CHAPTER III

PRELIMINARY INVESTIGATION ON THE ROOT OF GYNOCARDIA ODORATA. R. Br.

Extraction and isolation of different materials.

Air dried and powdered root of Gynocardia odorata was extracted with benzene and the neutral part was separated routinely. (Details of the extraction is given in the experimental portion vide Chapter IV). Chromatography of the neutral part and elution of the chromatogram with solvents of increasing polarity resulted in the isolation of solid materials in four fractions.

Fraction	Nature	m.p. ^{o}C
A	Solid	299-301^{o}
B	Solid	132-3^{o}
C	Solid	126-28^{o}
D	Solid	212-15^{o}

Preliminary investigation of fraction A.

The fraction A was purified by repeated crystallisations from chloroform and methanol and yielded crystals of constant m.p. 304-5^{o}. It was identified as Odolactone[44] (m.m.p, CO-IR and CO-TLC).

Preliminary investigation of fraction B

The solid on repeated crystallisations from chloroform and methanol yielded crystals of constant m.p. 136-7°. It was identified as β-sitosterol (m.m.p., CO-IR and CO-TLC).

Preliminary investigation of fraction C

The fraction C on rechromatography and crystallisation from acetone afforded plate like crystals of m.p. 131-2°, $[\alpha]_D$ - 72°.

Elemental analysis lead to the molecular formula $C_{20}H_{28}O_2$, which was confirmed by mass spectrometry (M^+ 300). IR showed the presence of a carbonyl moiety and a double bond appearing peaks at 3020, 1725, 1600, 1150, 880, 820 cm^{-1}. TNM showed characteristic yellow colouration indicating the presence of unsaturation. It gave a single spot in TLC plate but GLC showed that this fraction is a mixture of two compounds with 80:20 composition. Due to paucity of the sample it prevented the author to separate the mixture by chemical methods.

Preliminary investigation of fraction D

The fraction D on rechromatography and on repeated crystallisations from ethyl acetate and MeOH yielded needle shaped crystals m.p. 218°, $[\alpha]_D$ -50°.

Elemental analysis lead to its molecular formula $C_{20}H_{30}O_3$ which was confirmed by its mass spectrometry (M^+ 318). IR

showed peaks at 1730, 1720, 1140 cm^{-1} indicating the presencee of carbonyl moiety. It also showed peaks in the region 3320-3240 (broad) cm^{-1} indicating thereby the presence of -OH group. With TNM it gave no characteristic yellow colour, showing the absence of double bond. The compound did not respond to the characteristic test for ketonic and aldehydic carbonyl groups (DNP, Oxime, Osazone derivatives). Thus the peaks at 1730, 1720 cm^{-1} may be due to the presence of ester, acyl or lactone carbonyl group.

The homogeneity of the compound was confirmed both by TLC and GLC experiments.

Literature survey showed that this compound is a new one and is christened as hydroxy Odolide.

On dehydration with $POCl_3$/Pyridine, the compound furnished a solid $C_{20}H_{28}O_2$, m.p. 131-2°, IR 3020, 1725, 1600, 1150, 880, 820 cm^{-1}. The physical properties (m.m.p., CO-TLC, IR and $[\alpha]_D$) of the compound were found identical with that of the mixture found in fraction C. The isolation of two isomeric compounds on dehydration of a single hydroxy compound indicated that the components of the mixture from fraction C contained the same structural moiety related to fraction D with an olefinic double bond at two different positions giving rise to the isomeric unsaturated components of fraction C.

Since hydroxy Odolide appears as single compound and is structurally related to the mixture of compounds viz. Odolide

and Iso Odolide (components of fraction C), first attention of structural elucidation is turned over on this compound.

SECTION A

Elemental and Spectrometric Analysis of hydroxy-Odolide (component in fraction D)

1. Elemental analysis

Elemental analysis of hydroxy Odolide suggested the molecular formula to be $C_{20}H_{30}O_3$ which indicated that the compound isolated belonged to the diterpene group.

2. Mass spectrum analysis of hydroxy Odolide

The mass spectrum of the compound (Fig. 4) showed the molecular ion peak at 318 (M^+) along with other peaks at m/z 300, 285, 272, 260 (base) and 257.

Appearance of a sharp peak at m/z 300 indicated elimination of water from molecular ion ($M^+ - CH_3$) and the existence of the fragment $C_{20}H_{28}O_2$. A small peak appeared at m/z 285. This was due to elimination of methyl from the fragment m/z 300 ($C_{20}H_{28}O_2$). Appearance of a peak at m/z 272 was for the existence of the fragment $C_{18}H_{24}O_2$. The base peak at m/z 260 indicated the presence of the fragment $C_{17}H_{24}O_2$ and the peak that appeared at m/z 257 was due to the existence of the fragment $C_{17}H_{21}O_2$.

MASS SPECTRUM ; 2
SAMPLE:R-GO-R-40,DR.B.P.PRADHAN
NOTE :24/9/85.
R.T. 0'08" TIM 0.0 RIC 1000.0
BASE PEAK : M/E 260.0 INT. 235.8

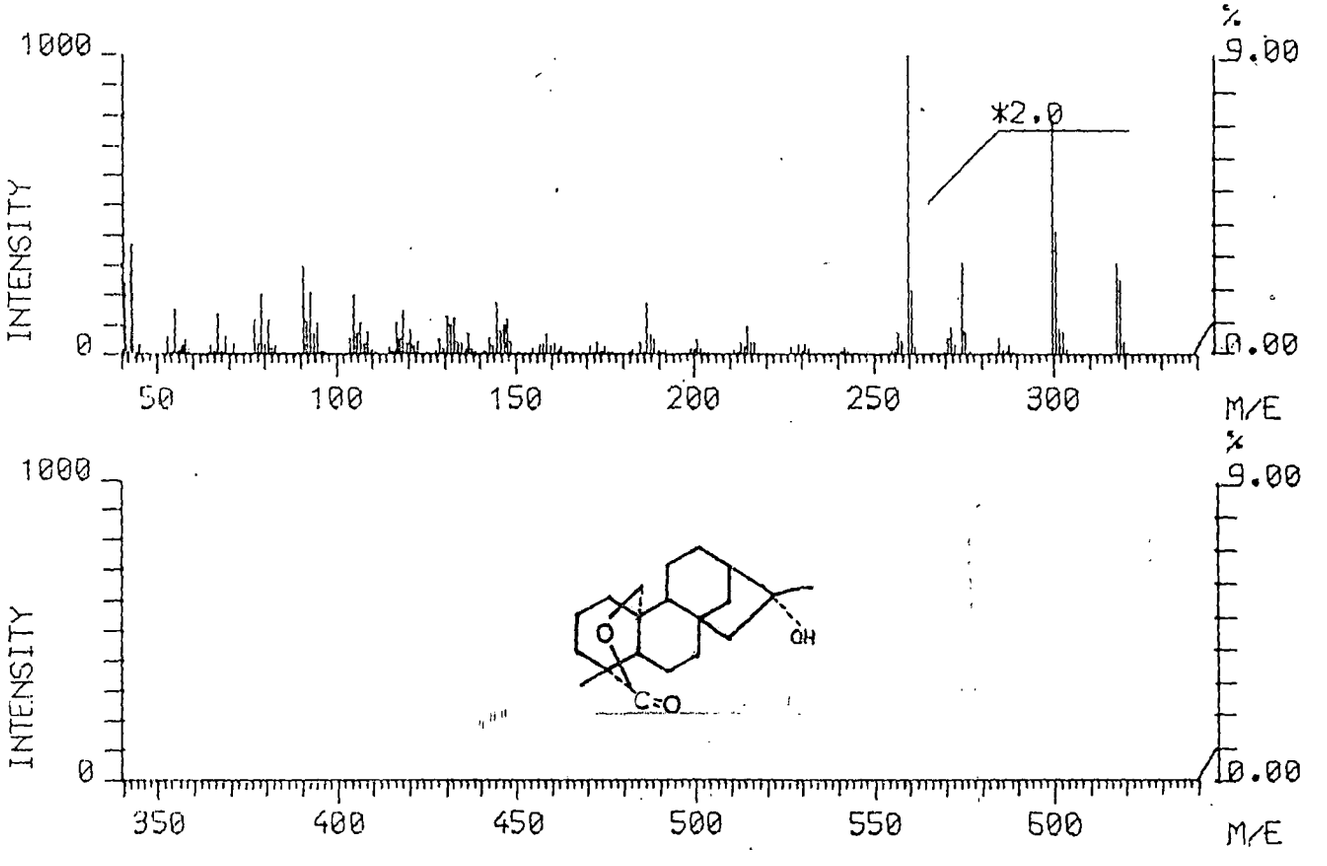


Fig. 4 Mass spectrum of hydroxy odolide, 1_c

3. Infrared spectrum of hydroxy Odolide

The infrared absorption spectrum of hydroxy odolide (Fig. 1) was examined in KBr with nujol mulling. The broad peak in the region 3320-3240 cm^{-1} indicated the presence of hydroxy group in the compound.

The peaks at 1730, 1720 and 1140 cm^{-1} indicated the presence of carbonyl moiety.

4. Ultraviolet absorption spectrum of hydroxy Odolide.

The ultraviolet absorption spectrum of hydroxy Odolide was recorded in the region 220-260 nm using water free methanol (with a few drops of chloroform) as solvent. No characteristic absorption was observed showing the absence of characteristic group absorbing in the region.

5. ^{1}H NMR spectrum of hydroxy Odolide.

The ^{1}H NMR of hydroxy Odolide was studied in $CDCl_3$ at 300 MHz. The spectrum (Fig. 2) showed two tertiary methyl groups at δ0.87 and 1.33 ppm as singlets, two protons centred at δ 4.01 and 4.12 ppm which are coupled with each other with geminal coupling constant 14 Hz thereby appeared as doublets. The peak (that appeared) in the region at δ 2.1 - 2.14 ppm probably as AB quartet indicated methylenic protons (2H) at C-15 which is also typical of Kauranoids.

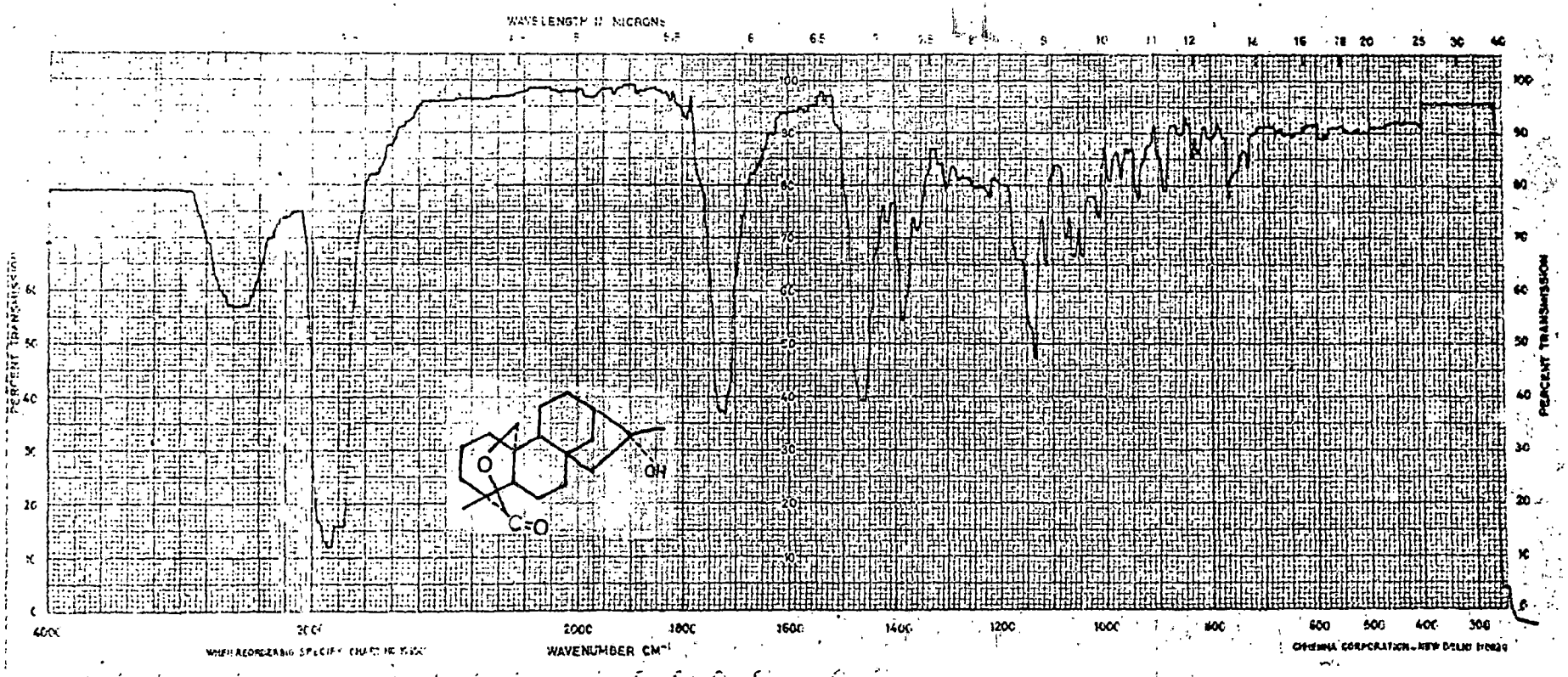


Fig. 1 IR spectrum of hydroxy odolide, 1_c

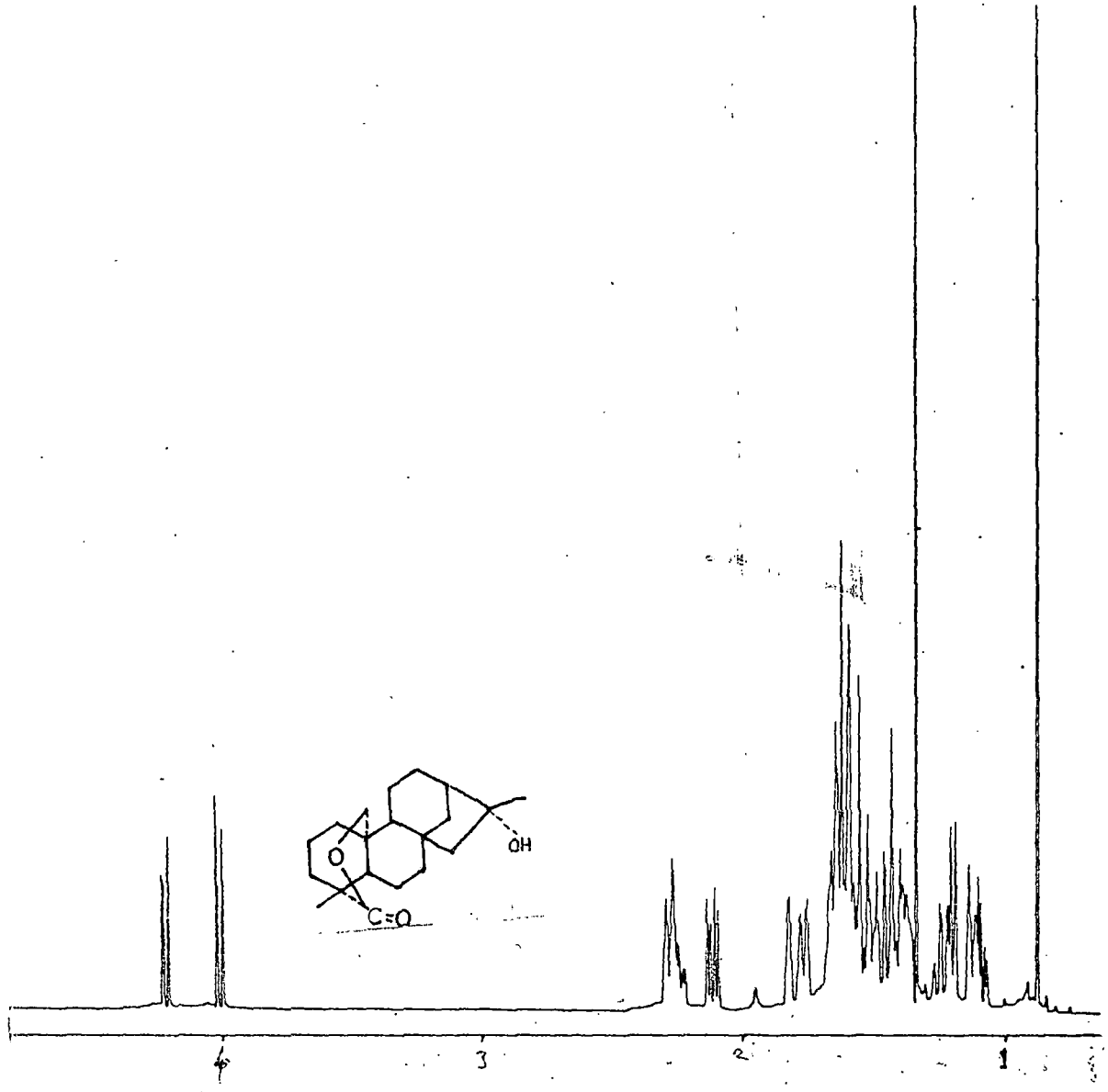


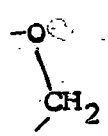
Fig. 2 ^{1}H NMR spectrum of hydroxy odolide, 1_c

6. ^{13}C NMR Spectrum of hydroxy Odolide

The ^{13}C NMR spectrum along with APT of hydroxy odolide is shown in Fig. 3. It shows 2-CH_3, 10-CH_2, 3-C-H groups and five non protonated carbons. The triplet at 76.51 ppm is due to a methylene carbon which bears oxygen. The singlet at 174.78 ppm is due to lactone carbonyl carbon and the singlet at 79.25 ppm is attributed for carbon bearing the another oxygen. Thus the hydroxy odolide is tertiary in nature. The number of different groups in hydroxy odolide and their ^{13}C chemical shift values are tabulated (Table 3) below.

Table 3

Number of different groups and ^{13}C shift values of hydroxy Odolide.

Groups	Number	^{13}C chemical shifts (ppm)
$-CH_3$	2	23.58, 24.30
$-CH_2$	9	18.72, 20.54, 22.48, 23.90, 35.24, 39.10, 39.66, 40.57, 56.55
	1	76.51
$>CH$	3	49.19, 49.66, 53.14
$-C-$	3	32.89, 44.66, 47.65
$HO-C-$	1	79.25
$>C=O$	1	174.78

Total number of carbons = 20, protons = 30, oxygen = 3

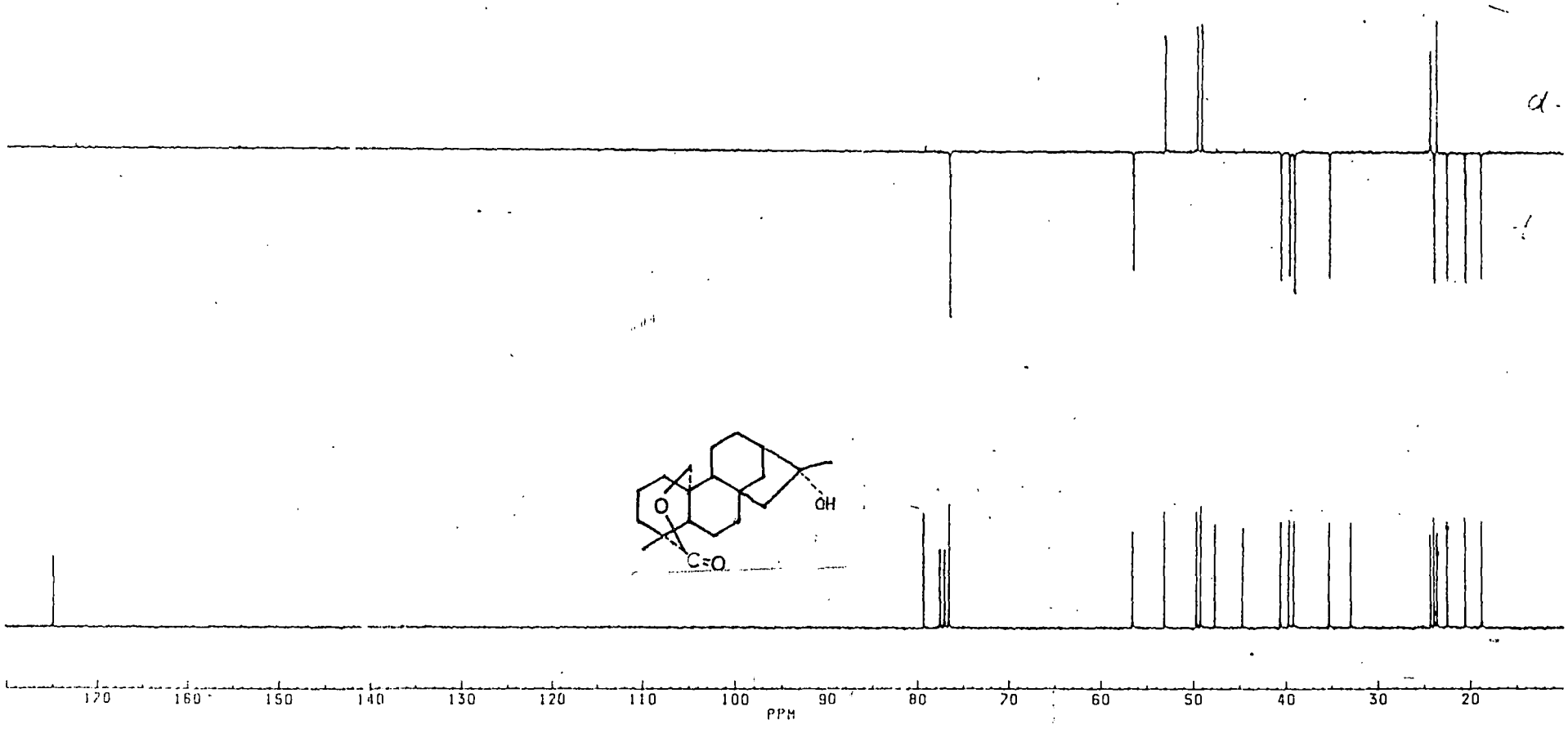


Fig. 3 ^{13}C NMR spectrum of hydroxy odolide, 1_c

This establishes the formula $C_{20}H_{30}O_3$, for hydroxy odolide which is in agreement with results of elemental analysis and mass spectrum.

SECTION B Structure elucidation of hydroxy-odolide (composition of fraction D) from spectral analysis.

Molecular formula of hydroxy Odolide

Elemental analysis and mass spectrum of hydroxy Odolide showed that t he molecular formula of the compound is $C_{20}H_{30}O_3$. This is also supported by the appearance of peaks characteristic of different functional groups in ^{13}C NMR (vide Table 3). Since the molecule contains only 20 carbon atoms the absorption at 1730, 1720 cm^{-1} must be due to lactone carbonyl group (intra-molecular ester group). From the position of IR absorption peak, it is indicative that the lactone ring must be a six membered one. The Jones' oxidation of hydroxy Odolide was performed in order to establish whether the hydroxy is primary, secondary or tertiary one. The resulting product on analysis was found to be the starting material indicating the tertiary nature. Thus the hydroxy group (-OH) is attached to a tertiary carbon atom.

Functional nature of oxygen atoms in hydroxy Odolide.

The IR spectrum (Fig. 1) of hydroxy Odolide showed the presence of hydroxy group (band at 3320-3240 cm^{-1}) and a δ-lactone moiety (band at 1730, 1720 and 1140 cm^{-1}), accounting the

functional nature of all the oxygen atoms in the compound. The nature of oxygen functions is also established by the appearance of three peaks in the low field region of ^{13}C NMR at 174.18 ppm (six membered lactone carbonyl carbon), 79.78 ppm (for a carbon bearing the hydroxyl group) and 76.51 ppm (for a carbon bearing the lactone oxygen).

Nature of Carbon Skeleton of hydroxy Odolide

Hydroxy Odolide has the molecular formula $C_{20}H_{30}O_3$ and contains one hydroxyl function and a δ-lactone moiety and no double bond (negative TNM colouration).

Replacement of the hydroxyl group by one hydrogen atom and two oxygens of the lactone moiety by four hydrogen atoms gives the molecular formula $C_{20}H_{34}$. An open chain saturated C-C skeleton containing 20 carbon atoms should have a total of 20 x 2 + 2 = 42 free valencies. Thus it is obvious that in hydroxy Odolide the number of valencies yet to be satisfied is 42-34 = 8, necessitating the presence of $\frac{8}{2}$ = 4 rings, and so the hydroxy Odolide is tetracyclic. The hydrocarbon $C_{20}H_{32}$ is equivalent to the general formula $(C_5H_8)_n$ with n = 4 appears to be tetracyclic diterpene. Therefore, the carbon-carbon skeleton of hydroxy Odolide can be fitted in either of the skeleton of Kaurane, Stachane, Gibbane, Ericacane and Tigliane (vide Chapter II, Table 1).

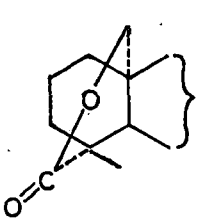
The peaks at §0.87 and 1.33 ppm in ^{1}H NMR are due to two tertiary methyl groups. The doublets appeared at §4.01 and 4.12 ppm representing two protons coupling with each other with geminal coupling constant 14 Hz attributed for the protons on the same carbon bearing the lactone oxygen. Therefore, one of the skeletal methyl group has been converted to lactone carbonyl carbon. Hence the skeleton of hydroxy Odolide must contain four methyl groups. Since Ericacane and Tigliane bear five methyl groups in their skeletons, therefore the hydroxy Odolide should not have Ericacane and Tigliane skeletons. Now we have to decide which of the skeleton between kaurane and stachane would fit in the hydroxy-Odolide.

APT showed the presence of five non-protonated carbons in hydroxy odolide, one of which is lactone carbonyl carbon. The non-protonated carbon that appeared at 79.25 ppm is attributed for the carbon bearing the hydroxyl group. So, out of five non-protonated carbons of hydroxy odolide there exist three non-protonated carbons in its skeleton. Among the proposed skeletons kaurane has three non-protonated carbons, but stachane has four such non-protonated carbons. Therefore, the carbon skeleton of hydroxy odolide is that of kaurane.

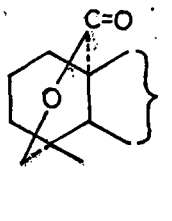
Position of lactone carbonyl and lactone oxygen attachment in hydroxy Odolide.

Out of the four primary carbon atoms of kaurane skeleton two are involved in the formation of §-lactone moiety of hydroxy

Odolide. It is only possible by using C-19 and C-20. Thus two possible structures viz. P and Q of hydroxy Odolide may be proposed .

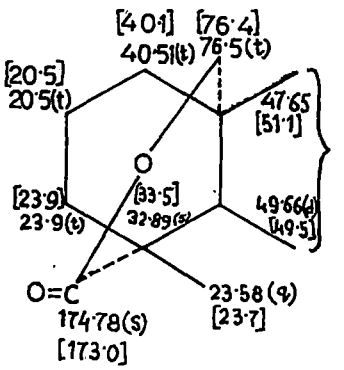


P



Q

There is a reported compound named potamogetonin[36], the lactone part of which is identical with the partial structure P. The ^{13}C shift values of lactone moiety part of potamogetonin[36] have striking resemblance comparable with that of some peaks of hydroxy Odolide as shown below



[The ^{13}C shift values in the square brackets represent that of potamogetonin].

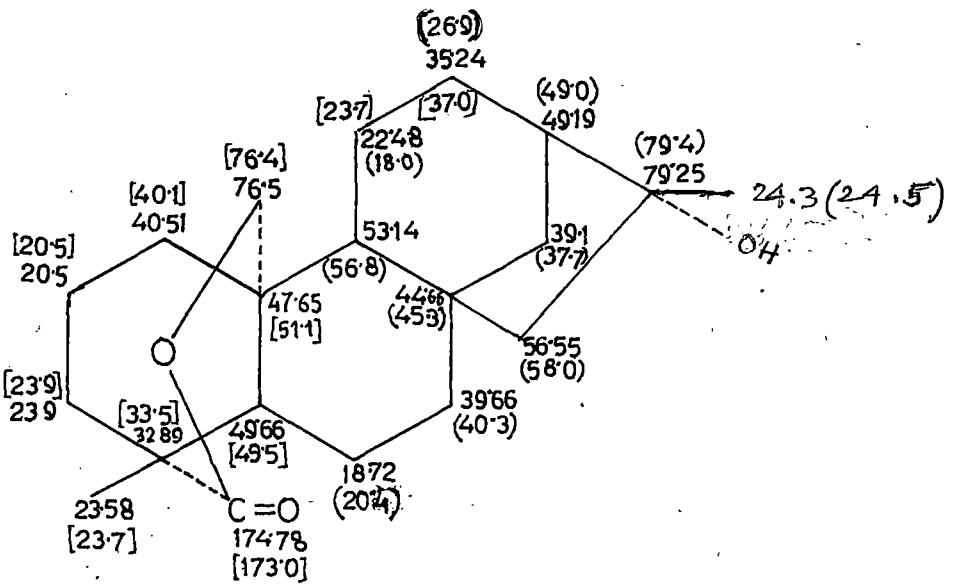
These close similarities of the ^{13}C shifts confirm the assignment of δ-lactone moiety of hydroxy Odolide as shown in P.

Position of the hydroxyl group in hydroxy Odolide.

The hydroxy group as evident in the IR spectrum by the peak at 3320-3240 cm^{-1} has been found to be resistant to oxidation by Jones' reagent. Thus the tertiary nature of hydroxy group is evident which is also supported by ^{1}H NMR that showed no resonance beyond 3-5 ppm except for the double doublet at δ4.01-4.12 ppm. Since the hydroxy group is tertiary in nature (evident from ^{1}H NMR and APT) it is attached to either C-5, C-9, C-13 or C-16 of the Kaurane skeleton. As there is no secondary methyl group in the hydroxy Odolide the hydroxy group must be at C-16. The position of hydroxy group at C-16 not only explains the existence of two tertiary methyl groups but also explains the downfield shift of the C-16 methyl at δ1.33 ppm in ^{1}H NMR.

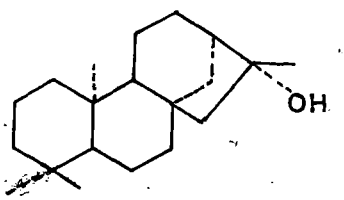
Conclusion

The structure of hydroxy odolide is established as



The data in the square bracket and parentheses indicate the ^{13}C shift values to that of potamogetonin and (-) ent-16 α-kauranol.

It is to be mentioned in this connection that the total ^{13}C shift values of hydroxy odolide have been assigned in the above structure by comparison with the ^{13}C shift values of potamogetonin (23) and (-) ent-16 -kauranol[36,45].



(-)ent-16 α-Kauranol

A comparative data of ^{13}C NMR spectra of hydroxy Odolide, potamogetonin and (-)ent-16 α-kauranol are given in the following table 4.

Table 4

^{13}C NMR spectra of hydroxy Odolide, potamogetonin and (-)ent-16α-Kauranol.

No. of Carbon atom	Compound		
	Hydroxy Odolide	Potamogetonin	(-)ent-16 -α kauranol
1	40.57	41.1	42.0
2	20.5	20.9	18.6
3	23.9	25.8	42.0

Table 4 (Contd..)

4	32.89	33.5	33.2
5	49.66	49.5	56.2
6	18.77	28.1	20.4
7	39.66	36.1	40.3
8	44.66	145.2	45.3
9	53.14	51.7	56.8
10	47.65	51.1	39.3
11	22.48	23.7	18.0
12	35.24	37.0	26.9
13	49.19	125.1	49.0
14	39.1	110.0	37.7
15	56.55	142.6	58.0
16	79.25	138.9	79.4
17	24.30	76.4	24.5
18	23.58	23.7	33.5
19	174.78	173.0	21.6
20	76.51	108.3	18.0

SECTION C Chemical proof of the structure of hydroxy-Odolide

The structure of hydroxy odolide was confirmed by converting the compound to a known compound 16∝-hydroxy-(-) kauran 19-oic acid[47a,b,c].

The lactone ring was opened by Li-EDA by the same procedure adopted by Pradhan et al[46]. The hydroxy Odolide was dissolved in ethylene diamine and to the solution Lithium metal was added in portions. The mixture was then refluxed for 2 hours under nitrogen atmosphere. Excess lithium was destroyed by adding ammonium chloride and then acidified with dilute HCl. It was extracted with ether. The ether solution was treated with dilute NaOH to separate neutral and acid part. The alkali layer on acidification with HCl gave white solid. It was filtered, washed with water and dried. The dried mass was dissolved in minimum quantity of benzene and poured on silica gel column developed with petroleum ether. The fraction on elution with 100% benzene showed single spot on TLC plate. On repeated crystallisations from chloroform-methanol gave amorphous solid m.p. 281-2°, $[\alpha]_D$ - 92°. It was analysed for $C_{20}H_{32}O_3$ and was found identical with 16∝-hydroxy-(-) kauran 19-oic acid[47a,b,c] [m.p. IR (Fig. 5)].

The acid on esterification with diazomethane in the usual procedure gave an ester which on crystallisation from chloroform-methanol afforded fine needle shaped crystals m.p. 155-7°.

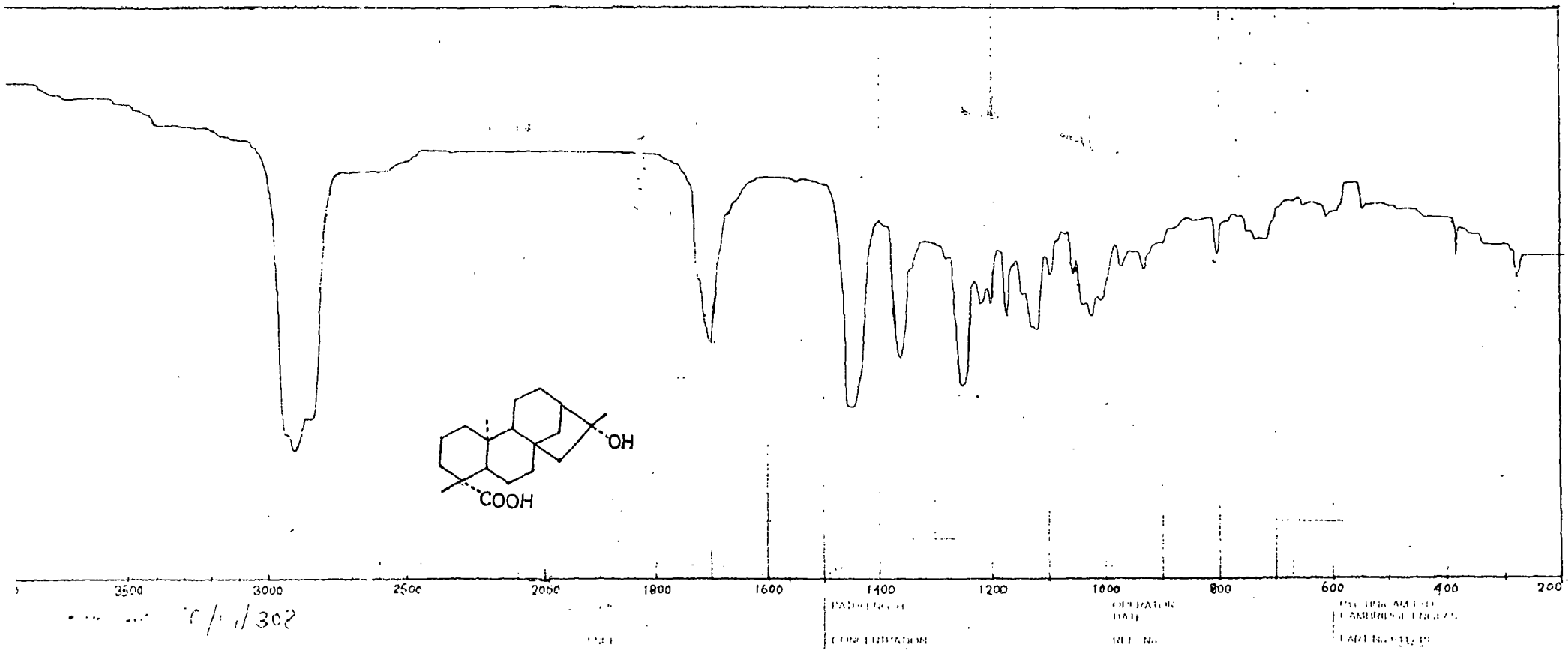


Fig. 5 IR spectrum of 16α-hydroxy-(-)-Kauran-19-oic acid

Elemental analysis and mass spectrum data (M^+ 334) showed the molecular formula of the ester to be $C_{21}H_{34}O_3$. IR spectrum (Fig. 6) of this compound showed peaks at 3340-3240 cm^{-1} for hydroxyl and 1720 cm^{-1} for carbomethoxy group. IR spectrum thus gave an information that a hydroxy ester was formed from a hydroxy acid which in turn formed from hydroxy odolide (a compound isolated from natural source) by reduction with Li-EDA. This inference is further supported by the analysis of ^1H NMR spectrum (Fig. 7) of the hydroxy ester.

^1H NMR analysis:

^1H NMR of the hydroxy ester (Fig. 7) exhibited signals for three tertiary methyls at δ0.738, 0.896 and 1.360 ppm as singlet. The down field shift of the methyl proton at δ 1.360 ppm indicated it to be situated on a tertiary carbon atom at C-16 carrying the hydroxyl group. Peak appeared in the region at δ 2.23-2.30 ppm as AB quartet indicated methylenic protons (2H) at C-14 was also typical of kauranoids. A sharp singlet appeared in the downfield region at δ3.67 ppm integrable for three protons was for the carbomethoxy group.

Mass spectrum analysis

Mass spectrum of the ester (Fig. 8) showed molecular ion peak at 334 (M^+) with other peaks appeared at m/z 316, 301, 276 and 257.

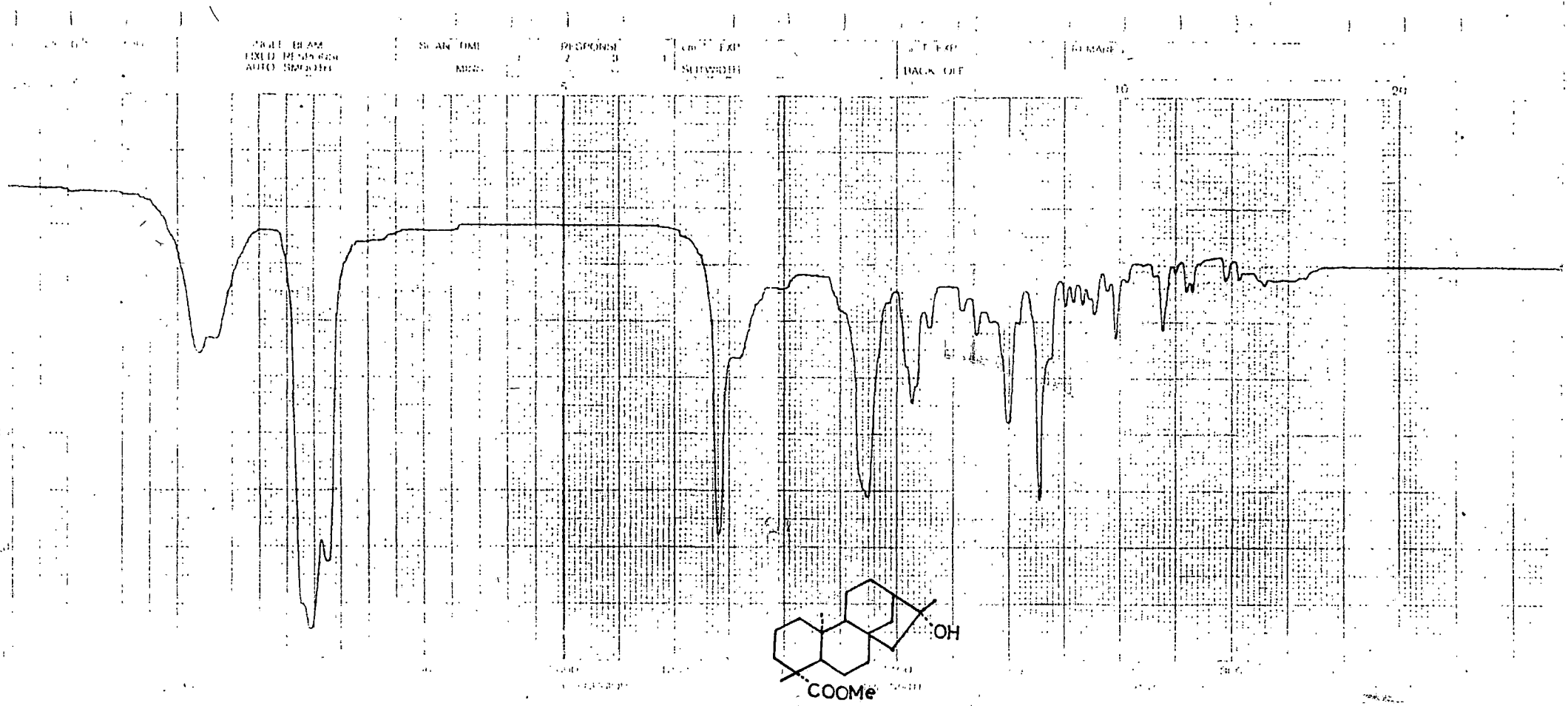


Fig. 6 IR spectrum of hydroxy ester 1_d

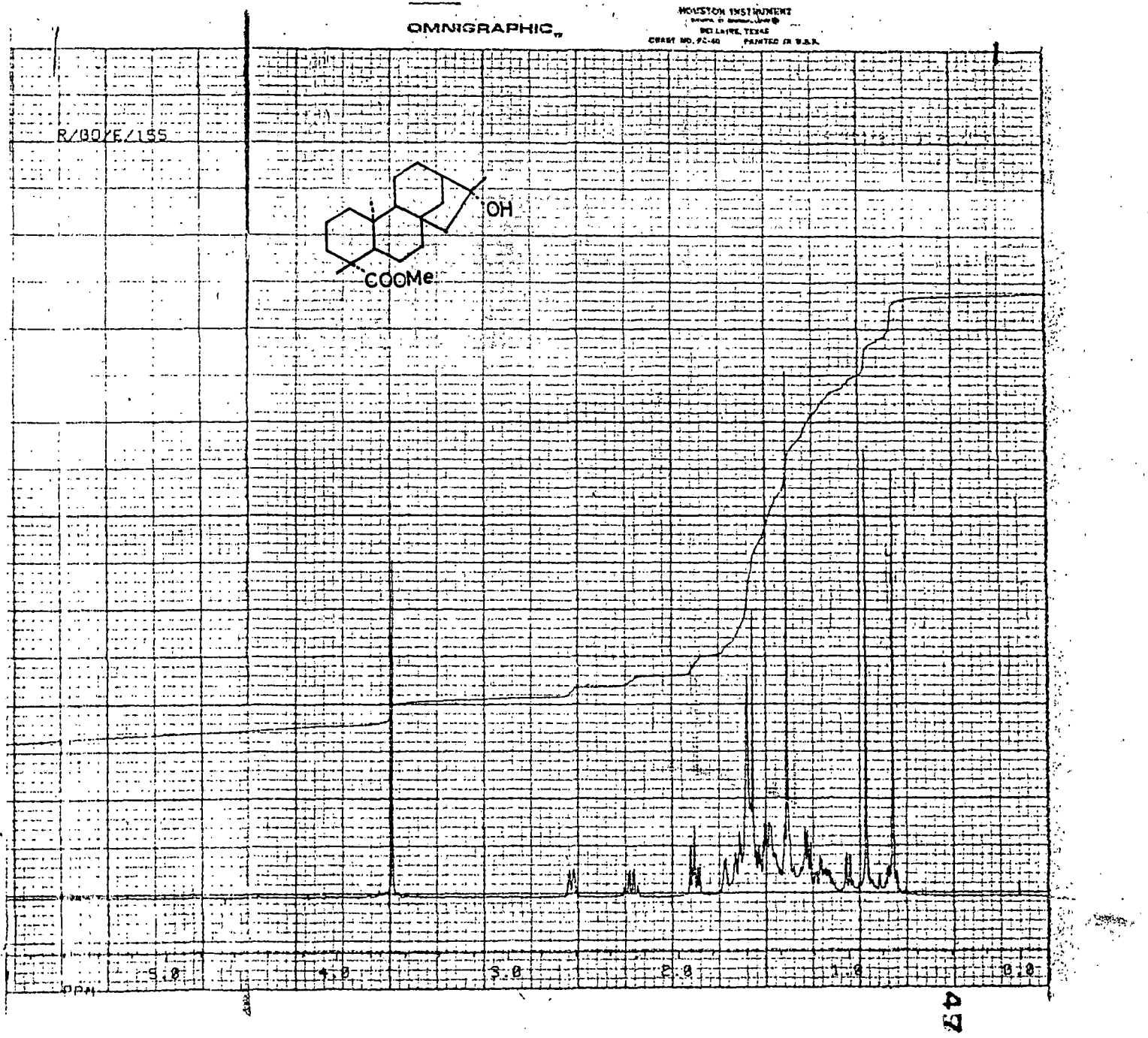


Fig. 7 ^1H NMR spectrum of hydroxy ester 1$_d$

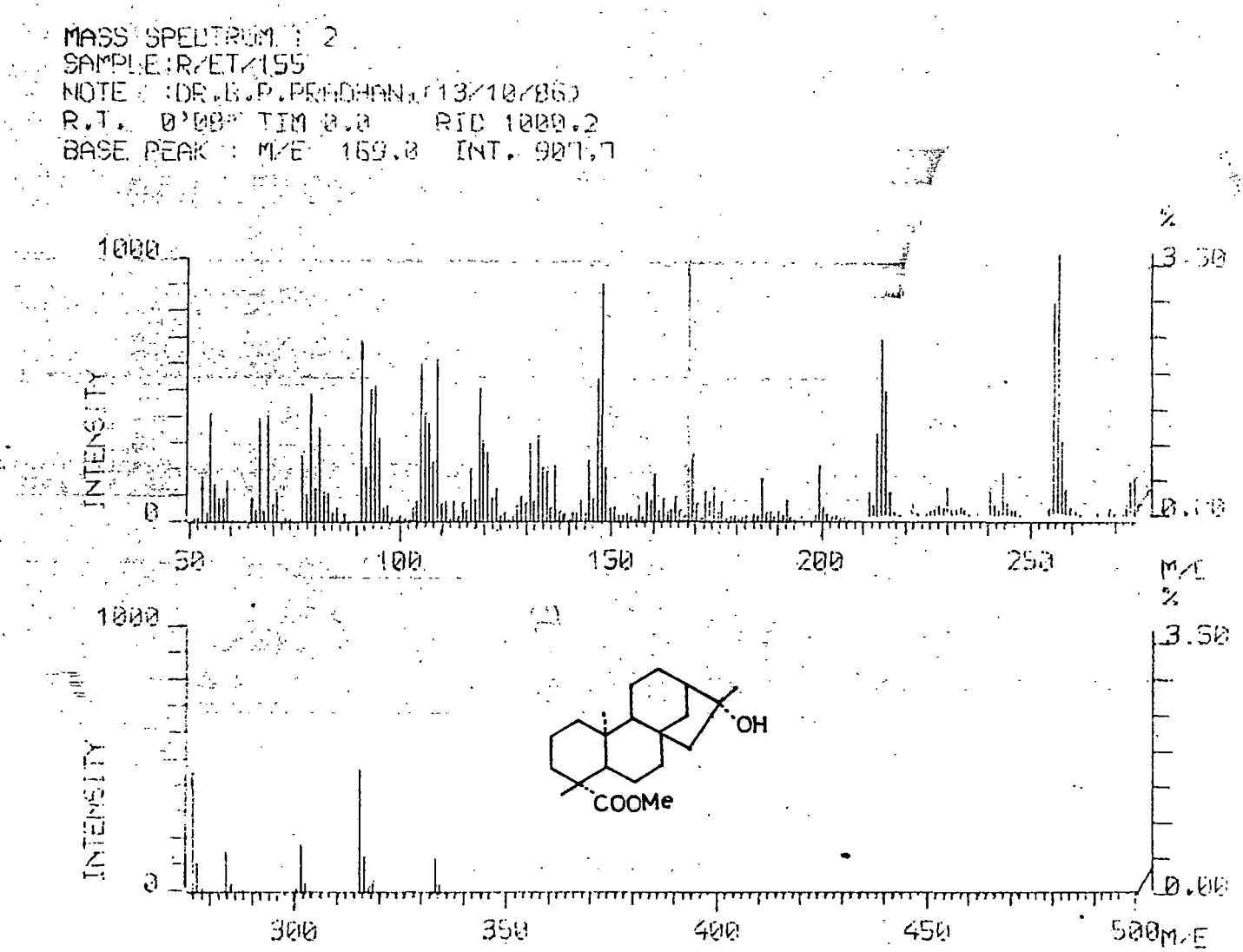
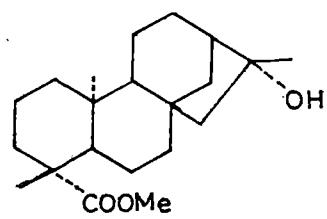


Fig. 8 Mass spectrum of hydroxy ester I_d

A prominent peak appeared at m/z 316. It may be due to the elimination of water from the molecular ion ($M^+ - H_2O$) and is due to the existence of the fragment $C_{21}H_{32}O_2$. Another prominent peak appeared at m/z 257 and this may be due to the existence of fragment $C_{17}H_{21}O_2$.

Thus all the above data (viz. IR, ^1H NMR, Mass) of the esterified product $C_{21}H_{34}O_3$ would be compatible with the ester of 16α-hydroxy (-)-kauran-19-oic acid. Hence the prepared ester is nothing but the ester of 16α-hydroxy-(-) kauran-19-oic acid. Hence the structure of the esterified profuct should be I_d as follows.



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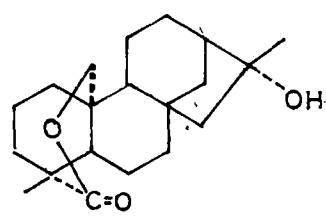
Again, since the acid was obtained from the hydroxy odolide by Li-EDA reduction, therefore it is confirmed that the carbonyl oxygen of lactone must be linked with C-19.

Therefore from physical and chemical evidences, the structre of hydroxy Odolide I_c should be assigned as 16α-hydroxy -ent-kauran-19 $\longrightarrow$ 20 olide as follows:



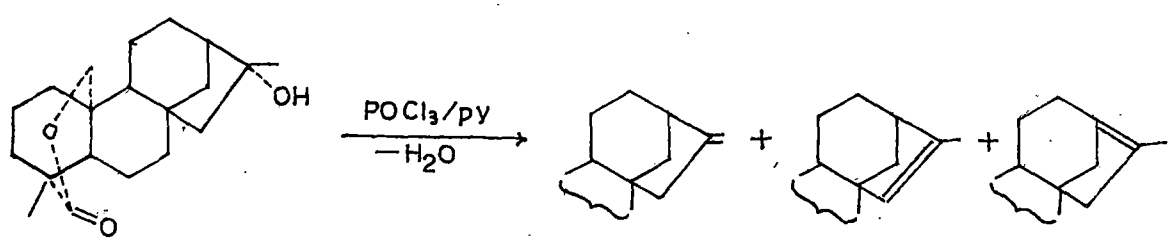
Ic

Hydroxy Odolide (16α-hydroxy-ent-kauran-19 $\longrightarrow$ 20 lide).

SECTION D : Structure elucidation of the components in fraction C

Preliminary investigation of fraction C gave an information that fraction C is an isomeric mixture of two compounds which is evident from GLC (Fig. 9). Furthermore, $POCl_3$ - Pyridine dehydration of hydroxy Odolide (Ic) gave a mixture of two double bond isomers identical with the mixture of two compounds in fraction C (mmp, CO-IR, CO-TLC). These two isomeric mixture of compounds have so close polarity that it was not possible to separate the mixture by chromatography.

Hydroxy odolide on dehydration with $POCl_3$-Py may give the three possible products having structures a, b and c as follows:



Ic a b c

The formation of c is not possible because bridge-head carbon can not have the double bond (Cf Bredt's rule). Hence the formation of c from hydroxy odolide can easily be excluded. Now the only two possibilities left may be a and b. So in the fraction C there may be two isomeric compounds having structures

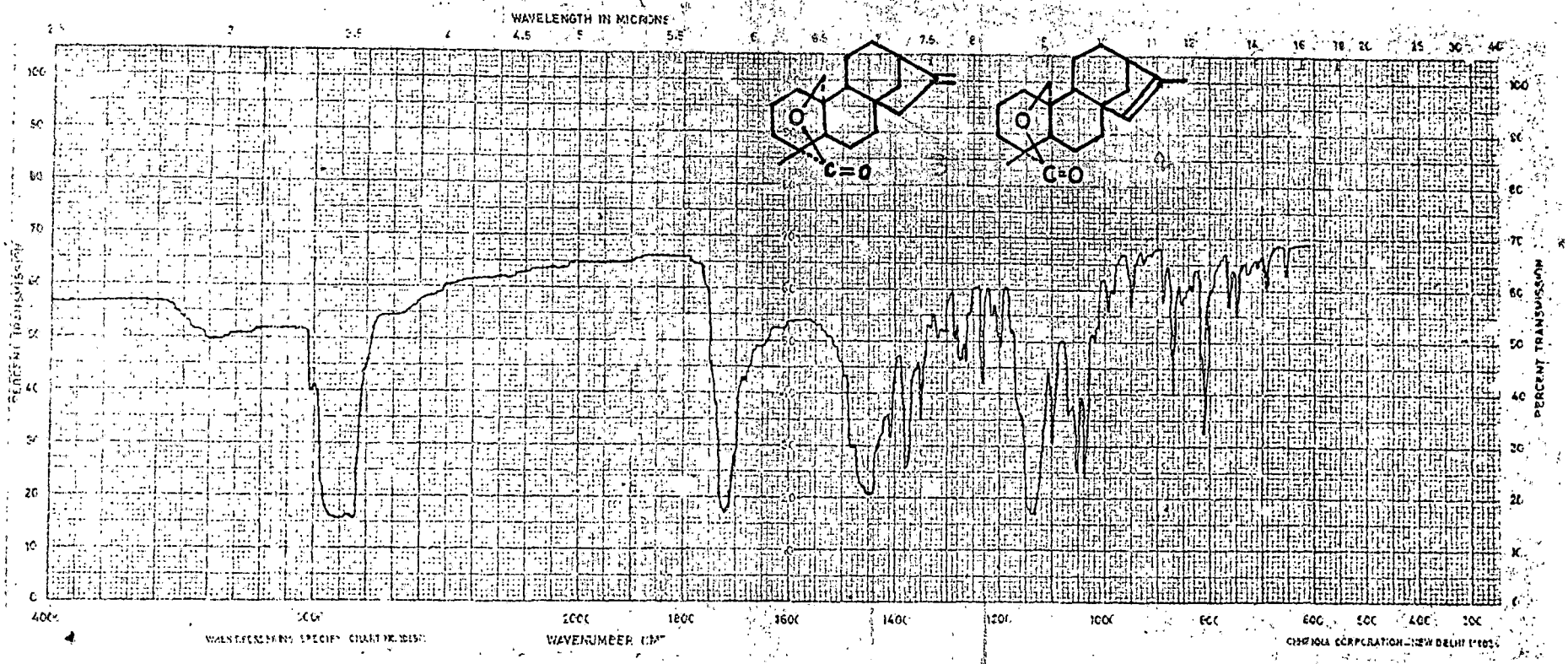


Fig. 9 IR spectrum of odolide, iso odolide, I_a, I_b

a and b respectively. The components in fraction C are named Odolide and iso-Odolide.

The structure of Odolide and iso-odolide in mixture (components of fraction C) was arrived at from studies of spectral analysis.

Study of ultraviolet absorption spectrum

The UV absorption spectrum of Odolide and iso-Odolide (components of fraction C) was recorded in the region 220-260 nm using water free methanol (with a few drops of chloroform) as solvent. No characteristic absorption was observed showing the absence of characteristic group absorbing in the region.

Study of Infrared absorption spectrum

The IR absorption spectrum was examined in KBr disc with nujol mulling. The IR spectrum (Fig. 9) showed peaks at 3010, 1725, 1600, 1150, 870, 820 cm^{-1} peaks showed at 1725, 1150 cm^{-1} indicated the presence of oxygen function as δ-lactone moiety. The presence of two sharp peaks at 820 and 870 cm^{-1} indicated the presence of exocyclic methylenic double bond ($=CH_2$) that is supported by the peak at 3010 cm^{-1} along with another olefinic double bond at 1600 cm^{-1} which is trisubstituted.

Study of ^1H NMR Spectrum

^1H NMR spectrum (Fig. 10) showed a sharp singlet at δ 0.904 ppm that was integrated for three protons was due to

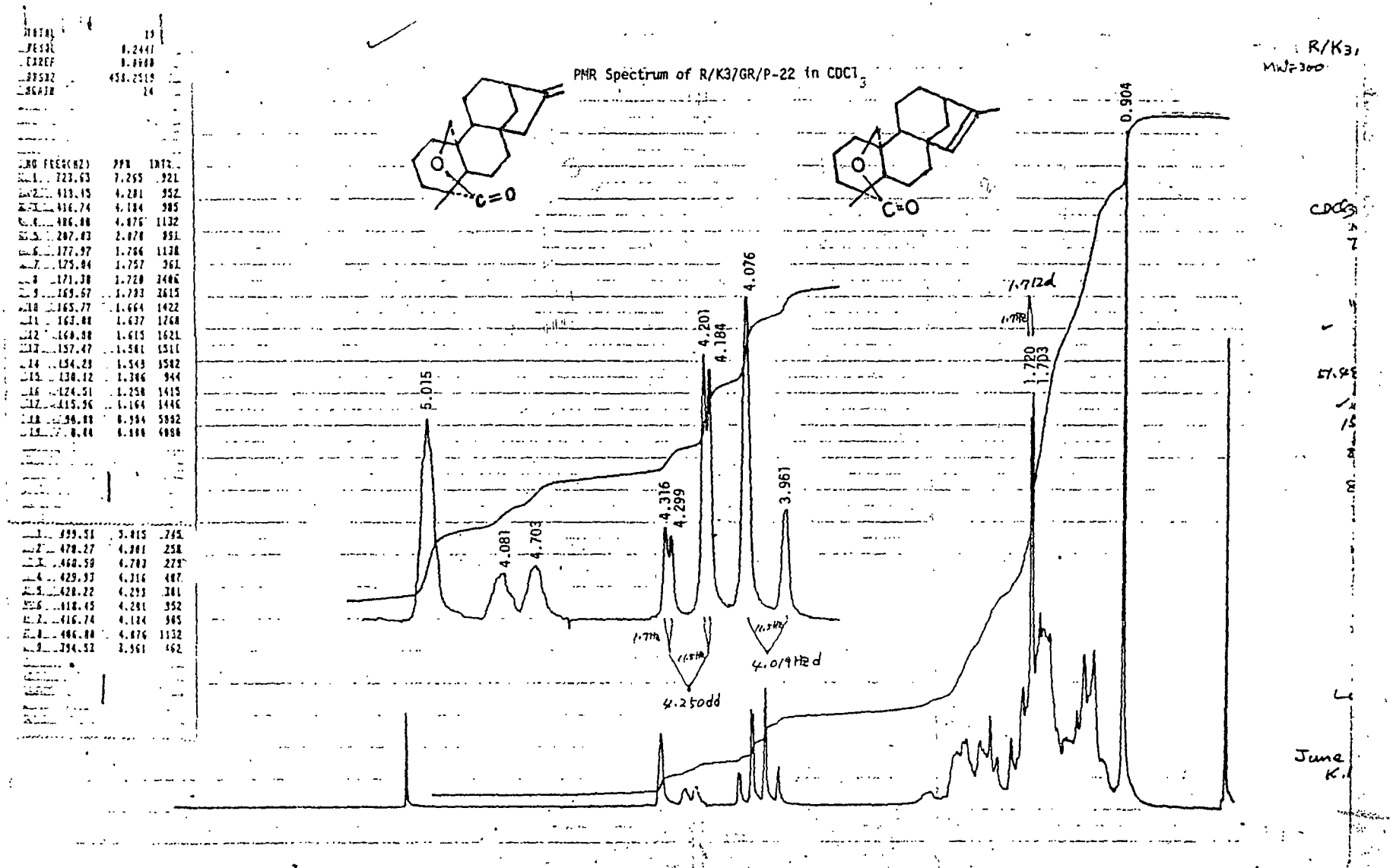


Fig. 10 ^{1}H NMR spectrum of odolide, iso odolide I_a, I_b

a tertiary methyl group at C-4. Two singlets that appeared at δ 1.703 and 1.720 ppm that was integrated for three protons indicated that both the peaks belonged to the same methyl group, downfield shift of which indicated it to be situated on a double bond. The separation of the two peaks was of 1.7 Hz. In the downfield region at the range of 3.9 and 4.4 ppm, there appeared an AB quartet, the peaks of which appeared at δ 3.967, 4.076, 4.184, 4.201, 4.299 and 4.310 ppm.

The latter four peaks were doublets with 1.7 Hz and 11.5 Hz coupling constant, while the first two appeared with coupling constant 11.5 Hz.

Appearance of doublet at δ 4.703 and 4.881 and a singlet like at δ 5.015 ppm, the two of which integrated for 1.5 protons suggested it to be due to protons belonging to methylenic protons and a vinyl proton respectively, the nature of which could be explained by irradiation experiment (Fig. 11). Irradiation at δ 1.713 ppm sharpen the peak at 5.015, while irradiation at 4.250 did not change the peaks at 1.703 and 1.720 ppm showing that AB quartet centred at 4.1 ppm do not couple with the methyl group at 1.712 ppm. Similarly, irradiation at 5.015 ppm collapse the peaks at 1.703 ppm and 1.720 ppm to a singlet at 1.712 ppm. This suggested that the vinyl proton couples with a small coupling constant of 1.7 Hz at 1.712 ppm. These irradiation experiments showed that a vinyl methyl group is present in the molecule but the integration of

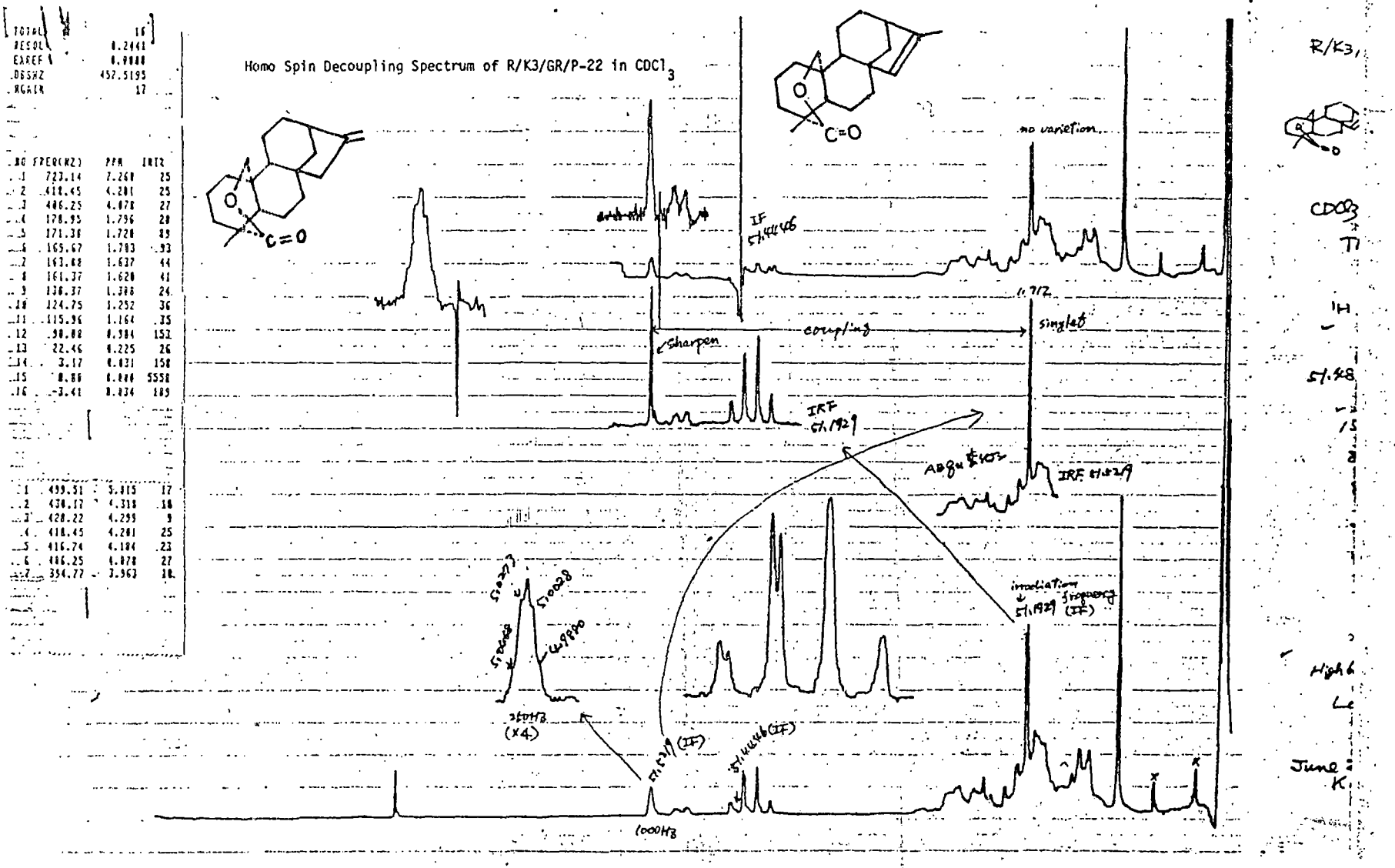


Fig. 11 Homo spin decoupling spectrum of odolide (I_a) and iso odolide (I_b)

the vinyl methyl and olefinic protons suggested that there is at least a mixture of two isomeric compounds. This is found to be true by Gas-liquid chromatography of the components in fraction C. GLC experiment (Fig. 12) showed this fraction C contains a mixture of two isomeric compounds in the proportion 80:20.

Study of mass spectrum

Mass spectrum (Fig. 13) of the components in fraction C showed molecular ion peak, relative intensity at 300 (M^+, 100%) with other peaks at m/z 272 (67.6), 257 (53.6), 224(21.2), 211 (42.1), 183 (20.5), 157 (11.9).

From the high resolution mass spectra, the molecular formula was found to be $C_{20}H_{28}O_2$.

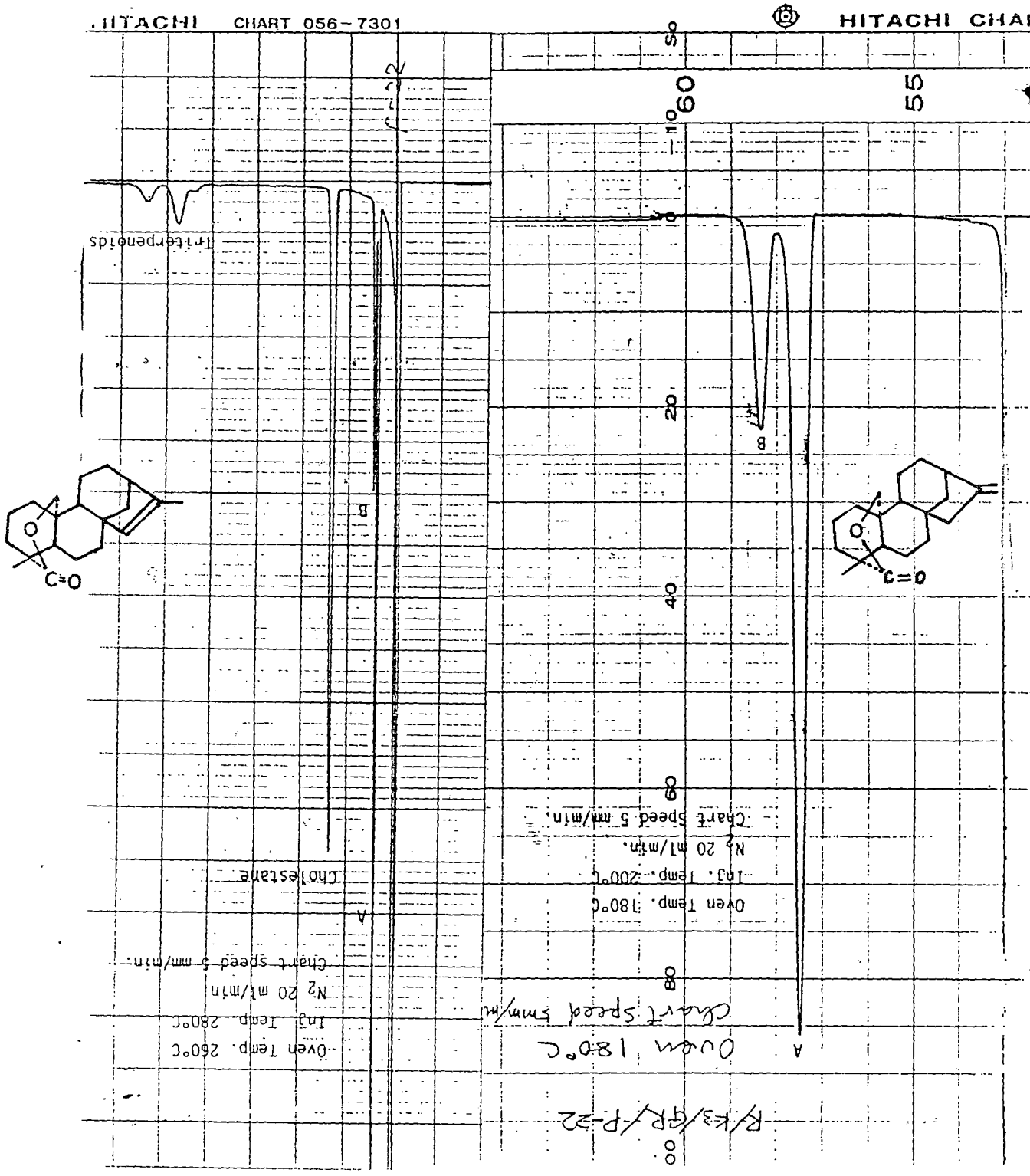
Appearance of a small peak at m/z 285 was due to the loss of methyl group from the molecular ion ($M^+ - CH_3$). A peak that appeared at m/z 272 was for the existence of the fragment $C_{18}H_{24}O_2$ and the one that appeared at m/z 257 was due to the existence of the fragment $C_{17}H_{21}O_2$.

^{13}C NMR spectral analysis

The most convincing proof for the structure of the compounds (Ia and Ib) was forthcoming from the ^{13}C NMR spectral analysis.

^{13}C NMR analysis (Fig. 14) showed a sharp triplet at 76.71 ppm which suggested that this carbon is connected with an oxygen atom that carries two protons with it. These two protons

Fig. 12 GLC of Odolide, iso odolide 1_a, 1_b



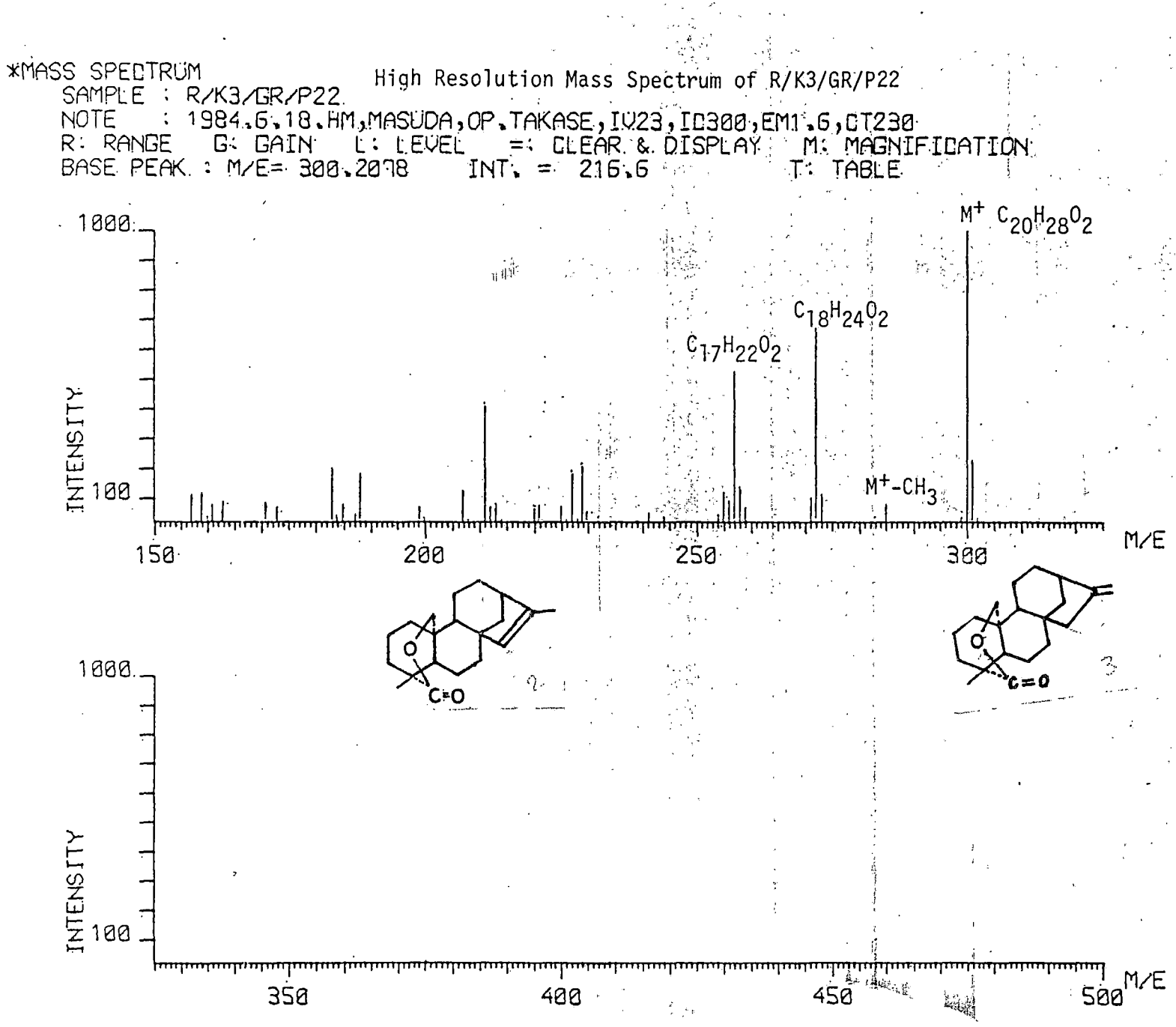


Fig. 13 Mass spectrum of odolide (I_a) and iso odolide (I_b)

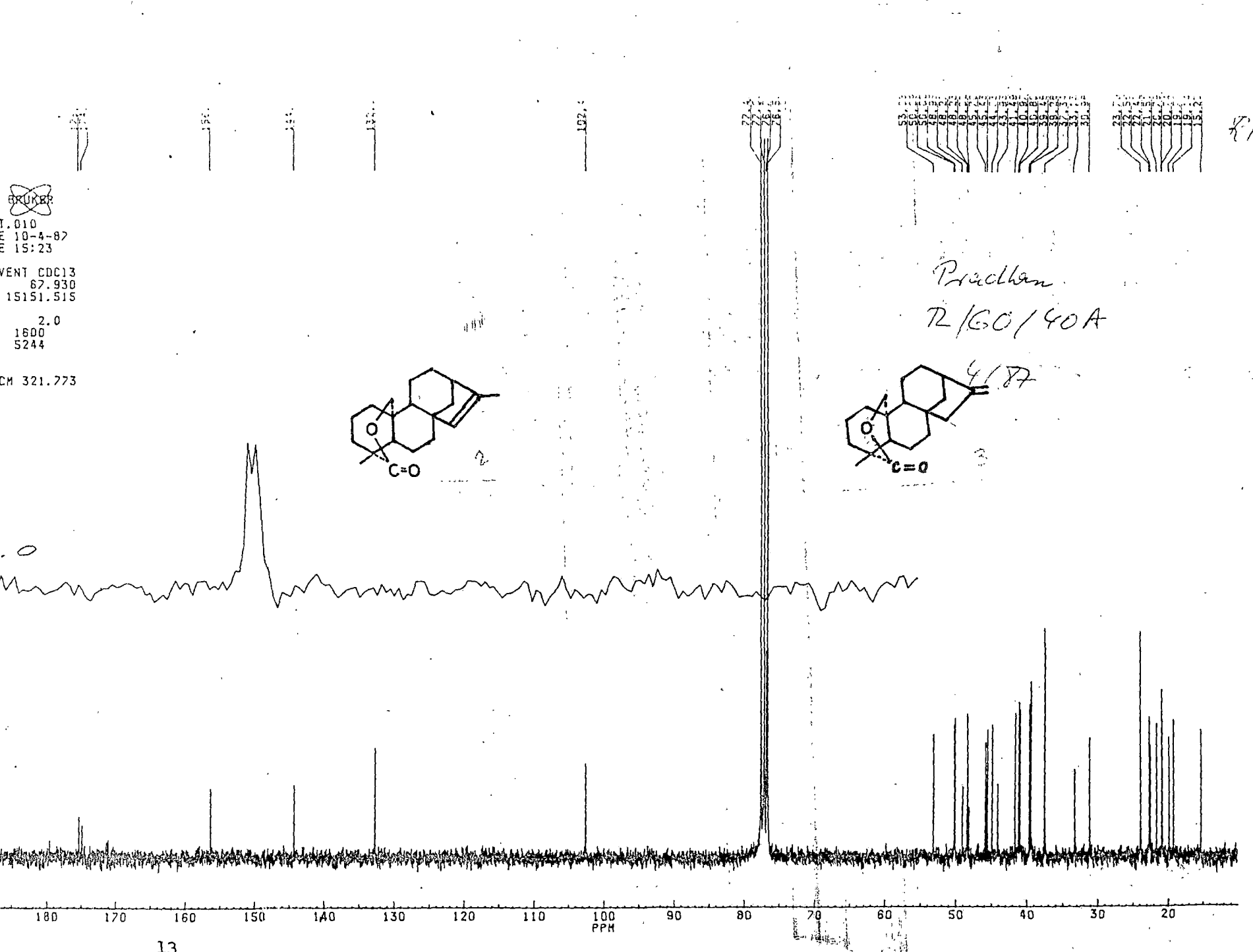
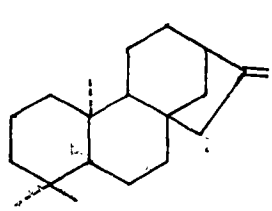


Fig. 14 ^{13}C NMR spectrum of odolide (I_a) and iso odolide (I_b)

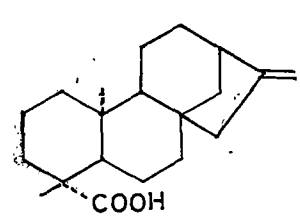
probably appeared as AB quartet centred at 4.1 ppm in the PMR spectrum. The appearance of two singlets at 175.2 and 174.7 ppm were probably due to the lactonic carbonyl group. The spectrum also showed two singlets at 156.2 and 144.1 ppm which must be due to a carbon without proton as olefinic carbon. The doublet that appeared at 132.6 ppm as due to the vinyl carbon and the triplet at 102.5 ppm as due to methylenic carbon.

From the molecular formula the compound is a diterpene with a lactone in it. The IR value suggested the lactone being a six membered one (δ-lactone).

A search of the literature showed that a Kaurane nucleus probably fit in the above compounds to explain the above observation with 19 th carbon being the lactonic carbonyl group attached to the 20th carbon. The 15-16, and 16-17 carbons possessing the olefinic double bond as isomeric mixture. The ^{13}C NMR peaks of odolide and iso odolide have been assigned in the following table 5 by comparison with the ^{13}C shift values of potamogeton[36] in (23), (-)-ent-kaur-16-ene[45] and (-)-ent-16-kauren-19-oic acid[48].



(-)-ent-Kaur-16-ene



(-)-ent-16-Kauren-19-oic acid

Table 5

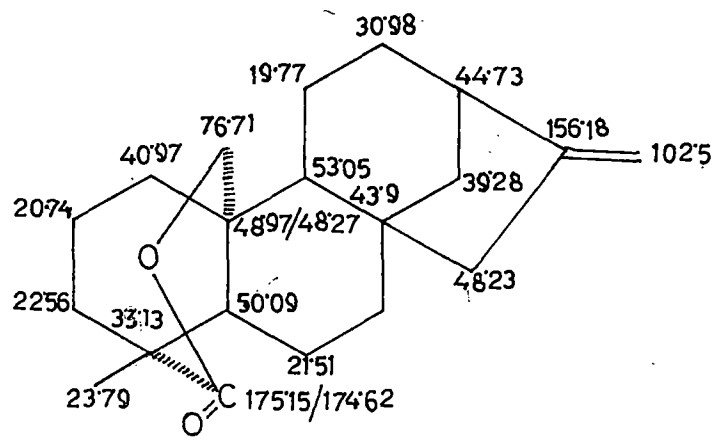
^{13}C NMR spectra of Odolide, iso odolide, potamogetonin (-)-ent-Kaur-16-ene and (-)-ent-16-Kauren-19-oic acid.

No. of carbon atom	Compound				
	Odolide	Iso-Odolide	Potamogetonin	(-)-ent-Kaur-16-ene	(-)-ent-16-Kauren-19-oic acid
1	40.97	40.80	41.1	41.3	40.6
2	20.74	20.79	20.9	18.7	19.0
3	22.56	22.42	25.8	42.0	37.7
4	33.13	33.13	33.5	33.3	43.7
5	50.09	50.02	49.5	56.1	55.0
6	21.51	21.51	28.1	20.3	21.7
7	37.34	41.49	36.1	40.4	41.2
8	43.9	43.9	145.2	44.2	44.1
9	53.05	53.05	51.7	56.1	57.0
10	48.97/ 48.27	48.27/ 48.97	51.1	39.3	39.6
11	19.77	19.14	23.7	18.1	18.3
12	30.98	30.98	37.0	33.3	33.0
13	44.73	45.70	125.1	44.2	43.7
14	39.28	39.48	110.0	39.9	39.6
15	48.23	132.59	142.6	49.2	48.9
16	156.18	144.10	138.9	156.0	155.6

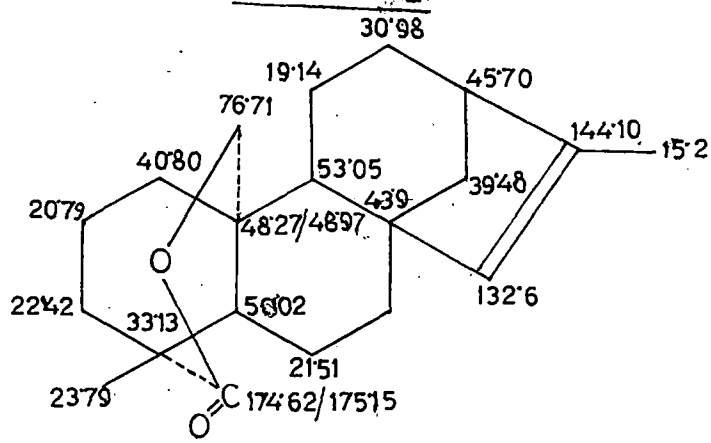
Table 5 (Contd..)

17	102.48	15.2	76.4	102.8	102.9
18	23.79	23.79	23.7	33.7	28.8
19	175.15/ 174.62	174.62/ 175.15	173.0	21.7	187.4
20	76.71	76.71	108.3	17.6	15.4

The total carbon shift assignment could best be fitted in the structures I_a and I_b given below be depicted for the compounds Odolide and iso-Odolide.



Odolide (I_a)



Iso-Odolide (I_b)

Thus from the spectral analysis, the structure of two isomeric compounds viz. Odolide and Iso Odolide (components of fraction C as mixture) may be assigned as (-)-ent-kaur-16(17)-en-19 → 20 olide and (-)-ent-kaur-16(15)-en-19 → 20 olide by assuming structure I_a and I_b as above.

Further proof of the above structures by chemical methods was impossible because of the paucity of the sample as mentioned earlier. However, further work is in progress for the isolation of the compounds.

SECTION E Biogenesis of Odolide, Iso-Odolide and Hydroxy Odolide.

The term biogenesis is the synthesis of natural products in the living organism. Biogenesis is a collection of hypothesis which have been proposed to describe the synthesis of natural products in the living organism. So biogenesis describes hypothetical transformations whereby natural products are synthesised in the living organism.

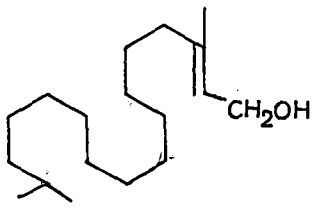
Chemical investigation on the benzene extract of the root of Gynocardia Odorata disclosed the presence of three new diterpenes of the Kaurane skeleton. It is, therefore, quite relevant to outline briefly the present day view regarding the biogenesis of tetracyclic diterpenes in general before to postulate about the biogenesis of odolide (I_a), Iso-Odolide (I_b) and hydroxy-odolide (I_c), the chemistry and spectral properties

of which have been discussed in the previous pages.

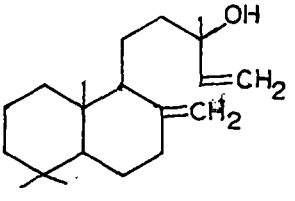
Biogenesis of tetracyclic diterpenes[49]

Because the diterpenes basically consist of four isopentane units combined, the possibilities for structural complexity become correspondingly greater, as reflected in some of the more prominent forms shown in the Chart I. Monocyclic forms are rare, and this may have some biochemical significance. However, acyclic, bicyclic, tricyclic, tetracyclic and pentacyclic forms are known. With the exception of abietic acid type resin acid[+], with an irregular sequence of four isopentane units, all of the higher plant diterpenes can be constructed from a phytol-like tetramer consisting of the four units linked head to tail. Ruzicka has suggested a hypothetical geranyl geraniol (or geranyl-linalool or geranyl myrecene) type of structure as a possible biogenetic precursor of the diterpenes[50] through ionic mechanism as indicated in Chart II.

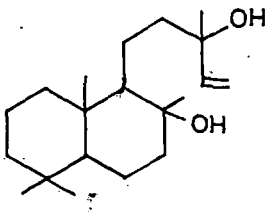
Chart I



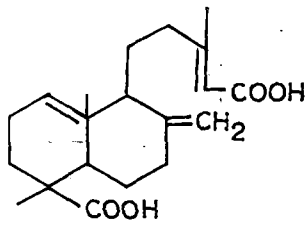
Phytol CI



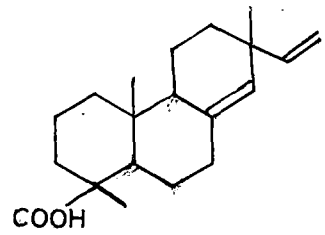
Manool CII



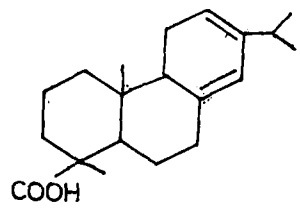
Schareol CIII



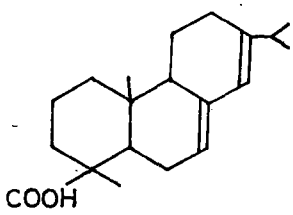
Agathic acid CIV



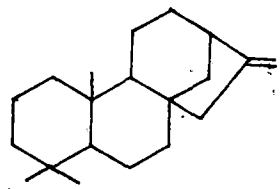
Dextropimaric acid CV



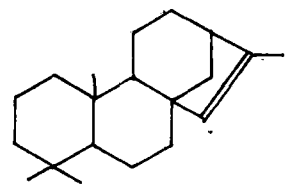
Levopimaric acid
CVI



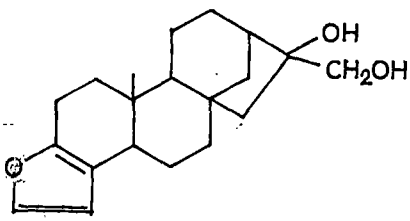
Abietic acid
CVII



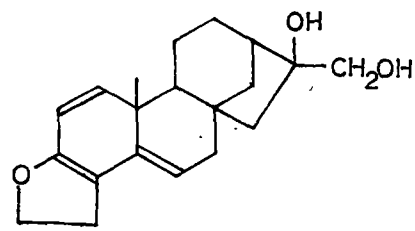
Phyllocladene
CVIII



Iso Phyllocladene
CIX



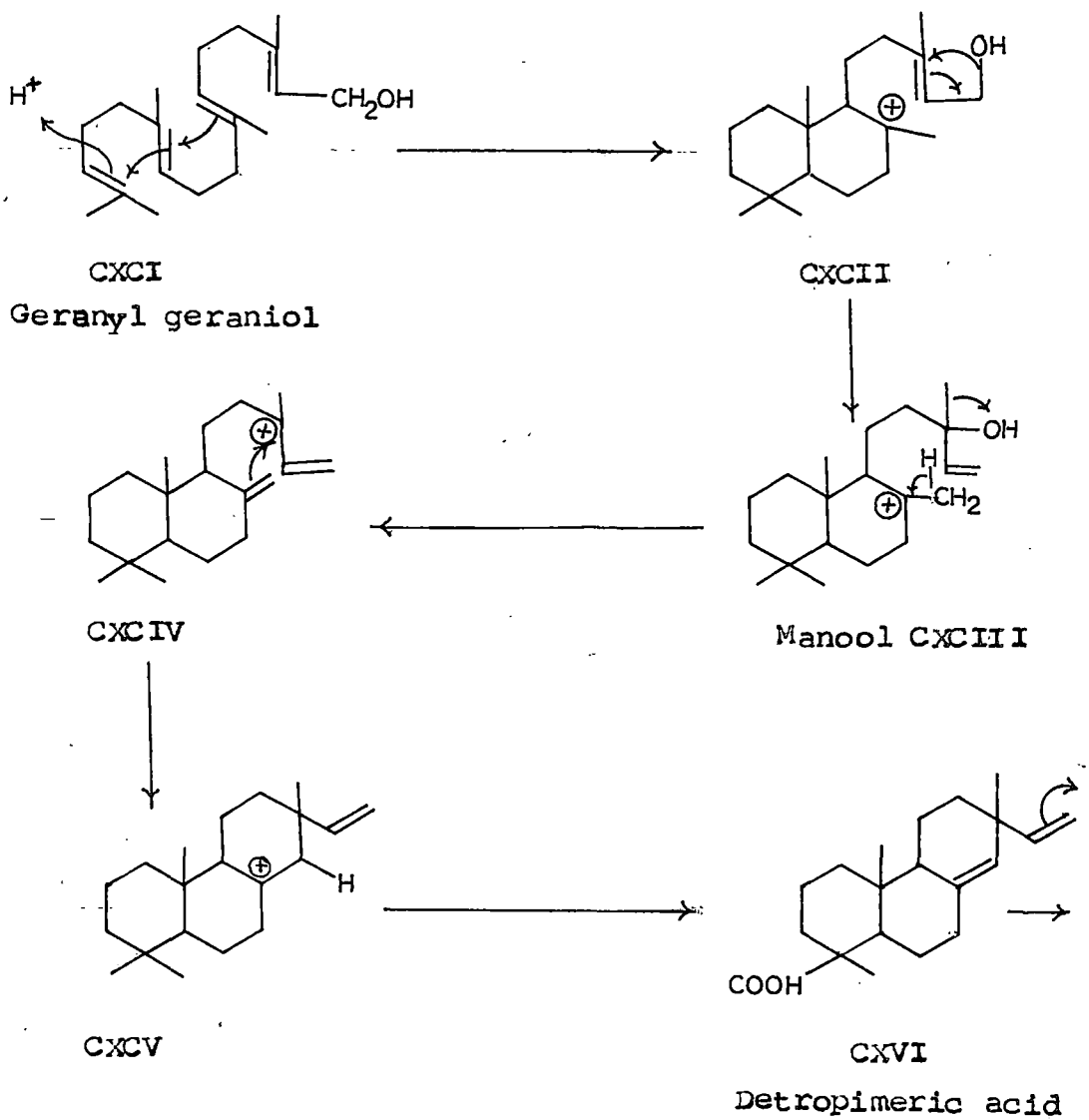
Cafestol
CX_a



Kahweol
CX_b

Ionic mechanism in the biogenesis of diterpenes; after Razicka[50]

Chart II



CXCI
Geranyl geraniol

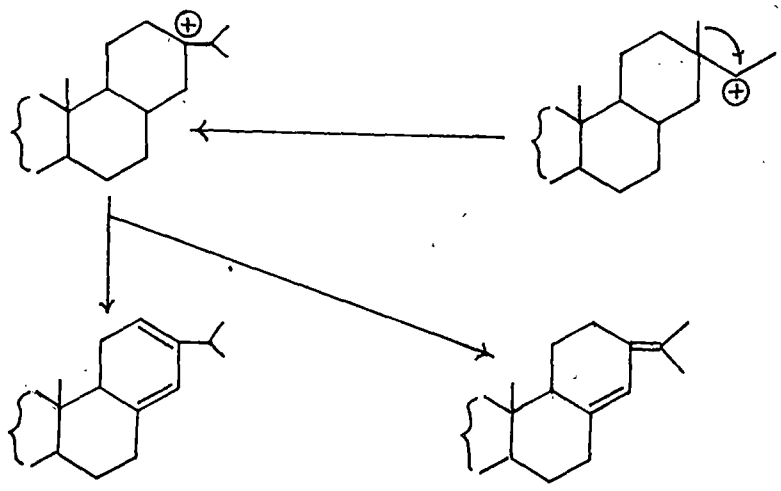
CXCII

Manool CXCIII

CXCIV

CXCV

CXVI
Detropimeric acid



Levopimaric acid
CXCVII

Neoabietic acid
CXCVIII

Because there is no substances analogous to the bicyclic and more complex diterpenes in animal tissues, their biogenetic origin presents a particularly challenging field for research. Our present concept of cyclic diterpene biogenesis following the suggestions of Ruzicka[50], involves cyclization of an initial isoprenoid, tetramer (e.g. the hypothetical geranyl geraniol) to an additional cyclic presursor from which all of the known diterpenes subsequently develop by methyl migrations characteristic of the terpene series (Chart III). Upto the present, more attention has been devoted to transformations that probably occur within the cyclised form than to the cyclisation mechanism itself. It is interesting that the majority of the presently

known cyclic diterpenes contain no hydroxyl at C-3 and possess time conventional 5α, 10β-configuration of rings A and B as in the steroid and triterpenes. An exception according to Djerassi appears to be the diterpenoid cassaine[51] which possesses the conventional steroid ring A/B configuration and hydroxyl at C-3. Cafestol and darutigenol[51] again possess the opposite wrong configuration (5β, 10α) andpotential hydroxyl group at C-3. The generation of the furan ring in cafestol and kahweol has been visualised as resulting from a Wagner-Meerwein rearrangement of a hydroxylated precursor[51]. It would appear that the full biochemical significance of these fascinating structural relationship can not be assessed at this time.

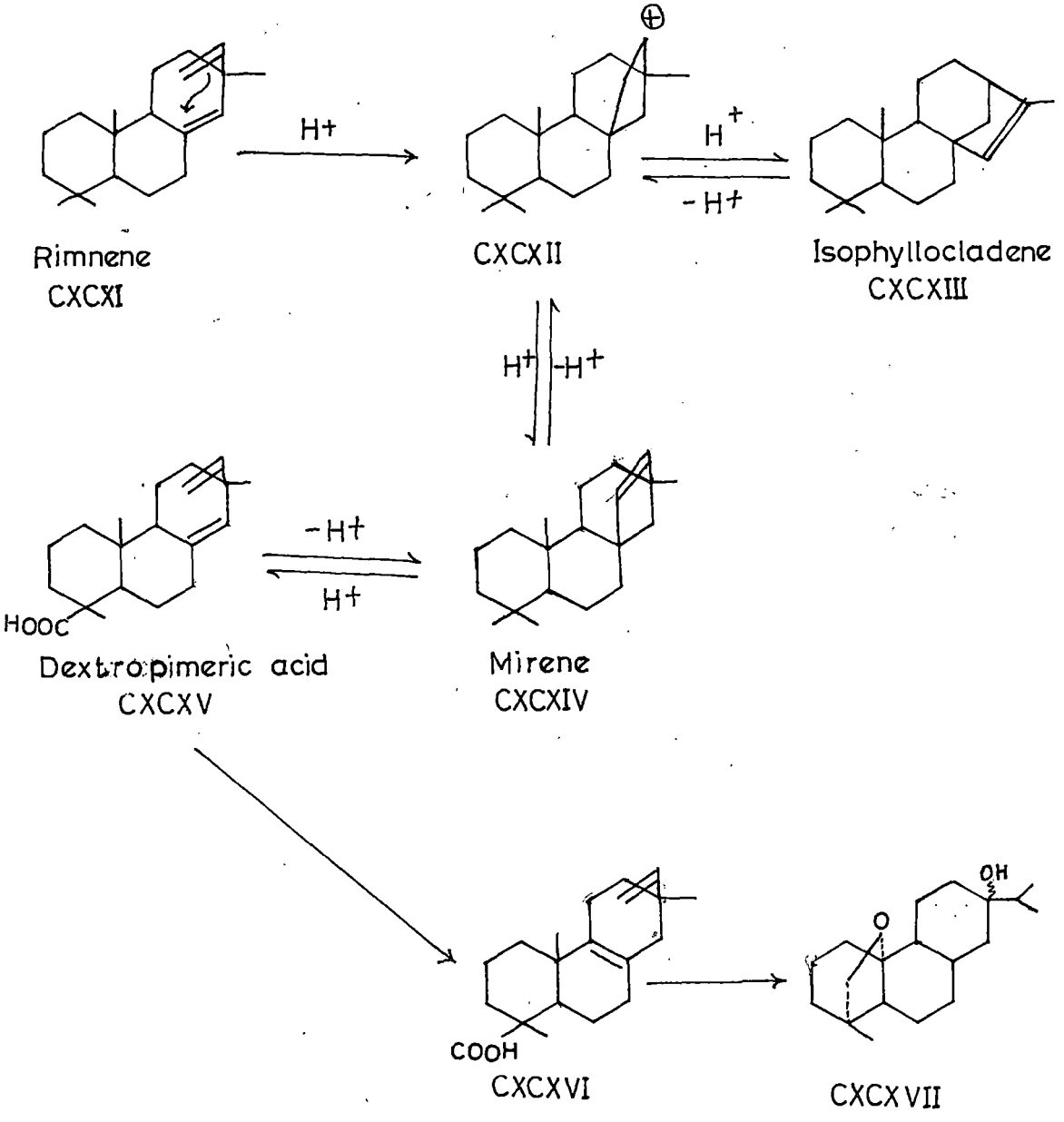
Considerable speculation has been aroused concerning the biogenesis of resin acids of the dextropomaric (pimarane) type, isomers of levopimaric acid (abietane) type and the tetracarbocyclic diterpene hydrocarbons of the phyllocladene class. Early suggestions that the resin acids are formed by cyclisation of phytol isomer[52] or by copolymerisation of isoprene and vinyl acrylic acid[+] were interesting but premature since knowledge of the structure of the resin acids was incomplete at that time. Since the stereochemistry of rings A and B are the same as the corresponding rings in the triterpenes[50], the speculations have largely dealt with the exocyclic isopropyl group of the abietane series and the origin of the bridged phyllocladene ring.

It will be noted that in acids of the pimarane type four isopentane units are combined in 'regular' formation, whereas in these of the abietane type that fourth unit is bound in an irregular manner (Chart I). Sandermann[52,53] originally suggested that both types of resin acids would arise from a common precursor through a final Wagner-Meerwein rearrangement of dextropimaric acid to levopimaric acid. The problem of biogenesis of these substances was reexamined by Wenkert[54]. According to this investigator acid catalysed rearrangement of the pimarane to the abietane or phyllocladene type should be especially influenced by the stereochemistry of the functional groups involved, in this case C-7 in particular Chart III. Pimaradienes may possess either a quasi-axial methyl and quasi-equatorial vinyl function or the opposite conformation. On mechanistic grounds it was concluded that the conformation which would most likely to be the one involved would be that in which the pimaridiene possessed the quasi-axial vinyl and quasi-equatorial groups: the transformation of rimuene to isophyllocladene and mirene was then envisioned as occurring as shown in Chart III. The acid catalysed transformation of dextropimaric acid to the abietane type was believed to occur as indicated in Chart III (CXCXV - CXCXVII) the mechanism being more complex because of the carboxyl of the dextropimaric acid. Biogenetic mechanisms were presumed to occur by the same

type of sequence. The general approach to this problem was latter modified[55] and the actual acid catalysed conversion of pimaradiene to an abietadiene demonstrated. It will be noted that by the suggested mechanism, the formation of the phyllocladene ring system occurs through migration of the C-6 carbon atom and not the angular C-20 methyl group. According to Briggs et al[56] a similar mechanism had been proposed earlier by Wilmshurst[57,58]. Briggs and associates provided additional evidence to substantiate the structural relationships between these complex diterpenes by establishing the absolute configuration of phyllocladene, mirene, rimuene, cupressene and kaurene, utilising the new tool of optical rotatory dispersion developed by Djerassi[59] (see also Wenkert and Beak[59a] and Djerassi et al[59b]). The final biogenetic scheme suggested was supported by the observation that phyllocladene and isophyllocladene co-occur in Araucaria excelsa and phyllocladus tri-chomanoides and the co-occurrence of ferruginol, sugiol (isomiropinic acid), isopimaric acid, phyllocladene, mirene and kaurene in podocarpus ferrugineus (the miropine), growing in Newzealand.

Chart III

Transformations in the resin acid series, according to Wenkert[55].



Rimnene
CXCXI

CXCXII

Isophyllocladene
CXCXIII

Dextropimeric acid
CXCXV

Mirene
CXCXIV

CXCXVI

CXCXVII

Biogenesis of the root products of Gyno Cardia Odorata

The three new diterpenes viz. Odolide (I_a), Iso-odolide (I_b) and hydroxy-odolide (I_c) are most probably bio-synthesised from dextropimaric acid as shown in the following Scheme IV. Dextropimaric acid is generated from the noble precursor Geranyl-Geraniol by mechanism suggested after Ruzicka[50] shown in Chart II,

Scheme IV