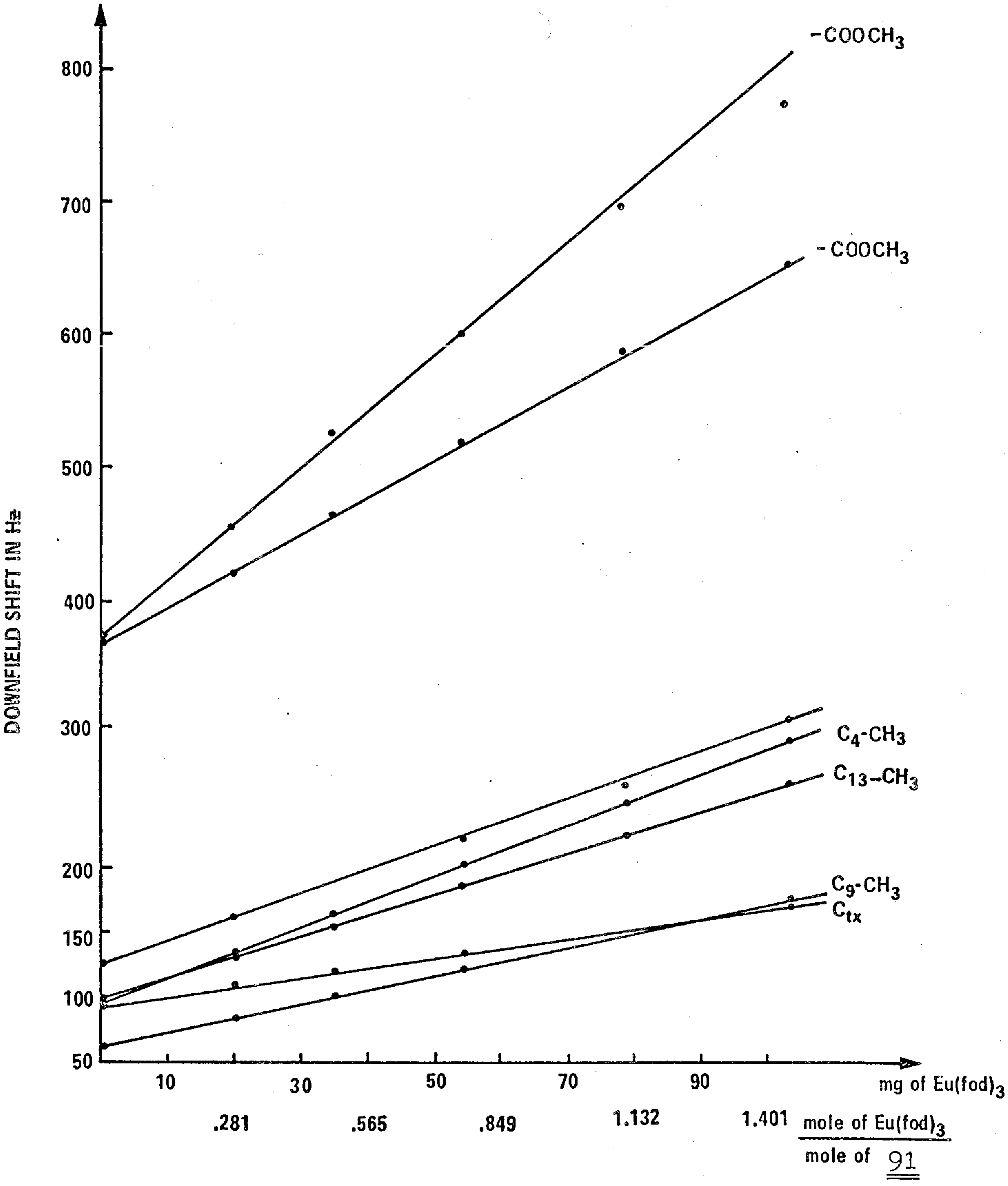


Figure 7: VARIATION IN INDUCED SHIFT WITH MOLAR RATIO (EU(FOD)$_3$) / (SUBSTRATE) FOR 91 IN CCl$_4$ SOLUTION

TABLE 4

Gradients in Downfield Shift in Hz per mole Eu(fod)$_3$ per mole of Substrate and Ratio of Gradients of Methyl Groups in Compounds 85, 90 and 91

Compound	Gradients $\left(\frac{\text{Downfield Shift in Hz}}{\frac{\text{mole Eu(fod)}_3}{\text{mole of Substrate}}}\right)$									
	C_4-CH_3	C_{tx}-CH_3[†]	C_9-CH_3	C_{13}-CH_3	$\frac{C_{tx}\text{-}CH_3}{C_4\text{-}CH_3}$	$\frac{C_{tx}\text{-}CH_3}{C_9\text{-}CH_3}$	$\frac{C_{tx}\text{-}CH_3}{C_{13}\text{-}CH_3}$	$\frac{C_9\text{-}CH_3}{C_{13}\text{-}CH_3}$	$\frac{C_4\text{-}CH_3}{C_{13}\text{-}CH_3}$	$\frac{C_4\text{-}CH_3}{C_9\text{-}CH_3}$
85	1.55	0.387	0.75	1.57	0.25	0.52	0.25	0.48	0.99	2.07
90	4.08	1.00	1.90	2.65	0.25	0.53	0.38	0.72	1.54	2.14
91	1.83	0.70	0.96	1.50	0.38	0.73	0.48	0.64	1.22	1.90

[†] C_{tx}-CH_3 = tertiary methyl in ring B

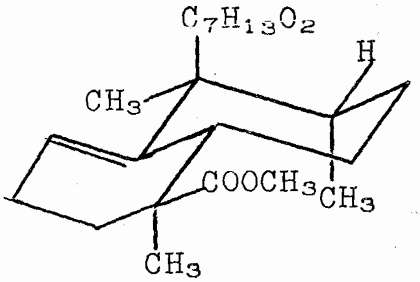
Confirming this assignment are the observed rates of change of chemical shifts of the quaternary and tertiary methyl resonances upon the addition of $Eu(fod)_3$ to the solution of 85 and its epoxide derivatives 90 and 91. When $Eu(fod)_3$ was added to solutions of these compounds in carbon tetrachloride in amounts varying from 0.28 to 1.1 molar ratios and the induced n.m.r. shifts of the quaternary and tertiary methyl resonances were plotted vs. $[Eu(fod)_3]/[Substrate]$, straight line plots (Figs. 5, 6, 7) were obtained. The gradients of these lines in Hz/mole $Eu(fod)_3$/mole substrate are given in Table 4.

It is observed that for 90 the rate of shift of all methyl resonances is much greater than for those of 85 or epoxide 91, supporting the earlier deduction that the epoxide and ester functions are on the same side of the molecule in this epoxide†. Significantly, when internal comparisons are made, the ratios of the rates of shifts $\frac{C_{tx}\text{-}CH_3}{C_4\text{-}CH_3}$, $\frac{C_{tx}\text{-}CH_3}{C_9\text{-}CH_3}$ and $\frac{C_{tx}\text{-}CH_3}{C_{13}\text{-}CH_3}$ for 91

---

†Close promixity between two or more polar groups should contribute to increased ability of the substrate to coordinate with the $Eu(fod)_3$ reagent resulting in larger shift gradients for the substrate hydrogens.

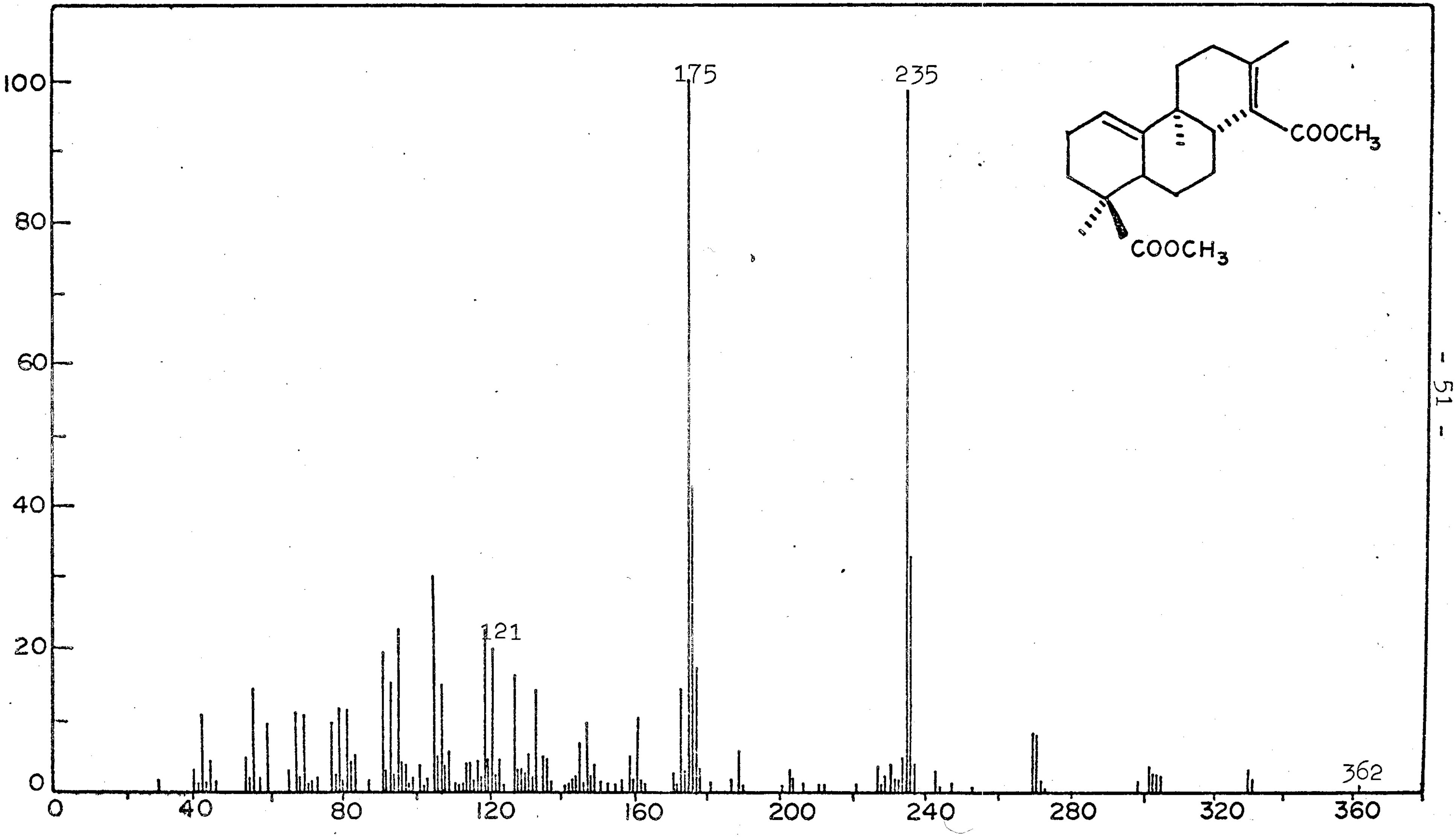
are greater than for 90 or 85. This observation is consistent only with the location of the C_{tx} methyl (tertiary methyl in ring B) nearer to the α-epoxide oxygen of 91 than the β-epoxide oxygen of 90.



i

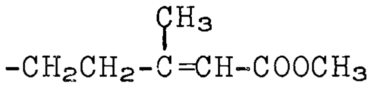
The partial structure, i, of 85 deduced at this point, leaves the $C_7H_{13}O_2$ fragment undefined except for the presence of one tertiary methyl ($>CHCH_3$) and a methylene carbomethoxy function ($-CH_2COOCH_3$). The structure of this fragment was deduced by chemical correlation of 85 with a second component of the pod resin. Thus 86, separated from the chromatographic fractions containing a mixture of 86 and 85 by GLC, exhibited spectra indicating a structure similar to 85 except for the presence of an additional double bond (M^{+}_{86} 362 vs. M^{+}_{85} 364; Fig. 8) which was conjugated with an ester (λ_{max}220, ε16354) and was responsible for the appearance of a vinyl methyl doublet (δ 2.19, J = 1.2 Hz) and a vinyl

Figure 8: Mass Spectrum of 86



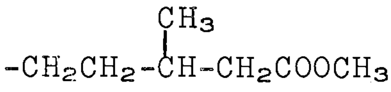
hydrogen (δ 5.73) resonance. Hydrogenation of 86 with Pd/$BaSO_4$ resulted in the disappearance of the vinyl methyl and hydrogen resonance and the formation of a product indistinguishable by n.m.r., i.r., u.v. and m.s. from 85. This establishes 86 as an unsaturated analog of 85 and requires the site of unsaturation to reside in the C_7 fragment. Since reduction of this unsaturation must a) remove the conjugation with an ester, b) produce a CH_2COOCH_3 function, and c) produce a $-\overset{|}{C}HCH_3$ group in the C_7 fragment, the structure of this fragment in 86 and 85 must be j and k respectively.

The stereochemistry of the double bond in j was determined by comparison of the chemical shift of the vinyl methyl of 86 with those of the α,β-unsaturated esters[46] as shown in Table 5. In 86 (containing j) the vinyl methyl resonance was observed at δ 2.19. This methyl is thus *cis* to the carbomethoxyl function.



$$-CH_2CH_2-\overset{CH_3}{\overset{|}{C}}=CH-COOCH_3$$

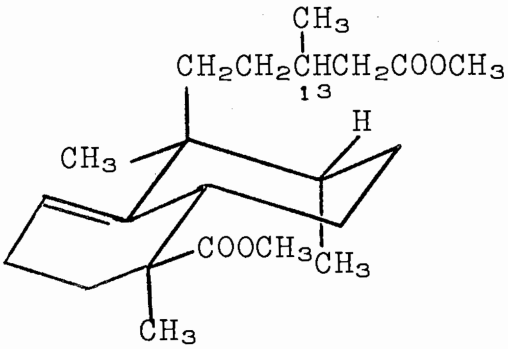
j



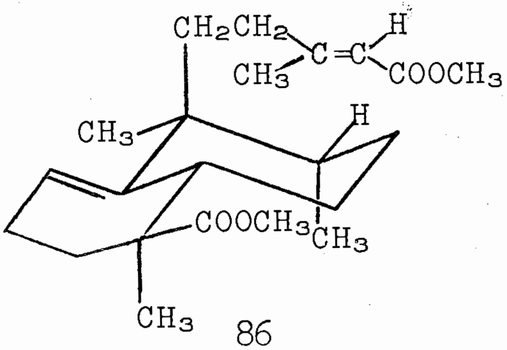
$$-CH_2CH_2-\overset{CH_3}{\overset{|}{C}}H-CH_2COOCH_3$$

k

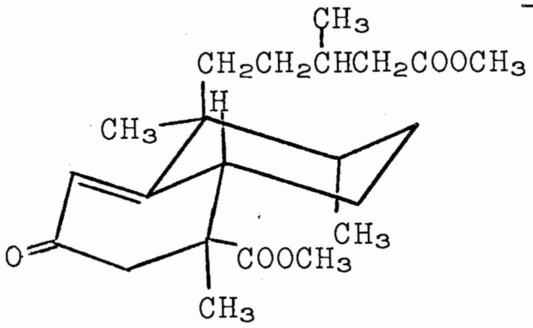
These observations allow formulation of structures for 85 and 86 but do not define the absolute stereochemistry of their decalin rings nor the configuration at C_{13} of 85. Comparison of the CD curves of 89 (Fig. 9) and 92 (Fig. 10) indicates an opposite configuration at C_5. Since 92 is known to be 5α, the hydrogen of 89 (and thus of 85 and 86) must be 5β. These diterpenes thus belong to the same antipodal class as 76.



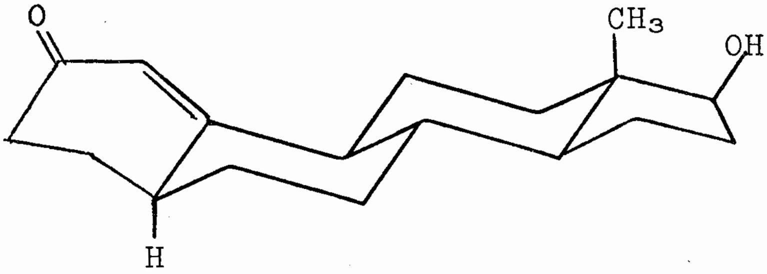
85



86

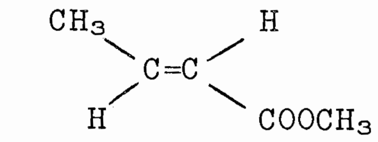
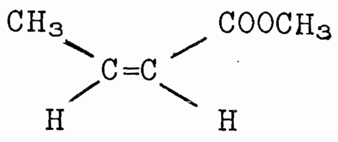
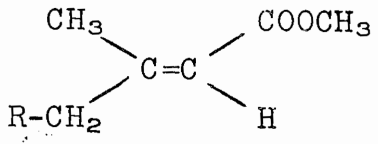
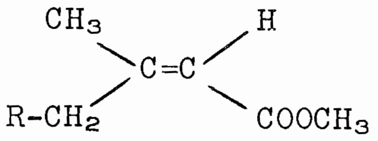
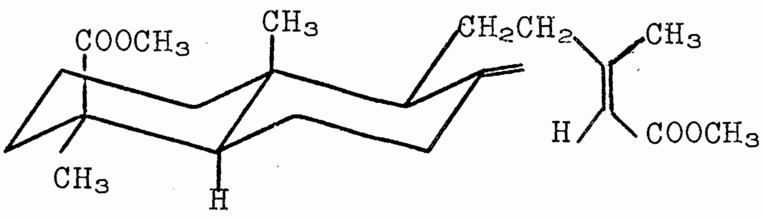


89



92

TABLE 5: Chemical Shifts of Vinyl Methyl Groups in Some α,β-Unsaturated Esters

Compounds	β-CH_3 δ
	1.88
	2.14
	2.19 - 2.29
	1.73 - 2.00
 Dimethyl agathate	2.12
86	2.19

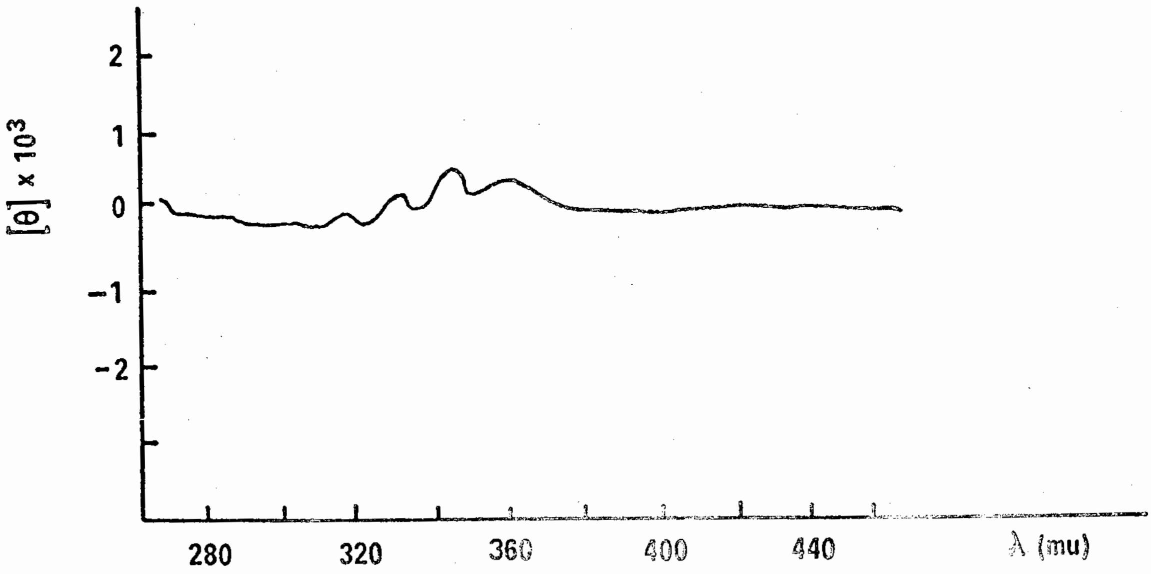


Figure 9: CIRCULAR DICHROISM CURVE OF 89

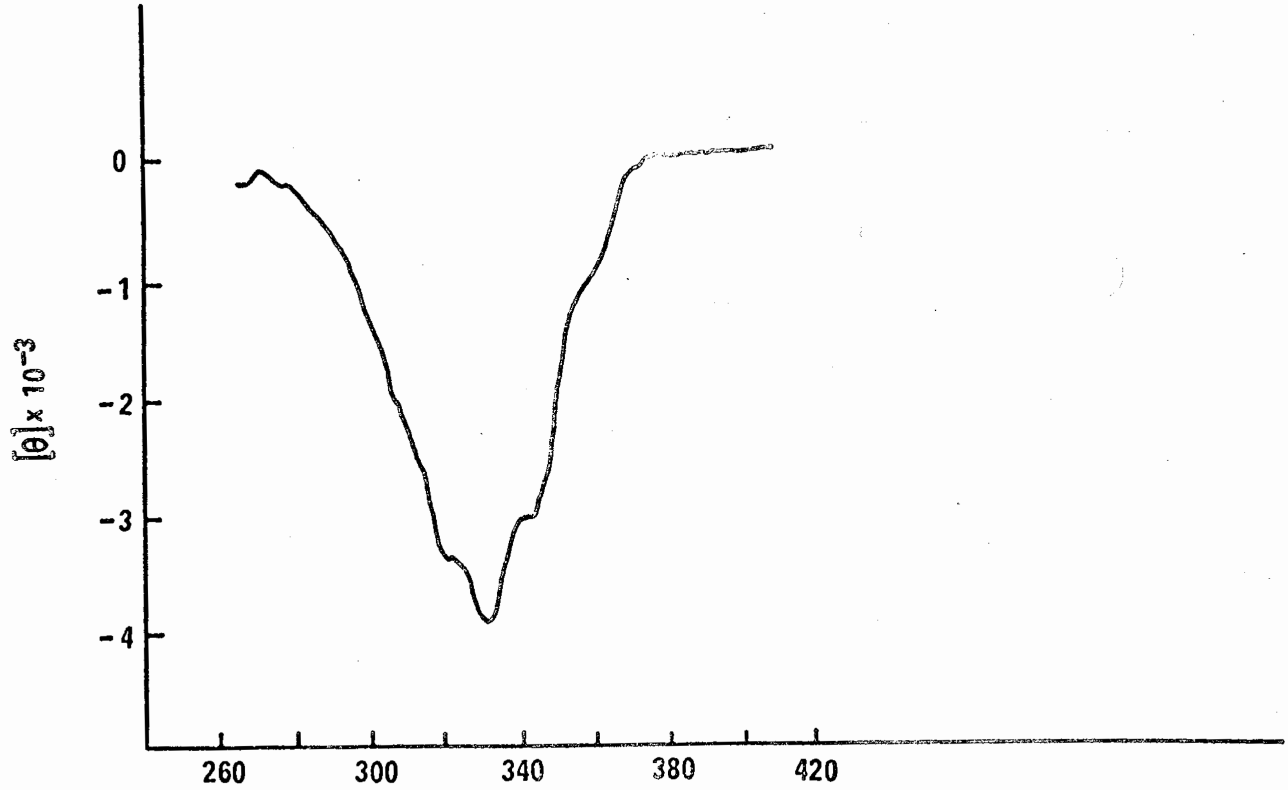
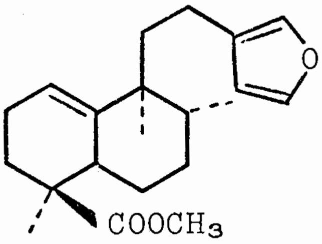


Figure 10: CIRCULAR DICHROISM (CD) CURVE OF 92

Structure of Ester 93

The spectral properties of 93 ($C_{21}H_{30}O_3$) suggested a bicyclic diterpene possessing a β-substituted furan ring. The n.m.r. of 93 revealed the presence of two quaternary methyl groups (δ0.97, δ1.13), a tertiary methyl doublet (δ0.83, J = 6.5 Hz), one carbomethoxy function (δ3.62), and a vinyl hydrogen (δ5.37). Three one-hydrogen triplets at δ6.15, 7.10 and 7.15[16] and infrared absorptions[47] at 1560, 1510 and 875 cm^{-1} indicated the presence of a β-substituted furan.

The mass spectral fragmentation pattern (Fig. 11) of 93 suggested the furan ring was attached to the side chain of a rearranged labdane skeleton. Thus while intense ions at m/e 81 and m/e 95 are also present in the mass spectrum of daniellic acid (Fig. 12), the base peak of 93 is m/e 235 (as was observed for 85, 86 and 89), indicating particularly facile cleavage of the furan containing side chain. The stereochemistry and absolute configuration of 93 was obtained by comparing with those of 76, 85, and 86.



93

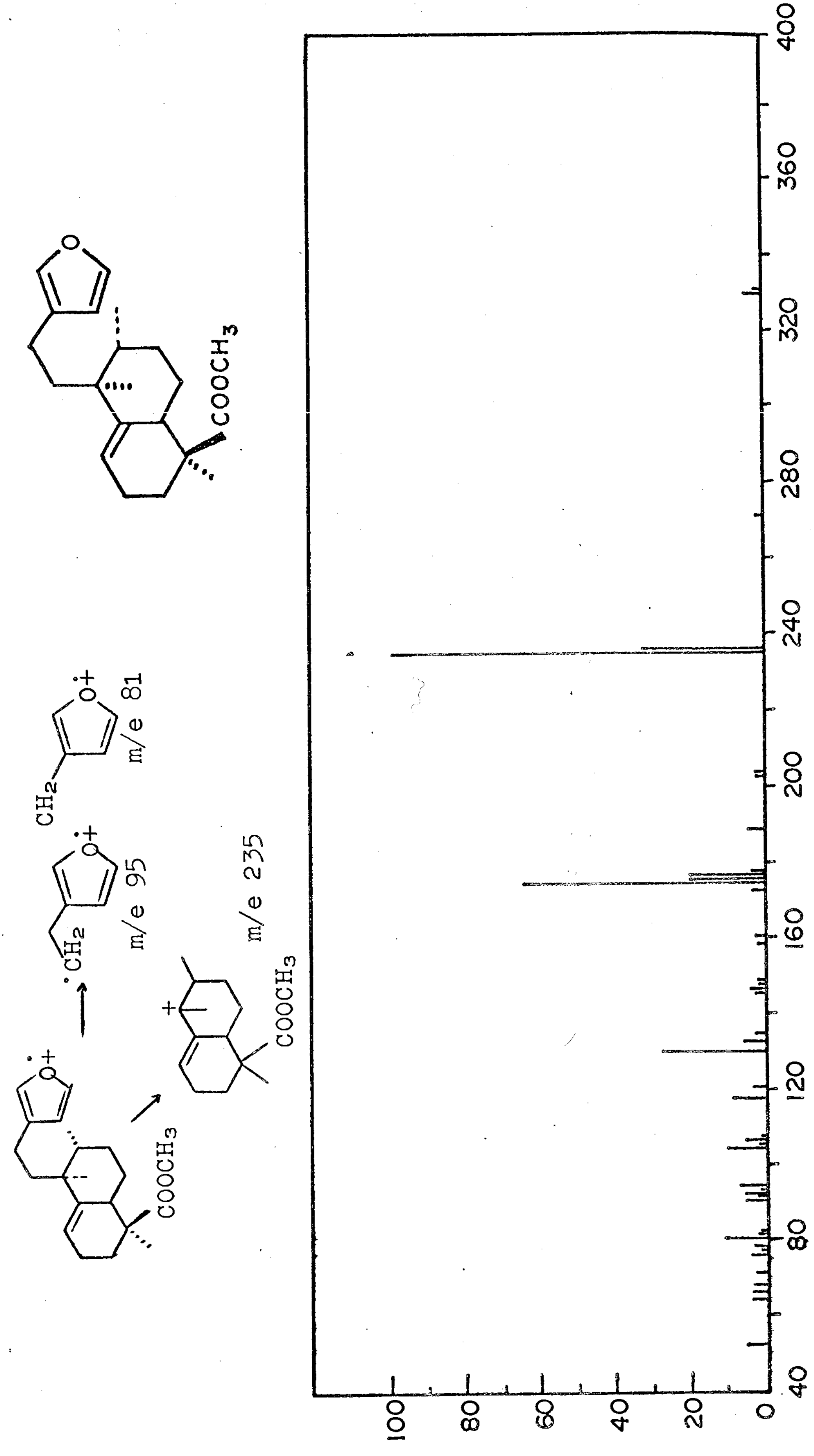


Figure 11: Mass Spectrum of 93

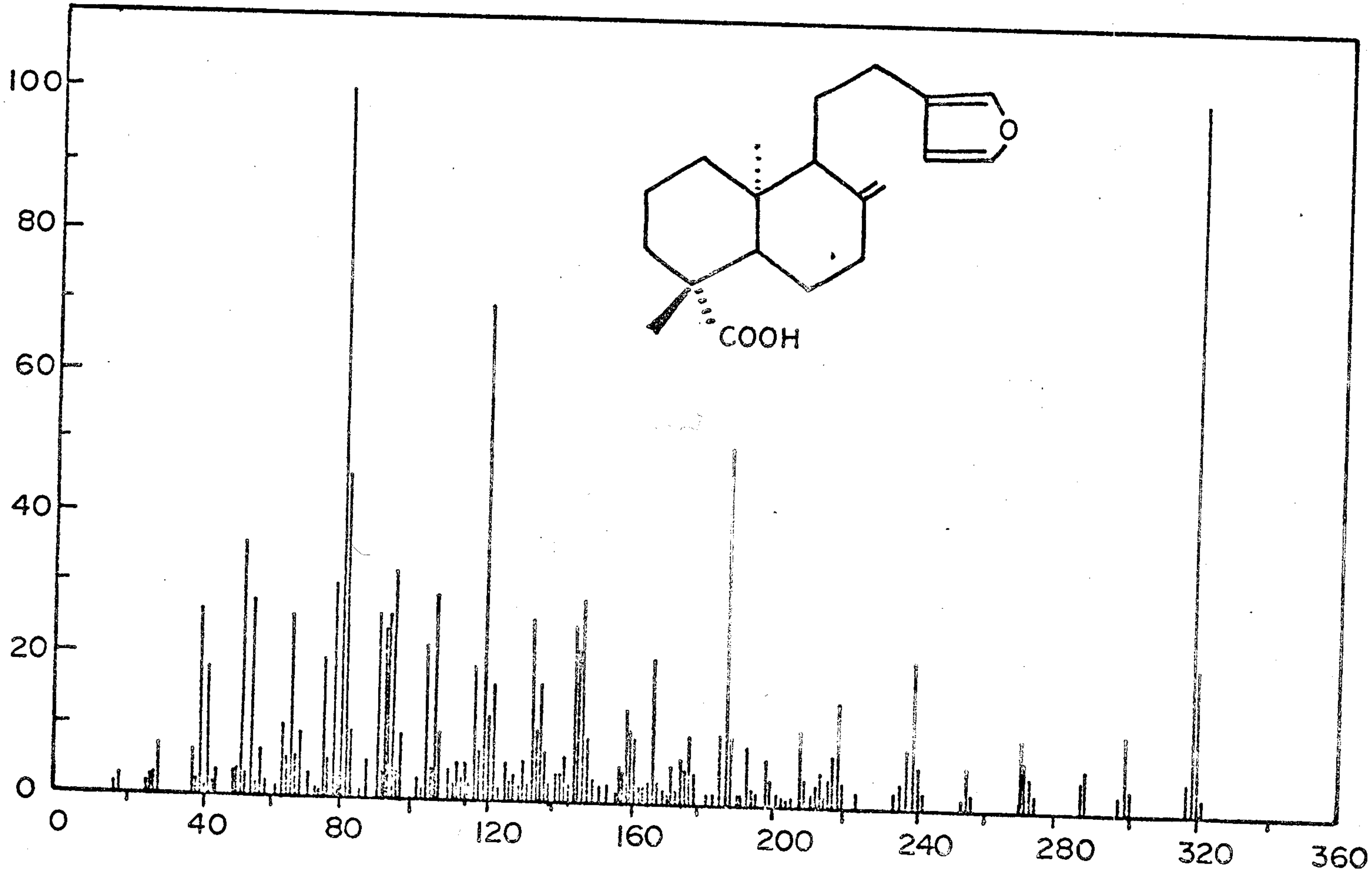


Figure 12: Mass Spectrum of Daniellic Acid

Sesquiterpene hydrocarbons of the seed pod resin were identified by comparative gas chromatographic retention indices[48]. The crude resin was found to contain cyclosativene, caryophyllene, α-Himachalene, selina-4(14),7(11)-diene as major sesquiterpene components and β-bourbonene, calarene, selina-4(14),7-diene, humulene, δ-cadinene, α-calocorene and α-muurolene as minor components, (Table 6).

Conclusion

The trunk resin of Hymenaea courbaril has been shown to contain bicyclic diterpenes possessing the labdane skeleton[29,30]. In the present study, the seed-pod resin of Jamaica-grown Hymenaea courbaril has been shown to contain three bicarbocyclic diterpenes (85, 86 and 93) possessing the rearranged labdane skeleton although one bicyclic diterpene with a normal labdane skeleton, 76, was also isolated from this source. This shows that diterpenes isolated from the pod are more highly rearranged than those isolated from the trunk of the same tree.

TABLE 6: Sesquiterpene Hydrocarbons in Seed-Pod Resin of Jamaica-Grown <u>Hymenaea Courbaril</u>

Compound	Percentage*
A	3.3 ± 0.1
B	0.85 ± 0.05
C	1.2 ± 0.1
Cyclosativene	17.3 ± 0.3
β-Bourbonene	1.1 ± 0.1
D	0.5 ± 0.2
Caryophyllene	13.5 ± 0.3
E	1.0 ± 0.1
Calarene	0.4 ± 0.3
F	1.8 ± 0.1
Humulene	1.6 ± 0.1
Selina-4(14),7-diene	0.3 ± 0.1
α-Himachalene	13.5 ± 0.4
G	1.2 ± 0.1
α-Muurolene	6.1 ± 0.2
δ-Cadinene	5.2 ± 0.1
H	3.98 ± 0.01
Selina-4(14),7(11)-diene	16.2 ± 0.2
I	7.4 ± 1.4
J	1.6 ± 0.1
K	1.6 ± 0.1

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* Mean of two determinations; % of sesquiterpene fraction is approximately 10 % of total terpene composition.

Chapter 3

EXPERIMENTAL

IR spectra were obtained neat or with KBr using a Perkin-Elmer 457 spectrophotometer. NMR spectra were obtained at 100 MHz or 60 MHz in $CDCl_3$ or CCl_4 using TMS as internal standard. Molecular weights and fragmentation patterns: Hitachi Perkin-Elmer RMU-7 double focusing mass spectrophotometer (I.E. = 70eV).

Extraction of Hymenaea courbaril seed pods

Dry seed pods of Jamaica-grown *H. courbaril* were pulverized and extracted repeatedly with benzene at r.t. until only minute amounts of extract were obtained. Evaporation of the benzene gave 57 g of dark resin from 950 g pods.

Treatment of this resin with excess aqueous Li_2CO_3 gave a water soluble fraction and an ether soluble fraction. The ether soluble fraction was separated and removal of the ether gave 22 g of neutral product.

The acidic portion of the extract was liberated by addition of 50% aqueous acetic acid to the lithium carbonate solution. Extraction of the acidified aqueous solution with ether followed by evaporation of the ether gave 28 g of acidic components which were methylated with ethereal diazomethane.

Isolation of Acidic Components

A portion (26 g) of the acidic fraction was chromatographed on Silica Gel (500 g). Elution with pet. ether:ether (9:1 to 7.5:2.5) gave 8.8 g of a mixture of 85, 86 and 93. These components were further separated by prep. t.l.c. on SiO_2/10% $AgNO_3$ eluting with EtOAC:pet. ether (3:7). The less polar 93 gave $[\alpha]_D^{25°}$ +53° (c = 0.25 $CHCl_3$); $\nu_{max.}^{film}$ 3120, 1725, 1560-1540, 1510, 1255, 1165, 1025, 875 and 780 cm^{-1}. NMR is as shown in Fig. 13.

Calcd. for $C_{21}H_{30}O_3$ (330): C, 76.32; H, 9.15. Found: C, 76.76; H,9.30. Mol. wt. by m.s. 330.

Separation of the mixture of 85 and 86 by prep.GLC on a 1.5% QF 1 on Chrom. G column at 210° gave 85; $[\alpha]_D^{25°}$ +31.4°(c = 0.77,$CHCl_3$); $\nu_{max.}^{film}$ 2940, 1725,1650, 1250,1190,1160,1150,1110 and 880 cm^{-1}; NMR is as shown in Fig. 14.

Calcd. for $C_{22}H_{36}O_4$ (364): C, 72.49; H, 9.96. Found: C, 72.59; H, 9.65 and 86 ; $[\alpha]_D^{25°}$ +0.694° (c = 0.288); ν_{max}^{film} 2948, 2872, 1720, 1644, 1225, 1150 and 920 cm^{-1}. λ_{max} 220 mμ, ε = 16354, NMR (Figure 15).

Continued elution with pet. ether gave about 15 g of more polar components which are unidentified.

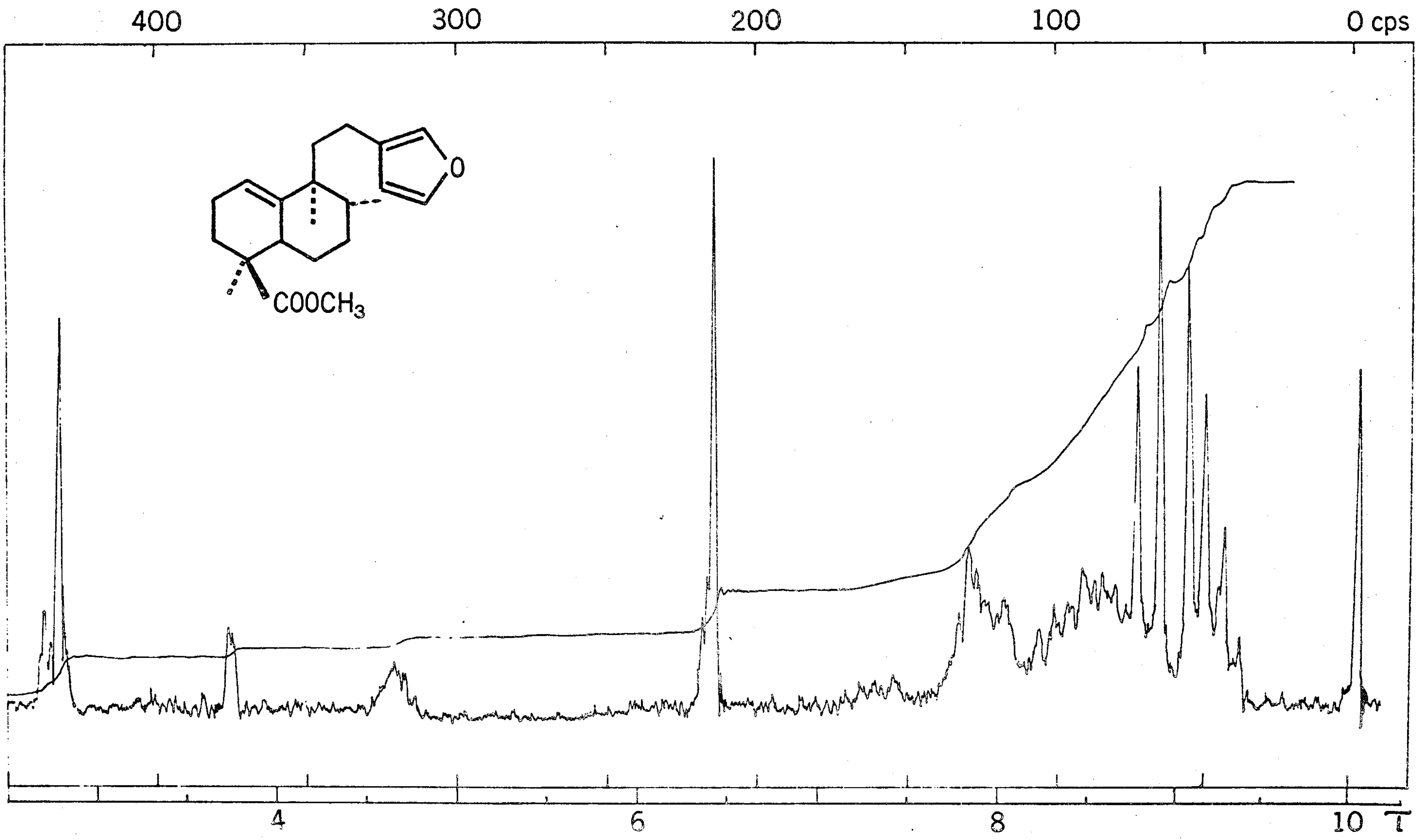

Figure 13: NMR SPECTRUM OF 93

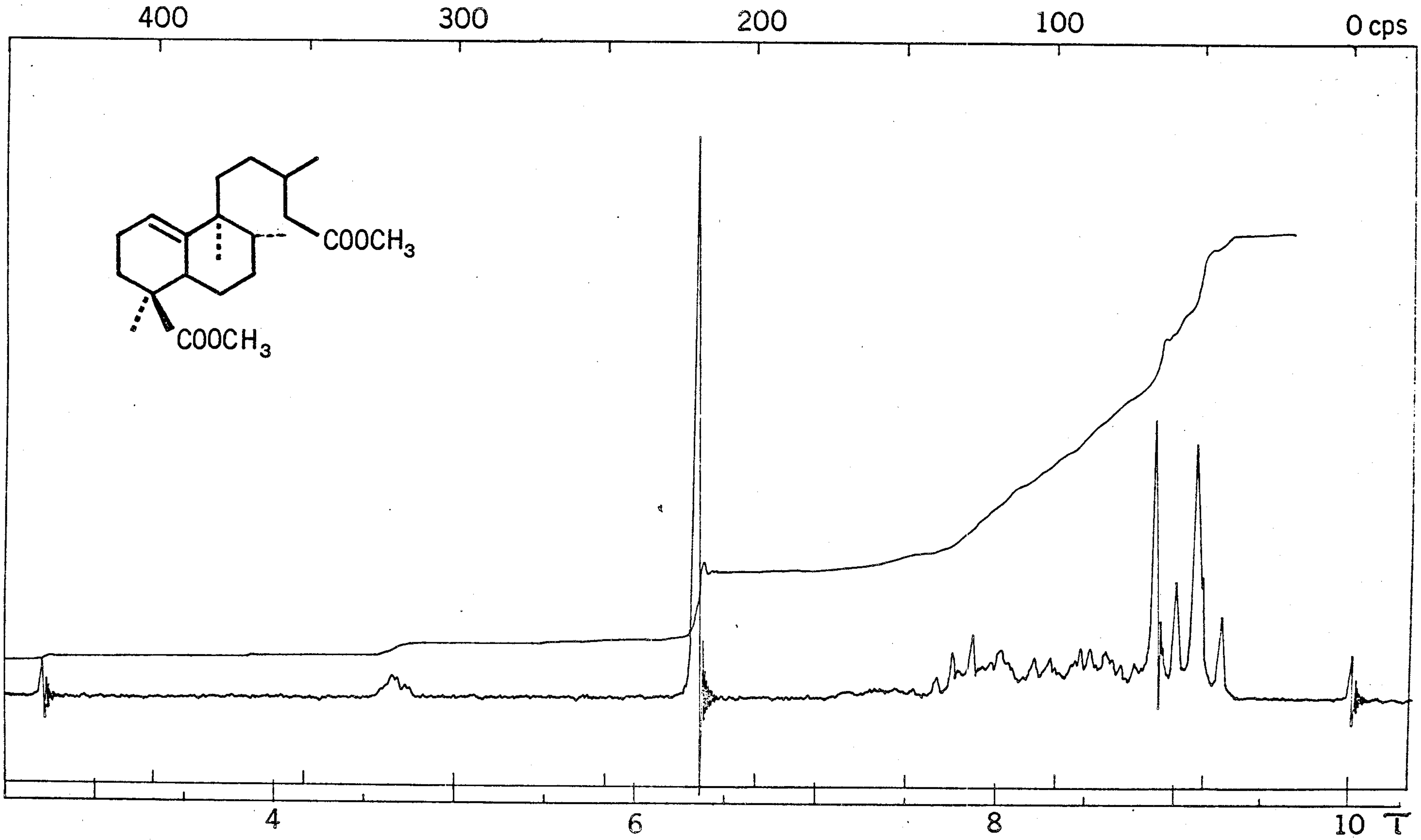

Figure 14: NMR SPECTRUM OF 85

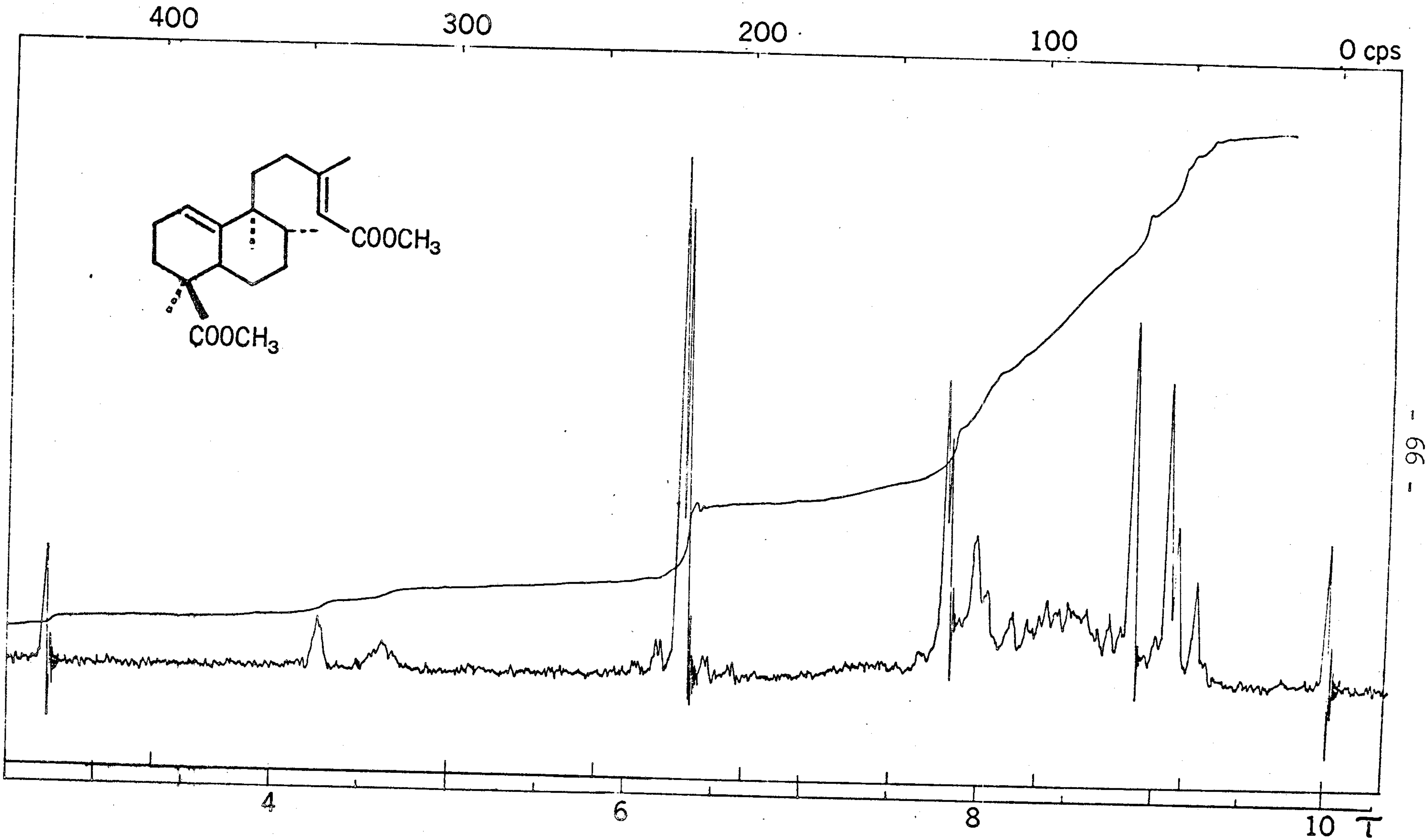

Figure 15: NMR SPECTRUM OF 86

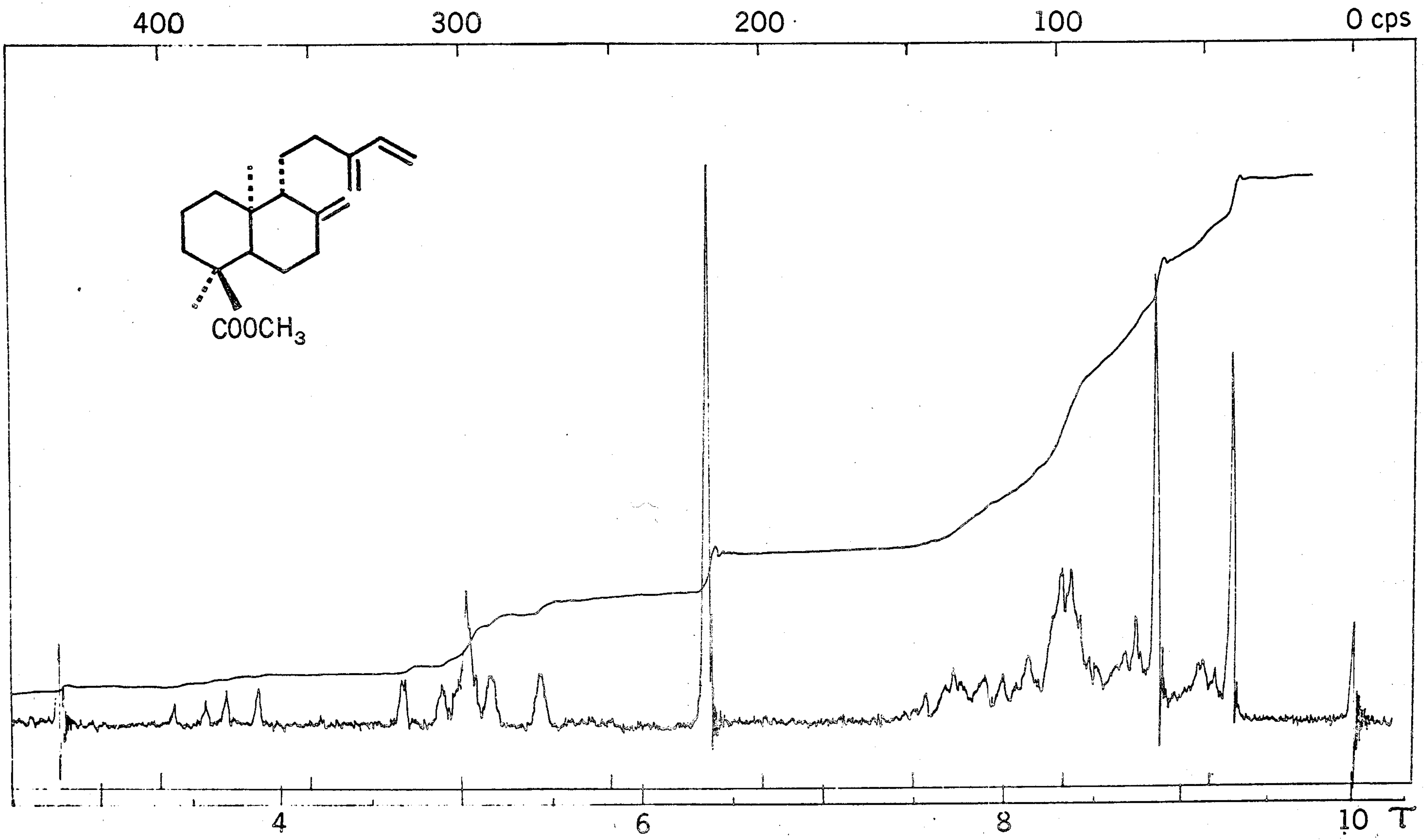

Figure 16: NMR SPECTRUM OF <u>76</u>

Isolation of Neutral Components

The neutral portion of the oleoresin was esterified with diazomethane and 21 g of the esterified neutral portion was chromatographed on 500 g Silica Gel. Elution with pet. ether:ether (4:1 to 2:1) gave 6.5 g of a mixture containing 76, 85 and 93. The separation of this mixture was effected by prep. t.l.c. on Silica Gel (10% $AgNO_3$). The major component was 76 $[\alpha]_D^{25^\circ}$ -17.5° (c = 0.4, $CHCl_3$); ν_{max}^{film} 3080, 2940, 2872, 1726, 1645, 1594, 1444, 1387, 1245, 1199, 1171, 1147, 1101, 989, 891, 759 and 715 cm^{-1}; λ_{max} 226 mμ (ϵ = 10000), NMR (Fig. 16). Calcd. for $C_{21}H_{32}O_2$ M.W. 316. Found: M.W. by mass spec. 316.

Ozonolysis of 76

A solution of 372 mg of 76 in 4 ml of EtOAc and 3 ml of glacial HAC was cooled to 0° and a stream of ozone and oxygen gas was passed through the solution for 2 hrs. To the cold reaction mixture was added 5 ml of 10% aq. NAOH and 2 ml of 30% H_2O_2. The reaction mixture was allowed to warm to r.t. The soln. was kept stirring overnight at r.t. then extracted with ether which was dried over $MgSO_4$ and evaporated to give 369 mg of an oil. Treatment of the oil with ethereal diazomethane (from 1.5 g of nitrosomethyl urea) and distilled *in vacuo* gave a viscous gum which was separated by prep. t.l.c. on Silica Gel impregnated with 10% $AgNO_3$ to give 94.7 mg of **76a** and an unidentified compound; ν_{max}^{film} 2940, 1710, 1444, 1390, 1250, 965 and 755 cm^{-1}; NMR ($CDCl_3$): δ 0.76 (3H,s), δ 1.19 (3H,s), δ 2.33 (2H; AB quartet, J = 6,6,13 Hz), δ 3.65 (3H,s), δ 3.69 (3H,s).

Base-Catalyzed Hydrolysis of Keto-Diester, **76a**

A solution of 94 mg of **76a** in 6 ml of 12% methanolic KOH was refluxed for 14 hrs. The methanol was evaporated *in vacuo*, the residue diluted with water and extracted with ether to remove traces of non-acidic material. The aqueous soln. was acidified and then extracted with ether . The solvent was removed and the residue triturated with dry ether to yield 74 mg of the keto dicarboxylic acid, **84**, crystallized from pet. ether:ethyl acetate (40:60) to give white crystals of **84**, m.p.211-212°, $[\alpha]_D^{25°}$ +11.67° (c = 0.48 in CH_3OH); ν_{max}^{film} 3500 (broad), 2940, 2874, 1700,1450,1390,1372,1248,1046 and 962 cm^{-1}; NMR ($CDCl_3$): δ 0.76 (3H,s), δ 1.19 (3H,s), δ 2.33 (2H,q.).

Reduction of 85 with LAH

To a soln. of 100 mg of 85 in 25 ml of ether was added 100 mg of LAH. The soln. was refluxed for 2 hrs. then worked up in the usual manner. Chromatography of the crude reduction product on SiO_2/10% $AgNO_3$ showed two components which were separated by prep. t.l.c. The principle product (58 mg) was a diol 87. NMR ($CDCl_3$): δ 0.78 (3H, d, J = 6Hz); δ 0.87 (3H, d, J = 6Hz); δ 0.84 (3H, s); δ 0.87 (3H, s); δ 3.34 (2H, AB quartet, J = 10.5 Hz); δ 3.60 (2H, t, J = 6.8Hz); δ 5.30 (1H, t, J = 3.5 Hz).

Acetylation of 87

The diacetate 88 was formed by allowing 87 to stand at r.t. with acetic anhydride in pyridine for 3 days. It was isolated by prep. t.l.c. as an oil, ν_{max}^{film} 1750, 1250 and 1140 cm^{-1}; NMR ($CDCl_3$): δ o.81 (3H, d, J = 6Hz); δ 0.87 (6H, s); δ 0.88 (3H, d, J = 6 Hz); δ 2.03 (6H, s); δ 3.85 (2H, AB quartet, J = 10.5 Hz); δ 4.09 (2H, t, J = 6.8 Hz) and δ 5.32 (1H, t, J = 3.5 Hz).

Allylic Oxidation of 85

A soln. of 300 mg of 85 in 1.5 ml of acetic acid and 2 ml of benzene containing 2.5 ml of acetic anhydride, 150 mg of Na_2CrO_4, 250 mg of NaOAc was stirred for 3 days at 45°. The solvent was evaporated *in vacuo* and the residue chromatographed directly on 30 g of SiO_2/10% $AgNO_3$. Elution with 250 cc. of 10% ether:90% pet. ether gave 30 mg of unreacted 85. Elution of the column with 250 cc. of 3:7 mixture of ethyl ether: pet ether gave 203 mg 89; ν_{max}^{film} 1740, 1680 and 1620 cm^{-1}; NMR ($CDCl_3$): δ 0.80 (3H, d, J = 7 Hz), δ 0.95 (3H, d, J = 6 Hz), δ 0.99 (3H, s), δ 1.23 (3H, s), δ 2.48 (2H, AB quartet, J = 16, 16, 28 Hz), δ 3.02 (1H, AB quartet, 5, 5, 11.5 Hz), δ 3.60 (6H, s), δ 5.79 (1H, s), (Fig. 17); $[\alpha]_D^{25°}$ +105° (c = 1.1, $CHCl_3$).

Calcd. for $C_{22}H_{34}O_5$ (378): C, 69.81; H, 9.05. Found: C, 70.00; H, 8.92. Mol. wt. by mass spect.: 378.

Epoxidation of 85

A soln. of 325 mg of 85 and 200 mg p-nitroperbenzoic acid in 15 ml of $CHCl_3$ was allowed to stand at r.t. for 1 hr. After this time t.l.c. analysis showed that

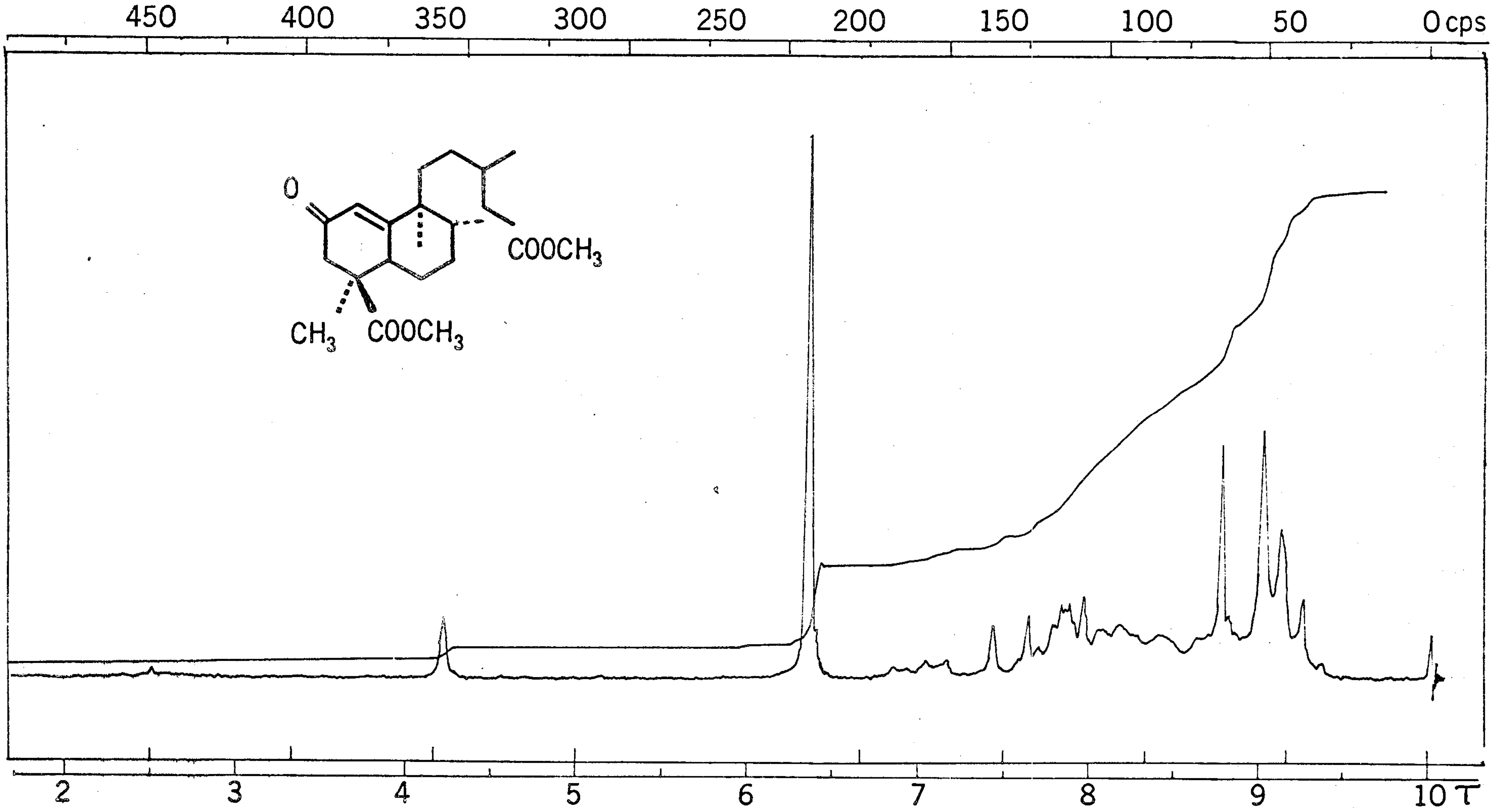

Figure 17: NMR SPECTRUM OF 89

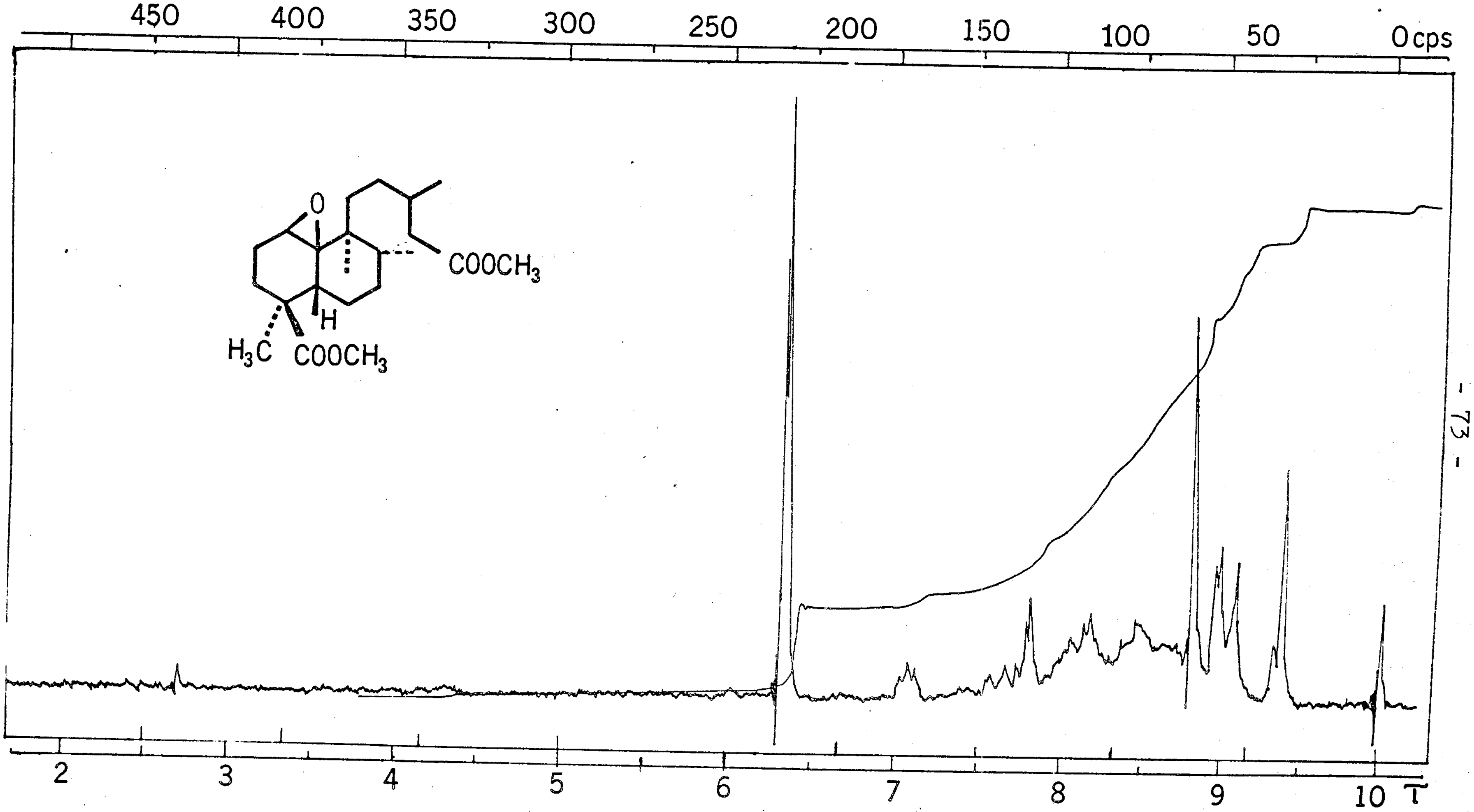

Figure 18: NMR SPECTRUM OF 90

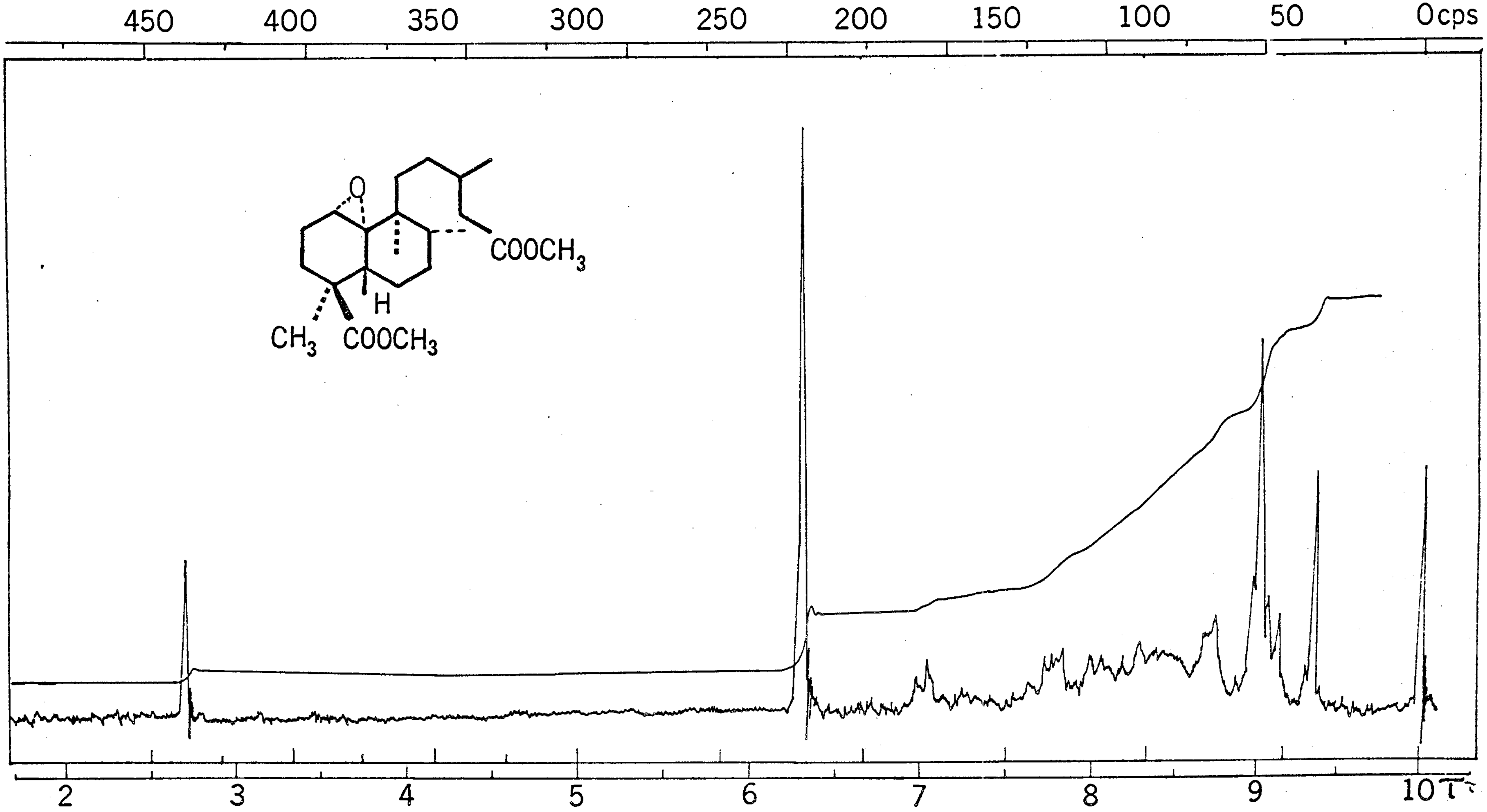

Figure 19: NMR SPECTRUM OF 91

the alkene was consumed. Prep. t.l.c. of the crude reaction mixture (SiO_2/10% $AgNO_3$) gave 107 mg 90 and 92 mg of 91 (more polar) as oils. 90: $[\alpha]_D^{25°}$ -15° (c = 1.4, $CHCl_3$); NMR ($CDCl_3$): δ0.65 (3H, s), δ 0.98 (3H, d, J = 6 Hz), δ1.16 (3H, d, J = 11 Hz), δ1.20 (3H, s) δ2.92 (1H, t, J = 2.5 Hz), δ3.63 (3H, s), δ3.65 (3H, s), (Fig. 18). Mol. wt. calcd. for $C_{22}H_{36}O_5$, 380. Found: m.s. 380.

91: $[\alpha]_D^{25°}$ +57° (c = 0.9, $CHCl_3$); NMR ($CDCl_3$): δ0.70 (3H, s), δ0.93 (3H, d, J = 6 Hz), δ0.99 (3H, d, J = 6 Hz), δ1.01 (3H, s), δ2.94 (1H, d, J = 4 Hz), δ3.68 (6H, s), (Fig. 19). Mol. wt. calcd. for $C_{22}H_{36}O_5$, 380. Found: m.s. 380.

Hydrogenation of 86

A soln. of 50 mg of 86 in 20 ml of ethyl acetate was shaken with 30 mg of $Pd/BaSO_4$ under 50 p.s.i.g. H_2 at r.t. for 18 hrs. After this time, excess $Pd/BaSO_4$ was filtered and the solvent evaporated in vacuo to give 30 mg of a compound whose IR, NMR and mass spectra were identical with those of 85.

Preparation of the Diethylene Ketal derivative of 89

A soln. of 18 mg (0.048 mmole) 89, 0.07 ml diethylene glycol (3 drops) and 4 ml p-toluenesulfonic acid in 17 ml of dry benzene was refluxed for 70 min. Benzene was then slowly distilled, fresh benzene being added to the reaction mixture at frequent intervals to maintain the initial volume. After 1.5 hrs. a total of 40 ml of distillate had been collected. The benzene soln. containing the product was washed with conc. $NaHCO_3$ soln. then with H_2O until neutral. The soln. was then dried over anhyd. $CaCl_2$ and evaporated to give 8.7 mg 89a, mol. wt. by mass spect. 466. ν_{max}^{film} 2940,2880,1730, 1670,1460,1435,1378,1238,1150 1010, 948, 850 and 730cm^{-1}.

Identification of Sesquiterpene Hydrocarbons

Sesquiterpene hydrocarbons of seed-pod resins were identified by comparative gas chromatographic KOVAT's indices[48] using α-Copaene and γ-Cadinene as standards[49].

The analyses were performed using a Varian-aerograph 1400 unit on 20' x 1/8" stainless steel columns, introducing the sesquiterpene fractions as 5% solutions in n-decane.

The method of calculating KOVAT's indices[48, 50] using two sesquiterpenes, A and B as standards is shown below:

$$I_x = (I_{std\ B} - I_{std\ A}) \times \left(\frac{\log t'_R(x) - \log t'_R(std\ A)}{\log t'_R(std\ B) - \log t'_R(stdA)}\right) + I_{std\ A}$$

where I_x, $I_{std\ A}$ and $I_{std\ B}$ = KOVAT's indices of compound x, of standard A and standard B respectively

The schematic GLC trace is shown in Fig. 20.

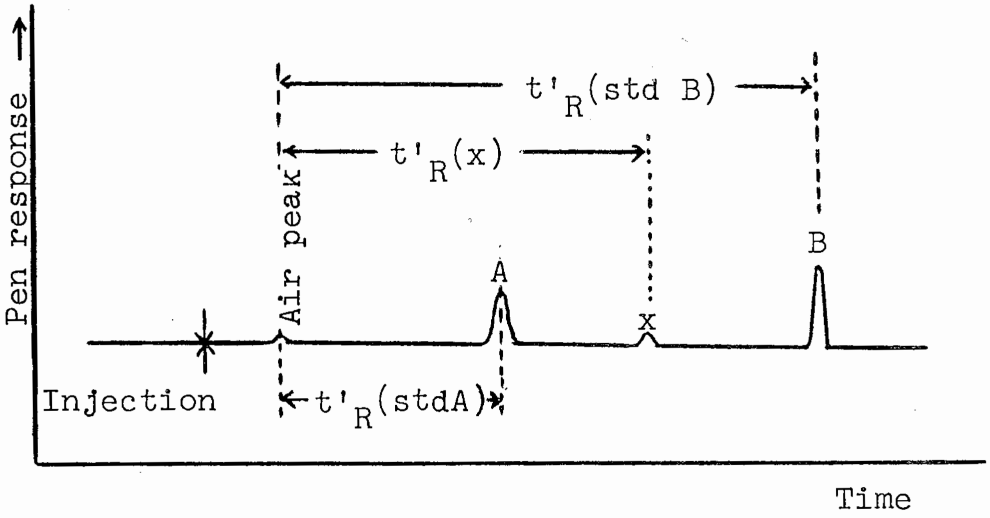

Figure 20: Schematic GLC trace

Using this method, the KOVAT's indices of sesquiterpenoids were obtained with three columns of different liquid phases (Apiezon L, DEGS and SE 30) using α-Copaene and γ-Cadinene as standards. The data obtained is shown in Table 7 and 8.

TABLE 7: KOVAT's Indices of Sesquiterpenes in Seed Pod Resins of Jamaica-grown Hymenaea Courbaril

Compounds‡	KOVAT's Indices*					
	Apiezon L, 155°c observed	Lit.	DEGS, 160°c Observed	Lit.	SE30, 130°c Observed	Lit.[48]
A	1363				1348.3	
B	1383.4					
C	1403.7					
Cyclosativene	1411.4	1411.9	1683.9	1684		
β-Bourbonene	1418.2	1418.3			1388	1386
m D	1441.4					
Caryophyllene	1451.5	1451.7			1417.4	1417.5
E	1459.2					
Calarene	1466.8	1466.0			1435.1	1435
F	1479.7					
Humulene	1489.1	1487.2			1448.9	1446.8
Selina-4(14)-7-diene	1493.4	1491.9	1852.2	1852.5		
α-Himachalene	1507.3	1508.0			1872	1870
G	1519.1					
α-Muurolene	1531.7	1531.3			1492.7	1495
δ-Cadinene	1547.3	1546.4			1504.6	1504
H	1555.5	1554.9¶				
Selina-4(14)-7(11)-diene	1572.8	1572	2019.3	2018		
I	1579.2	1580#				
J	1603.3					
K	1611.4					

* These indices are an average of three determinations and the precision are ±0.9

‡ Compounds A -K are some unidentified components.

¶ Kovat's index of γ-cadinene[48]

Kovat's index for selina-3,7(11)-diene[48]

TABLE 8*: KOVAT's Indices of A Chromagraphed Fraction of Sesquiterpenoids in Seed-Pod Resins of Jamaica-Grown *Hymenaea Courbaril*

Compounds	KOVAT's Indices			
	Carbowax 20-M, 165°		Apiezon-L, 190°[47]	
	Observed	Lit.	Observed	Lit.
α-Muurolene	1754	1753.5	1547.5	1548
δ-Cadinene	1782	1781	1560.5	1560
α-Calcorene	1928	1927	1589	1590
α-Copaene		1511.3		1433
γ-Cadinene		1792.3		1573

* Data provided by Prof. N.H. Anderson, Department of Chemistry, Univ. of Washington, Seattle, Washington.

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APPENDIX

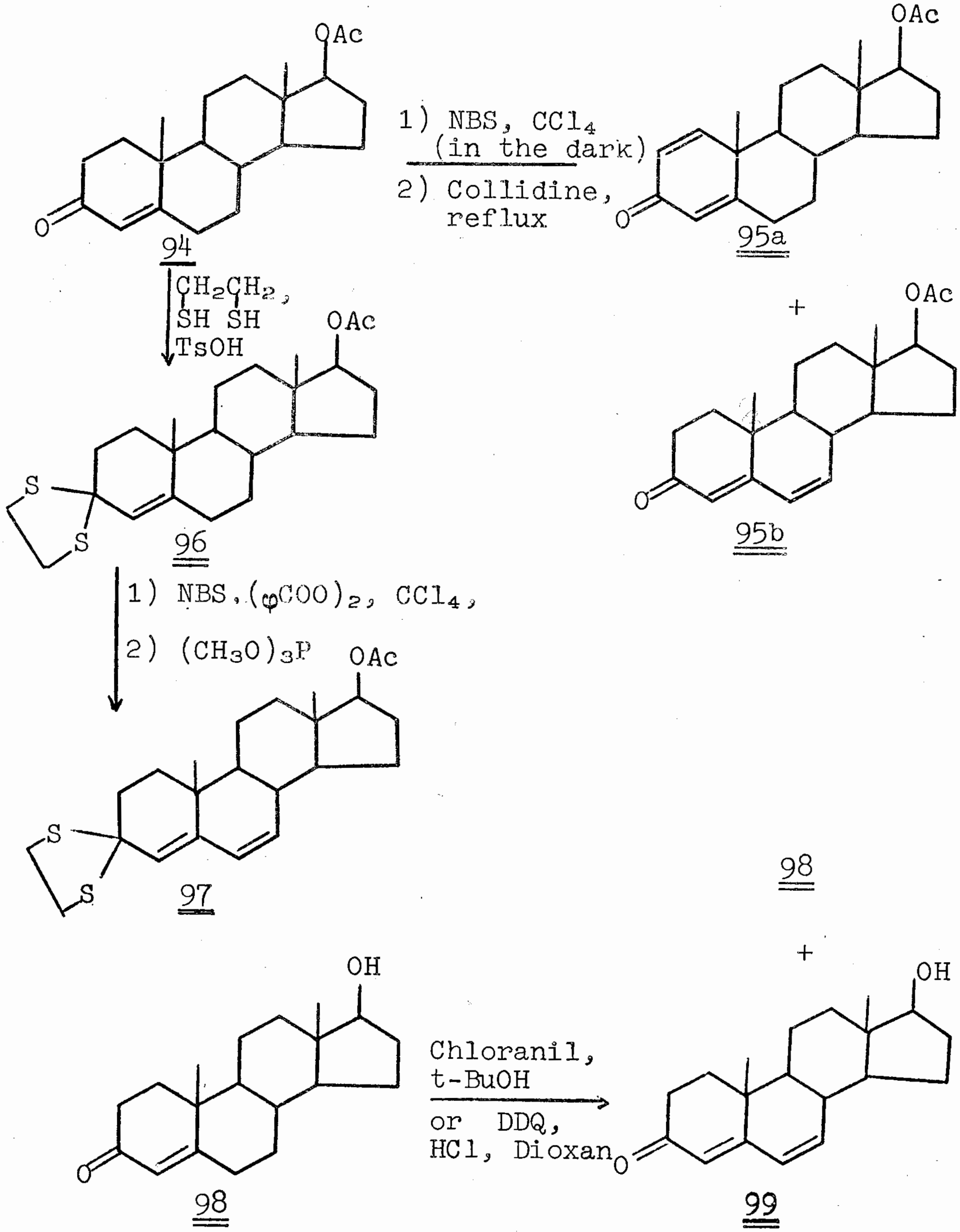
Attempted Extension of the Conjugation of Ring A of 89 to Ring B for Assignment of the Ring B-tertiary Methyl Group Location:

The introduction of ring B unsaturation was attempted by: a) NBS bromination followed by dehydrobromination[1], b) dehydrogenation with chloranil (tetrachloro-1,4-benzoquinone)[2] and c) dehydrogenation with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone)[3]. These reagents were chosen because of the relatively high yields reported for unsaturation-extension reactions in related steroidal systems[1-3].

When the model compound 4-androsten-17β-acetoxy-3-one, 94, was reacted in the dark with NBS (N-bromosuccinimide) in carbon tetrachloride followed by refluxing in collidine, a mixture of $\Delta^{1,4}$-3-one, 95a, (λ_{max} 244 mμ) and $\Delta^{4,6}$-3-one, 95b, (λ_{max} 280 mμ) was obtained. This reaction, when carried out with the thioketal derivative, 96, using benzoyl peroxide as the initiator and trimethyl phosphite for dehydrobromination gave a mixture of $\Delta^{4,6}$-diene, 97, and the starting material, 96.

The reaction of 4-androsten-17β-ol-3-one, 98, with chloranil in t-butanol or DDQ and HCl in dioxan gave

4,6-androstadiene-17β-ol-3one, 99, and the starting ketone.



From the above reactions carried out with model compounds, the reaction with NBS in the absence of light follow by dehydrobromination gave the highest yield of the corresponding $\Delta^{4,6}$-diene. Compound 89 was therefore subjected to this reaction sequence. The UV spectrum of the crude reaction mixture obtained in the dehydrobromination step gave no evidence of extended conjugation. At this stage these studies were discontinued in favor of location of the ring B methyl group in question by spectroscopic means.

Experimental

Bromination of 4-Androsten-17β-acetoxy-3-one, 94

Equimolar quantities of NBS (38 mg) and 4-androsten-17β-acetoxy-3-one (70 mg, 2.12×10^{-4} mole) were refluxed in the absence of light in CCl_4 soln. for 35 min. whereupon all the reagent had been consumed. Filtration, evaporation of the solvent gave 65mg 94a.

Dehydrobromination of 94a

A soln. of 65 mg 94a in collidine was refluxed for 1/2 hr. The soln. was cooled and dilute HCl was added until the soln. was neutral to litmus. The soln. was extracted with ether . The ether extract was washed with water to remove any excess HCl present. The soln. was then dried with anhyd. $MgSO_4$ and then evaporated in vacuo to give 40 mg of yellowish crystals; m.p. 120 - 125°c; λ_{max} 244 mμ and 280 mμ. NMR analysis showed the presence of a mixture of the starting ketone and a diene, 95b (from the signals of C_{18} and C_{19} methyl groups).

Preparation of Thioketal derivative of 4-Androsten-17β-acetoxy-3-one, 96

A mixture of 68.8 mg (0.2085 mmole) of 4-androsten-17β-acetoxy-3-one, 94, 1 ml of acetic anhydride, 67 mg of ethanedithiol and 19 mg of p-toluenesulfonic acid was stirred at r.t. White solid separated in 5 min. After 1.5 hrs., the reaction mixture was poured into water. The soln. was then filtered and the white crystals were washed with water until the washing was neutral to litmus. The solid was collected and then washed with methanol to give 81.2 mg 96 (95.6% yield); m.p. 209 - 211°c. NMR ($CDCl_3$): δ0.8 (3H,s), δ1.17 (3H,s), δ2.02 (3H,s), δ3.33 (4H,t; J = 2.5 Hz), δ4.6 (1H,t; J = 8 Hz), δ5.50 (1H,s).

Allylic Bromination of 96

A soln. of 36.9 mg (0.0909 mmole) of 96 in 3 ml CCl_4 was treated with 20 mg (0.1124 mmole) NBS and refluxed under irradiation for 20 min. when hydrogen bromide began to evolve. After this time, the mixture was cooled, the succinimide formed was removed by filtration and CCl_4 was removed by evaporating in vacuo to give 36 mg of yellow oil, 96a.

Dehydrobromination of 96a

A soln. of 36mg 96a, 3ml xylene and 0.3 ml trimethylphosphite was refluxed for 1 hr. The soln. was cooled to r.t. and the solvent and excess trimethylphosphite was removed by heating to 25°c under vacuum leaving an oil which failed to crystallize from several solvent systems. UV: λ_{max} 220 mμ, 252 mμ, 275 mμ and 355 mμ. NMR shows a mixture of starting ketone, 94, and a diene, 97.

Dehydrogenation of 4-Androsten-17β-ol-3one, 98 with DDQ

200 mg of 98 and 124 mg DDQ were dissolved in 6 ml dioxan, dry HCl was bubbled through for a few seconds and the mixture was left overnight. Ether and sodium sulphite soln. were added, followed by sodium bicarbonate soln. and water. Evaporation in vacuo left 184 mg of a

yellowish oil: λ_{max} 245 mμ and 281 mμ. NMR analysis revealed the presence of the starting ketone and a minor amount of diene, 99 (from the C_{18} and C_{19} methyl signals). The mixture resisted separation.

Dehydrogenation of 4-Androsten-17β-ol-3-one, 98 with Chloranil

A mixture of 150 mg 98 and 277 mg chloranil was heated with stirring in 17 ml of refluxing t-BUOH for 3 hrs. The excess chloranil was filtered and the filtrate taken to dryness. The residue was dissolved in $CHCl_3$ and the soln. washed with water, 5% NAOH and again with water. Evaporation of the $CHCl_3$ afforded 92 mg of a yellowish oil which resisted separation: UV 245 mμ and 281 mμ.

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