CHAPTER - III

PURE LAC RESIN

INTRODUCTION

Lac resin is a complex mixture of polyesters of varying molecular sizes and has been separated[1] into "soft" and "hard" resin (so called "pure resin" of Tschirch and Ludy) fractions by solvent extractions. Both of these fractions are fairly complex mixtures of several polyester species. Since "hard resin" accounts[1] for ~70% of total lac resin, it appeared to be the right raw material for further purification with ultimate objective of isolating a homogeneous lac resin fraction for structural elucidation.

After considerable exploratory work by Sukh Dev and co-workers[2](including attempted column chromatography over silica gel, ion-exchange resin, and gel filtration) a procedure involving fractional precipitation(solvent system; benzene-dioxan) was successfully worked out by them for the isolation, in ~12% yield, of a subfraction of "hard" resin, which appeared to be essentially homogeneous to tlc and was termed "pure lac resin". These workers monitored the separation by tlc, determination of neutralization equivalents and tlc/paper chromatography of acids resulting from saponification. This resin, a pale coloured amorphous powder, had the following characteristics: m.p., 102-104°; $[\alpha]_D^{30}$ +63.4(EtOH); U.V., λ_{max}^{EtOH} 225 nm($E_{1cm}^{1\%}$, 134); IR(KBr),3448 cm^{-1} (OH), 1724, 1250 cm^{-1}(COOR), 1631 cm^{-1}(olefinic linkage); molecular weight(osmometric method), 2095±110; neutralization equivalent, 990±50.

The above pure lac resin, which, from its method of isolation, corresponds to the major part of lac resin of high molecular weight, was considered[2] to constitute the "backbone" of seed-lac and had been subjected to further structural investigations with a view of throwing light on the nature of the lac resin.

Sukh Dev and co-workers arrived[3] at a structure(Fig.3 of PART B/CHAPTER-I) for the pure lac resin by carrying out glc determination of methyl ester of lac acids after formylation of free -OH group. By this method, the resin was found to be based on terpenic acids(essentially jalaric acid) and aleuritic acid in a 1:1 ratio. This conclusion was further supported by a comparison[3] of 220 MHz PMR spectra of pure lac resin derivatives with suitable reference samples.

It was also stated[3] that out of every two molecules of lac resin, on the average, one of these contains one laccijalaric unit. It was not considered necessary that the molecular species constituting "pure lac resin" should be sequence wise homogeneous or even in terms of jalaric/laccijalaric acid ratio, structure in Fig.3 of PART B-CHAPTER-I should represent the average situation. Moreover, depending on the age of the lac sample and the care taken during its collection, significant oxidation of the aldehyde, apparently, occurs and thus, it is not important to establish the aldehyde content, though, in view of the PMR(220 MHz) data one could state that out of four terpene acid units, two have aldehyde function.

As a continuation of this work, we wanted to isolate the above mentioned "pure lac resin" in a state of purity and study its ^{13}C NMR, Field Ionization mass, and 400 MHz PMR spectra in order to get an insight into its structure.

ISOLATION OF PURE LAC RESIN

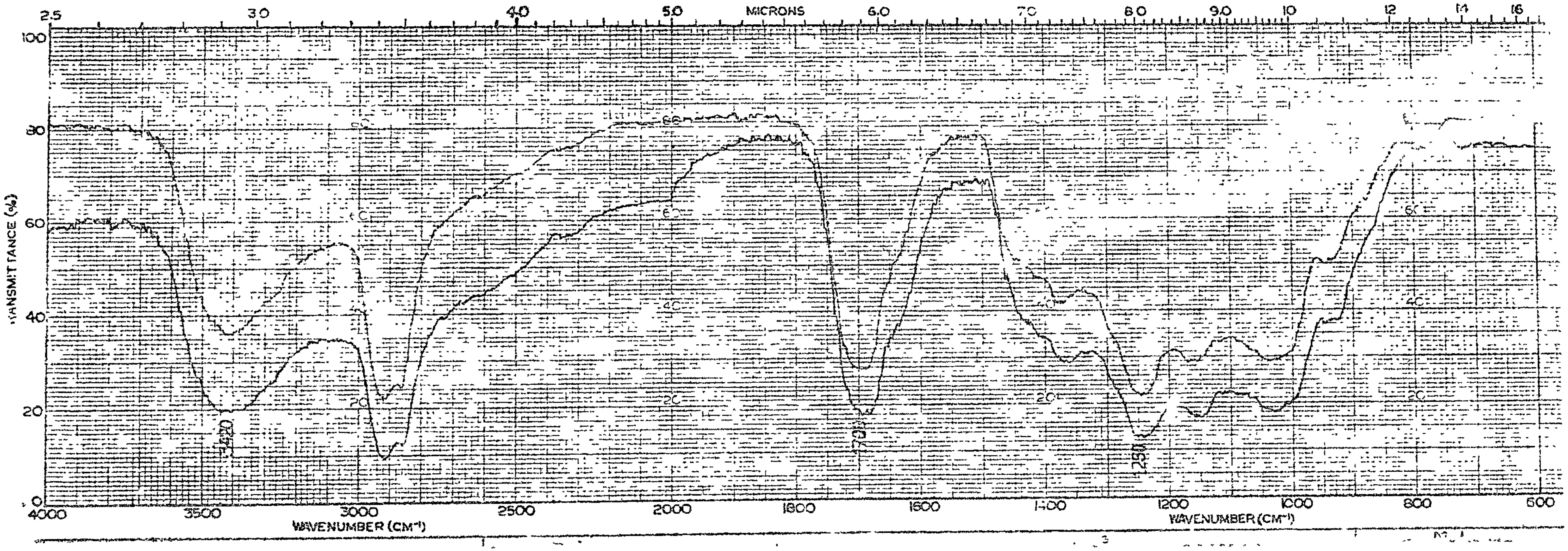
For the isolation of the pure lac resin, Palas seed lac was separated into "hard" and "soft" resin fractions by following a reported procedure[1]. This "hard" resin was subjected to gel permeation chromatography(gpc) using a set of 4 columns of styra gel having the porosity of 1000, 500, 200, and 100A^o at 30^{o}C. THF was used as eluant with a flow rate of 2 ml/minute. The calibration was done with polystyrene standard sample. Under these conditions, hard resin showed two peaks; one corresponding to molecular weight 1.95×10^3(~30%) and the other 7.6×10^3(~ 70%).

The above hard resin was subjected to further purification by using solvent precipitation method(i.e dissolving the resin in dioxan and precipitating it with benzene). During the course of this precipitation, we isolated, in 5.3% yield(based on hard resin), a resin which, by gpc(under the conditions described above), was essentially a homogeneous material and corresponded to 7.61×10^3 molecular weight peak. This resin was pale yellow in colour and has the following characteristics: m.p., 115-120^{o}C; $[\alpha]_D^{26}$, -36^o (EtOH); U.V., λ_{max}^{EtOH} 220 and 234 nm; IR(KBr)(Fig.1),3420 cm^{-1} (OH), 1700, 1250 cm^{-1}(COOR), 1630 cm^{-1}(olefinic linkage).

With a view of isolating the resin of MW$\sim$2000, the residue, left after removal of above resin(MW 7.61×10^3), was further subjected to the above precipitation process but, once again, instead of the desired resin (MW$\sim$2000), we isolated, in $\sim$8% yield(based on hard resin), a resin, homogeneous to gpc(under the earlier described conditions), which registered a molecular weight of 4.8×10^3(polystyrene standard). This resin, pale yellow in colour, has the following characteristics: m.p.,104-106°; $[\alpha]_D^{26}$ + 76°(EtOH); λ_{max}^{EtOH} 230 nm; IR(KBr)(Fig 2), 3420 cm^{-1}(OH), 1700, 1250 cm^{-1} (COOR), 1630 cm^{-1}(olefinic linkage).

IR AND PMR(90 AND 400 MHz) SPECTRA OF THE RESINS

The IR(Figs. 1 and 2) and PMR(Figs. 3 and 4) spectra of both the resins(i.e. corresponding to M.Wts. 7.61×10^3 and 4.8×10^3) are identical and essentially superimposable. This means that the repeating unit, i.e.jalaric ester-II[4], is the same in both the resins but their number is different. From calculations, this number appears to be 8 for the resin of molecular weight 4.8×10^3 and 14 for the resin of molecular weight 7.61×10^3. In the PMR spectra of both the resins, the ratio of the protons $C{=}C\underline{H}$, $C{=}C{-}C\underline{H}OCOR$, $C\underline{H}OCOR'$, and $C\underline{H}OH + C\underline{H_2}OH$ is 1.3:1.3:1:7.4, respectively, which is



——— FIG.1: IR SPECTRUM OF THE RESIN OF MW 7.61 x 10^3

– – – – FIG.2: IR SPECTRUM OF THE RESIN OF MW 4.8 x 10^3

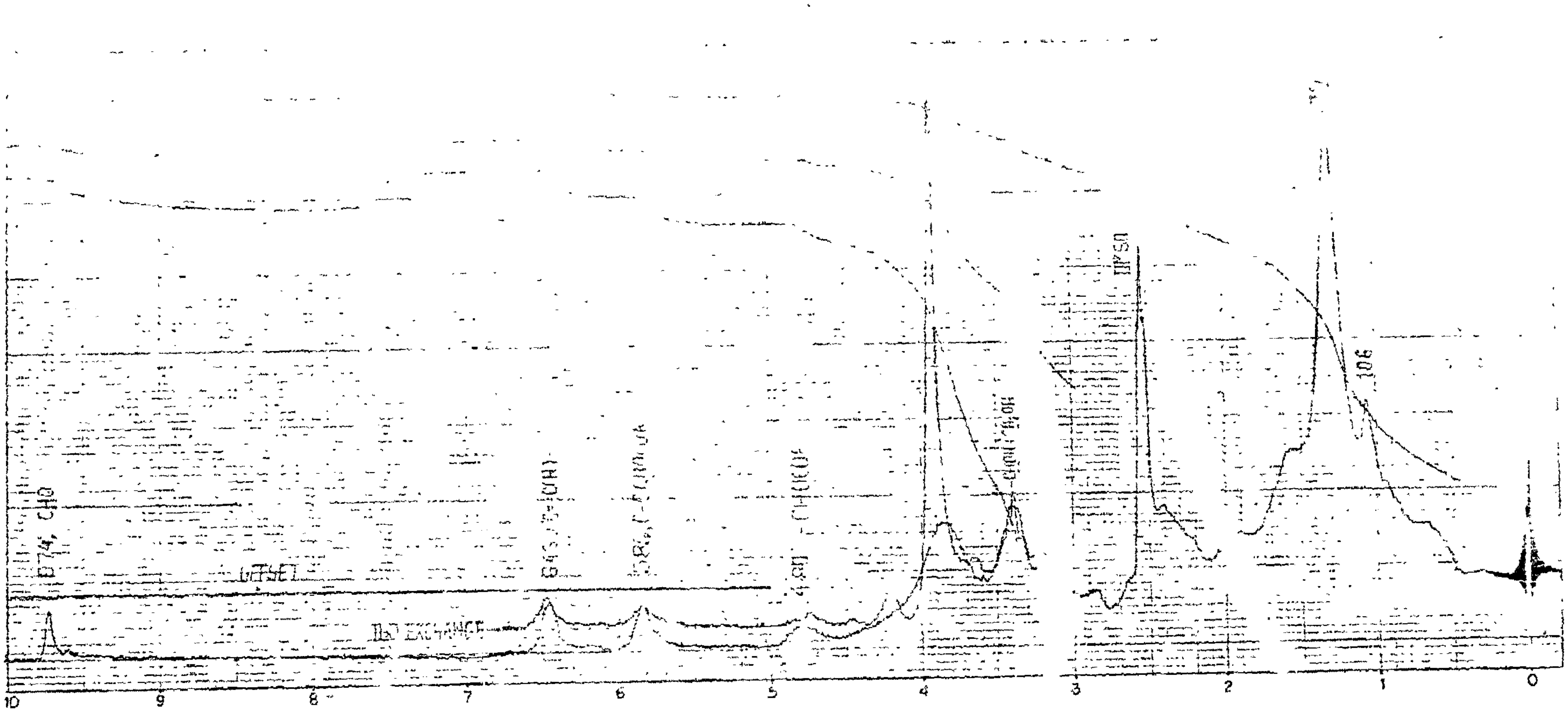


FIG.3: PMR SPECTRUM OF THE RESIN OF MW 7.61 x 10^3

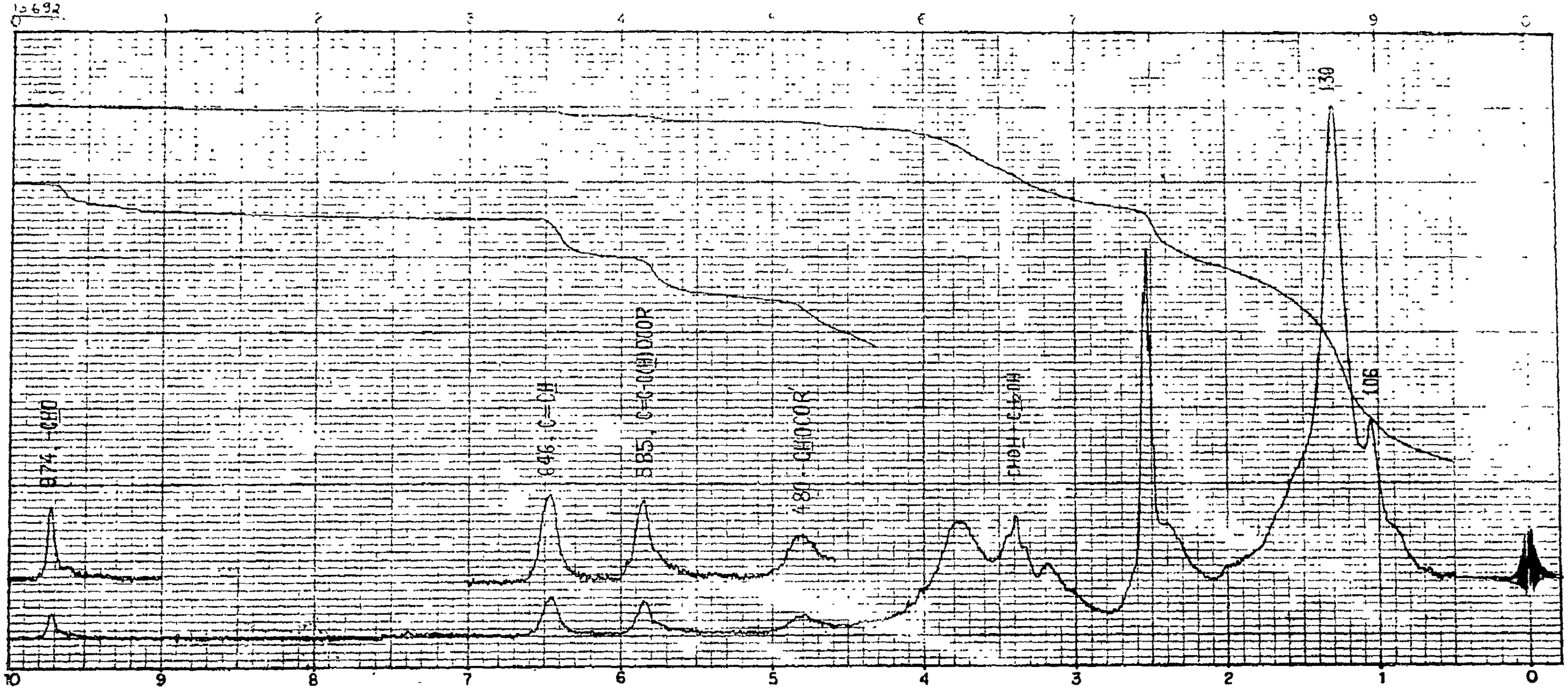


FIG.4: PMR SPECTRUM OF THE RESIN OF MW 4.8 x 10^3

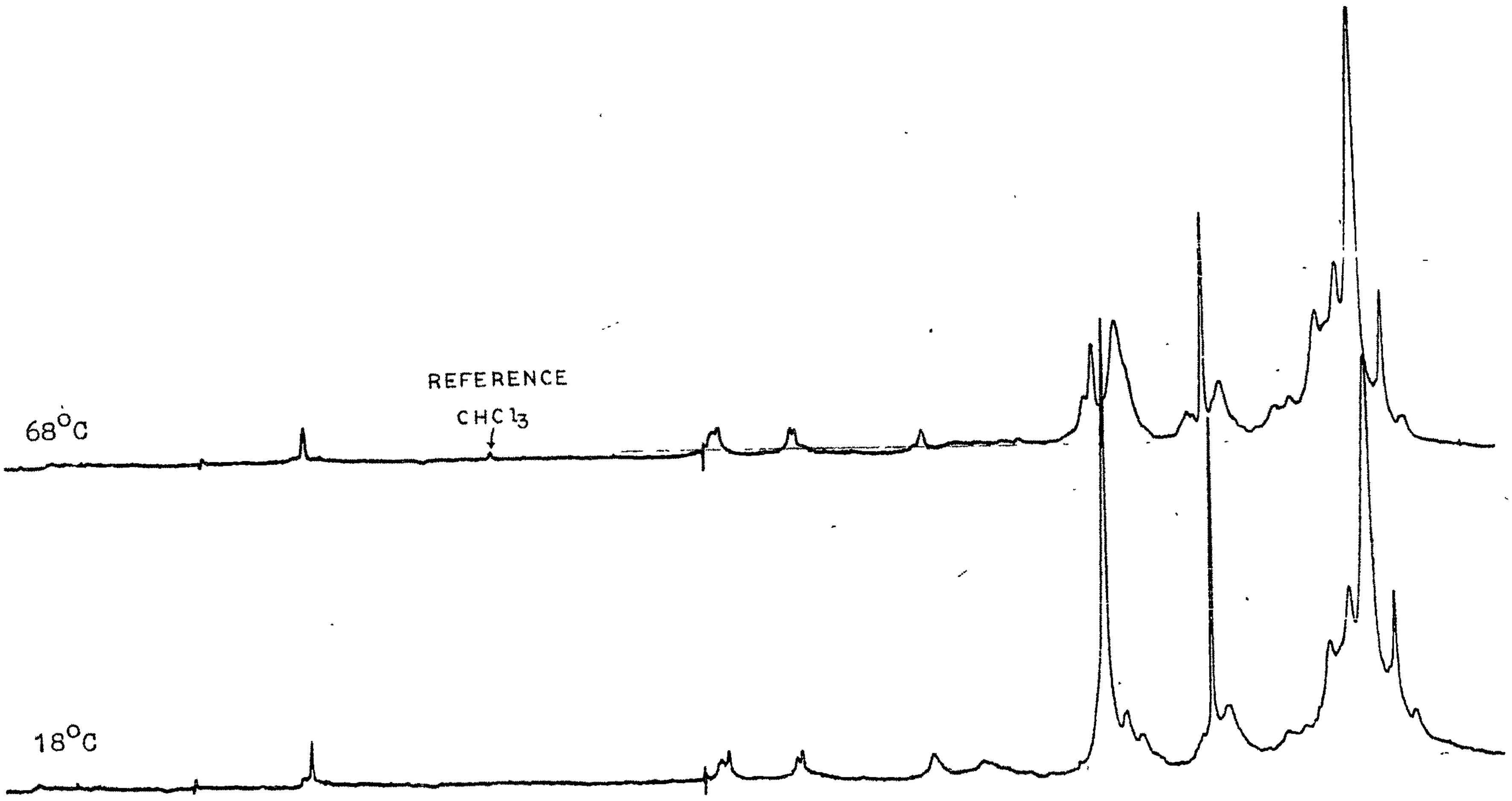


FIG.5: 400 MHz PMR SPECTRUM OF THE RESIN OF MW 7.61 x 10^3

identical with the same ratio derived[3] earlier from the 220 MHz PMR spectrum. The 400 MHz PMR spectrum(Fig.5) of the resin of MWt. 7.61×10^3 also bears the same ratio(as described above). This ratio is explainable only by a structure similar to the one shown in Fig.3 of PART B-CHAPTER-I.

^{13}C NMR SPECTRA OF THE RESINS AND SOME OF ITS CONSTITUENTS

In the structure shown in Fig.3 of PART B-CHAPTER-I, the primary hydroxyls of the sesquiterpenic as well as of the aleuritic acid part are free and so, in the ^{13}C NMR spectra of the resins, the positions of the ^{13}C signals for the carbons bearing these hydroxyls should be almost the same as those in the ^{13}C NMR spectra of dimethyl epishellolate and aleuritic acid.

Furthermore, since the allylic hydroxy group of the sesquiterpene part undergoes esterification in the resin, the position of the ^{13}C signal for this allylic carbon bearing the ester function in the resin should correspond to a signal for the same crabon in the ^{13}C NMR spectrum of the diacetate of epishellalic acid.

To test the validity of our reasoning, ^{13}C spectra of the two resins(Figs. 6 and 7) along with those of dimethyl epishellolate (Fig.8), <u>erythro</u>- aleuritic acid(Fig.9),

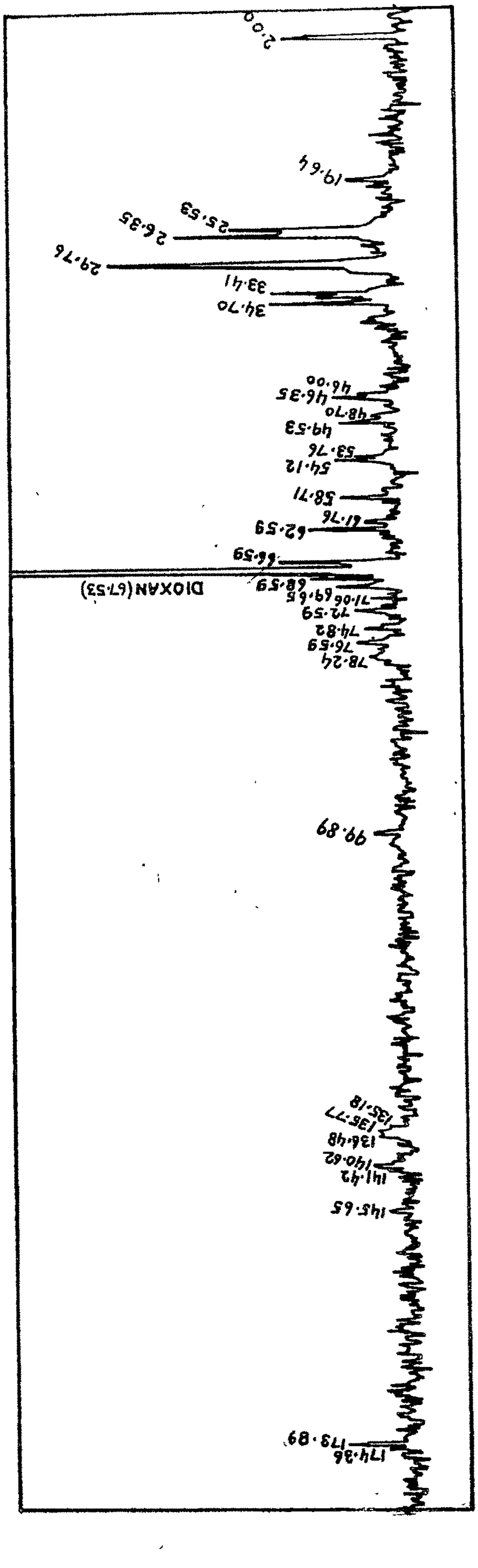


FIG.6: ^{13}C NMR SPECTRUM OF THE RESIN OF MW 4.8×10^3

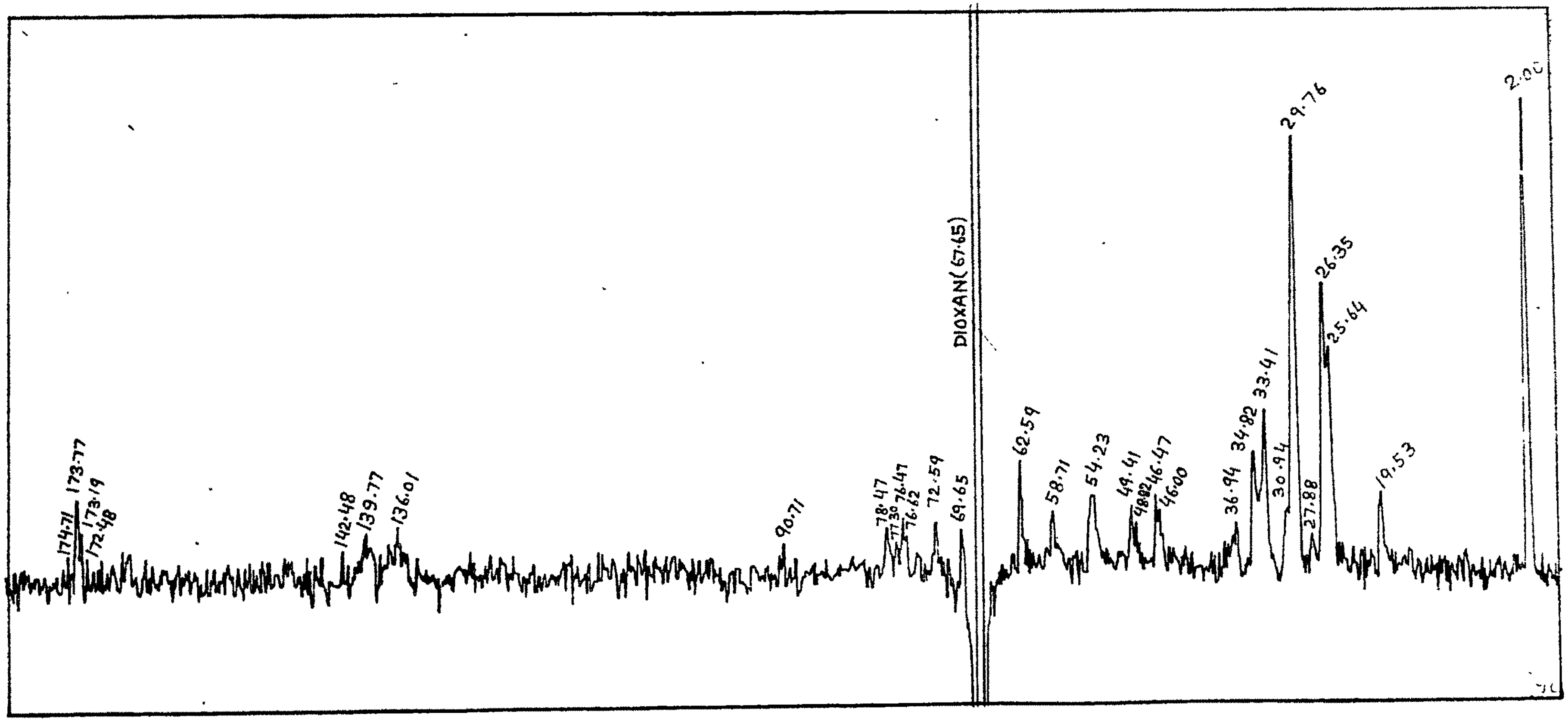


FIG.7: ^{13}C NMR SPECTRUM OF THE RESIN OF MW 7.61 x 10^3

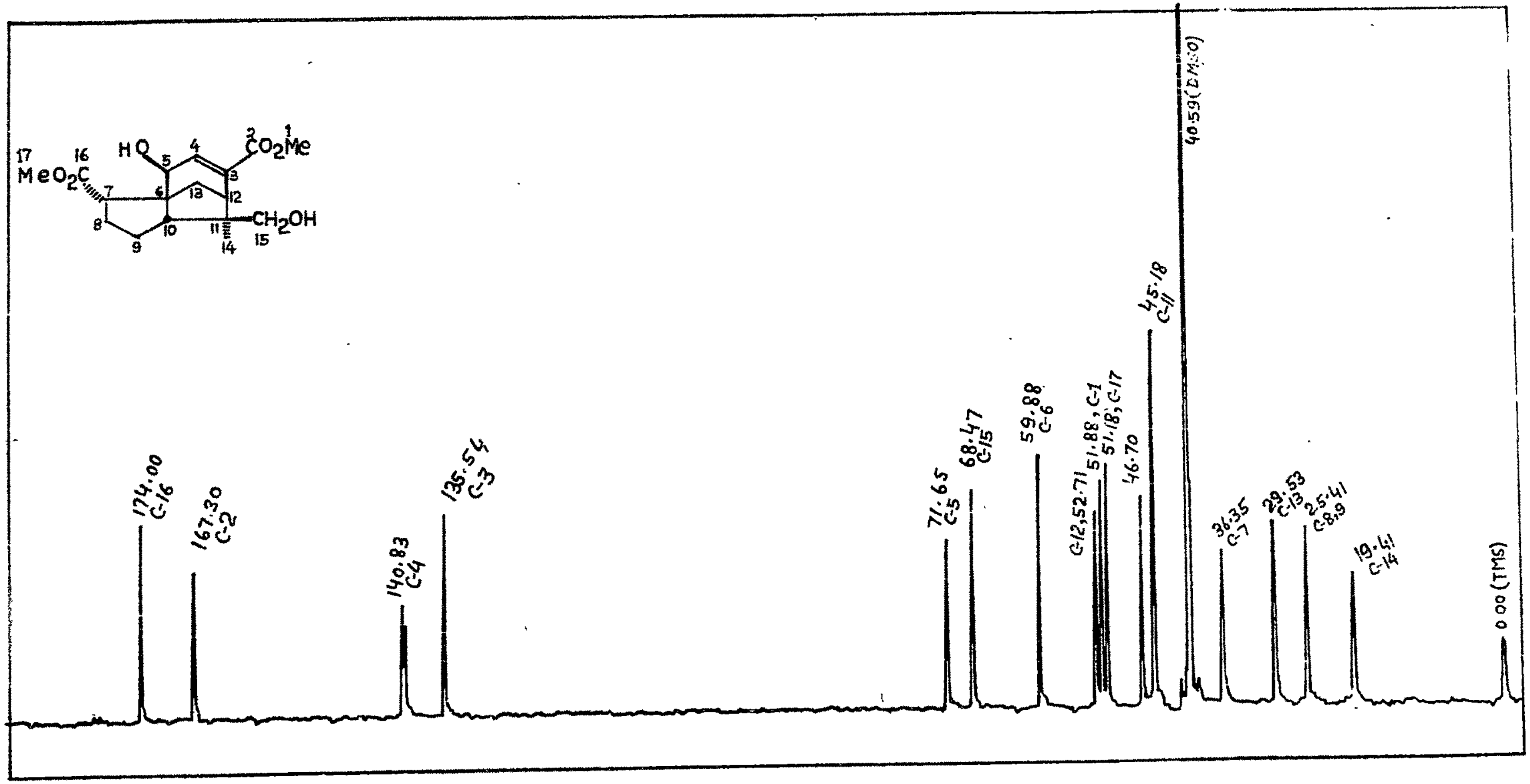


FIG.8: ^{13}C NMR SPECTRUM OF DIMETHYL EPISHELLOLATE

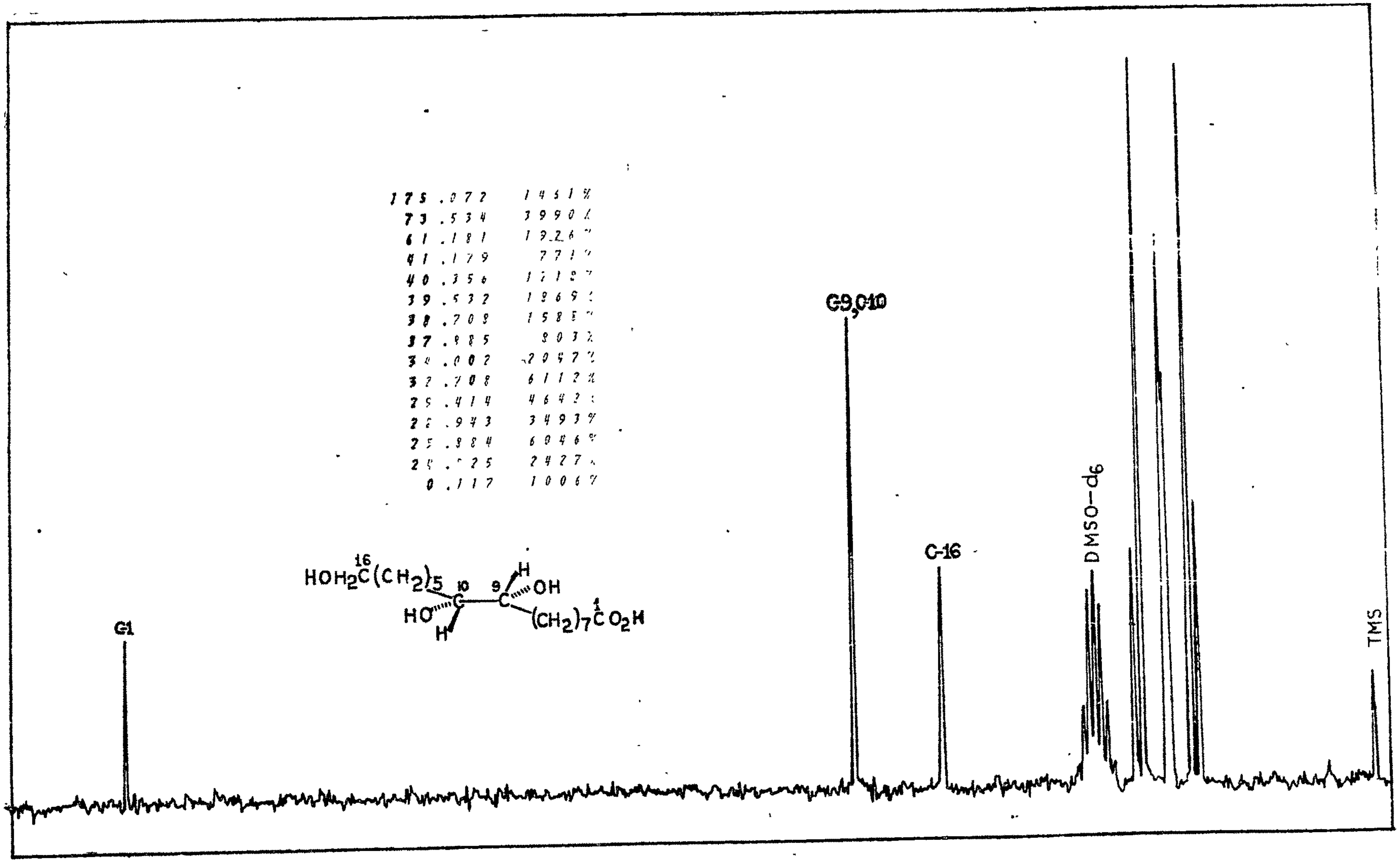


FIG.9: ^{13}C NMR SPECTRUM OF ERYTHRO ALEURITIC ACID

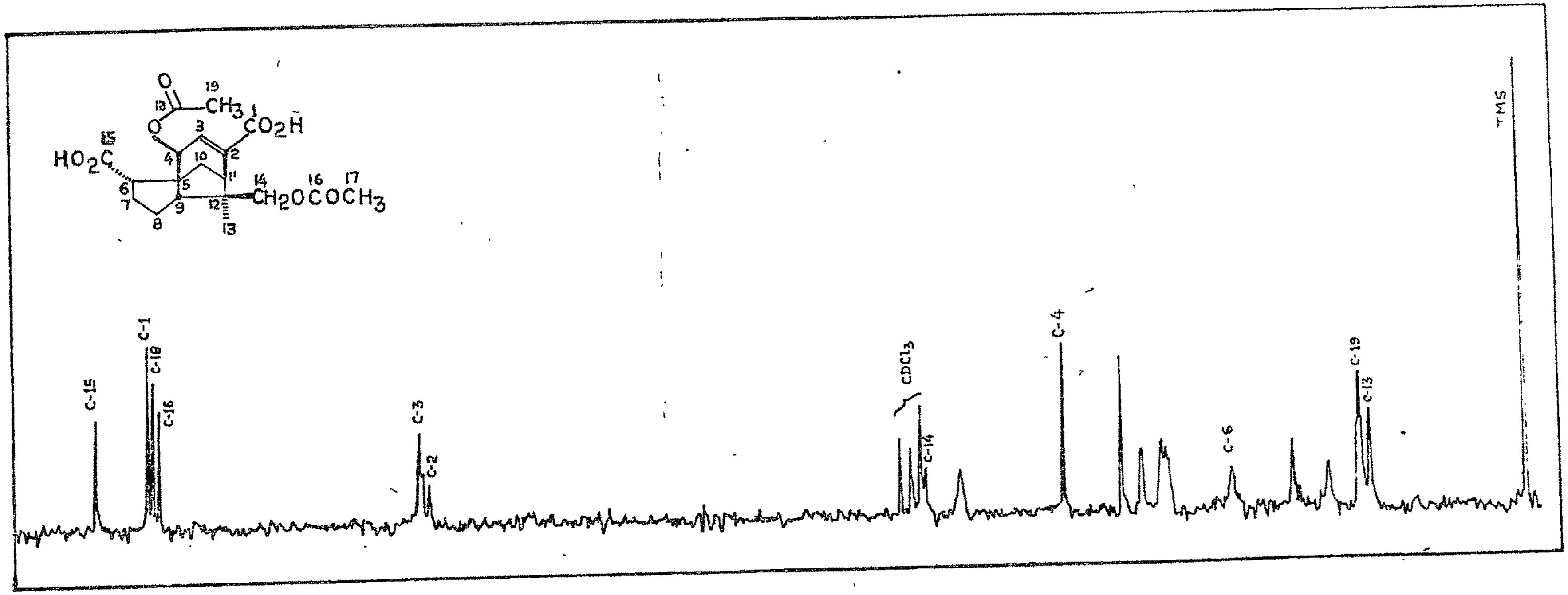


FIG.10: ^{13}C NMR SPECTRUM OF EPISHELLOLIC ACID-DIACETATE

and epishellolic acid-diacetate(Fig.10) were recorded. The position assignments for important carbons are done and these for diemthyl epishellolate, erythro-aleuritic acid, and for epishellolic acid-diacetate are given in Table-1.

As the ^{13}C NMR spectra of the two resins were almost identical, comparison with any one would solve the problem. We have compared the resin of molecular weight 4.8 x 10^3.

The ^{13}C signal at 68.59 ppm in the ^{13}C NMR spectrum of the resin (Fig.6) corresponds to the C-15 signal(at 68.47 ppm) in the ^{13}C NMR spectrum of diemthyl epishellolate. Similarly, the ^{13}C signal at 62.59 ppm in the spectrum of the resin corresponds to the C-16 signal (at 61.18 ppm) of erythro-aleuritic acid.

As shown in Table-1, the C-9 and C-10 carbons of erythro-aleuritic acid appear together at 73.53 ppm. After the C-9 -OH undergoes esterification, the calculated value for C-10 is 63.53-68.53 ppm (α-effect of -OH group= +5-10 ppm). The presence of a ^{13}C signal at 66.59 ppm in the ^{13}C NMR spectrum of the resin of molecular weight 4.8 x 10^3 shows further that the proposed esterification[3] of the allylic carboxyl group of the sesquiterpene part with the C-9 -OH of aleuritic acid is really borne out.

Because of solubility problem, the Field Ionization mass spectra of the resins could not be recorded.

TABLE - 1

^{13}C CHEMICAL SHIFTS OF SOME OF LAC ACIDS AND THEIR DERIVATIVES[a,b]

COMPOUND	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19
1. Dimethyl epishellolate	51.88[c]	167.30	135.54	140.83	71.65	59.98	36.35	25.41	25.41	46.70	45.18	52.71[c]	29.53	19.41	68.47	174.00	51.18[c]		
2. Erythro-aleuritic acid[d]	175.07	34.00	24.82	29.41	29.41	28.94	25.88	32.76	73.53	73.53	32.70	25.88	28.94	25.88	32.70	61.18			
3. Epishellolic acid-diacetate[e]	171.89	137.06	138.24	58.12		37.17							19.64	75.41	178.24	170.48	20.82	171.18	20.82

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a) for the numbering of the carbons, which is arbitrary, see the structures shown on the respective ^{13}C NMR spectra.

b) chemical shifts in ppm downfield from TMS

c) may be interchanged

d) natural, m.p. 100-101 °C

e) this acetate was prepared by Ac_2O-pyridine treatment of epishellolic acid

EXPERIMENTAL

The general remarks given in the experimental of PART A-CHAPTER-II hold good here also.

Waters Associates 200 Gel Permeation Chromatograph was used for the gpc purposes. The eluant (THF) was stabilized by 2,4-ditertiary butyl phenol. The concentrations of the solutions injected were 0.1-0.4%(w/v). The injection time was 1 minute.

^{13}C NMR spectra were recorded on Zeoul FX-100 spectrometer. The ^{13}C NMR spectra of dimethyl epishellalate, aleuritic acid, and epishellolic acid-diacetate were recorded in DMSO, DMSO-d_6, and $CDCl_3$, respectively, using TMS as the internal standard. The ^{13}C NMR spectra of the resins were recorded in dioxan at 70°C.

HARD RESIN

Palas seed lac (200 g, 100 mesh) was stirred with distilled water(1500 ml) for 30 minutes. The aqueous part was decanted and the process of washing repeated(another 7 times). The washed lac powder was air dried (3-4 days) at room temperature and the material (194 g) thoroughly dispersed in 78% aqueous EtOH(800 ml) with mechanical stirring(60 minutes). The alcohol insoluble material was again treated with two further portions of 78% EtOH (300, 200 ml) as above.

The total EtOH extract was concentrated under reduced pressure (~200 torr) to 1/5 its volume. The concentrate was cooled and with vigorous stirring slowly diluted with ether(2500 ml). The precipitate was collected by straining through cloth and the product was triturated with ether (300 ml x 4) to furnish an insoluble material(137 g), which was soxhleted(20 hours) with dry ether to finally yield the "hard" resin (127 g).

PURE LAC RESIN (MW 7.61 x 10^3)

Palas "hard" resin (50 g) was dissolved at room temperature (~35°C) in dry dioxan (700 ml) and the solution slowly diluted with dry benzene(450 ml). The mixture was left aside for 30 minutes, after which the precipitated resin(9.2 g,fraction 1) was collected(by decantation). To the mother liquor more benzene(50 ml) was added. The mixture was set aside as above and the precipitate (2.8 g, fraction 2) collected. This operation was repeated twice by successive additions of 50 ml benzene (fraction 3, 1.4 g; fraction 4, 1.8 g) and then thrice again by addition of 100 ml of benzene each time(fraction 5,4.4 g; fraction 6, 2.6 g; fraction 7,2.3 g). The last mother liquor was allowed to stand at 10±1° for 24 hours and the precipitate (12.9 g, fraction 8) collected. The mother liquor was next diluted with 200 ml of benzene and the precipitated resin(3.4 g, fraction 9) collected after 2 hours at ~35°C. The filtrate from fraction 9 was further diluted with benzene(400 ml) and allowed to stand (24 hours) at ~10° and the resin (0.6 g, fraction 10) collected. The filtrate was next diluted with more benzene (600 ml) and the separating resin (1.6 g, fraction 11) collected after 24 hours at ~10°. The final filtrate was freed of solvents at 40-50 mm to give a gum (5.4 g, fraction 12).

The above process was repeated twice and the results are summarized in the following table:

FRACTIONAL PRECIPITATION OF HARD RESIN(50 g/BATCH)
(YIELDS IN GMS)

FRACTIONS	1	2	3	4	5	6	7	8	9	10	11	12
BATCH NO.1	9.2	2.8	1.4	1.8	4.4	2.6	2.3	12.9	3.4	0.6	1.6	5.4
BATCH NO.2	9.3	0.6	1.4	2.5	3.9	2.2	1.6	10.9	5.7	0.5	1.8	8.1
BATCH NO.3	12.2	0.8	1.4	1.4	3.7	3.0	2.5	10.1	4.4	1.2	2.3	6.3
REPORTED YIELDS	6.0	3.2	1.8	3.4	12.1	6.1	2.5	7.0	1.5	1.5	2.1	2.6

Fractions 4-7 were pooled(31.9 g) and the well powdered material (24 g) extracted by mechanical stirring with EtOAc (1200 ml x 3) and then with 25% dioxan in EtOAc(1200 ml x 3). The insoluble material was titurated with dry ether to give a granular powder(19 g). This product was dissolved in dioxan (132 ml, 35°C) and then precipitated by addition of dry benzene(132 ml). After standing at 35°C for 30 minutes, the product(14.8 g) was collected. This material was again extracted, as above, with 25% dioxan in EtOAc(450 ml x 3) and the insoluble product(13.2 g) redissolved in dioxan(127 ml) and precipitated by addition of benzene(127 ml). The product (11.5 g) was reddish in colour and hence was redissolved in dioxan(156 ml). On addition of dry benzene(70 ml), a dark red precipitate(2.5 g) was thrown out and this was rejected, to the filtrate more benzene (200 ml) was added and the light buff coloured precipitate(8.0 g) collected. This product was again taken up in dioxan(100 ml), diluted with 50 ml benzene to remove a reddish precipitate(1.5 g) and further diluted with more benzene (130 ml) and the precipitate collected, titurated with dry ether and dried in vacuum at ~35°C to give a very pale coloured amorphous powder(6.0 g, ~5.3% based on hard resin, m.p. 115-120°C, MW, 7.61×10^3).

PURE LAC RESIN (MW 4.8 x 10^3)

The above fractions 1-3, 8-10 and others obtained while further purifying the mixed 4-7 fraction in the above experiment were mixed and powdered well. This material(50 g) was dissolved at room temperature(~35°) in dry dioxan(700 ml) and the solution slowly diluted with dry benzene(380 ml) till precipitation just started. The mixture was left aside at room temperature for ~30 minutes, after which the precipitated resin (10.9 g, fraction 1) was collected by decantation. To the mother liquor, more benzene (50 ml) was added. The mixture was set aside as above and the precipitate(0.9 g; fraction 2)collected. This operation was repeated twice by successive addition of first 50 ml and then 60 ml benzene(fraction 3; 2.2 g; fraction 4, 3.8 g) and then thrice again by the addition of first 120 ml, then 150 ml, and finally also 150 ml benzene(fraction 5, 6.4 g; fraction 6, 7.9 g; fraction 7, 2.5 g). The last mother liquor was allowed to stand at 10±1°C for 24 hours and the precipitate(7.5 g, fraction 8) collected. The solvent was removed from the mother liquor at reduced pressure(40-50 mm) and the material (7.3 g) recovered.

Fractions 4-7 were mixed, powdered well, and dried at room temperature(~35°) under reduced pressure(30 mm). This material (19.8 g) was extracted by mechanical stirring first with EtOAc(1000 ml x 3)and then with 25% dioxan in EtOAc(1000ml x3).

The isoluble material was titurated with dry ether to give a granular powder(17.3 g). This product was dissolved (35^o) in dioxan (125 ml) and precipitated by addition of dry benzene(125 ml). After standing at ~35^oC for 30 minutes,the product was collected and again extracted, as above, with 25% dioxan in EtOAc(450 ml x 3) and the insoluble product titurated with dry ether to give granular solid(10.3 g). This product was redissolved in dioxan(100 ml) and precipitated by addition of benzene(100 ml). The material(7.9 g) was redissolved in dioxan(110 ml); on addition of dry benzene (45 ml) a dark red precipitate(0.7 g) was thrown out and this was rejected, to the filtrate more benzene(145 ml) was added and the light buff coloured precipitate, so obtained, was titurated with ether. The product (6.0 g) was again taken up in dioxan(85 ml) and diluted with benzene (45 ml) to remove a reddish precipitate (0.2 g). More benzene(115 ml) was added and the precipitate titurated first with EtOAc and then with ether to give a granular solid which was dried in vacuum(40 mm) at room temperature to ultimately afford a pale coloured amorphous powder(5.8 g, m.p. 104-106^o, 8% yield based on hard resin, MW 4.8 x 10^3).

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