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CHEMISTRY OF LAC RESIN

A THESIS SUBMITTED TO THE POONA UNIVERSITY
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C O N T E N T S

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CHAPTER I

INTRODUCTION

C O N T E N T S

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Lac is one of the valuable gifts of Nature to man. The word lac is derived from Sanskrit word "Laksha"*. It is actually the secretion of a tiny insect Laccifer lacca[1] popularly known as lac insect. It was the first resin of insect origin to be investigated and hence attracted the attention of large number of investigators. Originally it was being cultivated for the production of its colouring matter. The far more valuable resin was not recognised until sixteenth century.

Lac Insect

The lac insect belongs to the family Lacciferidae[2], which constitutes a specialised and isolated group in the super family coccoidea of the order Hemiptera. The family Lacciferidae is divided into two sub-families: (1) Lacciferinae and (2) Tachardininae and is composed of about sixtyfive species. The common Indian lac insect belongs to the species Laccifer lacca. This is a tiny red insect (Fig.1) not larger than the smallest apple seed. This insect is specific in its parasitism and thrives

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*"Laksha" means "hundred thousand" an allusion to the myriads of minute lac insects which emerge at the swarming time and later exude the lac of commerce.

on certain trees which are called as lac hosts, most important amongst them are Ber (Zyzyphus mauritiana Lamk), Palas (Butea monosperma Lamk) and Kusum (Schleichera oleosa Lour). India, Burma and Siam are the main cultivators of this natural product and 90% of the world's production is obtained from the Indian cultivation.

Though the presence of resins is reported in the insect waxes[3] like Chinese insect wax and waxes from insects of Ceroplastes family, information regarding the detailed composition of these resins is not available.

Stick lac

The scale of the lac insect consists of an excretion which acts as a protective coating for the insects' body. This is an amber colored resinous substance known as lac. The insect lives most of its life under this resinous shield sucking the sap of the tree to which it has fixed itself. The exudation from the millions of insects gradually meets and joins until the entire branch is completely covered with the lac encrustation.

Lac is collected by cutting down the lac bearing twigs of the hosts and lac encrustations are separated from the twigs by scraping with a knife, the material thus

obtained is known as stick lac.

There is a marked difference in the colour of lac from different areas. Lac from West Calcutta is yellow or orange in colour, while the product available in the areas east and south of Calcutta is red. Assam produces lac of dark red colour. Material of similar shade is obtained from the cultivations in Siam.

While the life cycle of the insect produces two crops each year, there are numerous sub-divisions of the entire lac crop. The most important of them are:

Baisakhi crop of April
Jethwi crop of July
Katki crop of November
Aghani crop of February

There are probably 20 different varieties of raw lacs each having specific properties. Properties of lac from the same host tree varies from season to season and is attributed to the climatic differences, growth of the insects, thickness of the encrustations etc.

Seedlac and Shellac

The stick lac obtained directly from the host trees contain impurities like dye, fibres, animal remains and sand, so it needs refining before it could be utilised for commercial utilization. For this stick lac is crushed

and is cleaned by sieving out the dust and sand. The residue is then washed with fresh water when most of the floating impurities are removed and heavier particles which sink to the bottom are separated. The lac obtained is dried in shade and is known as Seedlac.

The average yield of seedlac from different hosts is reported to be as follows:

Host	Crop	Average yield of seedlac from sticklac %
Kusum	Aghani	68.7
Kusum	Jethwi	70.1
Ber	Katki	56.8
Ber	Baisakhi	63.5
Palas	Baisakhi	58.8
Palas	Katki	48.3

Seedlac thus obtained is a semi-refined product which still contains 3-7% of impurities like sand, wood-chips etc. which are removed either -

i) by a process of hot filteration or

ii) by solvent extraction.

The product thus purified is known as Shellac. Commercial shellac is available in many grades. Shellac contains 4 to 5.5% of wax, which is removed to obtain

dewaxed shellac. This dewaxed shellac is the main product of commercial importance.

Applications of Shellac

Shellac resin finds a large number of applications in industry, some of them are mentioned below:

1. For the manufacture of gramophone records.
2. As electrical insulating material.
3. In paper industry, printing inks etc.
4. In leather finishes, paints, rubber etc.

A large number of synthetic resins having specific properties are now commercially available. These in some applications are preferred to shellac, hence the foreign market for shellac is being affected considerably. In view of this fact, it was necessary to go deep into the study of the constitution of lac. This will give a clue to convert shellac into many more useful products.

CHEMISTRY OF LAC

Nature

Geoffrey and Lemery[4] considered shellac to be a wax but Gren[5] and Fourcroy[6] showed it to be a true resin which is a hard brittle solid. Shellac has no sharp melting point. According to Tamman[7] the range of melting point is between 30.5 - 56.5°C.

Nagel[8] reported the melting range to vary between

80-90°C.

Hatchett[9] was the first to study lac in some more details. The composition of sticklac, seedlac and shellac as reported by him is given in Table 1.

TABLE 1

Constituent	Sticklac %	Seedlac %	Shellac %
Resin	68.0	88.5	90.9
Dye	10.0	2.5	0.5
Wax	6.0	4.5	4.0
Gluten	5.5	2.0	2.8
Foreign bodies	6.5	-	-
Impurities	4.0	2.5	1.8

Funk[10] obtained from sticklac, a constituent which showed properties intermediate between a resin and wax and called it "Lac substance". This was corroborated by John[11]. According to him the composition of seedlac is as stated in Table 2.

TABLE 2

Constituents	% in seedlac
Resin including the portion insoluble in ether.	66.65
Lac substance	16.70

....contd.

TABLE 2 (Contd.)

Constituents	% in seedlac
Colouring matter	3.75
Extract (bitter principle)	3.92
Sticklac acid (Laccaic acid)	0.62
Insect remains (chiten) with colouring matter.	2.08
Waxy fat	1.67
Salts	1.04
Earth	0.62
Loss	3.96

Unverdorben[12] described the seedlac as composed of a mixture of resins having different solubilities in alcohol and ether, wax, coloring matter and lac substance similar to the one reported by Funk[10] and John[11].

The first systematic study of the components of lac was made by Tschirch and Farner[13]. They found that the major portion of sticklac was soluble in ethyl alcohol, methyl alcohol, acetic acid, alkalies, borax and soda solutions; partly soluble in ether, ethyl acetate, chloroform, acetone and completely insoluble in petroleum ether, benzene and toluene.

Tschirch in collaboration with Lüdy[14] made exhaustive

study of sticklac obtained from Indian source. Lac was subjected to successive extractions with the same solvents as used by Tschirch and Farner[13] but in a different order.

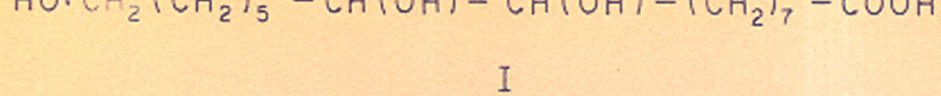
Schaeffer[15] analysed a number of samples of sticklac according to the method of Tschirch and Lüdy[14]. Seshadri and his coworkers[16] have reported a scheme of separation and claim to have achieved better separation.

Chemistry of Hard resin

Only after the isolation of more or less pure homogeneous fraction of lac resin called as hard resin, was complete, some progress could be made in the direction of its chemical nature. This fraction which was insoluble in ether but soluble in alcohol was the major portion of total resin from seedlac.

Tschirch and Farner[17] believed this fraction to be an ester, so they hydrolysed pure resin by passing steam for several weeks through its solution in 10% potash lye. The hot saponified solution was decomposed with dilute sulphuric acid and filtered hot, from the filtrate. On cooling an acid separated in 15% yield which after repeated crystallisations showed m.p. 101.5°C was named as aleuritic acid (I).

Harries and Nagel[18] hydrolysed pure resin by 5% KOH



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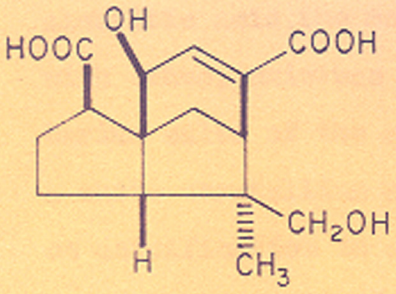

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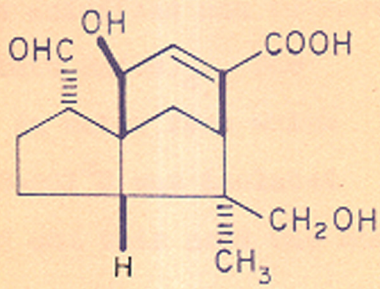

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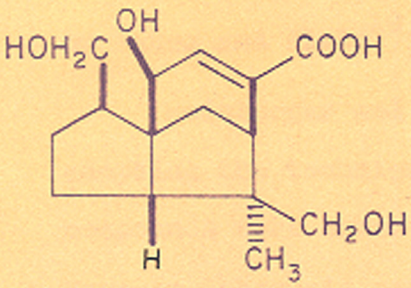

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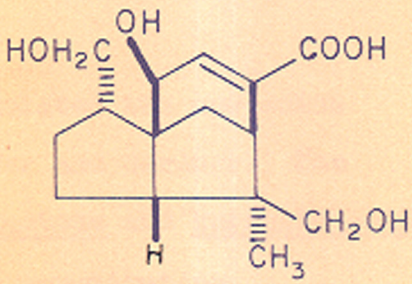

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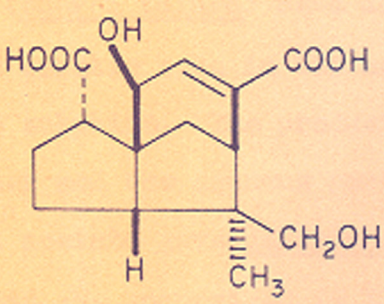

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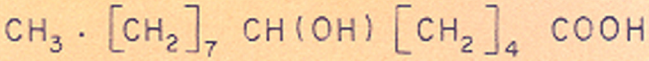


VII

solution at room temperature. After the removal of aleuritic acid, the mother liquor was acidified and extracted with ether, which was shaken with dilute $Ba(OH)_2$. The barium salts of the acids dissolved in water from which a white crystalline solid m.p. 199.5-201°C was isolated on acidification in a yield of 8-10% and this acid was named as shellolic acid (II).

Though the presence of this acid was confirmed later by most of the workers, the final structure was assigned by Yates and Field[19] in 1960.

Heinberger and Gardner[20] isolated two new acids adopting the technique of Schaeffer and Gardner. The compounds were named as laccolic lactone and kerrolic acid. However, they did not study their constitution.

Kamath and Potnis[21] isolated another acid in a 50% yield from Jalari (Shorea talura) seed lac. They saponified seed lac with 0.5N caustic soda in 50% aqueous alcohol in presence of sodium sulphite. The product was acidified in presence of ether and the aqueous portion after concentration and extraction with ethyl acetate gave jalaric acid (III). Properties of this acid are summarised in Table 3.

TABLE 3

Properties	Values obtained
Melting point	85-87°C
Acid value	200.4
Saponification value	229.4
Hydroxyl value	218.0
Melting point of 2:4-dinitrophenyl hydrazone	231-232°C with decomp.
Elementary analysis	C, 62.2; H, 7.2%.

They assigned the molecular formula $C_{15}H_{22}O_5$ to the acid, having one carboxyl, one carbonyl, and two hydroxyl groups. They could not reach to any conclusion regarding its structure. The structure and absolute configuration of Jalaric acid was established by Wadia, Bhaskar and Sukh Dev[22].

These workers[23] isolated two more new acids from the hydrolysed pure resin and named them as laksholic (IV) and epi-laksholic acids (V). They further pointed out the fact that these new acids along with shellolic and epishellolic acids (VI) are the artefacts obtained from the original aldehydic acid-Jalaric acid.

Apart from aleuritic acid a large number of other aliphatic acids have also been reported. Most important

amongst them is Butolic acid which was first isolated by Sen Gupta and Bose[24] and whose structure is now established as 6-hydroxy tetradecanoic acid (VII) which will be discussed in Chapter III.

In addition to the acids reported by the earlier workers some minor aliphatic constituents of shellac have been recently reported by Christie et al.[25] According to these workers about 7% of the total acids is composed of:

1) normal long chain fatty acids
2) unsaturated fatty acids of the same chain length.
3) Hydroxy acids C_{14}, C_{16} acids and
4) 9,10-dihydroxy C_{14}, C_{16} acids.

Palmitic and myristic acids were identified from the acids of hard resin; it appears that all the minor components as reported by Christie et al.[25] are probably the minor components of hard resin also.

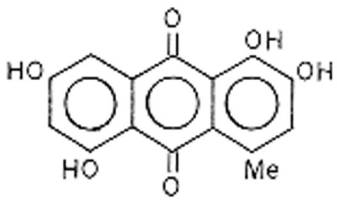
Colouring Matters

During the separation of stick lac into various components, besides resin, appreciable quantities of lac dye, odoriferous principle and wax are always obtained. Naturally, lac dye attracted the attention of many investigators.

Aqueous extract obtained from the washings of seed lac consists of organic and inorganic matter. Organic matter

contains sugar, albuminous matter and coloring matter. The latter is termed as laccaic acid (lac dye). It was first obtained by Schmidt[26] in more or less pure form in a yield of 2.5%. There have been many attempts to study the constitution but no one could arrive at any conclusion. Recently Bhide et al.[27] reported the isolation of a homogeneous pigment which is a purpuric derivative. They further showed that this pigment is a mixture of atleast three components and hence the structure of laccaic acid has not yet been finally established.

Another coloring matter was isolated from the ether extract of the resin to an extent of 1% by Tschirch and Farner[13] who named it as erythrolaccin. The constitution of this coloring matter was first studied by Tschirch and Lüdy[28]. This product was simultaneously studied by Venkataraman and coworkers[29] and P. Yates et al.[30] and is now proved to be 1,2,5,7 tetrahydroxy 4-methyl anthraquinone (VIII).



(VIII)

Wax

Shellac wax is present in stick lac to the extent of 5.5% The abundance of wax in the seed lac is next to the

resin. The lac substance (16.7%) obtained by Funk[10] seems to be a mixture of resin and wax, and the presence of this substance was supported only by John[11]. Later, such a substance has not been reported, and is due to the facts that the method of separation adopted by later workers gave a better separation of wax from the resin.

Shellac wax is used in many industrial applications. It is incorporated in paste polishes as it gives good gloss to the leather.

Lac insect secretes the wax in the form of thin white filaments along with lac resin. These filaments get embeded in the resin and thus form an essential minor constituent of lac.

Kauffmann[31] isolated the wax by dissolving shellac in hot soda solution and skimming off the soluble wax which floats on the surface as molten mass. m.p. 78-79°C.

He detected the presence of two alcohols $C_{28}H_{58}O$ and $C_{30}H_{62}O$.

Tschirch and Schaeller[32] isolated a hydrocarbon ($C_{25}H_{52}$) from the unsaponifiable fraction and named it as lakshadiacerin.

According to Subramanian[33], the composition of lac wax is as follows:

Free Acid component	3.1 per cent
Free Alcoholic component	26.4 " "
Anhydrohydroxy acid	1.6 " "
Esters	66.8 " "
Hydrocarbons	2.1 " "

The above work suggested that the alcoholic components are a mixture of n-primary alcohols possessing 26 to 34 carbon atoms.

The esters appear to be a mixture of atleast 15 different esters with a mean molecular weight of 718.5 and molecular formula $C_{49}H_{98}O_2$.

Faurot-Bouchet and Michael[34] reported the various constituents of wax obtained from the insect Tachardia lacca. The hydrocarbons (2%) isolated from the unsaponifiable were a mixture of heptacosane, nonacosane, and hentriacontane with even homologues in minute amounts as revealed by G.L.C. Octacosanol was the major constituent of the fatty alcohols.

The acids were composed of a mixture of C_{28}, C_{30}, C_{32} and C_{34} acids with traces of other homologues.

Odoriferous Principle (Neutral fraction)

The presence of odoriferous principle having a pleasant smell was first recorded by Tschirch and Farner[13]. According to them this substance could be obtained from direct steam-

distillation of seed lac.

Bose and Bhattacharya [35] showed it to contain wax, acidic portion, and an ester fraction probably lactonic in nature. No systematic study of this fraction has however been so far attempted.

Tschirch and Lüdy[28] also recorded the presence of salts, albuminous matter and sugars from the aqueous extract of stick lac. Glucose, arabinose and fructose were identified as their osazones.

This review of the work so far carried out on lac clearly indicates that many problems regarding (i) the separation of lac (ii) the composition of lac acids (iii) constitution of lac molecules are still unsolved. The present work deals with the first two subjects, the results of which would also help in getting a correct picture of lac molecule.

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CHAPTER II

ISOLATION OF 'HARD RESIN' FROM SEED LAC

C O N T E N T S

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ISOLATION OF "HARD RESIN" FROM SEED LAC

Introduction

The present work was undertaken with the purpose of gaining some insight into the chemical nature of lac resin. As has been described in the introductory Chapter, stick lac consists essentially of a complex mixture of lac resin, wax and coloring matters. The present Chapter deals with the separation of lac resin from the seed lac. After giving a summary of the previous methods described for its separation, the method finally adopted for the present work is discussed.

Previous Work:

Watchett[1] was the first to study the constituents of stick lac in some detail. He showed stick lac to consist of 'resin', wax, gluten, foreign bodies and some other impurities. Later Funk[2] isolated a constituent which had properties intermediate between resin and wax and named it as "lac substance" which was corroborated by John[3]. He could separate seed lac into resin, coloring matter, bitter principle, 'stick lac acid' (laccaic acid), wax, salts and insect remains.

Unverdorben[4] separated seed lac into wax, small quantities of oleic and stearic acids, a large portion of resin soluble in alcohol but insoluble in ether, a resinous material sparingly soluble in cold alcohol, a crystalline resin, 'brown extract', lac substance and an extractable coloring matter.

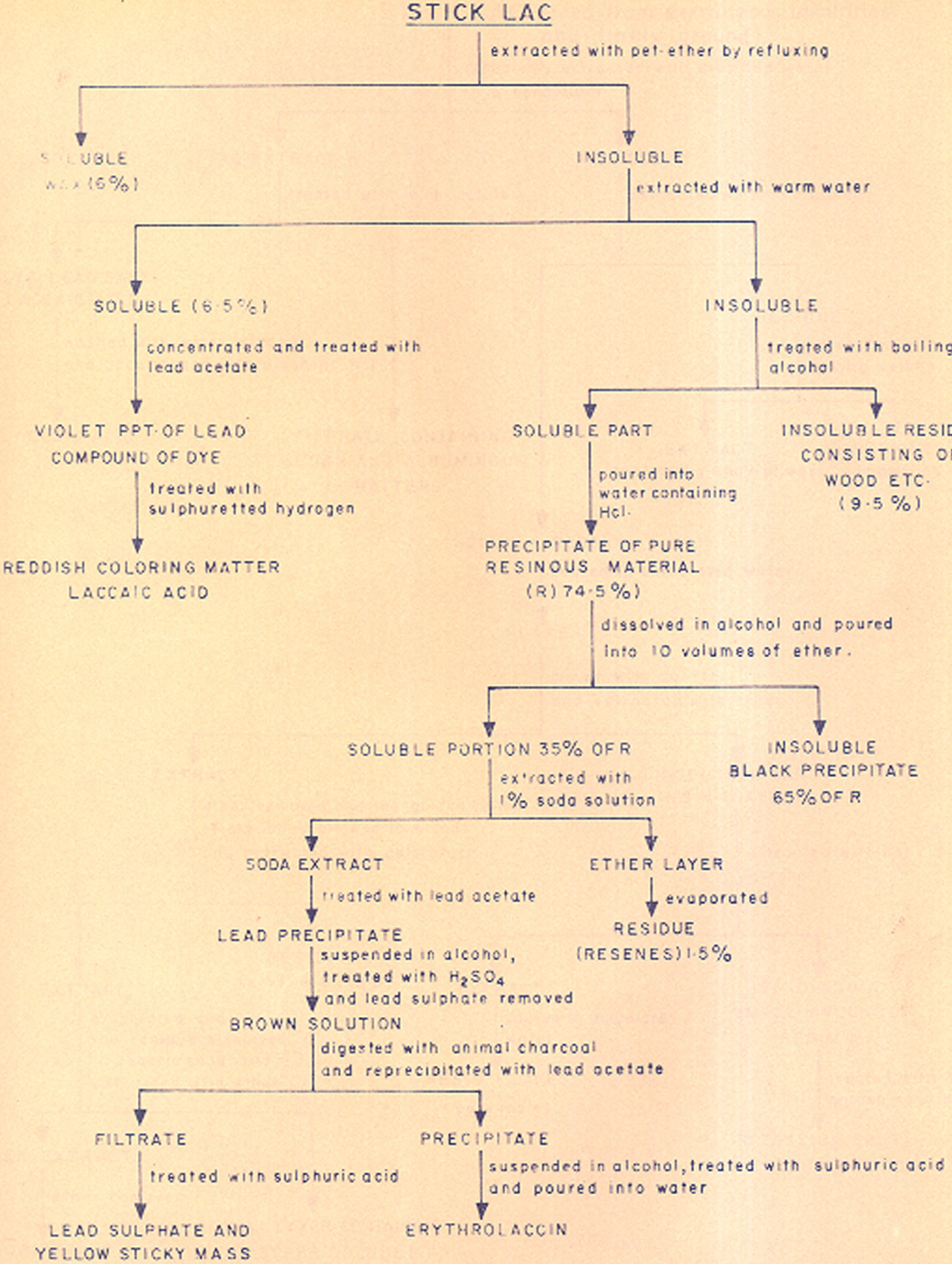
The first systematic study for the fractionation of stick lac was carried out by Tschirch and Farner[5]. The method of fractionation adopted by these workers is presented in Fig.1.

These workers for the first time, resolved total resin into (i) ether soluble portion later called by Nagel as soft resin and (ii) insoluble part called as pure resin.

Later on Tschirch, in collaboration with Lüdy[6], using a modified procedure made exhaustive study of stick lac obtained from Indian source. Lac was subjected to successive extractions with the same solvents as used by Tschirch and Farner[5] but in a different order as shown in Fig. II.

This modified scheme gave a better separation as the resin part was extracted out using cold alcohol in which wax is expected to be insoluble.

Verman and Bhattacharya[7] attempted to separate lac resin into two fractions by using solvents toluene, trichloroethylene, benzol and naphtha. Palit[8] used ethyl acetate for the same purpose while Bhattacharya and Gidwani[9], and Bhattacharya and Heath[10] used mild alkalies. Venugopalan and Sen[11] made use of acetone with and without urea. In most of these cases the separations were laborious and often



FIG·I SEPARATION SCHEME DUE TO TSCHIRCH AND FARNER[5]

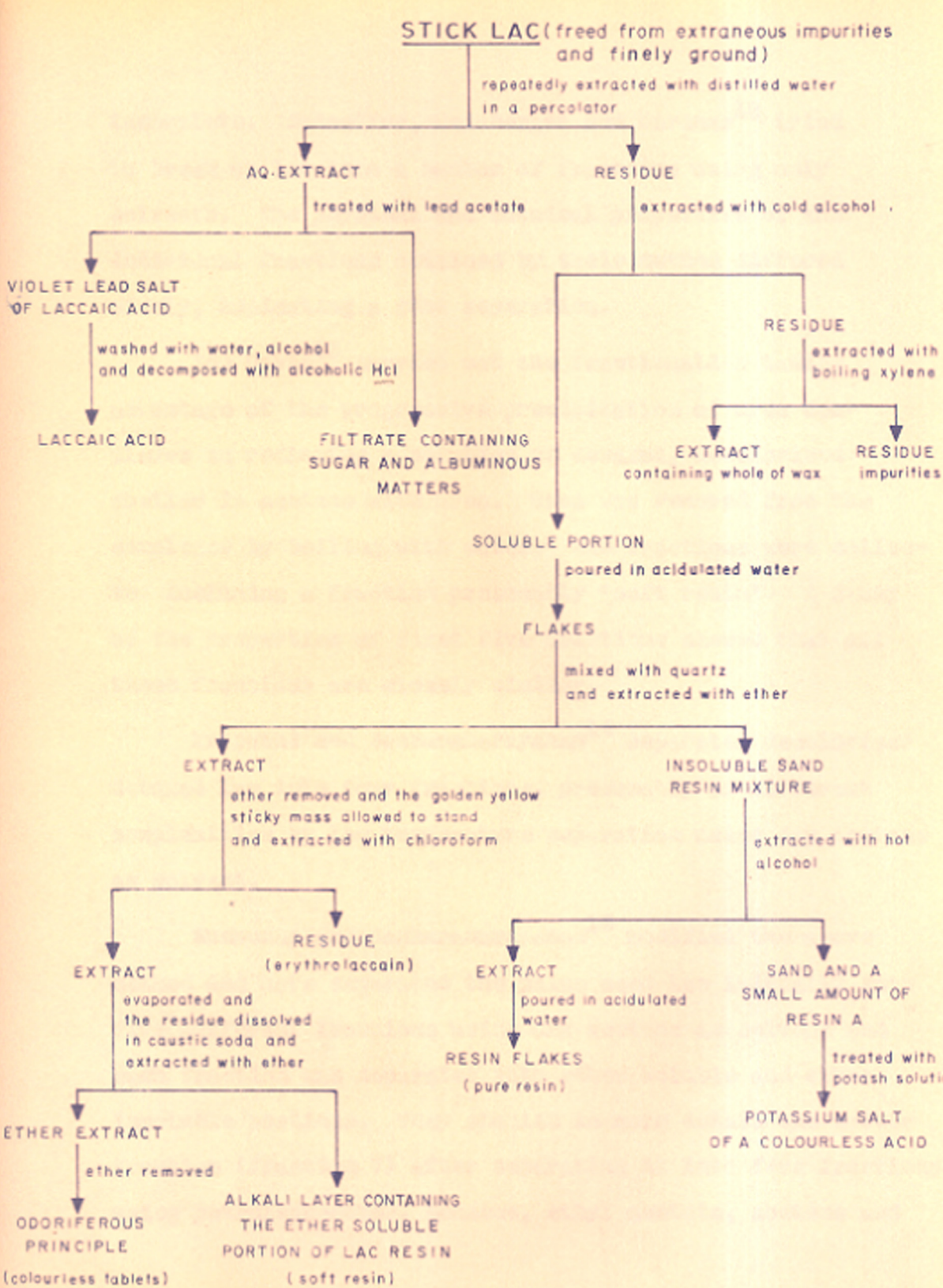


FIG. II. SEPARATION SCHEME DUE TO TSCHIRCH AND LÜDY[6]

incomplete. Schaeffer, Weinberger and Gardner[12] tried to break up lac into a number of fractions using only solvents. The physical and chemical properties of the individual fractions obtained by their method differed widely, indicating a good separation.

Sen Gupta[13] carried out the fractionation taking advantage of the progressive precipitation of urea complexes on refluxing a solution of dewaxed, decolorised shellac in acetone with urea. Urea was removed from the complexes by boiling with water. Six fractions were collected including a fraction presumably 'soft resin'. A study of the properties of first five fractions showed that all these fractions are closely similar.

Tripathi and Sankaranarayanan[14] separated decolorised dewaxed lac into four fractions, presumably of different complexities by low temperature separation using dry acetone as solvent.

Sambhamy and Sankaranarayanan[15] modified the above method and have separated the palas seed lac initially into five different fractions using dry acetone as solvent and each fraction was separated into ether soluble and ether insoluble portions. They studied in more detail the softer fraction (fraction V) after separating it into five fractions using petroleum-ether, benzene, ethyl acetate, acetone and

ethyl alcohol. However as the softer fraction is expected to be of a comparatively low molecular weight, the data obtained may not be helpful in giving additional information for the purer hard resin.

Present work:

A critical evaluation of the methods described in the literature showed that the method of Tschirch and Lüdy[6] (Fig.II) holds good promise to yield 'lac resin' essentially free from wax, colouring matters and other extraneous impurities. However, certain points in the scheme which required modification were apparent. Thus, it appeared likely that the employment of absolute alcohol for the separation of wax from resin would lead to the contamination of the resin fraction. This was confirmed by determining the solubility of lac wax in absolute alcohol. This value was found to be 0.6% at 28°C. This drawback has been overcome by the use of dilute 78% alcohol (d_4^{30} 0.838).

The scheme has been further modified so that the precipitation of hard resin from its alcoholic solution with water is avoided, as the resin precipitated with water is likely to retain some water which it may not be possible to remove easily.

Fig.III shows the separation scheme as finally adopted in the present work.

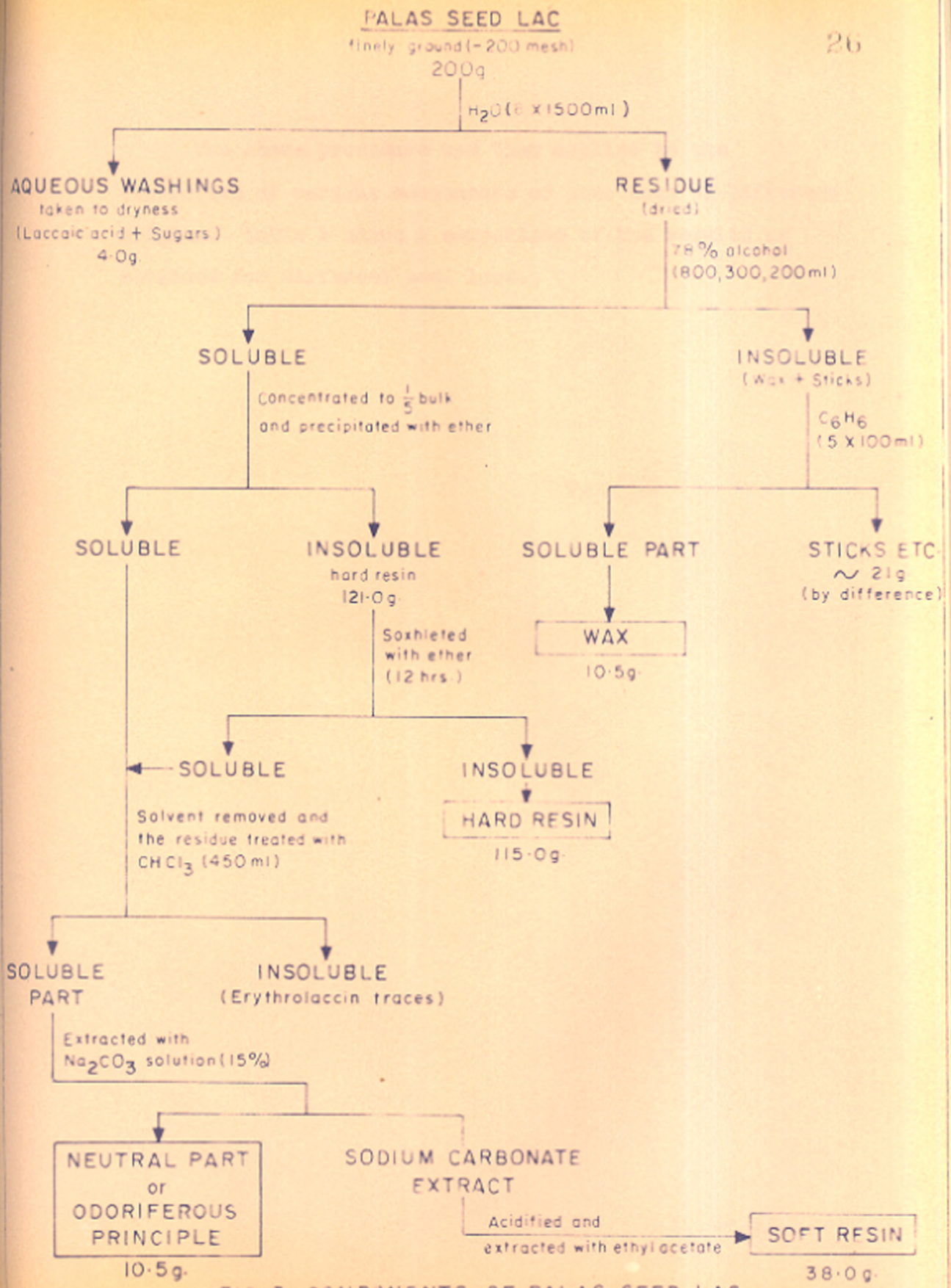


FIG 3· COMPONENTS OF PALAS SEED LAC·

The above procedure was then applied to the separation of various components of seed lacs of different origins. Table 1 shows a comparison of the results as obtained for different seed lacs.

TABLE 1 -COMPOSITION OF DIFFERENT SEED LACS

Seed lac		Approximate % composition			
Name	Host tree	Wax	'Hard resin'	'Soft resin'	Neutral fraction.
Ber	Zyzyphus mauritiana Lamk.	8.5	53.0	18.8	7.6
Kusmi	Schleichera oleosa Lour.	8.3	60.0	19.0	6.9
Jalari	Shorea talura	8.9	58.0	21.0	8.2
Palas	Butea monosperma Lamk.	5.8	57.5	19.0	5.2

E X P E R I M E N T A L

Supply of material:

The seed lac samples were supplied by the courtesy of Lac Research Institute, Namkum, Ranchi.

The physical appearance of the samples of the different varieties were as stated below:

Palas - Medium grain size, having dull red colour.

Kusmi - Grain size somewhat bigger than that of Palas, having shining orange colour.

Ber - Medium grain size with orange red shade.

Jalari - Grain size similar to Kusmi, colour - dull brown.

Separation of various components of Palas seed lac

200 gms of powdered Palas seed lac (200 mesh) was washed with water (1500 x 8 ml) till the washings were almost colourless. Mechanical stirrer was employed during the washing process. The material on drying in air for four days yielded 196 gms of almost pale coloured powdered material.

196 gms of the washed seed lac was extracted from 78% alcohol (800, 300, 200 ml) by using a mechanical stirrer. The extract was filtered through a fluted filter paper.

Alcohol insoluble residue which was assumed to contain stick and wax was extracted by boiling benzene (100 x 5 ml).

Benzene extract was filtered and solvent distilled off to give respectively 10.5 gms of wax and 1 gms of stick etc.

Total alcohol extract (1275 ml) was concentrated to 1/5 of its volume by distilling under reduced pressure, cooled to room temperature and was made homogeneous by addition of 200 ml of ether; 2.3 litres of ether was then added slowly with constant shaking when hard resin precipitated. The supernatant solvent layer was filtered through a fine muslin cloth. Residue was transferred into a mortar and titurated with ether (400 ml). Ether layer was decanted off and the solid product was titurated two times more to give 121 g. of powdered hard resin after drying in air for 12 hrs. and soxhleted using 500 ml of ether for 12 hrs. Solid product was dried in air for 3 days and powdered to yield 115 gms of hard resin.

All the ether extracts obtained during the separation of hard resin were combined and ether was distilled off. Residue was dissolved in 450 cc of chloroform and filtered. A very small quantity of residue (erythrolaccin) was obtained. Filterate was extracted with 16% sodium bicarbonate solution (500 x 2; 300 x 2; 200 x 2 ml). Sodium bicarbonate extract was acidified with 700 ml 1:1 phosphoric acid and extracted with ethyl acetate (500 x 3; 300, 200 ml). It was dried over sodium sulfate. The solvent from the ethyl acetate extract was distilled off when a product normally defined

as soft resin was obtained in a yield of 38.0 gms.

Chloroform layer obtained during the above procedure which contains neutral fraction was washed with water (100 x 3 ml). Dried over sodium sulfate and the solvent was distilled off yielding 10.5 gms of neutral fraction (odoriferous principle).

Separation of various components of other seed lacs

Different components from other seed lacs viz. Ber, Kusmi and Jalari were separated by following exactly the same procedure as described above using the same quantities of materials.

Solubility of wax

Absolute alcohol (100 ml) was added to finely powdered shellac wax (5.0 gms) isolated from Palas seed lac. The product was kept for 12 hours at 28°C with occasional shaking and was filtered off. The residue was washed with absolute alcohol (20 ml.). The solvent from the filterate and washings was distilled off yielding 0.6 gms of wax. A similar experiment was performed using 78% alcohol instead of absolute alcohol yielding 0.350 gms of soluble product.

SUMMARY

A procedure for the isolation of various components of Palas seed lac is described.

The procedure has been applied to Ber, Kusmi, and Jalari seed lacs.

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CHAPTER III

COMPONENT ACIDS OF HYDROLYSED HARD RESIN

C O N T E N T S

COMPONENT ACIDS OF HYDROLYSED HARD RESIN

In the previous Chapter we have described the separation of lac resin into hard and soft fractions, after it had been more or less freed from wax, coloring matters and any foreign bodies. The present Chapter relates to our study of the products obtained by the hydrolysis of hard resin ('Palas seed lac').

Previous work

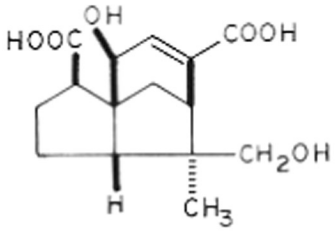
It has been shown by previous workers that hard resin on alkaline hydrolysis gives a complex mixture of acids from which a number of acids have been isolated and characterised. These have been briefly referred to in the introductory chapter (p. 8, 9, 10, 11, 12) and further details mostly of historical importance, have been recently reviewed[1]. Table 1 summarises the position, available at the time this work was undertaken about the percent occurrence of various acids in hydrolysed hard resin or shellac.

TABLE 1 - ACIDS REPORTED IN HYDROLYSED LAC

No.	Acid	Source	%
1	Aleuritic (I)	Hard resin	15[2], 30[3], 38[4]
2	Shellolic (II)	Hard resin	4-5[5], 9.2[4], 15[6]
3	Jalaric (III)	Seed lac	50[7], 25[8] (Jalari) (Kusmi)
4	Butolic	Shellac	2[6]

$$HO \cdot CH_2(CH_2)_5 - CH(OH) - CH(OH) - (CH_2)_7 - COOH$$

(I)



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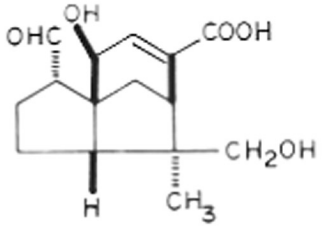

(III)

Present Work

A perusal of the methods followed by previous workers[1] for the isolation of lac acids* showed that their methods

*The term lac acid will be used to denote the acids obtained on hydrolysis of lac.

were aimed at isolating one or two specific acids from the lac hydrolysate and no systematic work, aimed at total analysis of the hydrolysate, has been described. The present study is an effort in this direction and in order to make the results more meaningful, component acids of hard and soft fractions have been studied separately. This work is expected to serve as a basis for the study of the pure lac resin*.

Base hydrolysis of Hard Resin

It has been rightly pointed out by Kamath[9] that it is quite conceivable that the acids produced on base hydrolysis of lac could be labile and could undergo base catalysed reactions during the hydrolysis. These authors presented evidence in support of their contention and the recent work of Nadia[10] et al. has established this by the isolation of pure jalaric acid which has been shown to be susceptible to Cannizzaro reaction.

In view of the above it was necessary to find the rate of hydrolysis of hard resin in order to determine the end point. The rate of hydrolysis of hard resin has been studied at 30, 50, 70°C (with 1.0 N alkali) and the results

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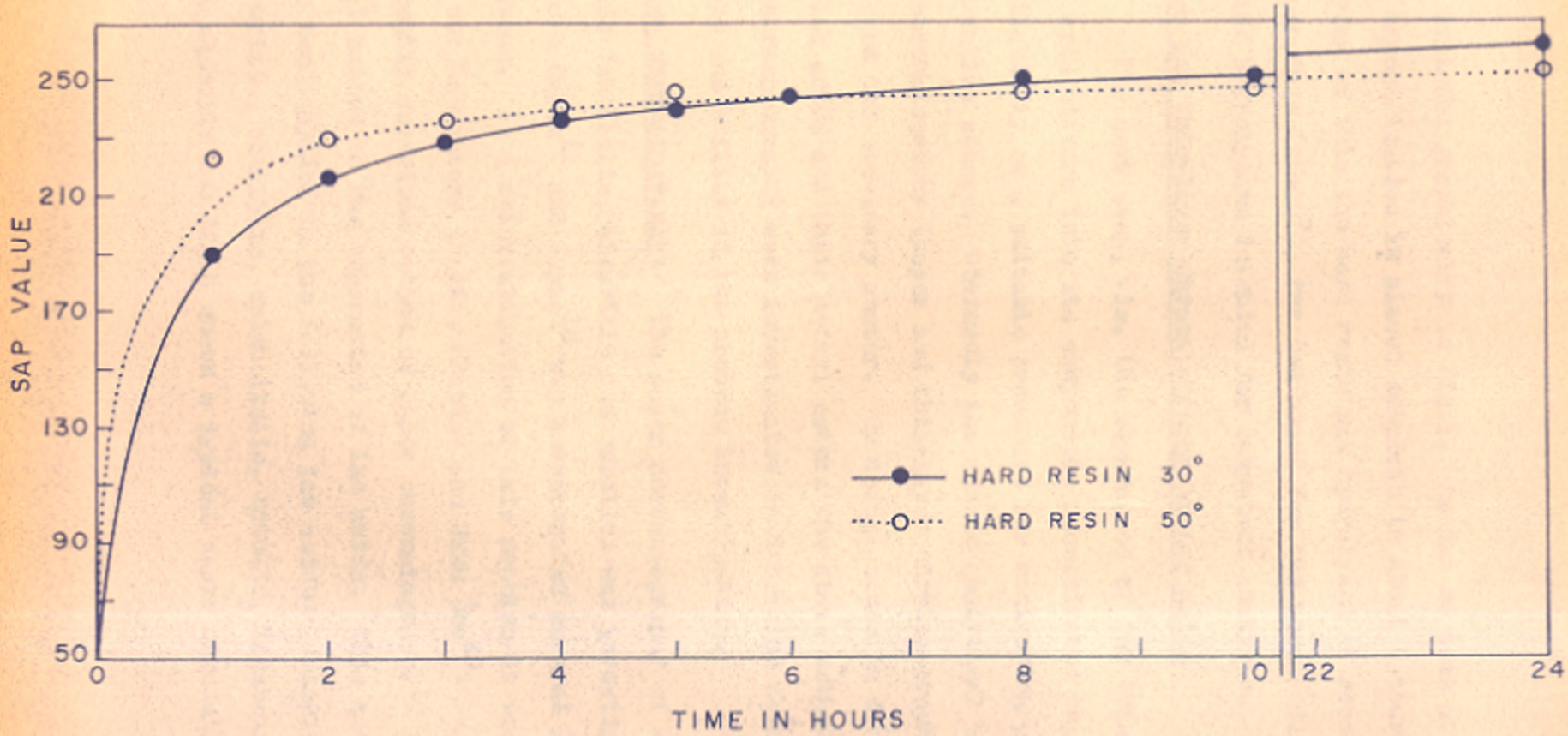
*Fractionation of hard resin into various fractions is being investigated separately by A.B. Upadhye of this Laboratory.



FIG·1· RATE OF HYDROLYSIS OF HARD RESIN

are depicted graphically in Fig.1. As can be seen at 30°C the saponification is almost complete in about 5 hours. In view of this the hard resin was hydrolysed at room temperature (25-30°C) for 5-6 hours for the preparation of the hydrolysate intended for component analysis.

Paper and Thin-layer Chromatography of Lac acids

The next step, viz. the separation of the total lac acid mixture into its components necessitated the availability of a suitable procedure for monitoring any separation scheme. Obviously the modern analytical methods of chromatography (paper and thin-layer chromatography) offered the necessary answer. By using authentic samples of lac acids and their methyl esters the above methods of chromatography were investigated to determine their scope and utility in the present investigation.

Paper chromatography: The paper chromatography of lac acids (shellolic, aleuritic and butolic) was investigated by Sen Gupta[11] and Gupta[12] who investigated several solvent systems. In a reinvestigation of this problem it was found in our Laboratory (Wadia, Bhaskar and Sukh Dev[10]) that the recently described method of paper chromatography[13] was well suited to the separation of lac acids. This method has been applied to the following lac acids: Jalaric, aleuritic, shellolic, epishellolic, butolic, laksholic and epilaksholic and Fig.2 shows a typical paper chromatogram

Fig.2 PAPER CHROMATOGRAM
(Individual lac acids)

PAPER: Whatman No.1.

SOLVENT FRONT: 14 cm

SOLVENT SYSTEM: Butanol-Ethanol-Buffer (35:35:30)

1 Jalaric acid; 2 Aleuritic acid; 3 Butolic acid;

4 Shellolic acid; 5 Epi-shellolic acid; 6 Laksholic acid;

7 Epi-laksholic acid.

of the individual lac acids while Table 2 summarises the R_f values.

TABLE 2 - PAPER CHROMATOGRAPHY OF INDIVIDUAL LAC ACIDS

(Solvent system: Butanol 35, Ethanol 15, Buffer[13]* 30)

Temperature: 28°C

No.	Acid	R_f value
1	Jalaric	0.67
2	Aleuritic	0.78
3	Butolic	0.85
4	Shellolic	0.52
5	Epishellolic	0.45
6	Laksholic	0.67
7	Epilaksholic	0.64

*7.20 g ammonium carbonate, 7.5 ml N $KMnO_4$, sp.gr. 0.88 in 95 ml water.

Thin-layer Chromatography (TLC): Because of the several advantages of TLC[14] over paper chromatography the TLC of lac acids and their methyl esters has been investigated. After considerable experimentation (vide experimental) a suitable solvent system could be found and Fig.3 shows a typical thin-layer chromatogram of several lac acids while Table 3 gives their R_{dye} values.

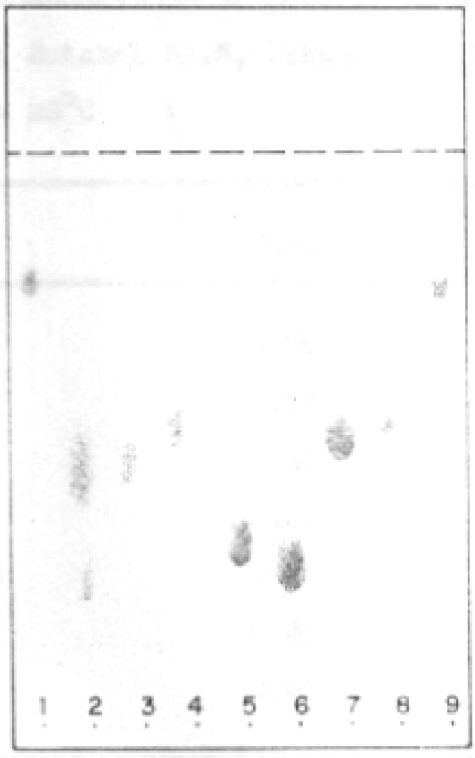


Fig.3 THIN LAYER CHROMATOGRAM
(Individual lac acids)

COATING: Silica gel-Plaster of Paris :: 100:15 (0.3 mm)

SOLVENT FRONT: 15 cm

SOLVENT SYSTEM: Butanol-Ethanol-Water (52.5:10:30)

1 Sudan III; 2 Jalaric acid; 3 Aleuritic acid;
4 Butolic acid; 5 Shellolic acid; 6 Epishellolic acid;
7 Epilaksholic acid; 8 Laksholic acid; 9 Sudan III.

TABLE 3 - THIN-LAYER CHROMATOGRAM OF INDIVIDUAL LAC ACIDS

(Solvent system: Butanol 52.5, Ethanol 35, Buffer* 30)

Temperature: 26°C

No.	Acid	R_{dye}
1	Jalaric	0.56
2	Aleuritic	0.57
3	Butolic	0.63
4	Shellolic	0.39
5	Epishellolic	0.32
6	Laksholic	0.66
7	Epilaksholic	0.63

*7.20 g ammonium carbonate, 7.5 ml NH_4OH sp.gr. 0.88 in 95 ml water.

Similarly a suitable solvent system was evolved for the thin-layer chromatography of the methyl esters of the lac acids. The results are shown in Fig.4 and Table 4.

TABLE 4 - THIN-LAYER CHROMATOGRAM OF INDIVIDUAL METHYL ESTERS

(Solvent system: Toluene 7, Ethyl acetate 4, Acetone 4)

Temperature: 28°C.

No.	Esters	R_{dye}
1	Methyl ketone methyl ester	0.48

....contd.

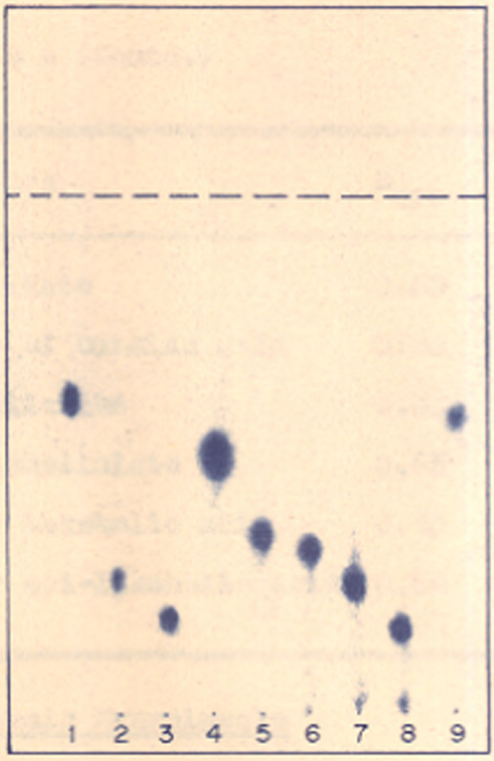


Fig.4 THIN LAYER CHROMATOGRAM
(Individual methyl esters)

PLATE: Silica gel-Plaster of Paris 100:15 (0.3 mm)

SOLVENT FRONT: 10 cm

SOLVENT SYSTEM: Toluene-Ethyl acetate-Acetone (7:4:4)

1 Sudan III; 2 Methyl ketone methyl ester; from Jalaric acid 3 Methyl aleuritate; 4 Methyl butolate; 5 Dimethyl shellolate; 6 Dimethyl epi-shellolate; 7 Methyl laksholate; 8 Methyl epi-laksholate; 9 Sudan III.

TABLE 4 (Contd.)

No.	Esters	R_{dye}
2	Methyl aleuritate	0.33
3	Methyl ester of butolic acid	0.90
4	Dimethyl shellolate	0.62
5	Dimethyl epishellolate	0.55
6	Methyl ester Laksholic acid	0.40
7	Methyl ester epi-laksholic acid	0.24

Analysis of Hard Resin Hydrolysate

As mentioned previously, the efforts of the previous workers in this field were directed essentially to the isolation of one or two particular components of the total hydrolysate (very often of the total shellac or seed lac) and no systematic attempt has been made for the complete analysis of the hydrolysate. One thing of significance is quite apparent from the previous data that the total lac acids fall distinctly into two groups.

Aliphatic acids (e.g. aleuritic, butolic, palmitic etc)

Terpenic acids (e.g. shellolic, jalaric etc.)

Further work aimed at the separation of the various components was organised keeping this fact in view.

Urea Adductation: Since long chain fatty acids are readily

adducted by urea[15,16] whereas cyclic molecules do not, segregation of the total acids into aliphatic and terpenic components by urea adductation was investigated in the first instance. Fractionation of total acids by urea was carried out in the usual manner both in acetone and methanol and the various fractions obtained this way after separation from urea were examined by paper chromatography. However, all the fractions were found to be complex mixtures and no satisfactory separation into aliphatic and terpenic components could be achieved by this procedure.

An experiment with pure aleuritic acid showed that it can be easily removed from its methanolic solution by urea adductation.

Fractional Distillation: High vacuum fractionation of the total methyl esters (diazomethane method) derived from the hard resin hydrolysate (10 days hydrolysis)* was next investigated. As shown in detail in experimental (Table 10) only 60% of the material could be distilled and the distillate was collected in three different fractions. Fig.5 gives

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*It has been shown (Wadia, Bhaskar and Sukh Dev[10]) that the action of diazomethane on jalaric acid leads to several side reactions, that is why the product from 10 days hydrolysis was investigated as this hydrolysate is expected to contain only negligible amount of jalaric acid.

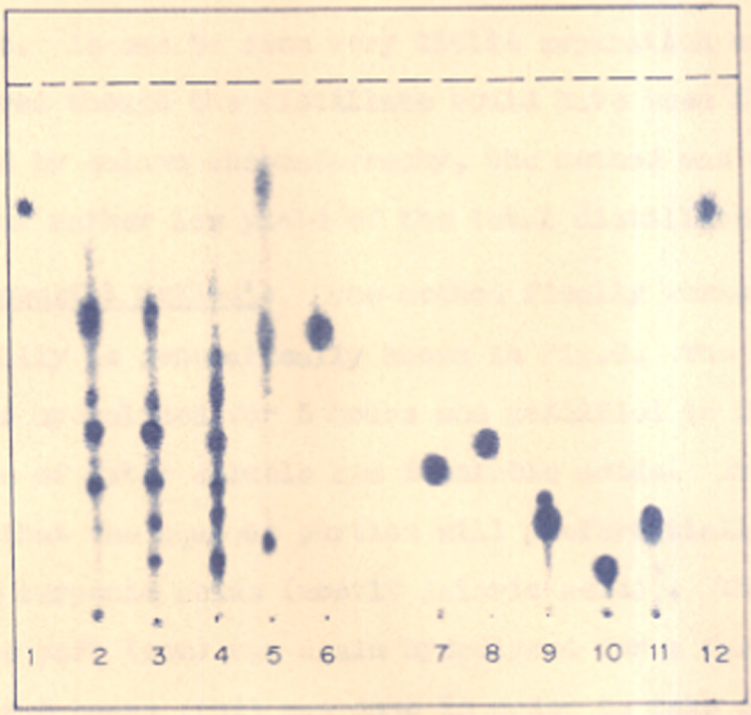

Fig.5 THIN LAYER CHROMATOGRAM
(Fractionation of Methyl esters
from hard resin hydrolysate).

PLATE: Silica gel-Plaster of Paris 100:15 (0.3 mm)

SOLVENT FRONT: 12 cm

SOLVENT SYSTEM: Toluene-Ethyl acetate:Acetone (7:4:4).

1 Sudan III; 2 Fraction b.p. 179-190°; 3 Fraction b.p. 200-230°; 4 Fraction b.p. 230-320°; 5 Undistilled fraction; 6 Methyl butolate; 7 Methyl aleuritate; 8 Dimethyl shellolate; 9 Dimethyl epi-shellolate; 10 Methyl epi-laksholate; 11 Methyl laksholate; 12 Sudan III.

the TLC of these fractions along with standard reference compounds. As can be seen very little separation could be achieved though the distillate could have been further separated by column chromatography, the method was abandoned because of rather low yield of the total distillate.

'The successful method': The method finally worked out successfully is schematically shown in Fig.6. The hard resin was hydrolysed for 5 hours and acidified to furnish a mixture of water soluble and insoluble acids. It was assumed that the aqueous portion will preferentially retain only the terpenic acids (mostly jalaric acid)*. The water insoluble part (gum) was again hydrolysed for a further period of 5 hours (this was done in order to take care of any inter-esterification occuring during the first work up, as well as any "dissolved" jalaric acid in the total acid mass) and worked up as before to furnish another lot of crude terpenic acids and the water insoluble acids. The crude terpenic acids were combined and amounted to 22.1% of the total acids. Paper chromatography as well as thin-layer chromatography (Fig.7 and 8) of this material showed that it consisted essentially of jalaric acid A contaminated

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*This has been demonstrated earlier by Sadia, Bhaskar and Sukh Dev[10] of this Laboratory, in connection with the work on jalaric acid.

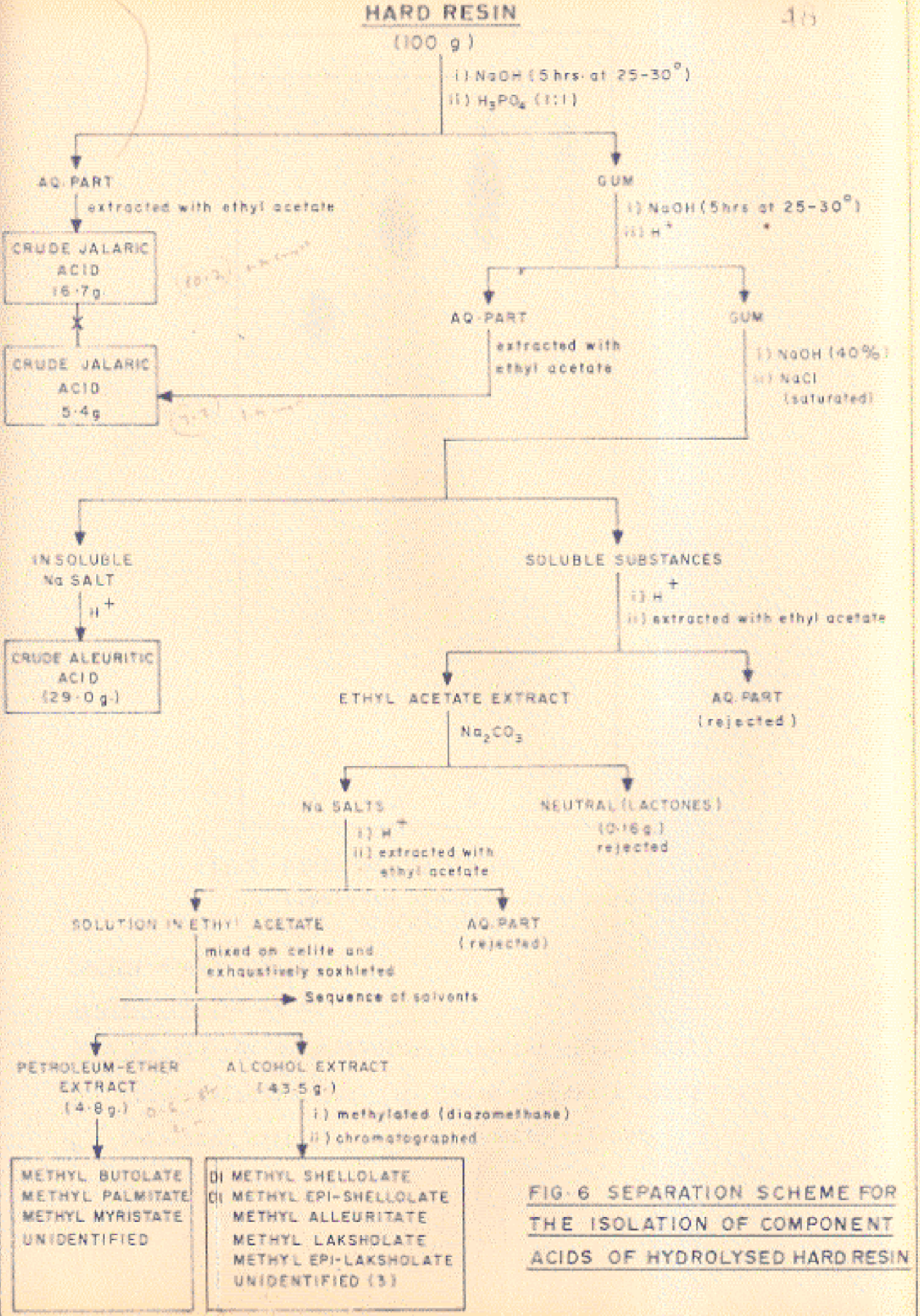


FIG. 6 SEPARATION SCHEME FOR THE ISOLATION OF COMPONENT ACIDS OF HYDROLYSED HARD RESIN

Fig.7 PAPER CHROMATOGRAM

(Isolated products from hara resin hydrolysate).

PAPER: Whatman No.1

SOLVENT FRONT: 14 cm

SOLVENT SYSTEM: Butanol-ethanol-buffer (35:35:30)

1 Crude jalaric acid; 2 Crude ulmaritic acid;

3 Pet.ether extract; 4 Alcoholic extract.

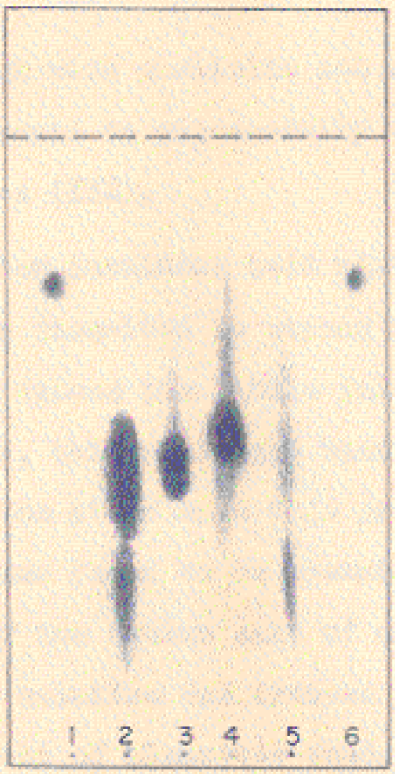


Fig.3 THIN LAYER CHROMATOGRAM
(Isolated products from hard resin hydrolysate)

PLATE: Silica gel-Plaster of Paris 100:15 (0.3 mm)

SOLVENT FRONT: 10 cm

SOLVENT SYSTEM: Butanol-Ethanol-Buffer (52.5:30:30)

1 Sudan III; 2 Crude jalaric acid; 3 Crude aleuritic acid;
4 Pet.ether extract; 5 Alcoholic extract; 6 Sudan III.

to some extent with shellolic and epishellolic acids. The product could be successfully crystallised to give JALARIC ACID-A (III).

The water insoluble acid mixture from the above treatment was dissolved in strong alkali and left aside for 24 hours (since the labile jalaric acid has been removed earlier, this prolonged base treatment is not detrimental. On the other hand this prolonged treatment with alkali has been found to be essential for the complete separation of the sodium salt of aleuritic acid (see below). The alkaline solution was treated with brine to precipitate the sodium salt of aleuritic acid. This is based on the earlier method of isolation of aleuritic acid as its sodium salt from hydrolysed shellac by Sen Gupta and Bose[17]. The sodium salts on acid treatment gave crude aleuritic acid (29%).

The paper and TLC of this product (Fig.7 to 9) showed that this consists mostly of aleuritic acid. The product on crystallisation from dilute alcohol gave pure ALEURITIC ACID (I) with a recovery of 95%.

The water soluble sodium salts from the above treatment were worked up to furnish a gummy mixture of water insoluble acids which were dispersed on celite and exhaustively soxhleted, first with petroleum-ether and finally with ethanol. The petroleum-ether extract (4.8%) on paper and thin-layer

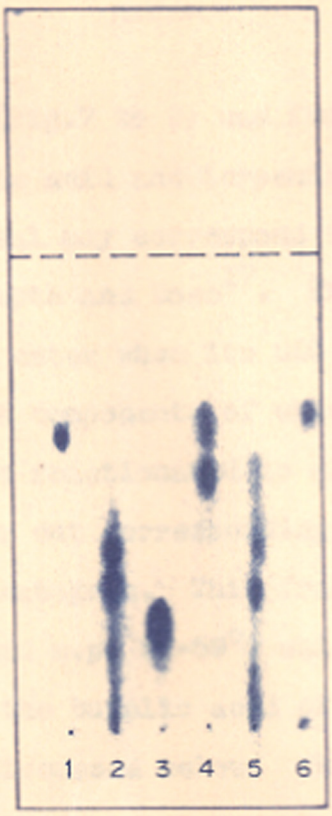


Fig.9 THIN LAYER CHROMATOGRAM
(Methyl esters of isolated products
from hard resin hydrolysate).

PLATE: Silica gel-Plaster of Paris 100:15 (0.3 mm)

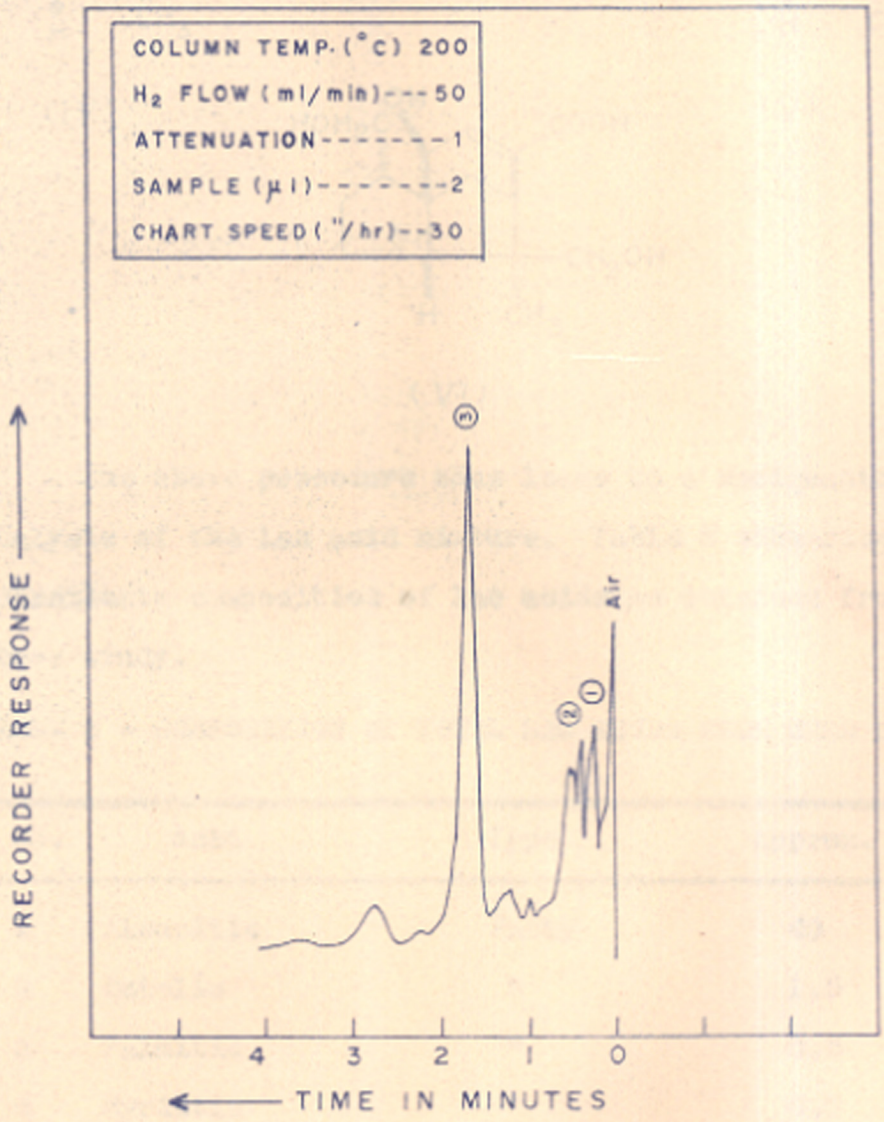
SOLVENT FRONT: 10 cm

SOLVENT SYSTEM: Toluene-Ethyl acetate-Acetone (7:4:4).

1 Sudan III; 2 Methyl ester crude jalaric acid;
3 Methyl ester crude aleuritic acid; 4 Methyl ester
pet.ether extract; 5 Methyl ester alcoholic extract;
6 Sudan III.

chromatography (Fig.7 to 9) was found to be essentially free of aleuritic acid and terpenic acids. It was suspected that this material may correspond to the butolic acid described by Sen Gupta and Bose[17]. The product was converted into its methyl ester when its GLC (Fig.10) showed it to consist of eight components of which one constituted ∽ 55%. The material was fractionated to give after a low boiling fraction a major cut corresponding to the major component of the G.L.Chromatogram. This fraction on hydrolysis readily furnished an acid m.p. 58-59°C which is considered to be identical with the butolic acid of Sen Gupta and Bose[17] and is separately discussed below. The lower boiling fraction appeared from its IR spectrum and was found to contain by mixed chromatograms with authentic samples, myristic and palmitic esters.

The acidic fraction extracted with ethanol (vide above) was shown by paper and T.L.C. (Fig.7 and Fig.8) to be still a complex mixture, but consisting essentially of terpenic acids. The material was esterified with diazomethane and the crude methyl esters systematically chromatographed on alumina. This yielded besides some other two unidentified components methyl esters of SHELLOLIC (II), EPISHELLOLIC (IV), LAKSHOLIC (V) and EPILAKSHOLIC (VI) acids.

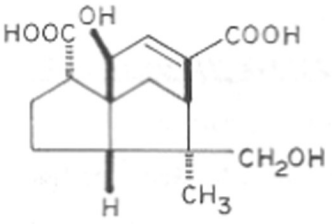


FIG·10 GL CHROMATOGRAM OF METHYLATED ACIDS OF PET ETHER FRACTION·

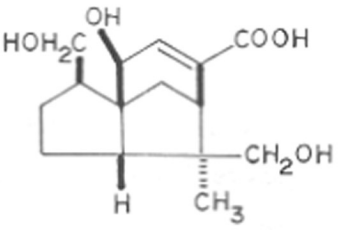
IDENTIFICATION:

① Methyl myristate ② Methyl palmitate

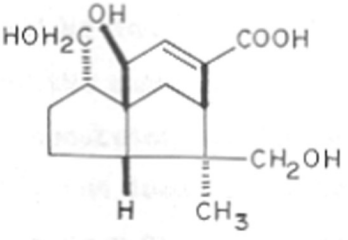
③ Methyl butolate



(IV)



(V)



(VI)

The above procedure thus leads to a semiquantitative analysis of the lac acid mixture. Table 5 summarises the approximate composition of lac acids as obtained from the above study.

TABLE 5 - COMPOSITION OF TOTAL LAC ACIDS FROM HARD RESIN

No.	Acid	Type	Approx. %
1	Aleuritic	Fatty	40
2	Butolic	"	1.5
3	Palmitic	"	0.5
4	Myristic	"	0.3
5	Shellolic	Isoprenoid	40
6	Epi-shellolic	"	
7	Jalaric	"	
8	Laksholic	"	
9	Epilaksholic	"	
10	Unknowns	-	Balance

Structure of Butolic acid

It has been mentioned above that during the systematic analysis of the lac acids an acid m.p. 58-59° could be isolated and it was considered that this acid is identical with the butolic acid of Sen Gupta and Bose[17]. Though a direct comparison of the samples could not be made and our method of isolation completely different from that of Sen Gupta and Bose[17], the identity of the two preparations appears to be evident from the comparison shown in Table 6.

TABLE 6 - PROPERTIES OF BUTOLIC ACID

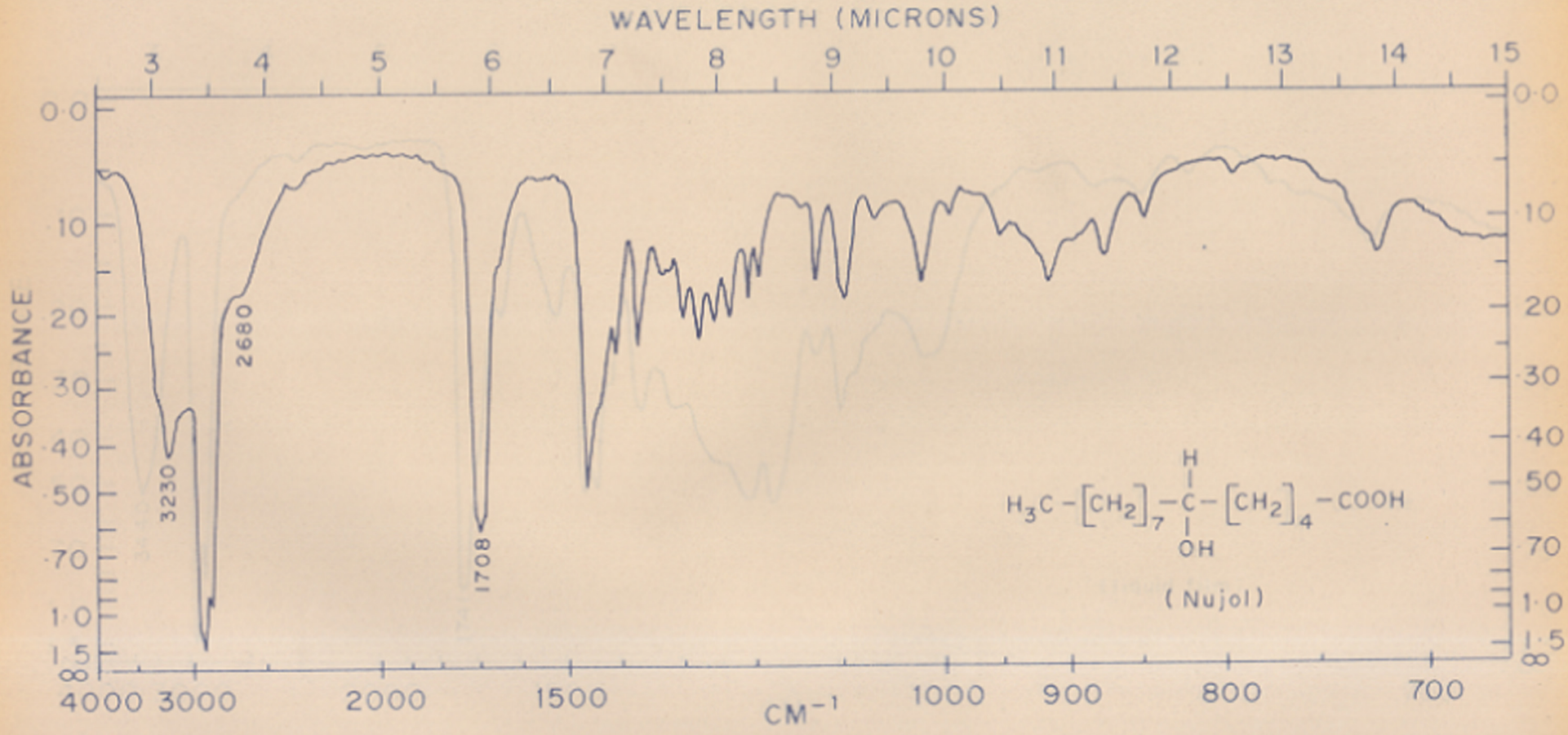
No.		Physical constants	
		Present work	Reported by Sen Gupta and Bose
1	Butolic acid		
	m.p.	58-59°	54-55°
	$[\alpha]_D$	-1.5°	-
2	Methyl ester		
	m.p.	26-27°	27-28°
	$[\alpha]_D$	-2.2°	-
	* n_D^{30}	1.4488	-
3	Keto acid from butolic acid		
	m.p.	70-71°	69.5-70.5°
4	Methyl ester		
	m.p.	23-24°	-
	n_D^{30}	1.4419	-

*Obtained by the chromic acid oxidation.

Sen Gupta and Bose[17] who called this acid as Butolic acid after the lac host Butea monosperma (Butea frondosa), tentatively formulated their compound as 6-hydroxy-pentadecanoic acid. However since no definite proof for the above structure have been put forward by these authors it was considered worthwhile to settle the structure in an unequivocal manner. The compound analysed for $C_{14}H_{28}O_3$ and its infra red spectrum (Fig.11) clearly showed it to be a hydroxy acid (νOH 3230 cm^{-1}, νCO 1708 cm^{-1}, νCOOH 2680 cm^{-1}) methyl ester: νOH 3440 cm^{-1}, νC=O 1741 cm^{-1}) of aliphatic type (Fig.12). The NMR spectrum of its methyl ester is shown in Fig.13 and this is fully consistent with its being a long chain fatty ester[18,19] with a secondary hydroxyl function. The results are discussed in Table 7.

TABLE 7 - NMR ASSIGNMENTS OF METHYL BUTOLATE

Signal	J in cps	WH	No.of protons	Assignments
Characteristic triplet centred at 52 cps.	5 cps	2 cps	3H	CH_3-CH_2
Broad signal at 77, 83 cps	-	5 cps	22H	$(CH_2)_x$
Essentially a doublet of superimposed triplets centred at 134 cps	7 cps	-	1H	$-H_2C-\overset{\overset{CH_2}{|}}{CH}-CH$
Singlet at 163 cps (conc.dependent 20%)	-	-	1H	$-\overset{|}{C}-OH$
Sharp symmetrical singlet at 216 cps	-	1.2 cps	3H	$-COOCH_3$



FIG· 11 IR SPECTRUM OF BUTOLIC ACID

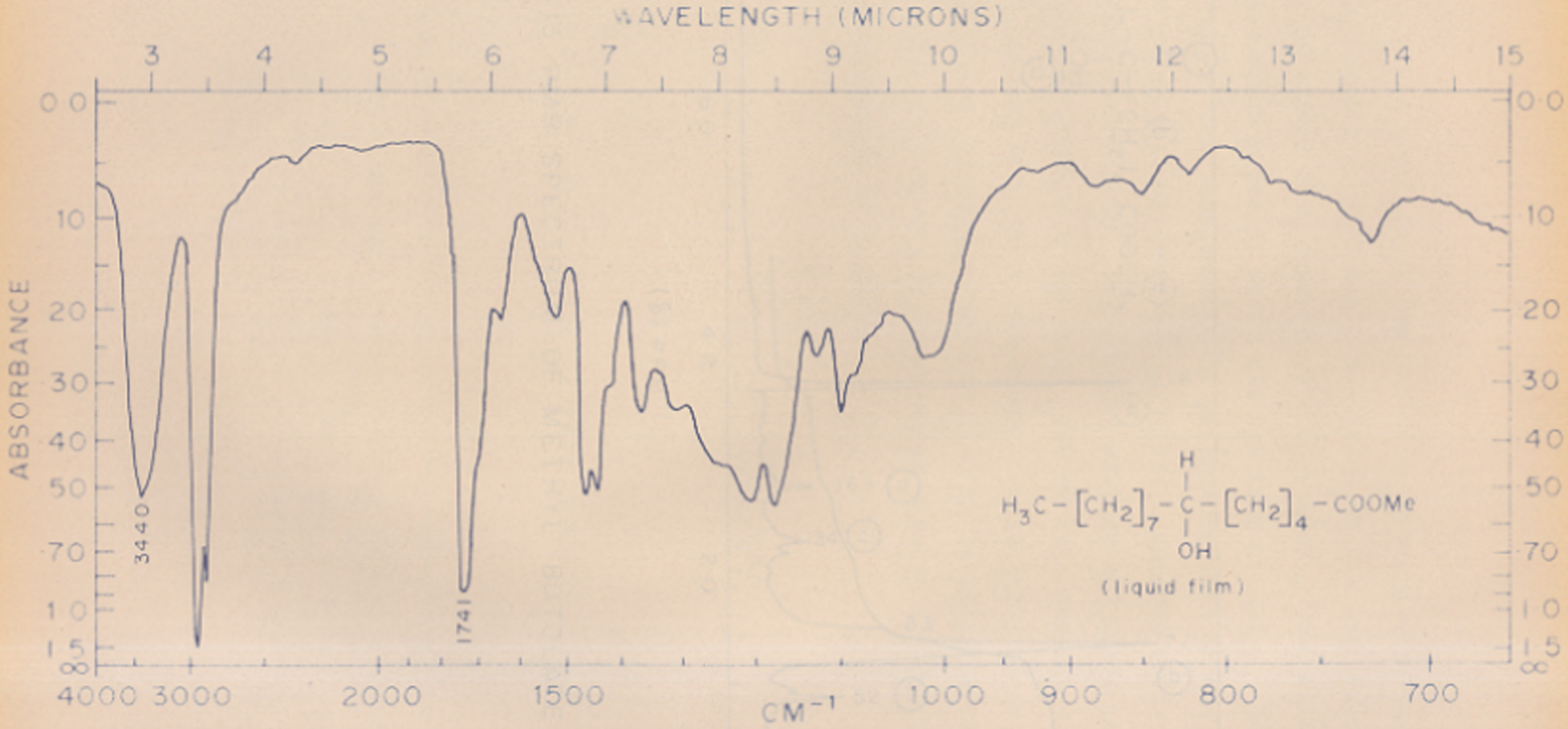


FIG-12. IR SPECTRUM OF METHYL BUTOLATE.

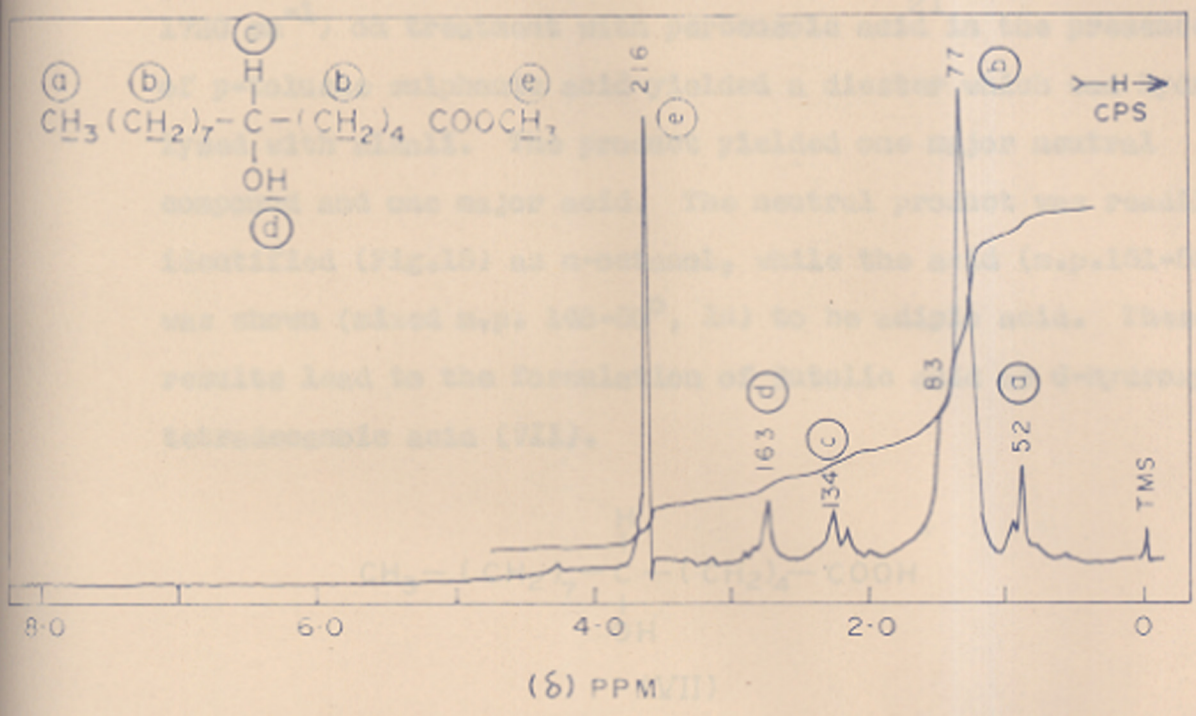
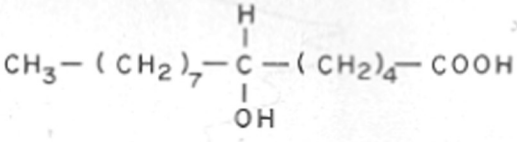
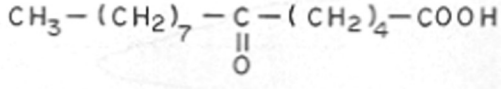


FIG. 13 PMR SPECTRUM OF METHYL BUTOLATE

The location of the secondary hydroxy function in the aliphatic chain was established as follows: Butolic acid on oxidation with chromic acid[20] yielded a keto acid (VIII) the methyl ester of which (IR spectrum Fig.14, C=O 1745 cm^{-1}, 1720 cm^{-1}) on treatment with perbenzoic acid[21] in the presence of p-toluene sulphonic acid yielded a diester which was hydrolysed with alkali. The product yielded one major neutral compound and one major acid. The neutral product was readily identified (Fig.15) as n-octanol, while the acid (m.p.151-53°) was shown (mixed m.p. 148-50°, IR) to be adipic acid. These results lead to the formulation of Butolic acid as 6-hydroxy-tetradecanoic acid (VII).



(VII)



(VIII)

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*While this work was in progress Christie, Gunstone and Prentice[22] also reported on the structure of Butolic acid. These authors also arrived at the same conclusion but by different method. These authors have also effected the synthesis of ± Butolic acid. At this stage we recorded our results in a preliminary communication[23].

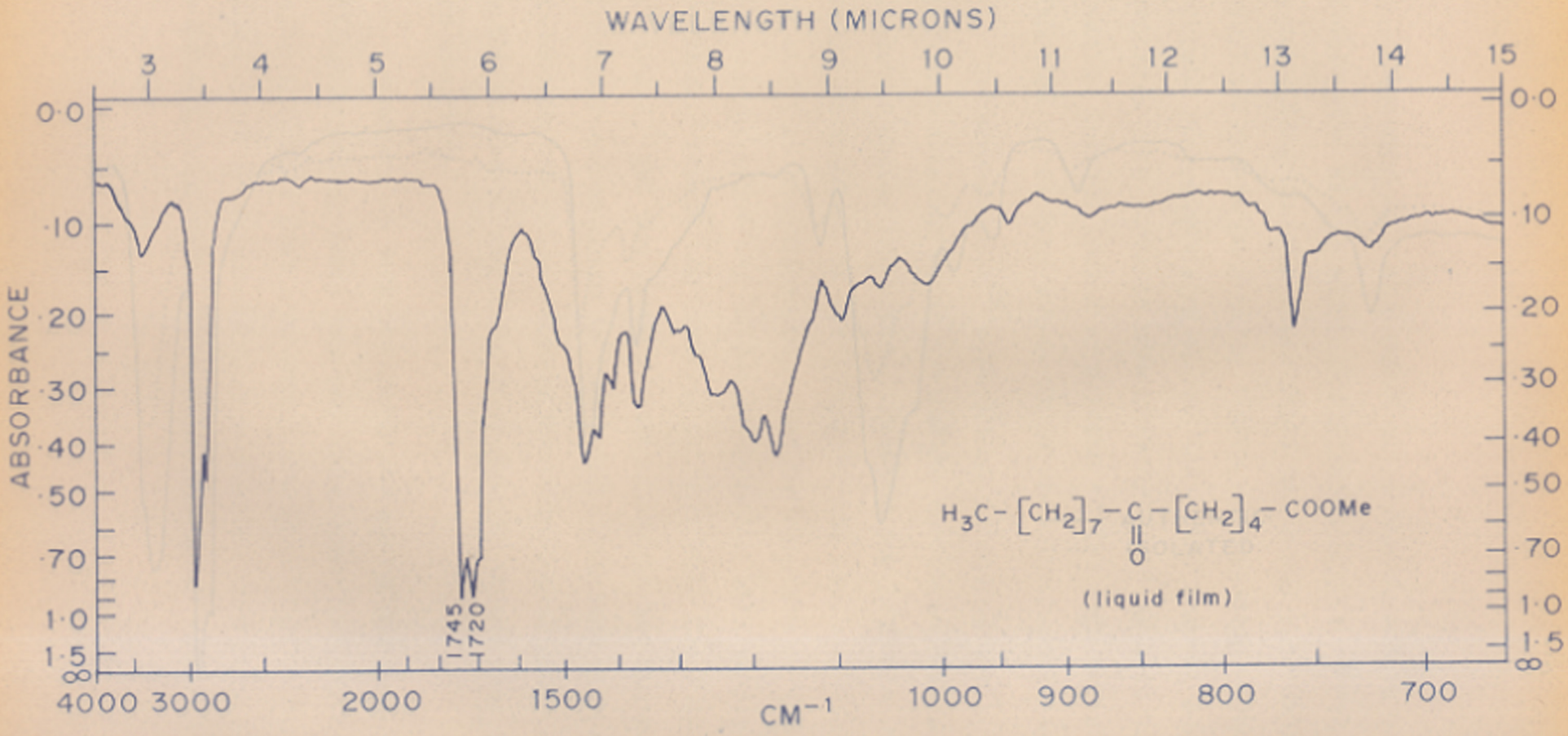


FIG. 14. IR SPECTRUM OF KETOESTER FROM BUTOLIC ACID

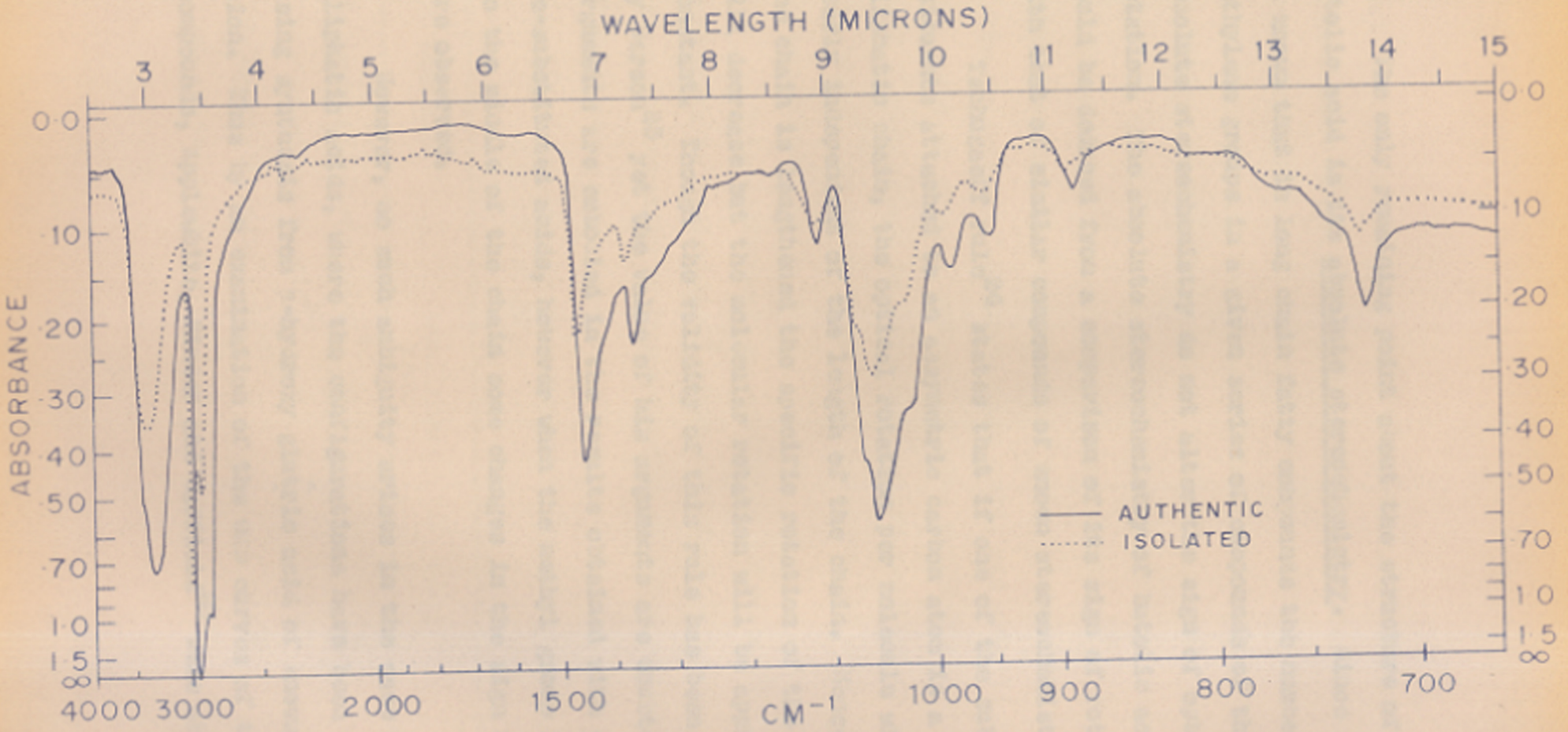


FIG. 15 IR SPECTRA OF n-OCTYL ALCOHOL

The only remaining point about the structure of butolic acid is its absolute stereochemistry. Since it is known that in long chain fatty compounds the number of methylene groups in a given series of compounds of the same absolute stereochemistry do not alter the sign of optical rotation. The absolute stereochemistry of butolic acid could be deduced from a comparison of its sign of rotation with that of similar compounds of known stereochemistry.

Tschugaeff rule[24] states that if one of the substituents attached to an assymetric carbon atom is a long aliphatic chain, the optical rotation per molecule will be nearly independent of the length of the chain. Hence if the chain is lengthened the specific rotation of the compound will decrease but the molecular rotation will be approximately constant. Though the validity of this rule has been questioned by Gerson[25] yet the value of his arguments are doubted[26]. These arguments are embodied in the results obtained with different Me-substituted acids, however when the methyl group is shifted to the middle of the chain some changes in the sign of rotation are observed.

However, no such ambiguity arises in the case of hydroxy aliphatic acids, where the configurations have been correlated[27] using synthesis from β-hydroxy glutaric acid of known configuration. Thus by an examination of the ORD curves of the saturated compounds, Applewhite, Binder and Gaffield[28] were able to show

that Dimorphecolic (9-hydroxy trans-trans 10-12-octadecandienoic acid) Lesquerolic (14-hydroxy cis eicosenoic acid) Densipolic acid (12-hydroxy cis-cis 9-15 octadecandienoic acid) all have the R-configuration on the basis of their similarity of ORD with that of 12-OH-octadecanoic acid prepared by catalytic reduction of Ricinoleic acid whose R-configuration has earlier been established[29]. The R-configuration for 9-OH-octadecanoic acid obtained by the catalytic reduction of Dimorphecolic acid, was established on the basis of similar properties with the R-9-OH octadecanoic acid - synthesised by Gunstone[30], also reported by Schoepfer and Bloch.[31] On these basis they assigned the R-configuration to Lesquerolic acid.

This assignment was confirmed later by its independent synthesis by Applewhite[32] from R-ricinoleic acid. Table 8 shows the rotation of hydroxy acids of known absolute configuration.

TABLE 8 - ROTATION OF HYDROXY ACIDS OF KNOWN ABSOLUTE CONFIGURATION

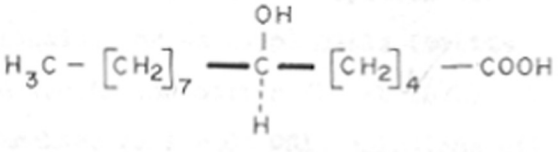
Compound	Configuration	$[\alpha]_D$	Solvent
9-OH-octadecanoic acid[28]	R	-0.17	MeOH
14-OH-eicosanoic acid[28]	R	-0.23	"
12-OH-octadecanoic acid[28]	R	-0.35	"
10-OH-Octadecanoic acid[31]	R	-0.16	"

...contd.

TABLE 8 (Contd)

Compound	Configuration	$[\alpha]_D$	Solvent
9-OH-octadecanoic acid[31]	S	+0.17	MeOH
10-OH-octadecanoic acid[31]	S	+0.15	"
3-OH-hexanoic acid[27]	R	-28	$CHCl_3$
3-OH-decanoic acid[27]	S	+20	"
		-3	EtOH
3-OH-nonanoic acid[29]	S	-3.4	EtOH
		+19.8	$CHCl_3$

Thus from the Table it is clear that butolic acid which is levorotatory has the R-configuration and can be represented as (IX).



(IX)

E X P E R I M E N T A L

The melting and boiling points are uncorrected, the former being determined in pyrex capillaries in an electrically heated apparatus. Pet. ether refers to the fraction having the b.p. range 40-60°. Rotations were measured in chloroform on a Perkin-Elmer Polarimeter (Model 141). Analytical GLCs were carried out on an Aerograph (Model A-350-B) or Perkin-Elmer Vapor Fractometer (Model 154-D) using succinic acid polyester of diethylene glycol (20%) or silicon SE-30 (20% and 1%) as stationary phases on Chromosorb W and employing hydrogen as carrier gas.

The UV spectra were taken on a Perkin-Elmer spectrophotometer (Model 380), in carbonyl-free methanol (unless specified otherwise). The IR spectra were recorded as smears (liquids) or as nujol mulls (solids) on a Perkin-Elmer Infracord spectrophotometer (Model 137B). The PMR spectra were determined in 10-20% CCl_4 solutions with tetramethylsilane (TMS) as internal standard on a Varian A-60 spectrometer. The signal positions are reported in cycles per second (Cps) units starting from TMS signal as zero.

The neutral alumina for column chromatography was prepared from commercial alumina (100-250 mesh) by the method of Evans and Shopee[33] and activated to grade I by heating with intermittent raking, at 450-460° for 6-8 hr. Suitable grades were prepared therefrom by mechanical shaking (4-6 hr)

with appropriate amounts of water[34] and the activities were tested by the Brockmann-Schodder[35] procedure.

Rate of Hydrolysis of Hard Resin

Hard resin (1.0 g) dissolved in neutral alcohol (5 ml) and titrated against potassium hydroxide solution (0.033N) gave an acid value[36] of 55.3.

Hard resin (5.0 g was dissolved in potassium hydroxide solution (27.0 ml, 1N) at room temperature (29°) and was immediately kept in a thermostat maintained at 30° ± 1°. After every one hour aliquots (2 ml) of the reaction mixture were withdrawn into a conical flask, diluted with ice cold water (100 ml) and titrated against hydrochloric acid (0.05N) using bromothymol blue (pH 6-7.6) as an internal indicator. Saponification values were calculated according to the following formula:

$$\frac{\text{Diff} \times 56{,}100 \times N \text{ of HCl}}{\text{Wt. of substance in aliquot}}$$

Likewise saponification values were calculated for different periods, and results plotted to give the rate of hydrolysis. Similarly the rate of hydrolysis was determined at 50° and 70°. Table 9 gives the data observed during the work while Fig.1 shows the plot of saponification values against time .

TABLE 9 - SAPONIFICATION VALUES OF HARD RESIN AT DIFFERENT TEMPERATURES

Time in hrs	Saponification value at 30°	50°	70°
1	189.0	234.7	241.5
2	217.4	230.7	246.3
3	229.0	236.3	257.5
4	237.5	239.7	262.2
5	240.6	242.8	264.8
6	244.7	243.6	265.2
8	249.2	246.2	261.0
10	250.9	247.1	-
12	253.0	250.0	-
24	263.1	253.9	262.6

Paper and Thin Layer Chromatography

Materials:

Authentic samples of lac acids and their methyl esters needed for the work were available in this Laboratory and were further purified as follows:

Jalaric acid: Crystallised from a mixture of tetrahydrofuran and ethyl acetate, m.p. 182-184°.

Epishellolic acid: Crystallised from water, m.p. 245-48°.

Shellolic acid: Crystallised from water, m.p. 205-207°.

Laksholic acid: Crystallised from chloroform-ethanol, m.p. 181-83°.

Epilaksholic acid: Crystallised from chloroform-ethanol, m.p. 201-203°.

Aleuritic acid: Repeatedly crystallised from dilute alcohol, m.p. 100-101°.

Product of esterification of Jalaric acid with CH_2N_2 (Methyl ketone methyl ester): Crystallised from benzene, m.p. 104-106°.

Dimethyl shellolate: Crystallised from benzene, m.p.149-151°.

Dimethyl epishellolate: Crystallised from benzene, m.p. 149-151°.

Methyl laksholate and methyl epilaksholate. Could not be induced to crystallise and hence were used as such.

Methyl aleuritate: Crystallised from acetone, m.p.71°.

Paper chromatography of the Lac acids

Paper chromatography of the authentic lac acids was run by the ascending technique. 1-10 µl of a 2% solution of the substance in ethanol was spotted at the starting points on Whatman paper (No.1). After equilibration (1 hr) in butanol-ethanol-buffer* (35:35:30) the paper was run a distance of 14 cms (~ 5 hr). The paper was dried in air (1 hr) and the spots were visualised by spraying with

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*7.20 g ammonium carbonate, 7.5 ml NH_4OH sp.gr. 0.88 in 35 ml water.

bromophenol blue solution* containing citric acid. The acids appeared (Fig.2) as light blue spots on a yellow background. R_f values for different acids were calculated and are given in Table 2.

Thin Layer Chromatography of the acids

TLCs were run on plane glass plates (10.0 x 15 cm) coated with silica gel-plaster of Paris (100:15; -400 mesh) using the apparatus and techniques of Gupta and Sudh Dev[57]. 1-10 µl of 2% solution of the substance in ethanol (dye: Sudan III was applied as 0.2% solution in acetone) was applied at the starting points. After a few minutes the plates were equilibrated (30 min) in the solvent system and run by the ascending technique (solvent front 10 cm). After development the plates were dried in air (30 min) and heated in an oven (110-120°) for 15 minutes. The dried plates were sprayed with concentrated sulfuric acid and again heated (130°) for 10 minutes. The acids appeared as reddish blue spots. In order to get a clear separation of the acids the following solvent systems were tried:

i) Benzene-methanol-acetic acid (1:2:1).

ii) Dioxane-ethyl acetate-acetic acid (15:25:2)

iii) Dioxane-ethanol-buffer+ (52.5:35:30).

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*Bromophenol blue (50 mg), citric acid (200 mg), water (100 ml).

+7.20 g ammonium carbonate, 7.5 ml NH_4OH sp.gr. 0.88 in 95 ml water.

The last solvent system was found to give the best results (Fig.3) and the R_{dye} values of the acids using this solvent system were calculated and are given in Table 3.

Thin-Layer Chromatography of Methyl esters

Authentic samples of the methyl esters were run (15 cm) using toluene-ethyl acetate-acetone (7:4:4) as the solvent system. Detection of spots (Fig.4) was made in the usual manner and the R_{dye} values were calculated which are given in Table 4.

Separation of Lac Acids

Urea adductation[15,16]

Hard resin (25.0g)was hydrolysed with sodium hydroxide (125 ml; 1:1) at 50° for 24 hours. The saponified product was acidified with aqueous phosphoric acid (40 ml; 1:1) and the acids were extracted with ethyl acetate (100 ml x 3) washed with brine (20 ml x 6), dried, filtered and freed of solvent yielding acids mixture (23.5 g). The above acids mixture (5.0 g) was treated with urea (9.5 g) in dry acetone (300 ml). The reaction mixture was refluxed on a waterbath (30 min) and was allowed to stand for 24 hrs at 15°. The solid adduct was filtered off and washed with dry pet.ether (150 ml) to yield fraction 1. Two layers were formed in the filterate which were separated and the solvent from each was distilled off to yield gummy residues

(fraction ii and fraction iii). The acids from the urea complex fractions were liberated by boiling with water containing few drops of hydrochloric acid. The fractions i) 0.080 g, ii) 2.240 g, iii) 1.92 g thus obtained were analysed by paper chromatography employing the usual solvent system Butanol-ethanol-Buffer*(35:35:30).

Aleuritic acid (2.0 g) was dissolved in methanol (15 ml) containing urea (8.0 g). The reaction mixture was refluxed on a waterbath for 30 minutes. After cooling and filtering two crystalline crops (i) 5.0 g and ii) 5.0 g) were obtained. After usual work up the first crop was shown to be only urea while the second crop yielded (0.92 g) of an acid m.p. 100-101°. Its mixed melting point with aleuritic acid was undepressed.

Fractionation of Methyl esters

Hard resin (5.0 g) was hydrolysed with sodium hydroxide solution (10 ml, 40%) at room temperature (30° ± 3°) for 10 days. The product was acidified with aqueous phosphoric acid (30 ml, 1:1) and was extracted with ethyl acetate (50 ml x 4), washed, dried and the solvent removed to yield 4.5 g of the hydrolysed product. The above product (4.5 g) was dissolved in ethanol (15 ml) and esterified with ethereal diazomethane. The resulting methyl esters were fractionally

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*7.30 g ammonium carbonate, 7.5 ml NH_4OH sp.gr. 0.88 in 95 ml water.

distilled under high vacuum. Three fractions were collected (Table 10).

TABLE 10 - FRACTIONATION OF METHYL ESTERS

Fr.No.	Temp.(bath, ^{o}C/5 x 10^{-4} mm)	Wt.	Remarks
1	170-190	0.456	Light yellow oil.
2	200-230	0.652	Crystalline solid.
3	230-320	1.656	Waxy solid.
4	-	2.012 (residue)	Hard waxy mass.

The fractions collected were studied by TLC (Fig.5) with reference esters using toluene-ethyl acetate-acetone (7:4:4) as solvent system.

'Successful Method'

Dried, powdered hard resin (100 g) was hydrolysed for 5 hours with sodium hydroxide solution (450 ml; 1.75N) at 25-30^{o}. The hydrolysed product was acidified with aqueous phosphoric acid (180 ml; 1:1) with mechanical stirring. The aqueous portion from the gummy mass was separated by filtering and the gummy mass was washed with water (300 ml x 3) by mechanical stirring and aqueous portion filtered. The washings were combined with the earlier aqueous filterate and extracted with ethyl acetate (500 ml x 2) washed with water (50 ml x 3), dried and solvent removed in vacuo

yielding crude jalaric acid (15.7 g, m.p. 80-142°) (Figs.7 and 8). The gummy solid acids were further hydrolysed for 5 hours and an additional quantity of crude jalaric acid (5.4 g) was separated by the procedure explained above. The crude jalaric acid (0.500 g, m.p. 80-142°) was purified by repeated (twice) crystallisations from tetrahydrofuran-ethyl acetate (1:8) to furnish jalaric acid as colorless micro crystals 0.405 g, m.p. 182-184°.

The total residual gummy solid from the above was again hydrolysed with sodium hydroxide solution (60 ml, 40%) for 24 hrs at room temperature (30°). Saturated saline solution (70 ml) was added and mixture allowed to stand at 0-10° for 12 hrs. The sodium salt of aleuritic acid which separated out was filtered, washed with ice cold saline (50 ml) and air dried (3 days) to yield crude sodium aleuritate (33 g). The filterate and the washings were combined and concentrated to half the volume. Storage at 0-10° for 24 hours yielded an additional amount of sodium aleuritate (1.50 g). Sodium aleuritate (34.5 g) was suspended in water (100 ml) and acidified with aqueous phosphoric acid (40 ml; 1:1). The liberated free acid was filtered and washed with ice cold water (20 ml x 2) to give (29.0 g) of aleuritic acid (Figs.7 and 8) m.p. 95-98°. Recrystallisation (1.0 g) from dilute alcohol gave pure aleuritic acid (0.950 g) as a crystalline solid

m.p. 100-100.5°.

The mother liquors after the removal of aleuritic acid were acidified with aqueous phosphoric acid (250 ml;1:1) and extracted with ethyl acetate (500 ml x 3), washed with sodium carbonate solution (500 ml x 2, saturated). The ethyl acetate portion was washed, and dried. After distilling off the solvent it yielded (0.16 g) of neutral fraction with pleasant odour.

The sodium carbonate extract was acidified with aqueous phosphoric acid (1500 ml; 1:1) and extracted with ethyl acetate (500 ml x 4), washed with water (100 ml x 2) and dried. Ethyl acetate extract was concentrated to half the volume (1000 ml) and dispersed on celite (200 g). The solvent was removed under vacuum and the dry powdered mass was transferred to a thimble and soxhleted with dry pet.ether for 100 hrs yielding a viscous light orange coloured fraction (4.8 g) Figs.7 and 8.

The pet. ether fraction (3.1 g) was methylated with diazomethane and after distilling of the solvent, the fraction was distilled (b.p. 70-130°/1 mm) furnishing (2.48 g) of a viscous light pale coloured liquid. Gas liquid chromatography revealed the presence of <u>methyl butolate</u> as a major component (see under butolic acid) which constituted (~ 55%) of the total fraction. The presence of Palmitic and myristic acid methyl esters were also confirmed

by taking mixed GLCs. G.L.Chromatogram also showed the presence of a few minor unidentified products (Fig.10).

The remaining acids from the celite were extracted with ethanol (500 ml) for 12 hr yielding (42.5 g) of an alcoholic fraction.

All the four fractions viz. crude jalaric, crude aleuritic, crude butolic (Pet.ether fraction) and alcoholic extract were studied by paper chromatography (Fig.7) and thin layer chromatography (Fig.8). Similarly, the four fractions isolated (vide above) were methylated (diazomethane) and studied by thin layer chromatography (Fig.9).

Chromatography of alcoholic extract: The alcoholic extract (13.0 g) was dissolved in ethanol (20 ml) and was methylated (diazomethane) to yield the methyl esters (13.3 g) which were chromatographed over neutral alumina. The results are tabulated in Table 11.

TABLE 11 - CHROMATOGRAPHY OF METHYL ESTERS FROM ALCOHOLIC EXTRACT

Wt. of the compound .. 13.5 g

Wt. of Al_2O_3 (neutral grade IV): 280 g

Column dimensions: 4.5 x 16 cms.

Fr.	Eluent	Ratio	Eluate (l)	Wt.of Fr. (g)	Remarks TLC*
A	Benzene	100	3.5	7.94	Streak
B	Benzene-Methanol	99:1	2.5	2.32	3 spots
C	Benzene-Methanol	95:5	3	0.64	3 spots
D	Benzene-Methanol	90:10	3	0.38	3 spots
E	Methanol	100	1.5	0.23	3 spots
F	Aq.Na_2CO_3 (15%)		2	0.51	4 spots+

*Thin layer chromatography was studied in a solvent system toluene-ethyl acetate-acetone (7:4:4).

+The fraction was studied by paper chromatography using a solvent system butanol-ethanol-buffer (35:35:30).

Fraction A: A long streak indicating a complex mixture.

Fraction B: Showed the presence of three components out of which two were identified as dimethyl shellolate and dimethyl epishellolate by comparing the R_f values with standard compounds.

Fraction C, D and E: Each fraction was a mixture of three components which were identified as methyl laksholate, methyl epilaksholate and methyl aleuritate by comparing the R_f values with authentic compounds.

Fraction F: The sodium carbonate extract was acidified with aq. phosphoric acid (400 ml, 1:1) and extracted with ethyl acetate (300 ml x 3) which on usual work up yielded a gummy mass (0.51 g). This product showed the presence of four components by paper chromatography which were identified as shellolic, epi-shellolic, laksholic and aleuritic acids by comparing the R_f values with authentic samples.

Rechromatography of Fraction A: The benzene eluted fraction from the above was dissolved in benzene (25 ml) and chromatographed over alumina. The results are given in Table 13.

TABLE 12 - RECHROMATOGRAPHY OF FRACTION A

Wt. of compound: 7.90 g

Wt. of Al_2O_3 (neutral gr.II): 160.0 g

Column Dimensions: 0.8 x 17 cms.

Fr.	Eluent	Ratio	Eluate (ml)	Wt.of fr. (g)	Remarks (TLC)*
A_1	Benzene	100	500 x 2	0.571	2 spots
A_2	Benzene-Methanol	99.5:5	50 x 5	2.403	3 spots
A_3	Benzene-Methanol	99:1	50 x 5	0.319	3 spots

.....contd.

TABLE 12 (Contd.)

Fr.	Eluent	Ratio	Eluate (ml)	Wt.of Fr. (g)	Remarks (TLC)*
A_4	Benzene-methanol	97:3	50 x 5	0.110	3 spots
A_5	Benzene-methanol	95:5	50 x 5	0.450	3 spots
A_6	Benzene-methanol	90:10	50 x 5	0.390	3 spots
A_7	Methanol	100	50 x 3	0.425	3 spots
A_8	Aq.Na_2CO_3 15%		1000	0.425	3 spots+

*TLC was studied in a solvent system toluene-ethyl acetate-acetone (7:4:4).

+The fraction was studied by paper chromatography using a solvent system butanol-ethanol-buffer (35:35:30).

Fraction A_1: This fraction showed the presence of two components by thin layer chromatography. A solid was isolated by concentration of the fraction which was crystallised into long needle shaped crystals (0.12 g) m.p. 178-79°.

IR spectrum: 1685, 758, 761 cm^{-1}.

PMR spectrum: Single peak at 196 cps.

Analysis: (Found: C, 42.34; H, 6.7; N, 23.2%).

The compound was not studied further.

Fraction A_2, A_3, A_4 and A_5: These fractions were obtained as solid products and were found to be similar by thin layer chromatography and hence were mixed together. On sublimation a colourless crystalline compound (0.12 g) m.p. 127-128° was obtained.

IR spectrum: ν^{OH} 3356 cm^{-1} $\nu^{C=O}$ 1764 cm^{-1}, 1695 cm^{-1} (amide-I band), 1418 cm^{-1} (CH_2 scissoring).

PMR spectrum: Triplet centred at 39 cps (3H, J = 3.5 cps, $-CH_2-\underline{H}_3$); A sharp doublet centred at 86 cps (3H, J = 2 cps, $-\overset{OH}{\overset{|}{\underset{H}{\underset{|}{C}}}}-\underline{H}_3$); an AB quartet centred at 125 cps (2H, J_{AB}= 4 cps, $\frac{J_{AB}}{J_B J_A}$ = 0.69, $\underline{H}_2-\overset{O}{\overset{\|}{C}}-$), a poorly resolved doublet centred at 233 cps (1H, J = 2 cps, $CH_3-\overset{OH}{\overset{|}{\underset{H}{\underset{|}{C}}}}-$); a sharp singlet at 287 cps (1H, C-O$\underline{H}$).

Analysis: (Found: C, 40.67; H, 5.78; N, 18.58%).

The residue dissolved in benzene (20 ml) deposited slowly a solid which was filtered and recrystallised yielding a crystalline product (0.300 g, m.p. 147-149°) and was identified as dimethyl shellolate by comparing its infra red spectrum with authentic sample and mixed melting point which was undepressed. The mother liquor showed the presence of dimethyl epishellolate and an unidentified component.

Fraction A_6 and A_7: These were found to be identical (TLC) hence dissolved in benzene (20 ml). On cooling a solid product was obtained which was filtered, this was shown to be methyl

alcuritate by comparing its R_f value with that of the authentic. On crystallisation from dilute alcohol granular crystals (0.21 g) were obtained which had m.p. 70-71, undepressed on admixture with authentic methyl alcuritate. The residue showed the presence of methyl laksholate and methyl epi-laksholate which could not be crystallised.

Rechromatography of fraction B: Benzene-1% methanol eluted fraction was dissolved in benzene (20 ml) and chromatographed over alumina. Results are given in Table 13.

TABLE 13 - RECHROMATOGRAPHY OF FRACTION B

Wt. of compound: 2.3 g

Wt. of Al_2O_3 (neutral gr.): 60.0 g

Column dimensions: 2 x 24 cms.

Fr.	Eluent	Ratio	Eluate (ml)	Wt. of fr. (g)	Remarks, (TLC)
B_1	Benzene	100	50 x 3	0.708	2 spots
B_2	Benzene-methanol	99.5:5	50 x 3	0.348	2 spots
B_3	Benzene-methanol	99:1	50 x 3	0.107	2 spots
B_4	Benzene-methanol	97:3	50 x 3	0.979	2 spots
B_5	Benzene-methanol	90:10	50 x 3	0.322	2 spots
B_6	Benzene-methanol	70:30	50 x 3	0.199	1 spot
B_7	Methanol	100	200	0.152	1 spot

*TLC was studied in a solvent system: toluene-ethyl acetate-acetone (7:4:4).

Fraction B_1 : This fraction showed the presence of two components by thin layer chromatography, one of them was obtained as crystalline solid (0.052) by concentrating the fraction which on crystallisation from benzene yielded a crystalline product (0.04 g) m.p. 178-179°, while the other could not be identified.

Fraction B_2 and B_6 : Both the fractions were found to be identical by TLC and hence were mixed together. The total product was dissolved in benzene (10 ml), a solid separated was filtered and recrystallised from benzene yielding a crystalline solid (0.130 g, m.p. 148-149°) which was identified as dimethyl shellolate by taking mixed melting point with the authentic sample which was undepressed. The remaining product which could not be crystallised was identified as dimethyl epishellolate by TLC.

Fraction B_4 and B_5 : These fractions were shown to be similar by TLC and were mixed together, all attempts failed to crystallise the product. Presence of two components was shown by TLC which were identified as methyl laksholate and methyl epilaksholate by comparing their R_f values with those of authentic compounds.

Fraction B_3 and B_7 : Although single spot by TLC, the product could not be induced to crystallise and was identified as methyl epilaksholate by comparison of R_f value.

HUTOLIC ACID

Isolation

From Hard resin: Pet. ether fraction (vide infra, 3.13 g) was methylated (diazomethane) and fractionally distilled under vacuum. Table 14 shows the properties of the fractions obtained.

TABLE 14 - FRACTIONAL DISTILLATION OF METHYLATED PET.ETHER FRACTION.

Fr.	Temp.(°C/1 mm)	Wt.of fr. (g)	Remarks
A	80-80	0.37	Colorless liquid.
B	80-110	1.12	Light yellow viscous liquid.
C	110-130	0.97	Yellow viscous liquid.
D	Undistilled	0.57	Reddish gummy mass.

These three fractions were investigated by GLC using column P (5' x 1"), temp. 200° using hydrogen as carrier. Fraction A was found to be rich in methyl palmitate and methyl myristate. The major component of fractions B and C could not be identified, and hence the two fractions B and C were mixed and the mixed fractions (1.50 g) were hydrolysed by refluxing with KOH solution (5 ml, 10%) for 5 hr. The product was acidified with aq. phosphoric acid (10 ml, 1:1) and extracted with ethyl acetate (40 ml x 3) washed, dried and freed from solvent to

yield an oily product which was crystallised from pet.ether to give crude butolic acid (1.25 g, m.p. 38-48°); two re-crystallisations from pet. ether gave colorless shining silky needles (0.35 g) m.p. 58-59° (TLC pure) which was identified as butolic acid $[\alpha]_D^{36}$ -1.3° ($CHCl_3$, c-10%), TAM test: negative.

IR spectrum: ν^{OH} 3230 cm^{-1}, $\nu^{C=O}$ 1708 cm^{-1}, ν^{COH} 2680 cm^{-1}.

Analysis: (Found: C, 69.13; H, 11.71. $C_{14}H_{28}O_3$ requires: C, 68.81; H, 11.55%).

Butolic acid from shellac (convenient method): Dewaxed blonde shellac (100.0 g) was hydrolysed with NaOH solution (100.0 ml, 40%) for 10 days, acidified with aq. HCl (150 ml, 1:1) and extracted with ethyl acetate (500 ml x 2). The extract was concentrated to half the volume and dispersed on celite (200 g), dried and soxhleted with pet. ether (500 ml, 100 hr) to yield crude butolic acid fraction (14.0 g). Crude butolic acid fraction (4.0 g) was methylated (diazomethane and distilled b.p. 50-150°/0.4 mm to yield light pale liquid (2.2 g). This ester on hydrolysis and work up gave pure butolic acid (1.7 g, m.p. 58-59°). This compound was found to be the same as that isolated from hard resin by taking mixed melting point which was undepressed.

Methyl Butolate: Butolic acid (0.20 g) was dissolved in ether (5 ml) and esterified using diazomethane. The product

on distillation afforded methyl butolate b.p. 120-122°/1 mm m.p. 26-27°, n_D^{30} 1.4488, $[\alpha]_D^{26}$ - 2.2° ($CHCl_3$, c-10%). TNM test: negative, GLC, TLC pure.
IR spectrum: ν^{OH} 3440 cm^{-1}, $\nu^{C=O}$ 1741 cm^{-1}.
Analysis: (Found: C, 69.43; H, 11.3. $C_{15}H_{30}O_3$ requires: C, 69.74; H, 11.70%).

Jones'oxidation:

Butolic acid (0.300 g, m.p. 58-59°) was dissolved in acetone (5 ml) and to this Jones reagent[20] (5 ml) was added drop by drop. The addition was stopped when the orange color persisted. The temperature of the reaction was maintained at 30-35°. The reaction mixture was allowed to stand for 3 hr at room temperature (30°) with occasional shaking. The product was diluted with water (10 ml) and extracted with ether (30 ml x 3), washed, dried and freed from solvent yielding a colourless solid (0.298 g, m.p. 64-69°) which on crystallisation from pet. ether gave pure keto acid (0.250 g, m.p. 70-71°), TLC pure. TNM test: negative.
Analysis: (Found: C, 69.15; H, 10.63. $C_{14}H_{26}O_3$ requires: C, 69.38; H, 10.87%).

Methyl ester of keto acid: Keto acid (0.245 g) was dissolved in ether (5 ml) and methylated with diazomethane. The product on distillation (b.p. 110-112°/1 mm) afforded a colorless liquid m.p. 23-24°, GLC and TLC pure, TNM test: negative, n_D^{30} 1.4419.
IR spectrum: $\nu^{C=O}$ 1720 cm^{-1}, 1745 cm^{-1}.

PMR spectrum: Characteristic triplet centred at (50 cps, 3H, J = 5 cps, J_H 1.6 cps $-\overset{\overset{H}{|}}{\underset{\underset{O}{\|}}{C}}-CH_3$); multiplet centred at (137 cps, 6H, CH_2-C-); sharp sym. singlet at (217 cps, 3H; J_H 1.2 cps, $\overset{\overset{X}{|}}{C}-O-CH_3$).

Baeyer-Villiger oxidation of keto ester:

Keto ester (0.200 g) was dissolved in chloroform (5 ml) and was allowed to react with freshly prepared perbenzoic acid solution[21] (24 ml .0094 g/ml, 2.5 moles) in presence of catalytic amount of p-toluene sulfonic acid (50 mg) at room temperature (30°). Side by side a blank experiment was also run. The reaction mixture was kept in a dark place and was swirled from time to time, aliquots of (0.5 ml) were withdrawn from both the experiments and titrated against standard $Na_2S_2O_3$ solution (0.1324) as per standard procedure. The results are summarised in Table 15.

TABLE 15 - BAEYER-VILLIGER OXIDATION OF KETO ESTER

No.	Time hr	PBA consumed ml	PBA consumed moles
1	0	-	-
2	38	2.3	0.621
3	58	2.9	0.784
4	82	3.4	0.92
5	107	4.2	1.13

After 107 hr, the reaction mixture was diluted with chloroform (40 ml), washed with saturated sodium carbonate solution (10 ml x 3), dried and freed of solvent under vacuo to yield the diester (0.190 g) which on distillation gave colorless liquid (0.150 g, b.p. 100-135°/2 mm). This product when examined by GLC showed chiefly a single product, IR $\nu^{C=O}$ 1754 cm^{-1}. The diester (0.130 g) was hydrolysed with KOH solution (5.0 ml, 10%) for 5 hr. The neutral portion from the saponified material was extracted with pet. ether (20 ml x 3). The aqueous portion after acidification with aqueous phosphoric acid (5 ml, 1:1) was extracted with ethyl acetate (20 ml x 4), washed, dried and freed of solvent to give a light pale colored solid (0.071 g). This product on crystallisation from acetic acid-benzene (1:8) gave colourless crystalline compound m.p. 149-152° which was identified as adipic acid by mixed melting point and superimposable IR spectra.

Neutral part:

The total pet.ether extract (60 ml) was washed, dried and freed of solvent to give a light yellowish oil (0.065 g). On distillation (0.051 g) of the product with a pleasant odor was obtained (b.p. 195-197°, n_D^{30} 1.4223). This was identified as n-octanol by GLC peak accentuation technique and superimposable IR spectra (Fig.15).

SUMMARY

The acids obtained by base hydrolysis of hard resin (Palas) have been prepared by a modified procedure.

Structure and stereochemistry has been assigned to butolic acid.

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CHAPTER IV

COMPONENT ACIDS OF HYDROLYSED SOFT RESIN

C O N T E N T S

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COMPONENT ACIDS OF HYDROLYSED SOFT RESIN

In Chapter II we have described the separation of lac resin into "hard" and "soft" fractions. Though the separation cannot be expected to be clear cut and some overlapping of the components of each fraction into the other is expected, the physical properties of the hard and soft fractions are sufficiently distinct to warrant their separate analysis. In order to see if the soft resin (Palas seed lac) fraction represents only the lower molecular weight part of the lac resin, or has some major difference in composition. Systematic analysis of the hydrolysate from the soft fraction was undertaken and this Chapter describes the results of such a study.

Previous Work:

Though the soft resin fractions obtained by earlier workers[1-3] have been prepared by methods which differ from that utilised by us and this could reflect in the acid composition, it is pertinent to give a brief survey of the results of previous workers. Here again none of the authors attempted to carry out a detailed systematic analysis of the soft fractions. Tschirch and Farner[4] were of the opinion that soft resin constitutes only a mixture of higher acids; they arrived at this conclusion as no solid component could be isolated. Tschirch and Ludy[5] have

attempted to isolate constituent acids of the saponified soft resin. For this investigation they used the usual technique of preparing the different metal salts of the acids. They reported the isolation of aleuritic acid as its potassium salt; the mixture of acids liberated from the insoluble calcium salts had m.p. 87° and according to them was a mixture of mono and dihydroxy palmitic acids. Gupta[6] failed to isolate these hydroxy palmitic acids from dewaxed soft resin and he claimed to have isolated palmitic acid, myristic acid and an unsaturated monohydroxy liquid acid. Bhowmick and Sen[7] could not isolate any of the acids described above. Potassium aleuritate failed to crystallise out, when the resin was allowed to react with 5% caustic potash. However from the above results it is clear that there has been not even general agreement regarding the constituents of soft resin.

Bhattacharya and Bose[8] separated the acids of the hydrolysed product by employing ether as the solvent. The ether insoluble fraction yielded aleuritic acid m.p. 94-95°. During the above procedure they isolated two more crystalline acids m.p. 125° and 134-135° which could not be identified. Fractionation of methyl esters of the total acids of soft resin by distillation under vacuum was also attempted, but without any inference. Sen Gupta[9] isolated jalaric acid to the extent of 20% from the hydrolysed soft resin.

The above results are summarised in Table 1.

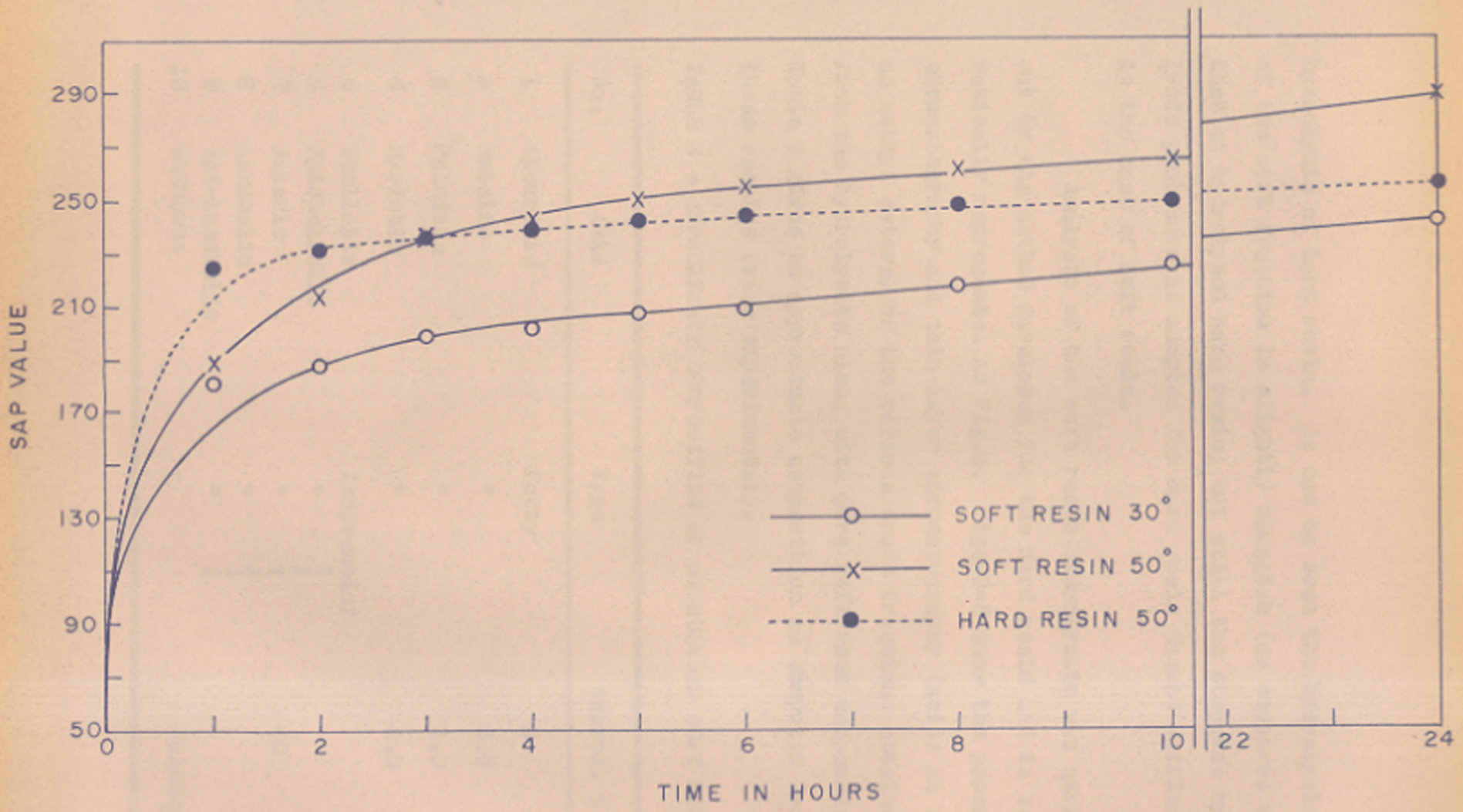
TABLE 1 - ACIDS REPORTED IN HYDROLYSED SOFT RESIN

No.	Acid	m.p.
1	Monohydroxy palmitic acid[5]	76.5 - 77°
2	Palmitic acid[6]	64°
3	Myristic acid[6]	58°
4	Unsaturated monohydroxy liquid acid[6]	-
5	Aleuritic acid[5,8,10]	100-100.5°
6	Butolic acid[10]	54-55°
7	Jalaric acid[9]	-
8	Unidentified acid[8]	125°
9	Unidentified acid[8]	134-135°
10	Unidentified acid[10]	148-149°

Present Work:

The procedure followed for the analysis of the soft resin fraction parallels closely to that worked out for the analysis of the hard resin fraction as described in Chapter III.

The rate of hydrolysis of soft resin has been studied at 30°, 50° and the results are graphically depicted in Fig.1 which also shows a comparison with the rate of



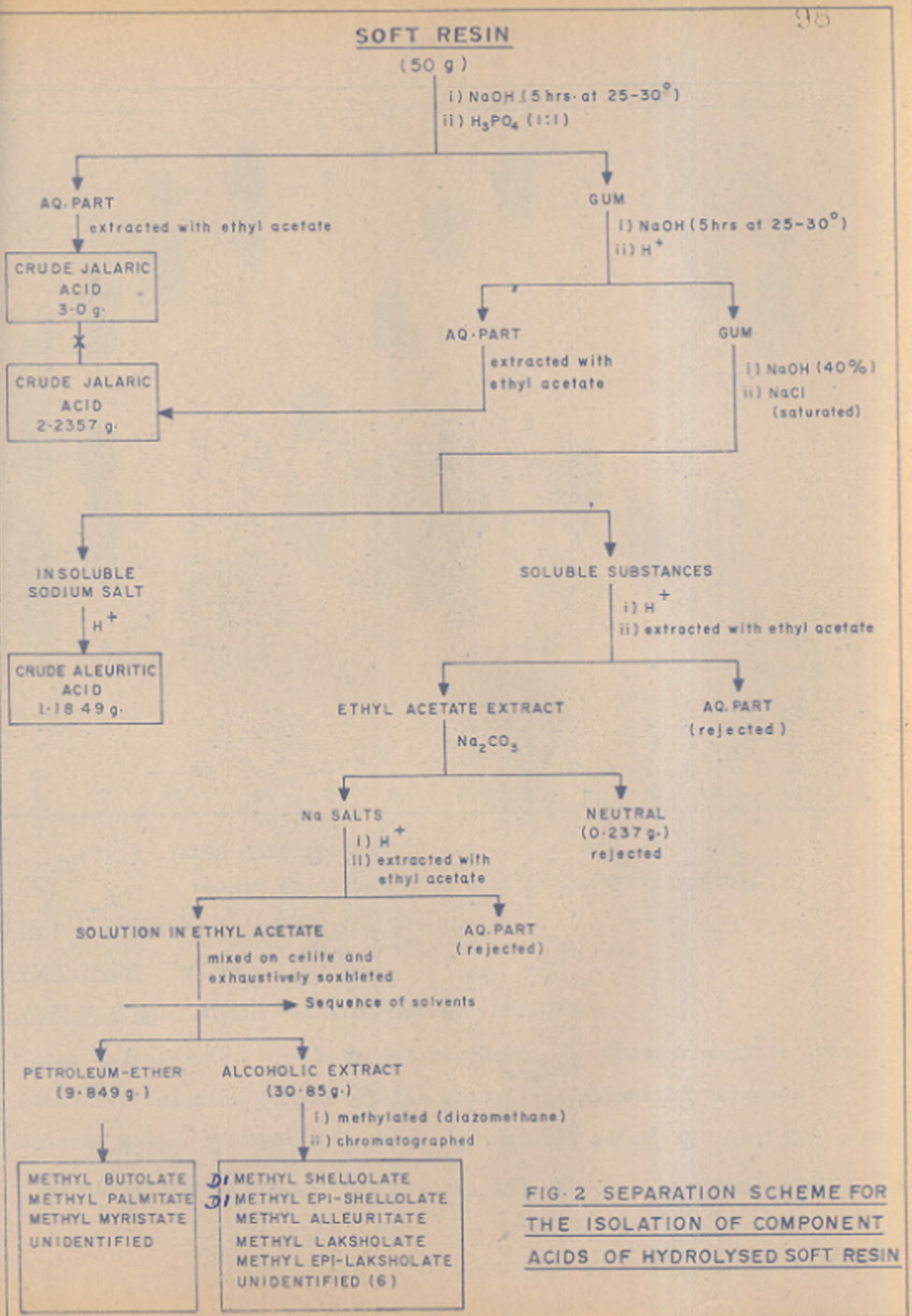
FIG·1 RATE OF HYDROLYSIS OF HARD AND SOFT RESIN

hydrolysis of hard resin. As can be seen the hydrolysis of the soft fraction is slightly sluggish (as compared with that of hydrolysed hard resin) but still the stepwise hydrolysis procedure as adopted for hard resin should suffice in the case of soft resin.

Analysis of the soft resin hydrolysate was carried out by the method developed for the hard resin and is schematically represented in Fig.2. Figs.3-5 show the paper chromatography and thin-layer chromatography (acids as well as methyl esters) of the various crude fractions obtained from the hydrolysate along with pure reference compounds. Table 2 gives an approximate composition as computed from these results (vide experimental).

TABLE 2 - APPROXIMATE COMPOSITION OF HYDROLYSED SOFT RESIN

No.	Acid	Type	Approx. %
1	Aleuritic	Fatty	3
2	Butolic	"	8.0
3	Palmitic	"	1.0
4	Myristic	"	1.0
5	Shellolic	Isoprenoid?	
6	Epishellolic	"	
7	Jalaric	"	30
8	Laksholic	"	
9	Epi-laksholic	"	
10	Unknowns	-	Balance



FIG·2 SEPARATION SCHEME FOR THE ISOLATION OF COMPONENT ACIDS OF HYDROLYSED SOFT RESIN

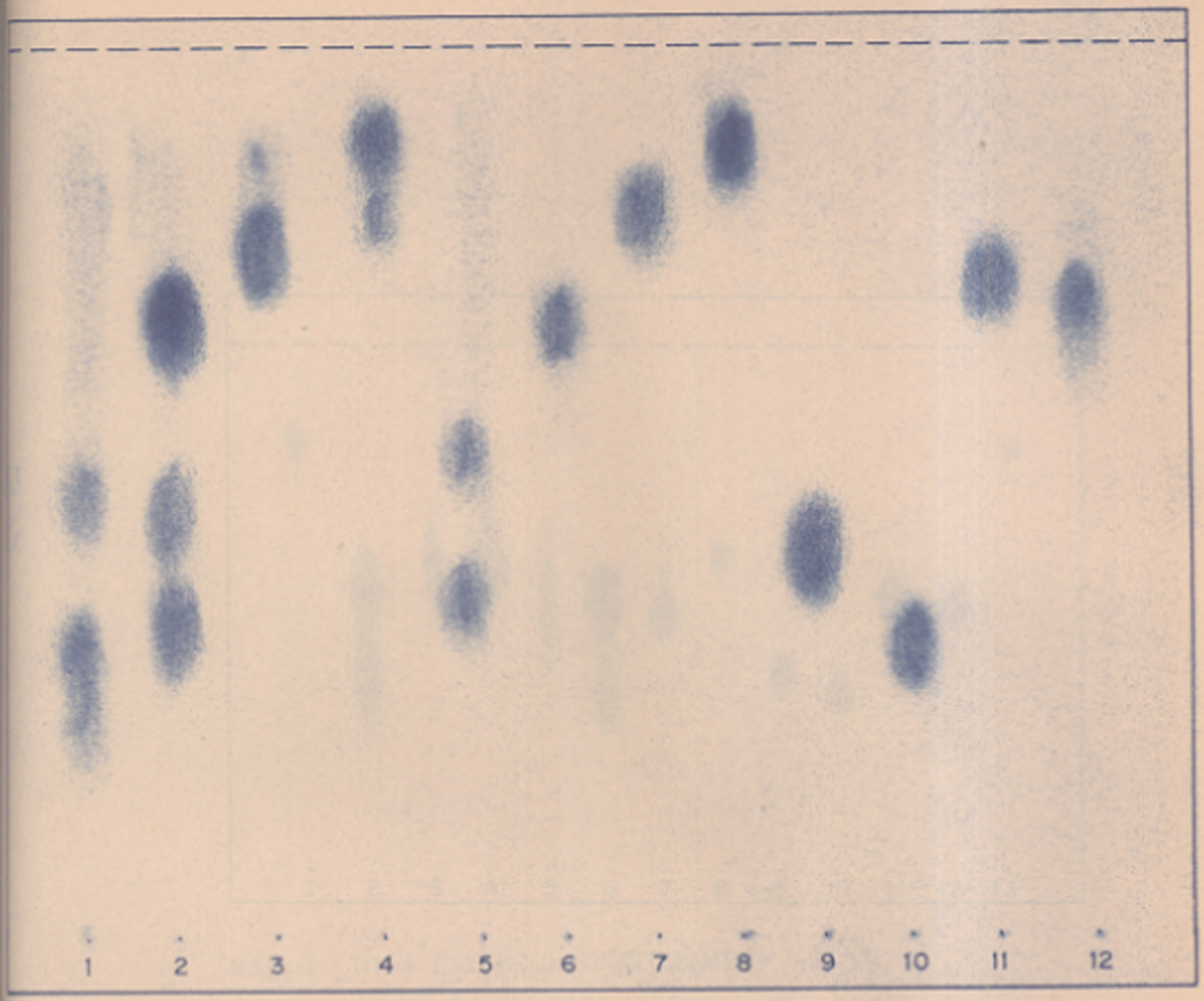


Fig.3 PAPER CHROMATOGRAM
(Acid fractions from soft resin hydrolysate).

PAPER: Whatman No.1

SOLVENT FRONT: 14 cm

SOLVENT SYSTEM: Butanol-Ethanol-Buffer (35:35:30)

1 Total hydrolysate; 2 Crude jalaric acid; 3 Crude aleuritic acid;
4 Pet.ether fraction (crude butolic acid); 5 Alcoholic extract;
6 Jalaric acid; 7 Aleuritic acid; 8 Butolic acid; 9 Shellolic acid;
10 Epi-shellolic acid; 11 Laksholic acid; 12 Epi-laksholic acid.

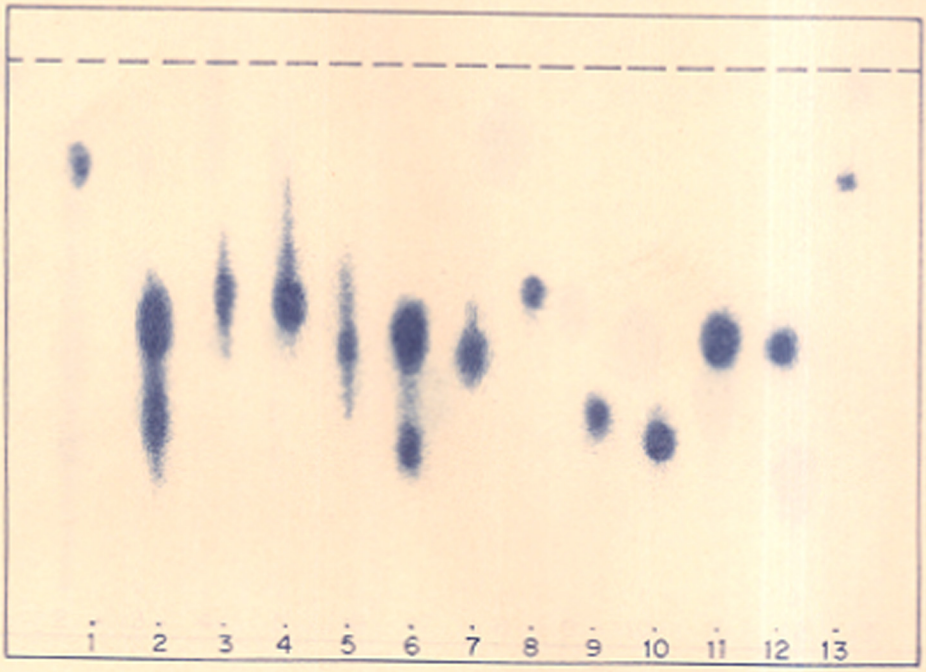


Fig.4 THIN LAYER CHROMATOGRAM
(Acid fractions from soft resin hydrolysate).

PLATE: Silica gel-Plaster of Paris 100:15 (0.3 mm)

SOLVENT FRONT: 10 cm

SOLVENT SYSTEM: Butanol-Ethanol-Buffer (52.5:35:30)

1 Sudan III; 2 Crude jalaric acid; 3 Crude aleuritic acid;
4 Pet.ether fraction (crude butolic acid); 5 Alcoholic extract;
6 Jalaric acid; 7 Aleuritic acid; 8 Butolic acid;
9 Shellolic acid; 10 Epi-shellolic acid; 11 Laksholic acid;
12 Epi-laksholic acid; 13 Sudan III.

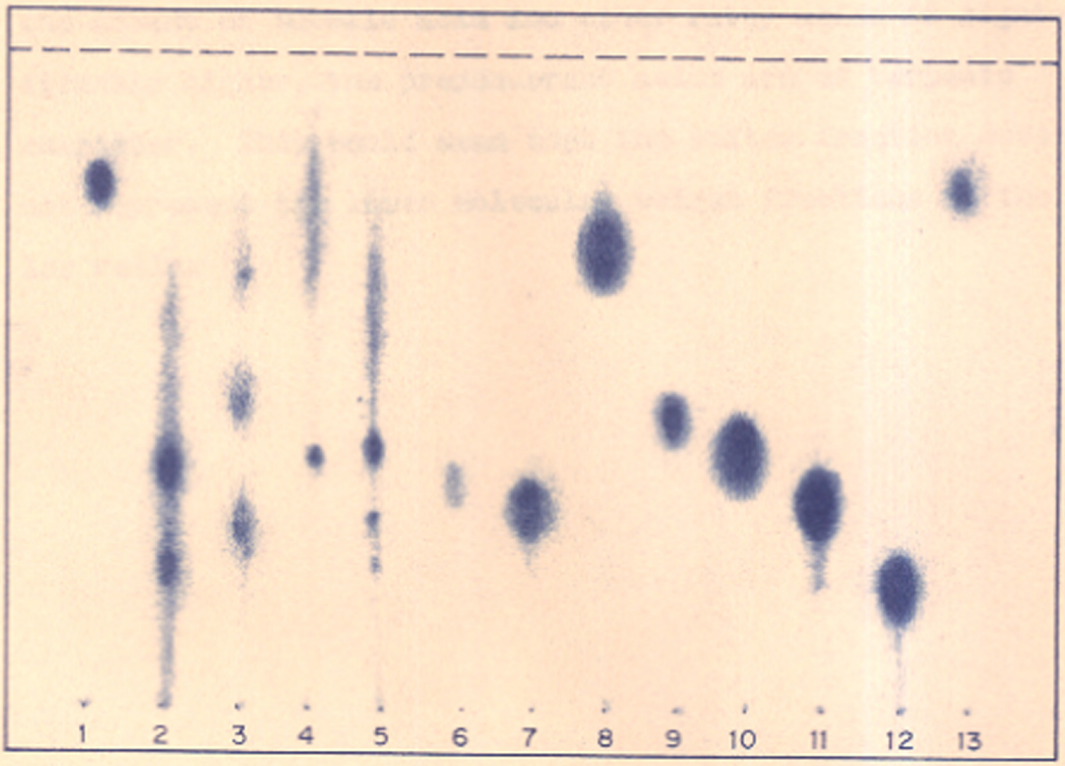


Fig.5 THIN LAYER CHROMATOGRAM
(Methyl esters of acid fractions from sort resin hydrolysate).

PLATE: Silica gel-Plaster of Paris 100:15 (0.3 mm)

SOLVENT FRONT: 12 cm

SOLVENT SYSTEM: Toluene-Ethyl acetate-Acetone (7:4:4)

1 Sudan III; 2 Methylated crude jalaric acid fraction; 3 Methylated crude aleuritic acid fraction; 4 Methylated pet.ether fraction; 5 Methylated alcoholic extract; 6 Methyl ketone methyl ester; 7 Methyl aleuritate; 8 Methyl butolate; 9 Dimethyl shellolate; 10 Dimethyl epi-shellolate; 11 Methyl laksholate; 12 Methyl epi-laksholate; 13 Sudan III.

As can be seen from these results, aleuritic acid is only a minor component of the soft fraction and though the amount of butolic acid and other fatty acids is significantly higher, the preponderant acids are of terpenic character. This would mean that the softer fraction does not represent the lower molecular weight fractions of the lac resin.

E X P E R I M E N T A L

For general remarks, see page 67 , Chapter III.

Rate of hydrolysis of soft resin

The acid value of soft resin was determined (as for hard resin, Chapter II) and was found to be 86.10

Soft resin (5.0 g) was dissolved in potassium hydroxide solution (27 ml; 1N) by warming. Warming was found to be essential because of the poor solubility at room temperature. The rate of hydrolysis was studied at constant temperatures 30°, 50° and 70°. The procedure adopted was exactly the same as described in Chapter III. Saponification values calculated for different periods are given in Table 3.

TABLE 3 - SAPONIFICATION VALUES OF SOFT RESIN

No.	Time (hr)	Saponification values		
		30°	50°	70°
1	1	180.9	188.5	198.5
2	2	186.88	213.9	230.6
3	3	198.90	234.8	241.7
4	4	201.90	242.30	249.5
5	5	207.80	249.80	253.2
6	6	209.40	254.30	258.8
7	8	216.90	259.70	265.4

.....contd.

TABLE 3 (Contd.)

No.	Time (hr)	Saponification values		
		30°	50°	70°
8	10	224.40	263.20	272.6
9	12	225.90	275.80	278.5
10	24	240.80	287.20	292.5

Components of hydrolysed soft resin

Soft resin (50.0 g) was dissolved in sodium hydroxide solution (225 ml, 1.75N) by warming on a waterbath and kept at room temperature (25-30°) for 5 hr. After the usual work up (vide Chapter III) crude jalaric acid (3.00 g) was obtained. The gummy solid acids were again hydrolysed for 5 hr and an additional amount of crude jalaric acid (2.25 g) was isolated. Pure jalaric acid was obtained in 30% yield by crystallising from tetrahydrofuran and ethyl acetate.

The solid gummy acids after the removal of crude jalaric acid were dissolved in sodium hydroxide solution (30 ml, 40%) and subjected to hydrolysis. After 24 hr brine (30 ml) was added and sodium aleuritate (1.024 g) thus separated was filtered off. The brine washing and filtrate were combined and concentrated yielding sodium aleuritate (0.10 g). Sodium aleuritate (1.12 g) was suspended in water (30 ml) and acidified with aqueous phosphoric acid (10 ml, 1:1) yielding aleuritic acid (1.0849 g, m.p. 93-96°) which

was further purified by crystallisation from dilute alcohol. The filtrate after the separation of sodium aleuritate was acidified with aqueous phosphoric acid (400 ml, 1:1) and extracted with ethyl acetate (500 ml x 2). This extract was washed with sodium carbonate solution (500 ml, 15%). The neutral fraction was obtained from the ethyl acetate extract as pleasant colorless liquid (0.24 g). The sodium carbonate extract was acidified with aqueous phosphoric acid (200 ml, 1:1) and extracted with ethyl acetate (500 ml x 2). The ethyl acetate extract was concentrated to half the volume and dispersed on celite (100 g). After drying, the product was soxhleted with pet. ether (300 ml) for 100 hrs yielding a viscous liquid pet.ether fraction (crude butolic acid, 9.849 g). The remaining acids on celite were extracted with ethanol (500 ml) yielding gummy solid (30.85 g).

Paper chromatography of the acids

All the above four fractions viz. crude jalaric acid, crude aleuritic acid, pet.ether fraction (crude butolic acid) and alcoholic extract, were spotted on paper along with standard acids (Fig.3) using the solvent system butanol-ethanol-buffer* (35:35:30).

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*7.30 g ammonium carbonate, 7.5 ml NH_4OH sp. gr. 0.88 in 35 ml water.

TLC of the acids and their methyl esters

The crude acid fractions obtained from the hydrolysate of soft resin were spotted on thin layer plates along with standard acids (Fig.4) using a solvent system: Butanol-ethanol-buffer* (5:1.5:3). Similarly their methyl esters were analysed by TLC (Fig.5) using a solvent system: toluene-ethyl acetate-acetone (7:4:4).

Crude butolic acid fraction:

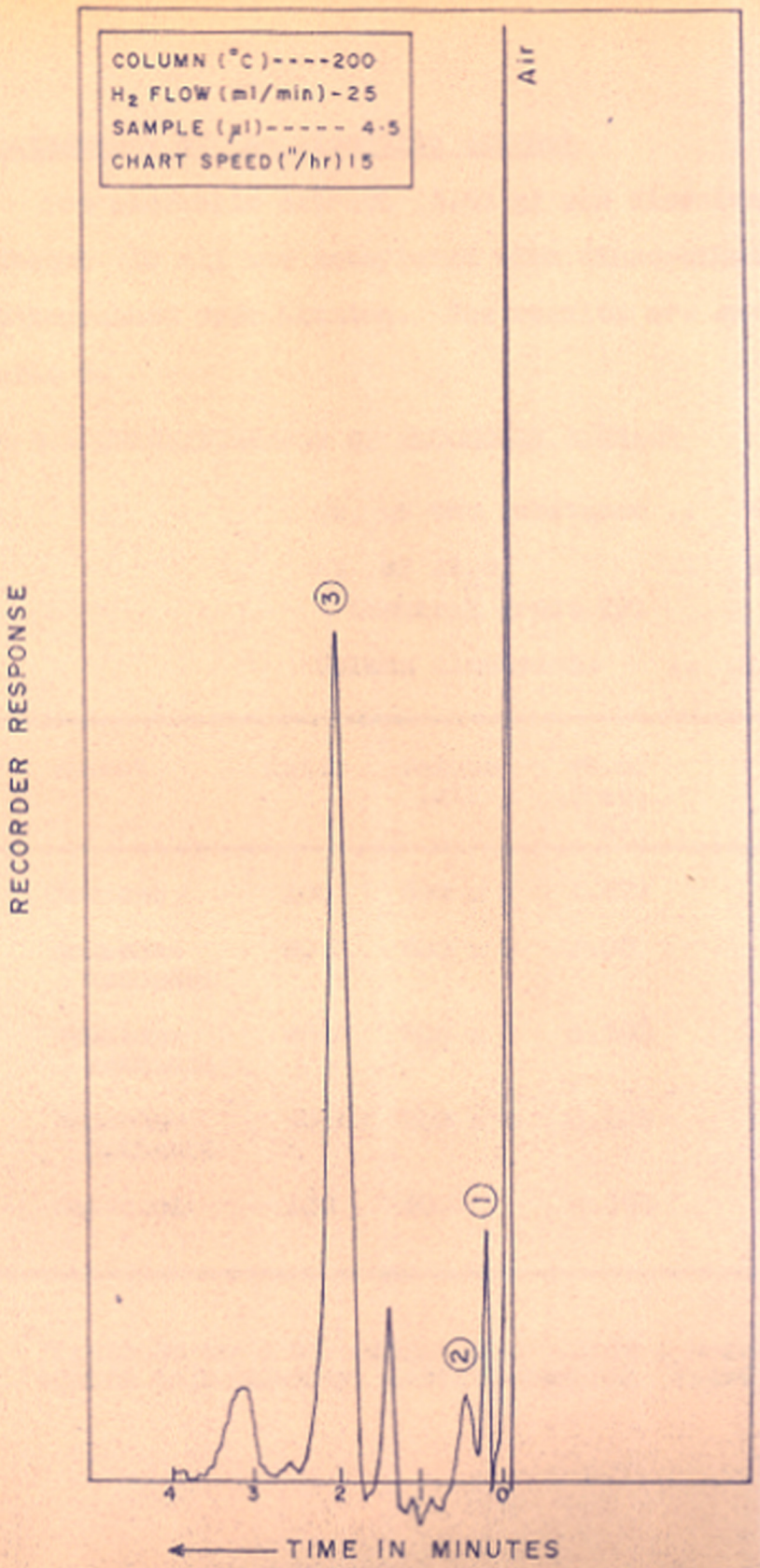
The crude butolic acid fraction (4.0 g) was methylated (diazomethane) and distilled (b.p.90-130/1 mm) yielding a light yellow liquid (1.4 g). This fraction was examined by GLC (Fig.6). The residue (undistilled) was shown to be a mixture of three unidentified products.

Methyl butolate, myristate and palmitate were identified by taking mixed GLCs. The percentage composition was calculated from the GLC data and is reported in Table 4.

TABLE 4 - % COMPOSITION OF THE METHYLATED PET.ETHER FRACTION

No.	Compound	%
1	Methyl butolate	8.3
2	Methyl myristate	8.2
3	Methyl palmitate	61.4
4	Unidentified	21.8

*7.2% ammonium carbonate, 7.5 ml NH_4OH sp. gr. 0.88 in 35 ml water.



FIG·6 G·L CHROMATOGRAM OF METHYLATED ACIDS OF PET·ETHER FRACTION

IDENTIFICATION :-

① METHYL MYRISTATE ② METHYL PALMITATE
③ METHYL BUTOLATE.

Chromatography of the alcoholic extract

The alcoholic extract (4.50 g) was dissolved in ethanol (10 ml) and methylated with diazomethane and chromatographed over alumina. The results are summarised in Table 5.

TABLE 5 - CHROMATOGRAPHY OF ALCOHOLIC EXTRACT

Wt. of the substance .. 4.5 g

Wt. of Al_2O_3 (neutral grade IV) .. 100.0 g

Column dimensions .. 2.4 x 85 cm

Fr.	Eluent	Ratio	Volume (ml)	Wt.of Frac. (g)	TLC*
A	Benzene	100	500 x 2	1.479	6 spots
B	Benzene-methanol	99:1	500 x 3	2.00	4 "
C	Benzene-methanol	95:5	500 x 3	0.593	3 "
D	Benzene-methanol	90:10	500 x 3	0.109	3 "
E	Methanol	100	500	0.044	3 "

*Fractions were investigated by using a solvent system toluene-ethyl acetate-acetone (7:4:4).

Fraction A: This showed the presence of six components by thin layer chromatography. Dimethyl shellolate, dimethyl epishellolate and methyl aleuritate were identified by comparing their R_f values with authentic compounds, while the remaining three could not be identified.

Fraction B: This showed the presence of four components by TLC. Dimethyl epi-shellolate, methyl aleuritate and methyl laksholate were identified by the comparison of their R_f values with those of authentic esters, while one component could not be identified.

Fraction C, D and E: Each fraction was shown to be a mixture of three components, the spot corresponding to methyl aleuritate was quite faint, methyl laksholate and methyl epi-laksholate were also identified by comparing the R_f values with authentic components.

Rechromatography of Fraction A:

The benzene eluted fraction dissolved in benzene (20 ml) was rechromatographed over neutral alumina. The results are given in Table 6.

TABLE 6 - CHROMATOGRAPHY OF FRACTION A

Wt. of compound .. 1.4 g

Wt. of Al_2O_3 (neutral, gr.IV) 50.0 g

Column dimensions .. 2.2 x 11 cm

Fr.	Eluent	Ratio	Vol. (ml)	Wt.of frac. (g)	TLC*
A_1	Pet.ether	100	25 x 6	0.1250	3 spots
A_2	Benzene	100	25 x 10	0.675	5 spots
A_3	Benzene-methanol	99:1	25 x 8	0.490	5 spots
A_4	Benzene-methanol	95:5	25 x 4	0.025	3 spots
A_5	Benzene-methanol	90:10	25 x 4	0.012	3 spots
A_6	Methanol	100	25 x 2	-	

*Fractions were investigated by using a solvent system toluene-ethyl acetate-acetone (7:4:4).

Fraction A_1: Showed the presence of three unidentified components. On concentrating the fraction, one of the components was obtained solid which was filtered and purified by sublimation yielding colourless crystalline product m.p. 127-128°. This compound was found to be identical with the one isolated from hard resin (p. 81), while the other two products could not be identified.

Fraction A_2 and A_3: Each fraction showed the presence of five components and were found to be similar (TLC), hence were mixed together. On concentration a solid product was isolated which on crystallization showed m.p. 178-179° and was found to be identical with the one isolated from hard resin (g. %o). The mother liquor was diluted with benzene and on storing a crystalline solid (0.12 g, m.p. 145-149°) was obtained which on crystallisation gave a solid m.p. 147-149° and identified as dimethyl shellolate by mixed melting point with the authentic sample and by comparing the R_f values of the two product. Mother liquor from the above crystallization was shown to contain dimethyl epi-shellolate and methyl aleuritate by TLC. Remaining one component could not be identified.

Fraction A_4 and A_5: Both the fractions showed the presence of three spots (TLC) and were found to be similar, hence mixed together. One of them was prominent and ascribable to methyl laksholate while the other two were identified as due to methyl epi-laksholate and methyl aleuritate.

SUMMARY

Separation scheme used for hard resin hydrolysate is applied for the isolation of acidic components from hydrolysed 'Soft Resin' (Palas).

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CHAPTER V

QUANTITATIVE ESTIMATION OF JALARIC AND ALEURITIC ACIDS

C O N T E N T S

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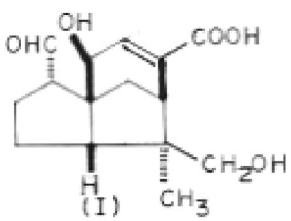
QUANTITATIVE ESTIMATION OF JALARIC AND ALEURITIC ACIDS

As has been stated earlier the work described in this thesis was undertaken to get some more definitive picture of the constituent lac acids and evolve methods which could later be effectively applied to pure lac resin, with the ultimate idea of determining its constitution. The work described so far has clearly shown that two major components of hard resin are jalaric acid and aleuritic acid. The present Chapter deals with procedures evolved for their quantitative estimation.

ESTIMATION OF JALARIC ACID

The characteristic features of jalaric acid (I) is the presence of aldehyde function in the molecule.



(I)

Since a detailed analysis of the hydrolysate from lac resin failed to show the presence of any other aldehydic constituent besides jalaric acid, an estimation of -CHO group would be a measure of jalaric acid.

Several methods[1-3] are available for the estimation

of aldehydic function as well as carbonyl group* [4] in general. It was considered desirable to select two different analytical methods, which could be employed in alkaline and acidic media respectively. This way it should be possible to assess any masked aldehydic functions as well. Of course, it is obvious that the reagents should be capable of attacking the aldehydic function before it has any chance of undergoing possible side reaction. The methods successfully developed for the purpose are described below:

1. Silver oxide method

A number of methods[6,7] involving oxidation with silver ion are available for the estimation of aldehydic group. Of these the method of Siggia and Segal[8] which is employed in alkaline solution was selected as it could be used under the alkaline conditions necessary for the hydrolysis of the resin. The method utilises the oxidation of aldehydic group by Tollen's reagent (silver oxide) and the determination of excess silver ions by potentiometric

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*Bhat, Kamath and Kadkarni[4] have determined the carbonyl value of various types of shellacs, employing hydroxylamine hydrochloride, sodium sulfite and alkaline hydrogen peroxide methods. Their results were re-assessed by Jen Gupta[5] who concluded that the carbonyl value of shellac ranges from 10-30.

titration with potassium iodide.

In order to harness this method for the estimation of jalaric acid in hydrolysed lac resin it was necessary to establish in the first instance the following facts:

i) Standardisation of the method with pure jalaric acid and determining the effect of concentration of alkali and reaction time.

ii) To determine the effect, if any, of the presence of the other major lac acid viz. aleuritic acid, on the estimation.

Table 1 shows the effect of ammonium hydroxide on the estimation while Table 2 shows the data when the sodium hydroxide concentration is varied. For these estimations

TABLE 1 - ESTIMATION* OF JALARIC ACID: EFFECT OF VARYING NH_4OH CONCENTRATION

No.	Amount of NH_4OH added		% Jalaric acid	
	ml	N	Found	Actual
1	0.4	9	86.8	~ 100
2	0.3	9	94.2	~ 100
3	0.6	4.5	90.72	~ 100

*$AgNO_3$ (10 ml; 0.1N), NaOH (0.5 ml; 5.357N). Reaction time: 16 hr.

TABLE 2 - ESTIMATION* OF JALARIC ACID: EFFECT OF VARYING $NaOH$ CONCENTRATION

No.	Amount of NaOH		% Jalaric acid+	
	ml	N	Found	Actual
1	0.4	5.987	86.8	~100
2	0.5	5.987	90.72	~100
3	0.5	5.987	91.80	~100
4	0.6	5.987	94.72	~100

*$AgNO_3$ (10 ml, 0.1N), NH_4OH (0.6 ml, 4.5N) Reaction time: 16 hr.

+Analytically pure jalaric acid.

analytically pure jalaric acid was employed. These results clearly show that the method is quite sensitive to ammonium hydroxide and sodium hydroxide. From this the optimum conditions, $AgNO_3$ (10 ml, 0.1N), NaOH (0.5 ml, 5.987N), NH_4OH (4.5N, 0.6 ml), Reaction time: 16 hr., were selected and the effect of prolonged reaction time determined (Table 3).

TABLE 3 - ESTIMATION** OF JALARIC ACID: EFFECT OF TIME

No.	Reaction time (hr)	% of Jalaric acid+	
		Found	Actual
1	8	77.8	~100
2	16	90.7	~100
3	24	90.8	~100

**$AgNO_3$ (10 ml, 0.1N), NaOH(0.5 ml, 5.987N), NH_4OH (0.6 ml, 4.5N).

+Analytically pure jalaric acid.

This data was necessary as the hydrolysis of lac resin is quantitatively complete at 30° only after 16 hr. As can be seen (Table 3) prolonged reaction time has no deleterious effect.

Finally the estimation of jalaric acid in synthetic mixtures of pure jalaric and aleuritic acids was carried out under the optimum conditions established above. Most unexpectedly the presence of aleuritic acid lead to distinctly high results (Table 4).

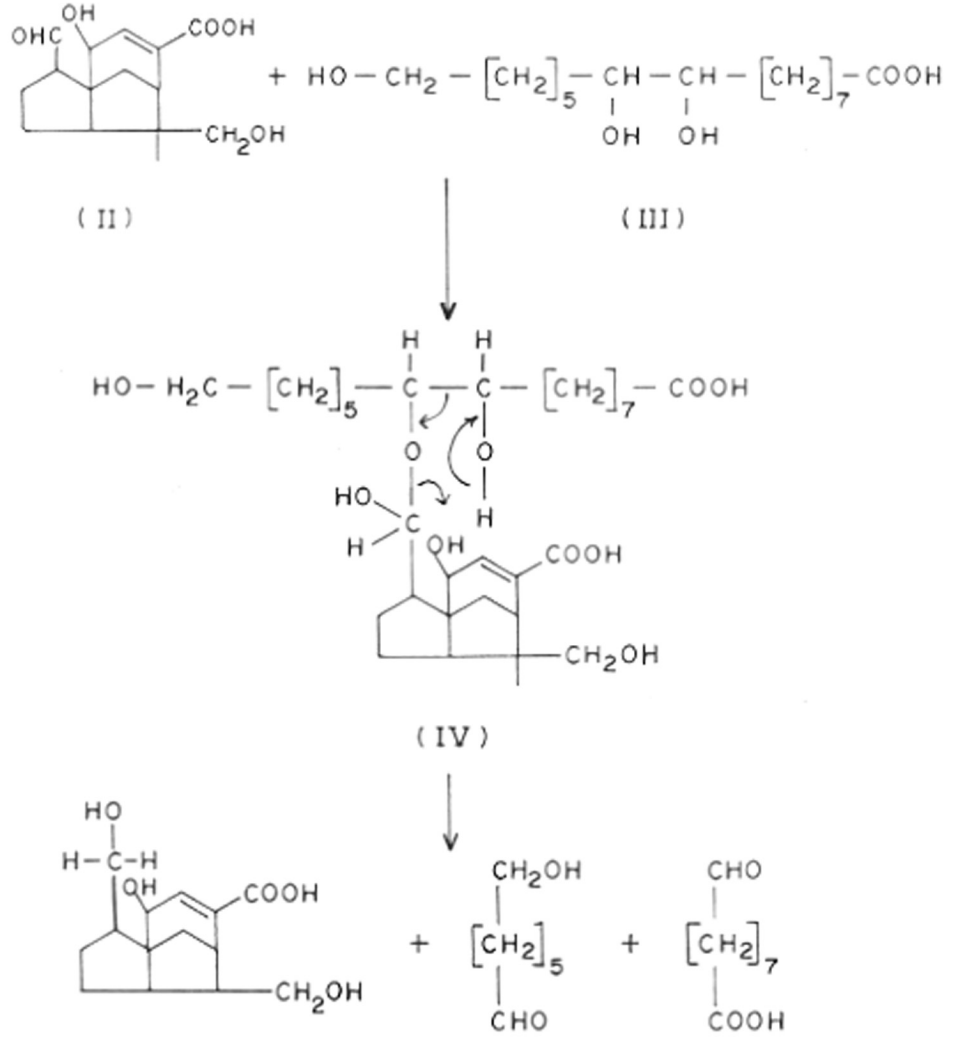
TABLE 4 - ESTIMATION* OF JALARIC ACID: IN MIXTURES OF JALARIC AND ALEURITIC ACID

No.	Composition of synthetic mixture		% Jalaric acid found	% Deviation.
	Jalaric acid+	Aleuritic acid+		
1	100	-	93	
2	30	70	53.5	77.5
3	47	53	73.5	56.2
4	46	54	69.8	50.5
5	63	37	88.6	32.3
6	0	100	-	-

*$AgNO_3$ (10 ml, 0.1N), NaOH (0.5 ml, 5.387N), NH_4OH (0.6 ml, 4.5N). Reaction time: 16 hr.

+Analytically pure samples.

This rather unexpected behaviour can be rationalised if one assumes a hemi-acetal formation with the hydroxyl function (of the α-glycol linkage), which then could cleave as shown (IV) to give two aldehyde units:



Support for this contention was forthcoming when an estimation of jalaric acid was carried out in the presence of ethylene glycol when again high value resulted (74.2%).

However since the above mechanism requires the simultaneous presence of free aldehydic and α-glycol linkages, the method could still be used for the estimation of jalaric acid in lac resin, as it would be shown under the aleuritic acid estimation most of the α-glycol linkages in lac resin are blocked. Table 5 gives the jalaric acid content of hard resin (Palas) and dewaxed shellac.

TABLE 5 - ESTIMATION* OF JALARIC ACID: JALARIC ACID CONTENT IN HARD RESIN AND DEWAXED SHELLAC

Sample No.	% of Jalaric acid	
	Hard resin (Palas)	Dewaxed shellac
1	32.7	25.7
2	31.3	24.7
3	33.7	26.3
4	34.3	28.1
Average	33.1	26.2

*$AgIO_3$ (10 ml, 0.1N), NaN (0.5 ml, 5.387%), NH_4OH (0.6 ml, 4.5N). Reaction time: 16 hr.

2,4-Dinitrophenylhydrazone method

Since 2,4-dinitrophenylhydrazone can react with the aldehyde function in strongly acidic solution it was thought worthwhile to examine this method for the estimation of jalaric acid units in the intact lac resin polymer. Furthermore any acetal linkages would also react with the reagent under the acidic conditions.

A number of methods utilizing 2,4-dinitrophenylhydrazine for the estimation of aldehydes and ketones have been described[9-11]. Obviously the gravimetric methods[11] are not applicable in the present case and the one employing[12] colorimetric determination of the 2,4-dinitrophenyl hydrazone derivative (formed in situ) appeared attractive. The method depends on the estimation of ϵ_{480} mμ of the alkaline solution of the 2,4-dinitrophenylhydrazone. The alkali treatment of the 2,4-dinitrophenyl hydrazone produces a very intense wine red colour presumably due to the formation of resonating quinoidal ion[12,13] (V).

(V)

It has been found[12] that the position of the absorption

maxima as well as the ε_{max} was nearly independent of the structure of carbonyl compound. The value of ε_{max} averages 2.72×10^4 at 480 mμ; besides, the colour formed is relatively stable though fading begins after several days. There is no need to isolate the pure phenyl hydrazone as the excess 2,4-dinitrophenyl-hydrazine reagent is converted into a very light yellow coloured substance on treatment with base which does not contribute to the ε_{480} ($\sim$ 0).

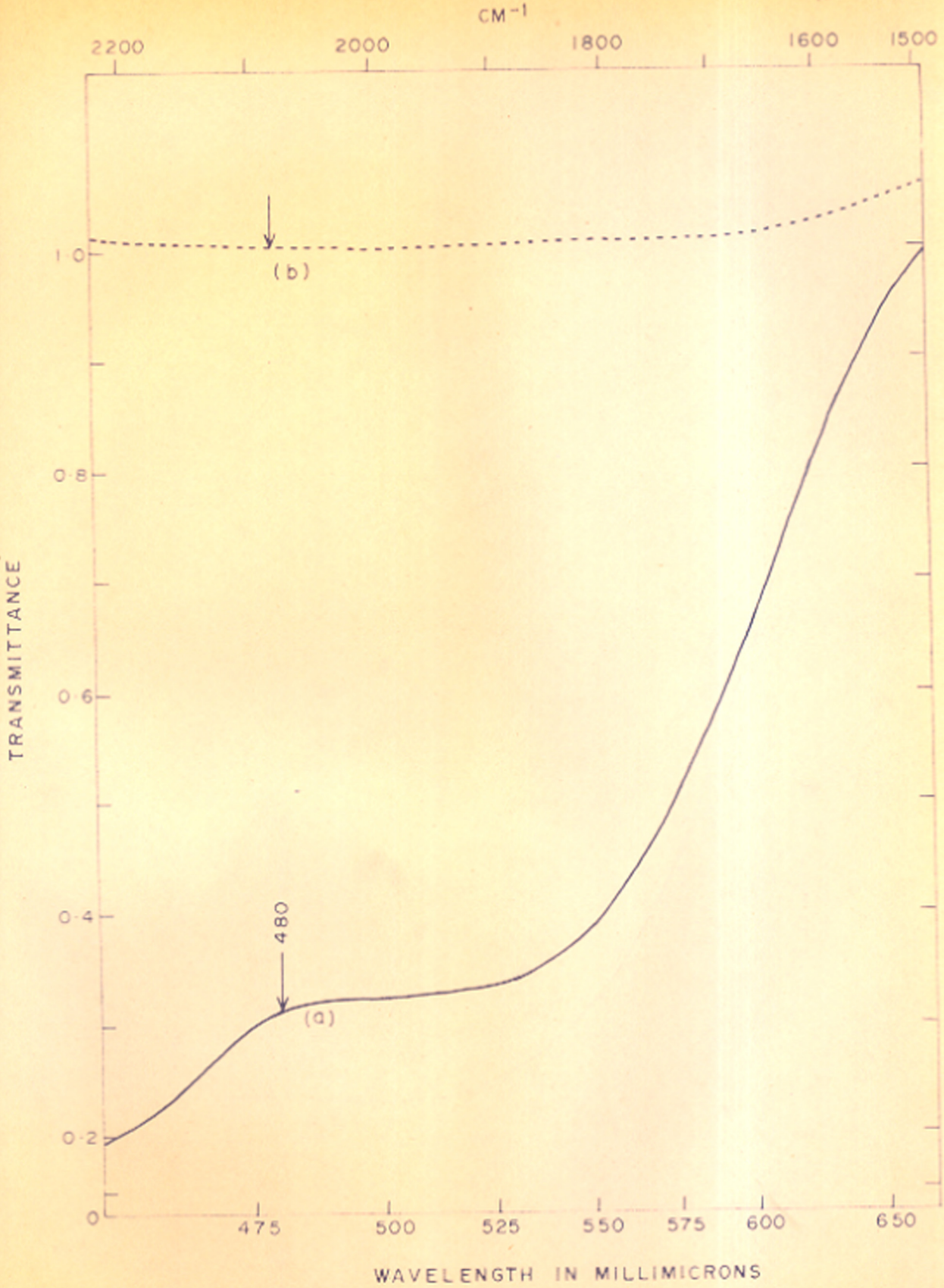
In the first instance the ε_{max} for the derivative resulting from jalaric acid was determined. Fig.1 gives the absorption curve for the alkaline solution of pure jalaric acid 2,4-dinitrophenyl hydrazone. The ε_{480} mμ was used to estimate the percentage of jalaric acid in hard resin (Palas).

Table 6 (Fig.2) gives the values obtained for jalaric acid as well as hard resin (Palas).

TABLE 6 - ESTIMATION OF JALARIC ACID: PERCENTAGE OF JALARIC ACID IN JALARIC ACID SAMPLE* AND IN HARD RESIN

No.	% Jalaric acid	
	*Jalaric acid	Hard resin
1	92.02	32.7
2	95.8	30.4
3	100.4	30.2
Average	96.7	31.1

*Analytically pure sample.



FIG·1 ABSORPTION SPECTRUM OF 2,4-D·N·P OF JALARIC ACID·
(a) 2,4-D·N·P OF JALARIC ACID + REAGENT (b) REAGENT·

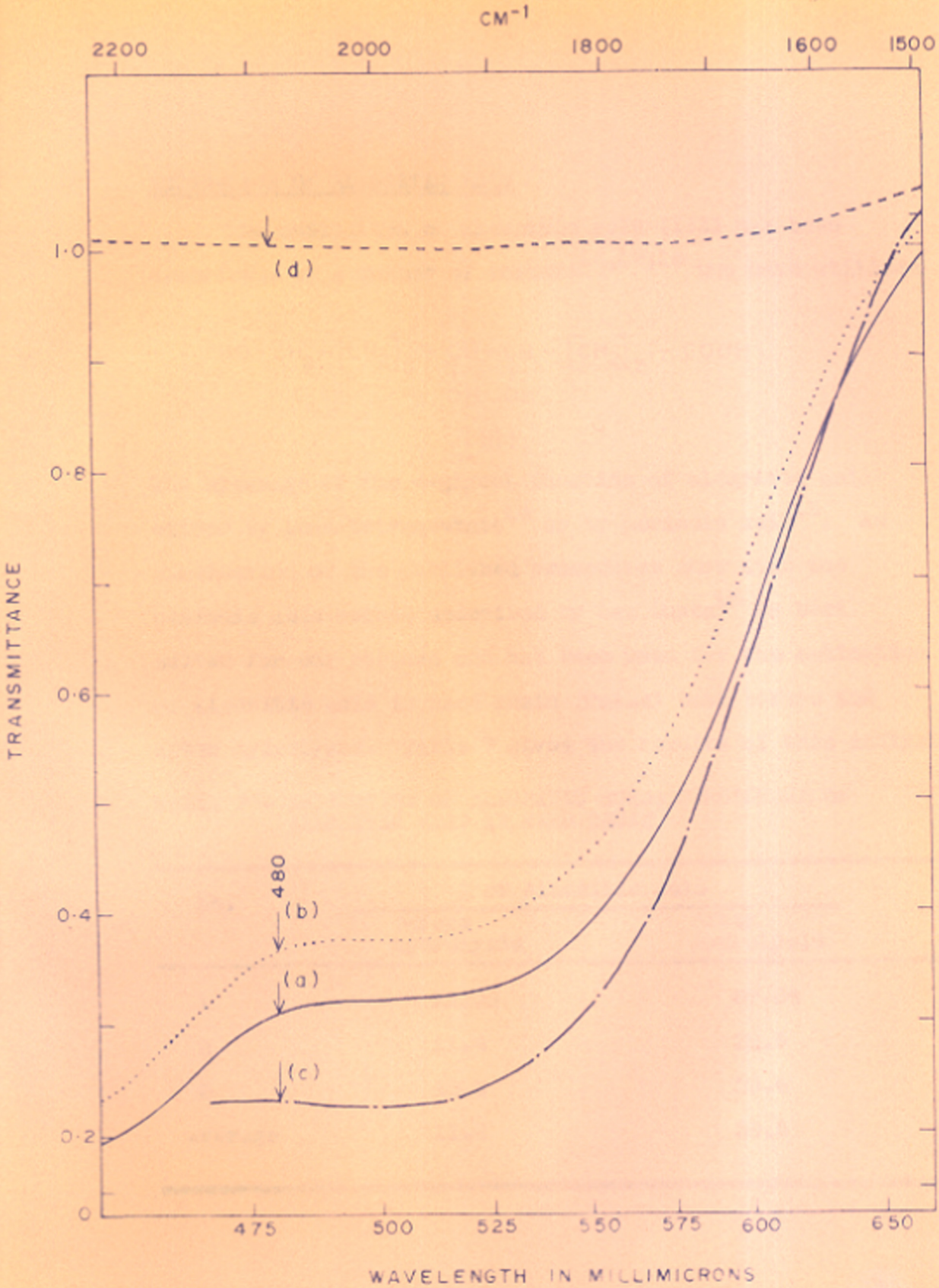
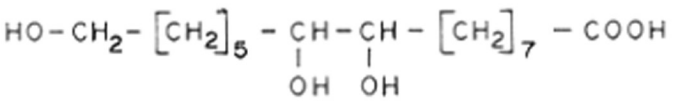


FIG. 2 ESTIMATION OF JALARIC ACID RESIDUES

(a) 2,4-D.N.P OF JALARIC ACID + REAGENT (b) JALARIC ACID + REAGENT (c) HARD RESIN + REAGENT (d) REAGENT.

ESTIMATION OF ALEURITIC ACID

The estimation of aleuritic acid (III) has been described, by a number of workers[14,15,16] who have utilised



(III)

the cleavage of the α-glycol function of aleuritic acid either by lead tetraacetate[14] or by periodic acid[15]. An examination of the published procedures show that the periodic acid method described by Sen Gupta[15] is best suited for our purpose and has been used for the estimation of aleuritic acid in hard resin (Palas) both before and after hydrolysis. Table 7 gives the results of this analysis.

TABLE 7 - ESTIMATION OF ALEURITIC ACID: PERCENTAGE OF ALEURITIC ACID IN HARD RESIN

No.	% of Aleuritic acid	
	Before hydrolysis	After hydrolysis
1	12.06	37.64
2	11.4	38.5
3	10.3	39.4
Average	11.2	38.5

ESTIMATION OF JALARIC ACID AND ALEURITIC ACID IN HARD AND SOFT RESINS PREPARED FROM DIFFERENT VARIETIES OF SEED LACS

The above methods were finally utilised for the estimation of jalaric and aleuritic acids in hard and soft resins prepared from four different types of seed lacs viz. Palas, Kusmi, Ber and Jalari. The results have been collected in Table 8 and in Table 9.

TABLE 8 - ESTIMATION OF JALARIC AND ALEURITIC ACIDS IN HARD RESINS

No.	Source of hard resin.	% of Jalaric acid* +	% of aleuritic acid*	
			Before hydrolysis	After hydrolysis
1	Palas	31.1	11.2	38.5
2	Kusmi	37.2	11.5	45.1
3	Jalari	37.2	12.2	38.9
4	Ber	41.0	11.6	38.8

*Analytically pure sample.

+2,4-Dinitrophenylhydrazone method.

TABLE 9 - ESTIMATION OF JALARIC AND ALEURITIC ACIDS IN SOFT RESINS

No.	Source of soft resin	% of jalaric acid*	% of aleuritic acid	
			Before hydrolysis	After hydrolysis
1	Palas	36.1	9.8	16.9
2	Kusmi	34.6	12.5	26.5
3	Jalari	34.1	11.7	28.6
4	Ber	36.8	17.1	23.4

*Analytically pure sample.

†2,4-Dinitrophenylhydrazone method.

CONCLUSION

As can be seen by comparison of the results recorded in Table 5 and in Table 6 the percentage of jalaric acid as determined in both hydrolysed and intact lac resin is essentially identical, thus showing that the aldehyde function is not masked in the lac resin.

From the results shown in Table 7 it is clear that the α-glycol linkage of aleuritic acid is essentially masked in the lac resin, as the value of aleuritic acid found before hydrolysis is nearly one-third of that found after hydrolysis. Thus atleast one hydroxyl function of the α-glycol linkage is involved in an ester linkage in

approximately two-third of the aleuritic acid molecules used in the construction of hard resin.

The results collected in Tables 8 and 9 show that the nature of the host tree has some effect, though not very significant on the composition of the polymer.

A comparison of the aleuritic acid contents of the soft and hard resins show that the percentage of aleuritic acid is significantly less in the hydrolysates from soft resin. However as has been shown (Chapter IV) in the case of soft resin from Palas seed lac the amount of aleuritic acid actually isolated was much smaller than what would be expected from the data in Table 9. This implies that the soft resin hydrolysate should contain some other acids with α-glycol linkage, as the method of estimation of aleuritic acid is only a measure of the α-glycol linkage.

E X P E R I M E N T A L

ESTIMATION OF JALARIC ACID

Silver oxide method

Materials:

Jalaric acid: Freshly crystallised sample (182-184°) from tetrahydrofuran and ethyl acetate. (It is quite difficult to get pure jalaric acid as it is normally contaminated with its oxidation product epishellolic acid).

Potassium iodide solution: (0.1N) was prepared from the AR reagent (J.T.Baker) after drying the sample at 180-190°.

$AgNO_3$ solution (0.1N): was prepared from the AR reagent (BDH) after drying at 80-90°.

Sodium hydroxide solution (5.387N): was prepared from the analytical reagent (E.Merck).

Ammonium hydroxide used was 4.5N.

ELICO pH meter (Model LI-10) fitted with silver and calomel electrodes and potassium nitrate bridge.

Procedure:

$AgNO_3$ solution (10 ml, 0.1N) was taken in a ground glass stoppered flask and sodium hydroxide solution (0.5 ml, 5.387N) was added, the turbid precipitate formed was dissolved by the addition of ammonium hydroxide solution (0.6 ml) and

jalaric acid (100.0 mg) was added. The reaction mixture was kept in dark for 16 hr at room temperature ($30^{\circ} \pm 2^{\circ}$) with occasional swirling. The contents of the flask were transferred into a beaker along with water washings (15 ml) of the reaction flask, and titrated against KI solution potentiometrically. The end point obtained was quite sharp and showed the presence of 30-32% jalaric acid in the sample (Table 2). This procedure was used to determine the effect of aleuritic acid on jalaric acid estimation by employing synthetic mixtures of the two acids (Table 4). Finally the amount of jalaric acid present in hard resin (Palas) and dewaxed shellac were determined (Table 5) by the method discussed above.

2,4-Dinitrophenyl hydrazone method

Reagents:

Carbonyl free methanol: This was prepared by refluxing AR methanol (Merck, 500 ml) with 2,4-dinitrophenyl hydrazine reagent (5.0 g) and concentrated HCl (1.0 ml) for 6 hr and fractionated. The solvent had b.p. 64.5 - 64.8°.

2,4-Dinitrophenyl hydrazine reagent: The reagent (BDH) was twice recrystallised from carbonyl free methanol and a saturated solution in the same solvent was prepared.

Potassium hydroxide solution: This was prepared by dissolving AR KOH (Merck, 10.0 g) in water (20 ml) and

diluting with carbonyl free methanol to a total volume of 100 ml.

2,4-dinitrophenyl hydrazine of jalaric acid: To the recrystallised reagent (0.4 g) concentrated sulfuric acid (2 ml), water (3 ml) and warm carbonyl free methanol (10 ml) were added. A solution of jalaric acid (0.5 g in 20 ml carbonyl free methanol) was added to the freshly prepared 2,4-dinitrophenyl hydrazine reagent and the reaction mixture was again warmed and left for 12 hours. Crystals of 2,4-dinitrophenyl hydrazone of jalaric acid were filtered, washed with methanol (10 ml) and recrystallised from carbonyl free methanol to give a yellow crystalline product m.p. 242-244°.

Procedure:

2,4-dinitrophenyl hydrazone of jalaric acid (4.72 mg) was weighed in a standard flask (25 ml) and made up with carbonyl free methanol. The solution (1.0 ml) was withdrawn into a measuring cylinder (25 ml), and treated with a saturated solution of 2,4-dinitrophenyl hydrazine reagent (1 ml) and concentrated HCl (one drop). A blank was also prepared by using methanol instead of the sample. The contents of both the cylinders were heated (50°) for 3 hours, cooled to room temperature (30°) and KOH solution (5 ml) added. The black colour thus formed changed to wine red immediately.

The instrument was adjusted to 100% transmittance with the blank at 480 mμ. ε at 480 mμ was 1.21×10^4 for the 2,4-dinitrophenyl hydrazone of jalaric acid. The ε_{max} in the case of jalaric acid sample was determined and the percentage of jalaric acid in the sample was calculated from the value obtained for 2,4-dinitrophenyl hydrazone of jalaric acid (92.02%, Fig.2). By adopting the procedure explained above the percentage of jalaric acid present in hard resins (Palas, Kusmi, Jalari and Ber) were determined (Table 8). Similarly the percentage of jalaric acid present in the different samples of soft resins (Palas, Kusmi, Jalari and Ber) were determined (Table 9).

ESTIMATION OF ALEURITIC ACID

Reagents:

Oxidizing reagent: Periodic acid (5.0 g) was dissolved in water (200 ml) and diluted with glacial acetic acid to a total volume of 1000 ml.

Potassium iodide (AR Merck, dried at 180-190°) - 20% solution.

Starch indicator solution (1%)

Standardised $Na_2S_2O_3$ solution: 0.007688

Aleuritic acid (100 mg) was dissolved in glacial acetic acid (5.0 ml), treated with the oxidizing reagent (20 ml) and the mixture was kept in the dark (20°-25°). After 30 minutes chloroform (5.0 ml) and KI solution (10.0 ml) were added to the reaction mixture and titrated against $Na_2S_2O_3$

using starch as indicator giving a value 99.82% for the sample of pure aleuritic acid. By following the above method, the percentage of aleuritic acid content in hard and soft resins derived from different seed lacs (Palas, Kusmi, Jalari and Ber) was determined (Table 8 and 9).

Aleuritic acid content in hydrolysed lac:

Hard resin (3.0 g, Palas) was dissolved in sodium hydroxide solution (6 ml, 40%) by warming on a water bath and left at room temperature ($30^{\circ} \pm 2^{\circ}$) for 24 hours. The reaction mixture was acidified with aqueous phosphoric acid (15 ml; 1:1) and extracted with ethyl acetate (30 ml x 2), washed with water (10 ml x 4) and dried. The isolated product was dried under vacuum (6-7 mm) for 3 hrs. Aleuritic acid content of the hydrolysed Palas hard resin was determined by the procedure described above. Similarly the hard resin samples isolated from different varieties of seed lacs (Kusmi, Jalari and Ber) were hydrolysed and the percentage of aleuritic acid in each was determined (Table 8). Finally the method was applied to determine the amount of aleuritic acid present in hydrolysed soft resins (Palas, Kusmi, Jalari and Ber) (Table 9).

SUMMARY

Methods for the estimation of jalaric acid in acidic and alkaline medium have been developed. The methods have been used to estimate the percentage of jalaric acid residues in hard and soft resins derived from different lacs.

The method for the estimation of aleuritic acid standardised by Sen Gupta has been applied to hard and soft resins from different seed lacs and their hydrolysates.

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R.G. Khurana

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