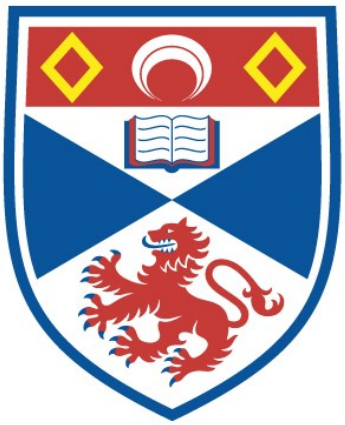
STUDIES OF SOME ALIPHATIC CONSTITUENTS OF SHELLAC

William Walker Christie

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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STUDIES OF SOME ALIPHATIC CONSTITUENTS OF SHELLAC

being a thesis

presented by

WILLIAM WALKER CHRISTIE B.Sc.

to the

UNIVERSITY OF ST. ANDREWS

in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY.

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DECLARATION.

I hereby declare that this Thesis is based on results of experiments carried out by me, that it is my own composition and that it has not been presented previously for a Higher Degree.

The research was carried out in the Chemical Research Laboratories of the United College in the University of St. Andrews under the direction of F.D.Gunstone D.Sc., F.R.I.C.

CERTIFICATE

I hereby certify that Mr. William Walker Christie has spent twelve terms at research work under my supervision, has fulfilled the conditions of Ordinance 16 (St. Andrews) and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Research Supervisor.

UNIVERSITY CAREER

I entered the United College of St. Salvator and St. Leonard, University of St. Andrews in October 1957 on the award of a Matheson Residential Scholarship. I pursued a recognised course for graduation in Science and graduated B.Sc. with First Class Honours in Chemistry in 1961.

I was admitted as a Research Student in October 1961 and was awarded a D.S.I.R. Studentship which I held until October 1964.

ACKNOWLEDGEMENTS

I wish to record my sincere thanks to Dr. F.D.Gunstone for his able guidance, constant interest and encouragement throughout this work. I am also grateful for his help and advice in many matters.

I wish also to thank Mr. R. Morris and the technicians of the Chemistry Department for their assistance from time to time.

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Finally, I would like to thank the D.S.I.R. for financial assistance throughout this work.

Paper published on part of this work.

Shellac. Part I. The Structure of Butolic Acid.

(W.W.Christie, F.D.Gunstone & H.G.Prentice. J, 1963, 5768.).

Paper submitted for publication.

Shellac. Part II. Some minor aliphatic constituents.

(W.W.Christie, F.D.Gunstone, H.G.Prentice & S.C.Sen Gupta.).

CONTENTS

SUMMARY

Studies of Some Aliphatic Constituents of Shellac.

The application of various chromatographic techniques to the mixed esters prepared from shellac has led to the discovery of ether-linked polymer fragments in shellac and the isolation and identification of a number of hitherto unrecognised aliphatic acids including saturated and unsaturated non-hydroxy acids, 6-keto-tetradecanoic acid, 6-hydroxytetradecanoic acid, 16-hydroxyhexadec-9-enoic acid, 16-hydroxyhexadecanoic acid, threo 9,10-dihydroxy-tetradecanoic acid and threo 9,10-dihydroxyhexadecanoic acid.

Appendix.

Lipolytic Studies of some Seed Oils containing Sterculic Acid.

The sterculic acid containing seed oils, Bombacopsis glabra, Sterculia parviflora and Sterculia macrophylla, were examined and subjected to hydrolysis with pancreatic lipase.

STUDIES OF SOME ALIPHATIC CONSTITUENTS OF SHELLAC.

INTRODUCTION.

1. The Production of Shellac.

Lac is the secretion of the insect Laccifer Lacca, a natural parasite on certain shrubs and trees in India and South East Asia. Shellac is the refined product prepared from lac.

In India, the insect larvae are deliberately introduced onto fresh trees with young shoots on which they settle and bury their proboscides into the twig tissues to reach the sap. They then begin very quickly to secrete a protective coating consisting of a deep red chitinous scale and a yellow-red resin. The coatings touch and coalesce until eventually the twig is covered with a continuous encrustation. The insects breathe and are fertilised through minute holes in the lac from which the new larvae in time emerge to repeat the cycle on fresh branches.

Lac is collected by cutting off the infected branches or "sticklac". In the more modern refining processes, these are crushed and sieved to remove the twigs, washed with water to remove the red dye and winnowed to separate the crude "seed-lac" from the smaller wood fragments. The finest quality shellac is prepared from the seed-lac by a solvent process, which removes the wax, and treatment with activated carbon, which decolourises it. Inferior grades of shellac are prepared by heat processes or by traditional native hand processes.

Shellac is a thermoplastic resin, possessing a wide range of thermal, mechanical and electrical properties. It finds many uses,

in particular for paints, varnishes and polishes, for electrical insulation and in moulding compositions.

2. The Resin Structure.

Lac is known to consist of a red dye (laccaic acid), a yellow dye (erythrolaccin), an odiferous principle, a wax and a resin. Structures have been reported for laccaic acid[1] and erythrolaccin[2,3] but doubt exists as to the correctness of both. The resin has the greatest commercial value and is the portion of interest in this study.

It is known to consist of a number of hydroxy and aldehydic mono and dibasic acids, described later, which are interesterified forming a cross-linked polymer. Extraction with ether yields a soft resin (~25 %) with an acid value of 100 and a molecular weight of about 550. The remainder is much harder and has an acid value of 55 and a molecular weight of ~2,000[4].

Studies on the alkaline hydrolysis of lac showed that after initial rapid reaction, the hydrolysis slowed down and almost ceased but recommenced on warming. This is explained[5] as being due to the formation of micelles which are broken down by thermal agitation. Whitmore, Weinberger and Gardner[6], however, explained the phenomenon as immediate neutralisation of the free acid groups followed by hydrolysis of easily saponifiable lactide or anhydride linkages. The final stage is the hydrolysis of the remaining ester

bonds.

Although most of the lac acids must be joined by ester bonds, it has been suggested that anhydride formation occurs on heating the lac during the refining process[6,7] and that lactide[8], lactone[6,9] and ether[9] linkages may also be present though little positive evidence for any of these has been put forward.

The known acids of shellac are polyfunctional and this condition is necessary for the formation of a polyester under heat treatment[10] and, in fact, on prolongued heating aleuritic and shellolic acids (alone or together) undergo condensation with the evolution of water to give clear yellow resins resembling the original shellac[8]. Hydroxy acids, having the hydroxyl carbon atom separated from the carboxyl group by four or more carbon atoms, however, do not form lactones[11], and even when the separation is more than twelve carbon atoms, lactonisation again becoming possible polyester formation is more likely[12,13]. Lactide formation is equally improbable[6]. Kamath and Mainkar, nevertheless, have obtained some evidence for the presence of hemi-acetal, ketal or acyloin groups in shellac[14] as, on alkaline hydrolysis, groups capable of reaction with sodium bisulphite, disappear.

Several hypothetical formulae for the shellac "molecule" have been put forward from time to time[9,15,16] but it seems much more likely that shellac consists of a complex mixture of polymeric entities than any simple single aggregate.

3. The Component Acids.

Until recently only two acids had been positively identified from shellac i.e. aleuritic and shellolic acids, both of which are comparitively easily isolated. Several other acids related to shellolic have now been claimed and it seems probable that shellolic acid is not a primary hydrolysis product. A number of other acids have been reported but not fully identified.

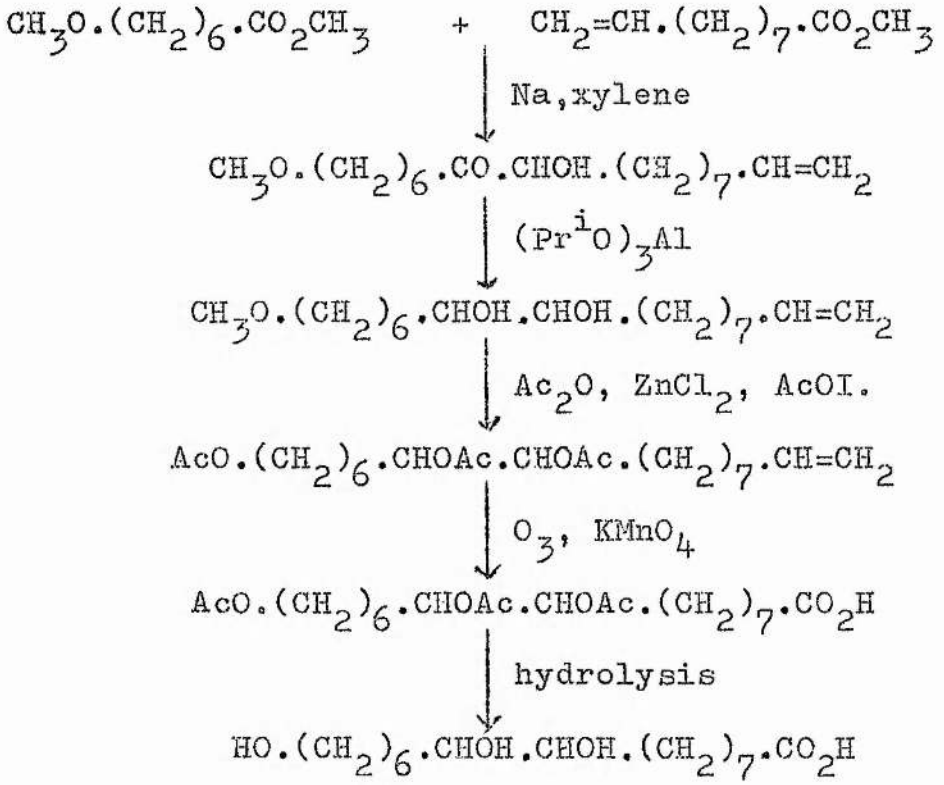
Aleuritic acid.

This is readily obtained from shellac mixed acids by precipitation of its sodium salt, in yields of 30-40%[17-19]. It was first isolated by Tschirch and Farner[20] in 1899. Harries and Nagel[17] (1922) showed it to be a trihydroxy palmitic acid (m.p. 100-101°; methyl ester, 69-70°), and Nagel[21] was later able to identify it as 9,10,16-trihydroxypalmitic acid-

$$HOCH_2.(CH_2)_5.CHOH.CHOH.(CH_2)_7.CO_2H$$

-since on oxidation with permanganate, nonanedioic acid and a C_7 monohydroxy acid, oxidisable to heptanedioic acid, were obtained. This structure has since been adequately confirmed[22,23] by other degradative procedures. Nagel[24] was also able to suggest a threo configuration for the vicinal hydroxyl groups. Further proof of this was obtained by Hunsdiecker[25], although it is still uncertain whether the acid is optically active.

Aleuritic acidwas synthesised by Baudart[26] by the following scheme -



Two forms were prepared by this procedure (m.p.s 131-132° and 102-104°), of which the lower melting isomer was identical with the natural acid, reaffirming that this has a threo configuration. An alternative synthesis of the erythro isomer has been described by Mitter et al.[28-30].

A number of isomers of aleuritic acid have been reported though none are described satisfactorily. Endemann[38] reported the isolation of 9,10,15-trihydroxypalmitic acid and Rittler[39] found 8,9,16-trihydroxypalmitic acid though the evidence presented by both is scanty. Other lower melting isomers have been reported[18,40] but the purity of the acids obtained is open to question.

Aleuritic acid has proved a useful starting material for the synthesis of a number of natural products, in particular

civetone[25,31-33], isocivetone and dihydrocivetone[33], epiambrettolic acid[34,35], ambrettolide[36] and 8-octadecenedioic acid[37].

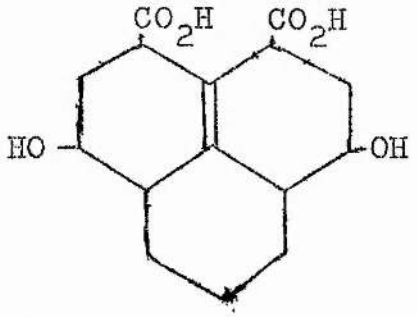
Shellolic and related acids.

Harries and Nagel[41] isolated an acid, designated "shellolic" acid, (m.p. 200-201°, decomp.) in the form of its dimethyl ester (m.p. 149°) from the hydrolysis product of hard resin and establishe that it was an unsaturated dihydroxy dibasic hydro-aromatic acid having the molecular formula $C_{15}H_{20}O_6$.

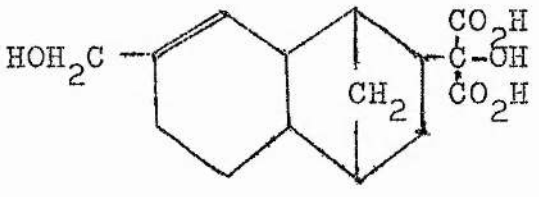
Other workers[18,26,40,42] were unable to repeat this isolation of the acid, though Gidvani[19] claimed an improved separation as the zinc salt and this method is now most often used[43,44]. Other methods of separation yielded products with entirely different properties from those of shellolic acid, suggesting to many[9,15,19,45,46] that shellolic acid is not a primary hydrolysis product. Nagel[41] isolated 4-6% shellolic acid from hard lac, Kirk et al.[47] obtained upto 3.6% from various lacs though Wright[48] claims yields in the region of 10%.

On the basis of rather slender chemical evidence, Nagel[41] assigned a hydro-aromatic structure (I) to shellolic acid which he later[9] changed to the tricyclic sesquiterpene (II).

(I)

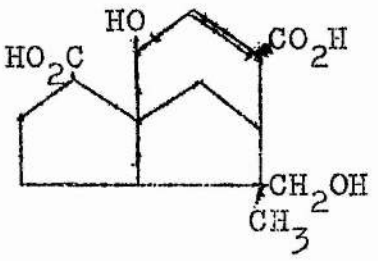


(II)

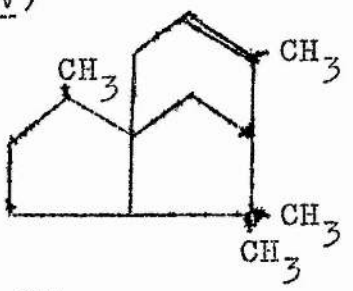


Yates And Field[43], however, by a series of degradative and synthetic reactions, were able to show that shellolic acid is a sesquiterpene (III) with the rare cedrene skeleton (IV)[49]. The only other naturally occurring compound related to this is cedrol (V).

(III)



(IV)



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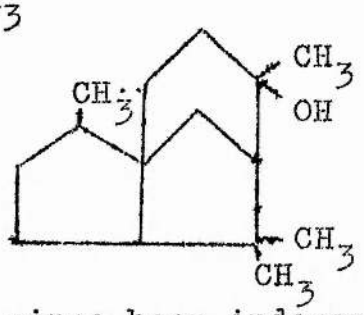
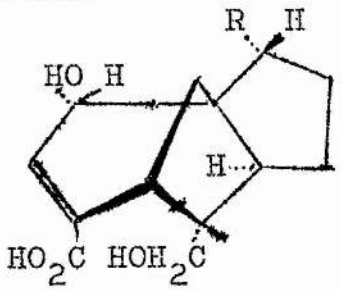
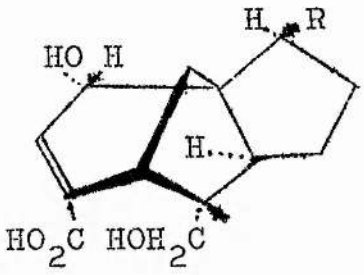


This structure has since been independently confirmed[44] and the stereochemistry elucidated by degradative studies[50], proton magnetic resonance[51] and X-ray analysis[52].

Kamath and Potnis[53] isolated an acid from Jalari sticklac of molecular formula $C_{15}H_{22}O_5$ and found it to be a dihydroxy monobasic acid having one aldehyde function. Sen Gupta[54] was also able to isolate it from Kusmi seedlac. Wadia et al.[55] isolated a pure sample of the acid ("jalaric acid - A") which was readily oxidised with silver oxide to shellolic acid (VI) and epishellolic acid (VIII). They deduced that epishellolic acid was formed by oxidation of jalaric acid - A (IX), while shellolic acid was formed by oxidation of jalaric acid - B (VII), the epimer of jalaric acid - A,

which is very labile and epimerises under the conditions of the reaction.





VI	R = CO_2H	VIII	R = CO_2H
VII	R = CHO	IX	R = CHO
X	R = CH_2OH	XI	R = CH_2OH

Two further acids have now been reported[56] in which the aldehyde group is replaced by a $-CH_2OH$ group (X, laksholic acid; XI, epilaksholic acid). These authors[56] suggest that shellolic and epishellolic acids are not the primary products of hydrolysis of lac resin but that they arise along with laksholic and epilaksholic acids by a Cannizzaro reaction of jalaric acid. In fact, when pure jalaric acid was subjected to alkaline conditions, all four acids were isolated from the products.

It seems likely that the shellolic acid isomer isolated by Kirk, Spoerri and Gardner[47] was epishellolic acid and that other isomers which have been claimed from time to time, including "lacolic lactone" [18,26] are mixtures of the above.

Other acids.

A number of other acids have been reported but not adequately confirmed. These include a monohydroxypalmitic acid[2], myristic and palmitic acids[56,57], an unsaturated acid[57], a tetrahydroxy acid of

molecular formula $C_{16}H_{32}O_6$ ("kerrolic acid")[18,40] and a monohydroxypentadecanoic acid ("butolic acid")[58].

4. Polyhydroxy Acids from other Natural Sources.

There is no other natural resin exactly analoguous to shellac, though suberin, the non-extractable portion of cork, is very similar in that it consists of a cross-linked polyester of several polyfunctional long-chain hydroxy acids. Polyhydroxy acids are, in fact, most often found in the outer coatings of plants such as leaf cuticles and waxes and tree barks.

Cork acids.

Suberin from Quercus suber or Betula verrucosa, on hydrolysis, yields a complex mixture of hydroxy mono and dibasic acids. The principal hydroxy acids are 9,10,18-trihydroxyoctadecanoic acid (phloionolic acid, XII)[59,60], 9,10-dihydroxyoctadecanedioic acid (phloionic acid, XIII)[60], 22-hydroxydocosanoic acid (phellonic acid, XIV)[61,62,63], 18-hydroxyoctadecanoic acid (XV)[64] and 18-hydroxyoctadec-9-enoic acid (XVI)[65]. Eicosanedioic acid (phellogenic acid, XVII)[66,67] and octadec-9-enedioic acid (XVIII)[65] have also been reported.

$HOCH_2.(CH_2)_7.CHOH.CHOH.(CH_2)_7.CO_2H$	XII
$HO_2C.(CH_2)_7.CHOH.CHOH.(CH_2)_7.CO_2H$	XIII
$HO.(CH_2)_{21}.CO_2H$	XIV
$HO.(CH_2)_{17}.CO_2H$	XV

$HOH_2C.(CH_2)_7.CH=CH.(CH_2)_7.CO_2H$ XVI

$HO_2C.(CH_2)_{20}.CO_2H$ XVII

$HO_2C.(CH_2)_7CH=CH.(CH_2)_7.CO_2H$ XVIII

The similar material from the bark of Pseudotsuga taxifolia (Douglas fir) is reported to contain 11-hydroxylauric acid (XIX) and saturated and unsaturated monohydroxy palmitic acids[68], while that of Abies concolor contains 13-hydroxytetradecanoic acid (XX)[69].

$CH_3.CHOH.(CH_2)_9.CO_2H$ XIX

$CH_3.CHCH.(CH_2)_{11}.CO_2H$ XX

Plant cuticle acids.

9,10,18-Trihydroxyoctadecanoic acid, 18-hydroxyoctadecanoic acid, 18-hydroxyoctadec-9-enoic acid, 10,16-dihydroxyhexadecanoic acid (XXI) and 10,18-dihydroxyoctadecanoic acid (XXII) have been isolated from a number of plant cuticles[70,71]. 10,16-Dihydroxyhexadecanoic acid and 9,10,18-trihydroxyoctadecanoic acid have also been isolated from olive leaves[72].

$HOH_2C.(CH_2)_5.CHOH.(CH_2)_8.CO_2H$ XXI

$HOH_2C.(CH_2)_7.CHOH.(CH_2)_8.CO_2H$ XXII

Other acids.

Carnauba wax (from Copernicia cerifera) has been shown to consist of a series of ω-hydroxy acids from C_{10}-C_{32}[73-75]. ω-Hydroxyacids, in particular 16-hydroxyhexadecanoic acid (juniperic acid)

and 12-hydroxydodecanoic acid (sabinic acid), are common constituents of conifer leaf waxes[13,76-81]. Tetrahydroxy acids, including 9,10,12,13-tetrahydroxyheneicosanoic acid (XXIII) and 9,10,12,13-tetrahydroxydocosanoic acid (XXIV) have been isolated from certain Norwegian lichen[82] but their function in the plant is unknown.

$CH_3.(CH_2)_7.CHOH.CHOH.CH_2.CHOH.CHOH.(CH_2)_7.CO_2H$ XXIII

$CH_3.(CH_2)_8.CHOH.CHOH.CH_2.CHOH.CHOH.(CH_2)_7.CO_2H$ XXIV

DISCUSSION.

This investigation was undertaken with the object of isolating and identifying the component acids of shellac other than aleuritic and shellolic acids which are now well known, and, if possible, obtaining a greater knowledge of the polymeric nature of the lac resin. Previous investigators had based their approach to the problem on the fractional crystallisation of various metallic salts - a technique which is laborious, chemically vigorous and gives poor separations. Chromatographic methods have now been developed for the isolation or analysis of most natural products - in particular, column adsorption chromatography, thin layer chromatography and gas-liquid chromatography are finding increasing use. These techniques were, therefore, applied to the mixed hydroxy-esters, prepared from the lac hydrolysate as will be described.

1. Chromatographic Techniques.

Column adsorption chromatography.

Trial separations of epoxy esters from dihydroxy esters by adsorption, partition or reverse-phase partition chromatography on silicic acid or silica gel have been reported[83] and it is concluded that there is little to choose between the methods on a small scale though adsorption chromatography is favoured for large quantities and as it is more convenient. Reports of other such separations of hydroxy esters are infrequent though Meakins and Swindells[72] isolated 9,10,18-trihydroxyoctadecanoic acid and 10,16-dihydroxy-

hexadecanoic acid from olive leaves by chromatography of the methyl esters on neutral alumina. Tulloch, Spencer and Gorin[84] separated methyl 17-hydroxyoctadecanoate from the corresponding 18-hydroxy ester on silicic acid.

Silicic acid is usually favoured as an adsorbent in lipid research as there are fewer side reactions than with alumina which causes saponification of triglycerides and autoxidation of unsaturated acids and rarely gives quantitative recovery[85]. However, the larger particle size of commercial alumina allows faster flow rates so larger quantities can be handled. A review of the preparation and properties of silicic acid as an adsorbent for lipid chromatography has recently been published[86]. Most workers stress proper activation and conditioning of the column before use[87,88] and the careful choice of solvent systems[89,90].

In this work, neutral alumina was generally used for initial comparitively crude large scale separations and silicic acid for more exacting work. Stepwise elution was favoured over gradient elution as more convenient and reproducible. With silicic acid columns, the flow rate was increased by applying pressure from a nitrogen cylinder to the top of the column.

Thin layer chromatography. (T.L.C.).

Since the technique of thin layer chromatography was developed and standardised by Stahl[91-95], it has become an essential tool in lipid analysis[96]. Its main advantages over paper chromatography are excellent sharpness of separation, high

sensitivity and the short time necessary to obtain the completed chromatogram. It has been shown that isomeric hydroxy and epoxy esters, differing only slightly in polarity, can be adequately resolved[97-99].

During this investigation, this technique was found very useful for the rapid monitoring of fractions from chromatographic columns, particularly when the esters were too polar for gas chromatographic analysis.

Gas-liquid chromatography. (G.L.C.).

Although G.L.C. has revolutionised the study of normal fatty acids, comparitively few long-chain hydroxy acids have been examined by this technique, probably because of their high polarity and low volatility. With hydrocarbon or polyester liquid phases, peaks tend to "tail" and the response is poor and becomes progressively worse as the number of hydroxyl groups in the molecule is increased. When silicone liquid phases, such as "SE-30" or "QF-1", became available, this problem was lessened though neither methyl aleuritate nor dimethyl shellolate give recognisable peaks with these phases. Most workers prefer to modify the hydroxy esters chemically by preparing the methoxy[100,101] or acetoxy[75,102] derivatives, thereby retaining the essential configurations of the molecules but greatly reducing their polarity.

At the start of this research, when only the liquid phase "Apiezon L" (hydrocarbon) was commercially available, methoxy

compounds were the most suitable because of their greater volatility. The method developed by Kishimoto and Radin[100] for 2-hydroxy acids (i.e. refluxing with methyl iodide and silver oxide) was not successful with polyhydroxy esters. A further modification of Purdie and Irvine's methylation procedure[103] has been described by Kuhn, Trischmann and Low[104] in which the sugar is dissolved in dimethyl formamide and treated with silver oxide and methyl iodide. This only effected partial methylation of a dihydroxy ester but, by changing the solvent to "sulpholane" -tetrahydrothiophene sulphone and refluxing the reaction mixture for 8 hours, complete methylation was achieved. A recent paper[105] recommends the use of a similar solvent - dimethyl sulphoxide - for carbohydrate methylations. A series of hydroxy esters i.e. methyl aleuritate, dimethyl shellolate, methyl threo and erythro 9,10-dihydroxyoctadecanoates and methyl 12-hydroxyoctadecanoate, was methylated for use as reference compounds.

When "SE-30" became available, the acetoxy derivatives were found most convenient for analysis by G.L.C. as they are easily prepared (in acetic anhydride at 100°C for 4 hours[84]) and as the original hydroxy ester or acid can readily be recovered. Again, a similar series of hydroxy esters was acetylated for reference purposes.

The relative retention times of esters on each of the columns in use (Ap.L., QF-1., SE-30.) were measured as "carbon numbers" (C.No.s)[106]. Where possible, these were determined on at least two of the columns for more complete characterisation.

2. Solvent Extraction of Shellac and Chromatographic Examination of the Mixed Esters.

Schaeffer, Weinberger and Gardner[18] claim to have isolated four new acids - lacolic lactone, kerrolic acid, a new isomer of aleuritic acid and a liquid acid - by solvent fractionation of shellac, followed by fractional crystallisation of various metal salts of the mixed acids. It was hoped that, in this study, solvent fractionation in conjunction with modern chromatographic methods might lead to the isolation and identification of more of the component acids of shellac and shed some light on its polymeric nature. It is now recognised that the latter aim was too ambitious.

Shellac was, therefore, extracted in a soxhlet extractor for 24 hours successively with the following solvents - petroleum ether (40-50°), diethyl ether, chloroform, ethyl acetate, acetone and methanol. Initial difficulties due to the shellac "gumming up" with the solvent were overcome and the extraction was completed. The yield of soluble material (63.7%) was much less than would be expected on the basis of the normal solubility of shellac in methanol (approx. 100%). This may be because the polymeric nature of the shellac was altered by the prolongued heating at temperatures from 50-80°C (i.e. for a total of 6 days).

Portions of each were hydrolysed and methylated and the methyl esters chromatographed on neutral alumina[72]. The esters were loaded in benzene, eluted with solvent mixtures of gradually increasing polarity and the fractions monitored by T.L.C. and where

possible by G.L.C. Most of the fractions were fairly complex mixtures and only in one case, the ether extract methyl esters - fraction 4, was there a single acid in large concentration. This was subsequently shown to be 6-hydroxytetradecanoic acid. Comparison of corresponding fractions in the various solvent extracts, showed a slight increase in the proportion of more polar esters from the later extracts but no real differentiation in kind was evident.

The small petrol extract consists entirely of normal saturated and unsaturated non-hydroxy fatty acids, some of which may be from residual wax in the shellac (commercially dewaxed). The fractions from the ether extract were examined in greatest detail, prior methylation of the hydroxyl groups of the esters being essential for their examination by G.L.C. At that time, it was sometimes possible to postulate structures of unknown esters from a knowledge of the carbon numbers of the reference esters. Later, it was possible to identify certain peaks positively.

6-Hydroxytetradecanoic acid.

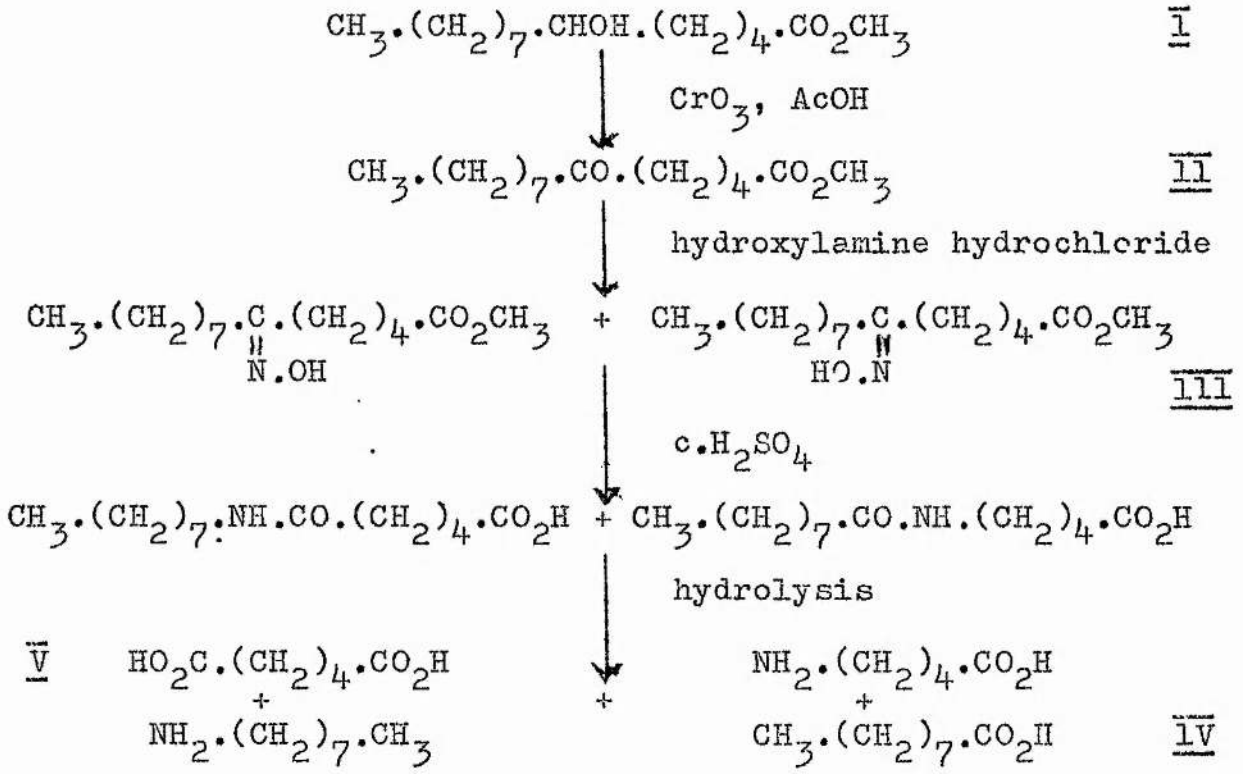
The ether extract - fraction 4 (7.5%) was a white waxy low melting solid shown by G.L.C. and T.L.C. to be largely a single ester (C.No. 16.2, 2½% Ap.L.; 15.8, 10% Ap.L.; 18.0, 10% QF-1.). Rechromatography gave a sample sufficiently pure for structure determination. It was shown to be 6-hydroxytetradecanoic acid on the following evidence.

i. After iodination-deiodination[107], the ester gave a product

which, from its chromatographic behaviour, must be methyl tetradecanoate.

ii. The hydroxy ester (I) was oxidised to a keto ester (II), the oximes of which (III) were submitted to a Beckmann rearrangement[108] and then hydrolysed. The products - a monobasic acid (nonanoic, IV) and a dibasic acid (adipic, V) were recognised by gas chromatography of the methyl esters.

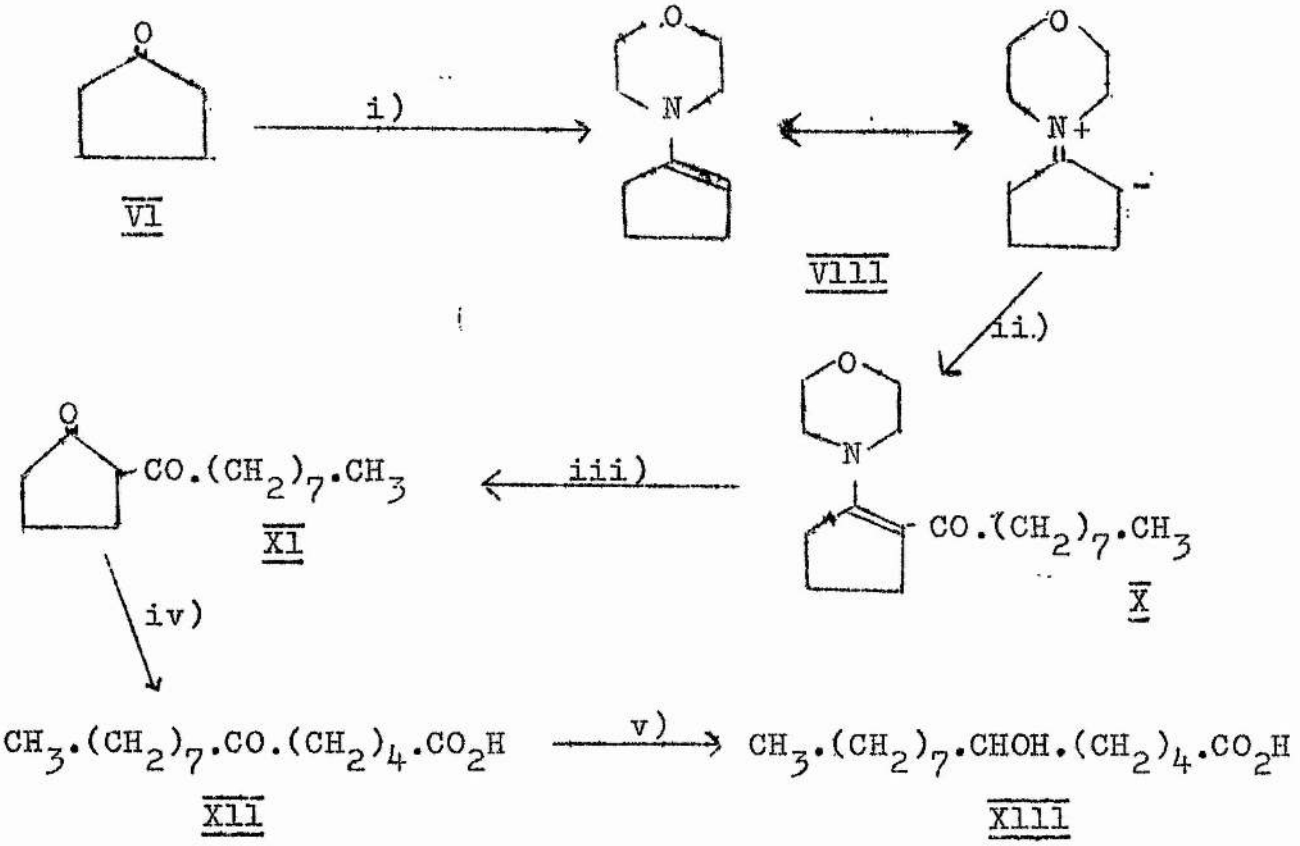
iii. The semicarbazone of the keto acid was prepared and gave a satisfactory analysis. The above information was confirmed by Prentice[109] who also obtained samples of the hydroxy and corresponding keto acids which gave satisfactory analyses.



iv. The (±)-hydroxy and corresponding keto acids were synthesised and shown by melting points and mixed melting points and by their

chromatographic behaviour to be identical in structure to the acids obtained from the natural source.

The method adopted was based on the procedure used by Hünig[110] for the preparation of 7-keto acids from cyclohexanone via the enamine. Using cyclopentanone, 6-keto acids can be obtained[111-113]. Condensation of cyclopentanone (VI) with morpholine (VII) gave the enamine (VIII), 1-morpholino-1-cyclopentene. This is activated at the 2-position so that acylation with nonanoyl chloride (IX) gave the 2-acyl enamine (X) which, on acid hydrolysis, gave the β-diketone (XI). Alkaline hydrolysis resulted in ring cleavage to give 6-ketotetradecanoic acid (XII). The hydroxy acid (XIII) was then prepared by borohydride reduction of the methyl ester followed by hydrolysis.



i) morpholine, VII. ii) $CH_3.(CH_2)_7.COCl$ (IX), NEt_3. iii) HCl. iv) K_2CO_3. v) a. MeOH,HCl. b. $NaBH_4$. c. KOH.

The natural acid was optically active ($[\alpha]_D^{19°}$ -0.9°.; $[M]_D^{19°}$ -2.3°). The racemic acid, obtained from the natural acid by oxidation to the keto acid and subsequent reduction with sodium borohydride, had a higher melting point than the natural acid.

The acid has not previously been recognised from any other source nor has it been synthesised, though the keto acid has been prepared by an alternative route[114] to that descibed above. Prentice[109] has shown the acid to be identical to butolic acid, isolated by Sen Gupta[58] and considered by him to be a monohydroxy-pentadecanoic acid (thanks to a kind donation of a sample of butolic acid from Mr. Sen Gupta). These results have now been independently confirmed[56].

A summary of the melting point data is set out in table 1.

Acid	-OH Acid	Keto acid	Semi-carb.
i. Isolated here.	58-58.5°	70-71°	129-131°
ii. Butolic acid.[58]	54-55°	69.5-70°	128-129°
iii. Racemic acid.	61.5-63°		
iv. Synthetic acid.*	65-66°	71-71.5°	128-130°

(* Lit[114]. Keto acid. 71.5°; Semi-carbazone. 130°).

Table 1.

Dihydroxy acids.

Rechromatography on silicic acid of material eluted after the 6-hydroxy ester gave a fraction which showed one peak on G.L.C. but two spots on T.L.C. Oxidation experiments and T.L.C. using plates sprayed with boric acid[99] indicated the presence of an acid with two vicinal hydroxyl groups and a mid,ω-hydroxy acid* (*i.e. a straight chain acid with one primary hydroxyl group and a secondary hydroxyl group somewhere in the carbon chain). Iodination - deiodination[107], oxidation and the preparation of the isopropylidene derivative[23] suggested that 9,10-dihydroxyhexadecanoic acid was present. Later work confirms this.

The iodination - deiodination experiment and the C.No.s before and after oxidation suggest a mid,ω-dihydroxy C_{14} acid. Attempts to find the position of the secondary hydroxyl group[70] were inconclusive and, while the evidence suggests that the 6 position is most likely, especially since a mono-hydroxy C_{14} acid has been isolated with the hydroxyl group in this position, the acid may be a mixture of isomers.

The samples of these acids were obtained rather laboriously in small quantities by repeated chromatography and insufficient material could be isolated to characterise them fully, especially in the second case where no similar acids were available for model experiments.

Although a higher proportion of the 6-hydroxytetradecanoic acid was present in the ether extract than in the others, thus

simplifying its isolation, it was evident that no real advantage had been gained by the solvent extraction. It was decided, therefore, to start again with the total lac esters in order to obtain larger amounts of individual esters to work on and so that a more accurate estimate of the proportions of the acids present might be made.

3. Chromatographic Examination of the Total Lac Esters.

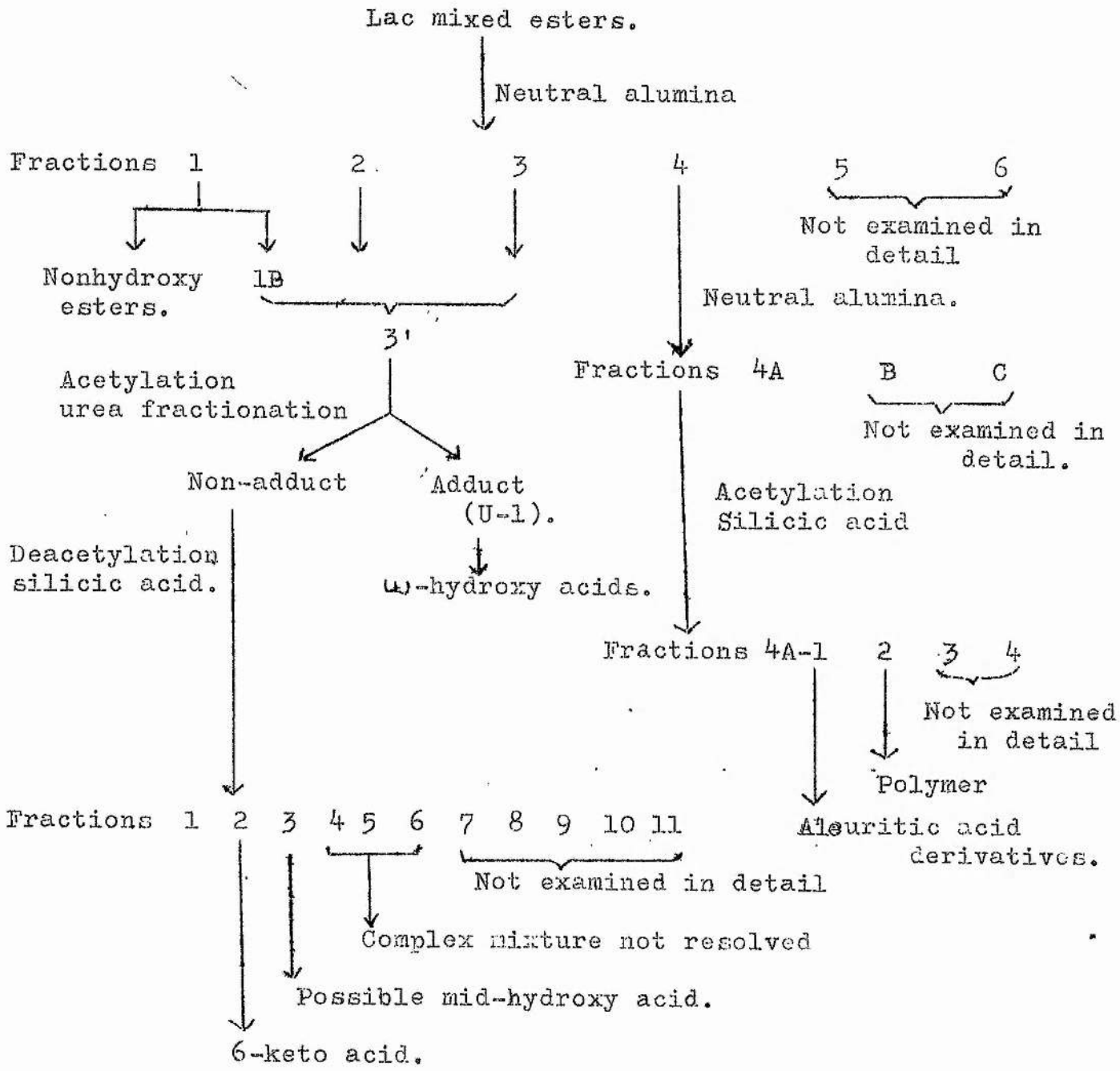
The methyl esters of a large quantity of shellac (approx. 100g) were prepared and chromatographed in two batches on neutral alumina. Six fractions were collected.

The non-hydroxy acids.

The first of these fractions was rechromatographed on neutral alumina to obtain the non-hydroxy esters. The unsaturated esters were separated from the saturated by chromatography on silicic acid impregnated with silver nitrate[115] and the individual C_{14}, C_{16} and C_{18} esters obtained by preparative G.L.C.

Von Rudloff oxidation[116] showed that the C_{16} and C_{18} acids had the double bond almost entirely in the 9 position with just a trace of the 8 isomer. The C_{14} acid was more complex, however, and, though in approximately half of the molecules the double bond was in the 9 position, there were significant amounts of the 5 and 6 isomers and minor amounts of the 7 and 8 isomers.

Separation Scheme 1.



The ω-hydroxy esters.

The remaining material from fraction 1, along with fractions 2 and 3, was acetylated and subjected to urea fractionation which would remove any ω-monoacetoxy esters[84]. The adducted esters were examined by G.L.C. which showed two peaks of C.No.s 19.2 and 19.5 (5% Ap.L.), in the approximate ratio 4:1. The hydroxy acids, from which these are derived, were shown to be 16-hydroxy-cis.9-hexadecenoic acid and the corresponding saturated acid on the following evidence.

i. On hydrogenation of the acetoxy esters, only a single peak of C.No. 19.5 (5% Ap.L.) was observed. Chromatography of the acetoxy esters on silicic acid impregnated with silver nitrate[115] yielded pure samples of each ester.

ii. Chain length determination showed that the acids were largely C_{16} with a minor amount of C_{14} (approx. 3%).

iii. Oxidation of the saturated hydroxy ester gave hexadecanedioic acid which was adequately characterised by the chromatographic behaviour and melting point of its dimethyl ester.

iv. The methoxy derivative of the unsaturated acid (XIV) was prepared and subjected to von Rudloff oxidation[116]. The methyl esters of the fragments, an ω-methoxy C_7 acid (XV) and a C_9 dibasic acid (XVI), were prepared and characterised by G.L.C.

$$\underset{\text{XIV}}{CH_3O.(CH_2)_6.CH{=}CH.(CH_2)_7.CO_2H} \xrightarrow[KMnO_4]{KIO_4} \begin{matrix} CH_3O.(CH_2)_6.CO_2H & \text{XV} \\ + & \\ HO_2C.(CH_2)_7.CO_2H & \text{XVI} \end{matrix}$$

v. Satisfactory analyses were obtained for the C_{16} acids and their methyl esters. The melting points of the saturated acid and its methyl ester were similar to those of the same compounds reported from another source.

vi. The infra red spectrum of the unsaturated acid did not show a prominent peak at 10.3μ corresponding to a trans double bond so it may be concluded that its configuration is cis.

vii. Some of this work was confirmed by Sen Gupta[118], who initiated this part of the study.

The chromatographic evidence suggests that a trace of 14-hydroxytetradecanoic acid is also present.

16-Hydroxyhexadecanoic acid has previously been isolated from conifer waxes[119]. The unsaturated acid has not been reported from any other source, though a similar acid with the double bond in the 7 position has been isolated from Hibiscus abelmoschus seed oil[120]. A monohydroxy C_{16} acid has been reported from shellac[2] as has an unsaturated hydroxy acid[57] but in neither case is a structure suggested.

Chromatography of the non-adducted esters.

The non-adducted esters were hydrolysed to the acids and re-methylated (fraction 3'). This was chromatographed in small lots on silicic acid, combining corresponding fractions. Fraction 3'-2 was shown to consist largely of 6-ketotetradecanoic acid. The remaining fractions were examined after methylation of the hydroxyl

groups and all were found to be complex.

It was hoped to use preparative G.L.C. of the methoxy esters for separation of the more complicated fractions, particularly fraction 3'-3, in which the chromatographic evidence suggested the presence of an unidentified mid-hydroxy C_{16} acid. However, it was found that even the methoxy esters were too polar for the instrument (Perkin Elmer Fractometer) and were not eluted.

6-Ketotetradecanoic acid.

Fraction 3'-2 (eluting solvent, benzene:ether 9:1) consisted largely of a single acid. Rechromatography gave a sample from which it was possible to identify the acid as 6-ketotetradecanoic acid as follows.

i. A major "hump" on the ester peak in the infra red spectrum was evident, suggesting that a further carbonyl group was present.

ii. Its behaviour on G.L.C. (C.No.s 15.4 (Ap.L.), 18.9 (QF-1).) was identical to that of synthetic methyl 6-ketotetradecanoate, examined earlier. Its C.No. was not changed on attempted methylation by the sulpholane procedure.

iii. Borohydride reduction gave a hydroxy ester of C.No.s 15.8 (10% Ap.L) and 18.0 (QF-1) i.e. the same as those of 6-hydroxy-tetradecanoate. It was possible to prepare the methoxy ester of C.No. 15.1 (Ap.L.).

iv. Chain length determination[107] on the hydroxy ester gave, as the main component, an ester indistinguishable in its chromatographic behaviour from methyl tetradecanoate.

v. The oximes of the keto ester were prepared and subjected to a Beckmann rearrangement followed by hydrolysis of the amides. The products, a monobasic acid (nonanoic) and a dibasic (adipic), were recognised by G.L.C. of their methyl esters.

vi. Samples of the acid and its semicarbazone were prepared which gave satisfactory melting points, alone and when mixed with the synthetic compounds.

The acid has not been reported from any other natural source, though it has been synthesised by Keskin[114] and earlier in this investigation.

Chromatography of fraction 4.

Fraction 4 (the largest fraction) was rechromatographed on neutral alumina giving three subfractions. The first of these (4A) was chromatographed again on silicic acid, giving four further subfractions. Meanwhile, Sen Gupta[118] had obtained encouraging separations by chromatographing the acetoxy derivatives of shellac esters on silicic acid. As SE-30 silicone was now commercially available as a liquid phase, it was possible to monitor the fractions by G.L.C.

Accordingly, the first of these fractions (4A-1) was acetylated and chromatographed on silicic acid, 11 fractions being recovered. The 8^{th} and 9^{th} of these appeared to be largely single acids. Fraction 4A-2 was treated similarly and a subfraction obtained which gave a single spot on T.L.C. but was not eluted on G.L.C. It is considered to be polymeric. The two acids from

fraction 4A-1 were decomposed by boron trifluoride/methanol complex to mixtures of products which included methyl aleuritate and it seems possible that these are monomeric lactones or cyclic ethers derived from aleuritic acid.

The polymer.

Fraction 4A-2(3) was shown to be a dimer made up of aleuritic acid units joined by ether linkages through the 9,10 or 16 positions. The evidence for this is that -

i. The acetoxy ester is apparently of high molecular weight as it is not eluted from a G.L.C. silicone column under extreme conditions.

ii. Chain length determination[107] on the hydroxy polymer gave an ester indistinguishable in its chromatographic behaviour from methyl palmitate.

iii. It was unaffected by alkaline hydrolysis but acid hydrolysis gave aleuritic acid as the principal product with two other acids which, from the C.No.s of their acetoxy esters, appear to be a vic-dihydroxy C_{16} acid and a mid-hydroxy C_{16} acid. These last two acids may be artefacts produced during the breaking of the ether linkage and may not be present in a similar form in the polymer.

iv. It was not possible to prepare an isopropylidene derivative of the hydroxy polymer or to obtain recognisable fragments on von Rudloff oxidation, indicating the absence of adjacent free hydroxyl groups in the polymer.

v. A molecular weight determination indicated that the fraction must be mostly dimeric.

No evidence for the presence of ether linked polymers in shellac has hitherto been presented though Nagel[9] has suggested that they may play some part in its structure. Most workers, however, have assumed that shellac is almost entirely a cross-linked poly-ester formed by interesterification of a mixture of hydroxy acids.

This conclusion raises important fundamental questions about our earlier experiments. The conditions used to prepare the mixed methyl esters of shellac (viz. methanolic hydrogen chloride) could give rise to a number of artefacts. Also, the presence of polymeric fragments of similar polarity to monomeric esters would increase the difficulty of isolating pure esters by chromatographic means.

It was, therefore, decided to conduct the separation of esters in the following stages.

i. Alkaline hydrolysis of ester bonds.

ii. Esterification of free acid groups with diazomethane.

iii. Separation of monomeric esters from ether linked polymeric esters by molecular distillation of the acetoxy derivatives.

iv. Separation of individual esters by adsorption chromatography.

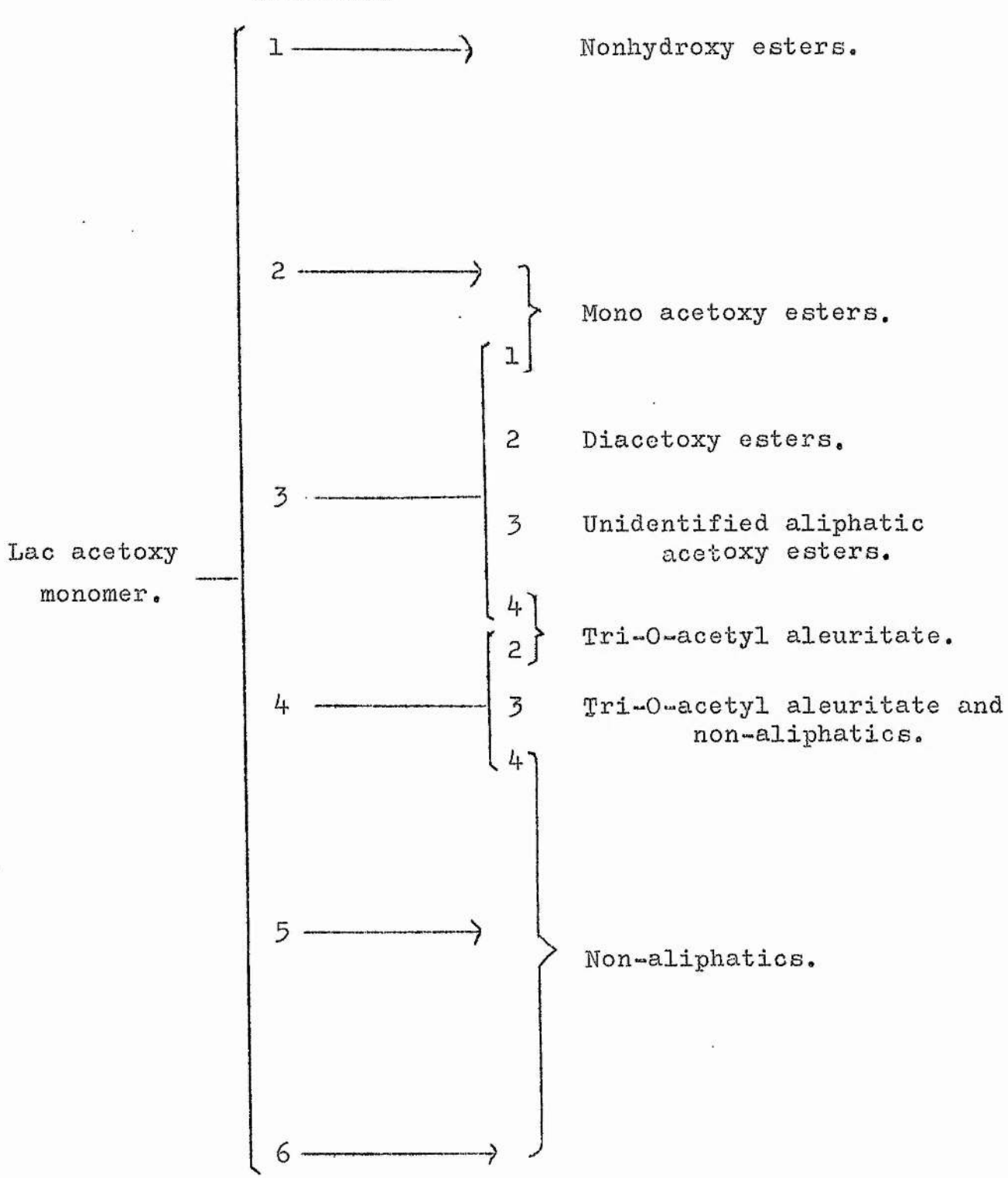
4. Chromatographic Examination of the Polymer - free Mixed Esters.

The shellac mixed acids, obtained as before, were methylated with diazomethane and the resulting mixed esters acetylated. On taking up the acetoxy esters in ether, a brown solid (approx. 2%) separated out and may be a polyhydric alcohol, carbohydrate or similar material. The determination of its exact structure was deemed to lie outside the scope of this study so it was not subjected to detailed examination.

Distillation separated the acetoxy esters into monomeric (67% of the recovered esters) and polymeric fractions. G.L.C. examination of the monomer gave peaks for all the acids already identified and for a large number of other unknown acids.

The monomeric acetoxy esters were chromatographed on silicic acid in five lots and corresponding fractions combined. G.L.C. showed that the first two (approx. 10%) of the six fractions collected contained the non-hydroxy and mono-hydroxy acids already identified. The third (approx. 35%) contained a large amount of aleuritic acid with a number of unknown components. This fraction was rechromatographed in two lots and four subfractions obtained of which the second consisted of two esters of C.No. 18.6 and 20.3 (SE-30). Rechromatography of this gave pure samples of each ester for structure determination. They were shown to be derived from threo 9,10-dihydroxytetradecanoic acid and the similar hexadecanoic

Separation Scheme 2.



acid as will be described. Fraction 3-3 was much more complex than fraction 3-2. On rechromatography, some simplification was achieved but no fraction had more than 50% of any single ester. Some of the more promising fractions were examined but did not contain any vic-dihydroxy acids which might be seperable as the isopropylidene derivatives.

It was felt that, if a suitable preparative G.L.C. instrument were available, some useful separations might be achieved. Further adsorption chromatography, however, would serve no purpose so the fractions were not examined in greater detail.

Threo 9,10-dihydroxytetradecanoic acid.

The acetoxy ester of C.No. 18.6 (SE-30) was shown to be derived from the above acid on the following evidence.

i. Iodination - deiodination of the hydroxy acid gave a product indistinguishable in its chromatographic behaviour from methyl tetradecanoate.

ii. Preliminary oxidation of the hydroxy ester with chromic oxide in acetone and sulphuric acid[70] indicated the presence of a glycol group and, in fact, the ester formed an isopropylidene derivative. The hydroxy acid was subjected to von Rudloff oxidation[116]. The major product - nonanedioic acid - was recognised by G.L.C. of its dimethyl ester (C.No. 11.8, Ap.L.; 14.6, QF-1.). The other product, presumably pentanoic acid, was not found, but would easily be lost in working up the reaction mixture because of the volatility of its methyl ester.

iii. When the hydroxy ester was chromatographed on a T.L.C. plate impregnated with boric acid[99] with methyl threo 9,10-dihydroxyoctadecanoate as standard, the two ran very close together. This would suggest that it, also, is threo in configuration.

iv. Samples of the hydroxy acid and its methyl ester were obtained which gave satisfactory analyses. The melting point is similar to that reported for the acid prepared synthetically[121].

The acid was not found to be optically active. It has not been reported from any other natural source though it has been synthesised[121].

Threo 9,10-dihydroxyhexadecanoic acid.

The acetoxy ester of C.No. 20.3 (SE-30) was shown to be derived from the above acid in an analogous manner to that of the similar C_{14} acid. It also was not optically active.

The acid has not been reported from any other natural source although it has been synthesised[122].

The remaining fractions.

Fraction 4 was rechromatographed on silicic acid and four sub-fractions collected. The first three contained tri-O-acetyl aleuritate but the fourth had two major components of C.No. 21.25 and 21.9 (SE-30). The hydroxyesters were subjected to iodination - deiodination but on G.L.C. examination of the product nothing was eluted and T.L.C. showed that a complex mixture of polar compounds were formed. It seems likely, therefore, that these are not aliphatic

hydroxy acids but may be shellolic, jalaric or related acids. Fractions 5 and 6 were found to contain similar material and were not examined further.

The Polymer.

This was not examined further because the high temperature during the distillation might have affected it so that any results could not be relied upon.

On the basis of these last separations, the following rough estimate can be made of the percentages of the known constituents of shellac.

Acid.	%.
Non-hydroxy acids.	0.5
6-ketotetradecanoic acid.	0.25
6-hydroxytetradecanoic acid.	5.0
ω-hydroxyhexadecanoic acid.	0.5
ω-hydroxyhexadec-9-enoic acid.	1.5
threo 9,10-dihydroxytetradecanoic acid.	3.0
threo 9,10-dihydroxyhexadecanoic acid.	1.0
aleuritic acid.	22.0
non-aliphatic acids.	24.0
unidentified aliphatic acids.	8.0
unidentified polyhydric compound.	2.0
polymer.	32.0

Table 2.

Conclusions.

This study, therefore, has resulted in the following discoveries on the composition of lac resin.

1. The presence of ether linkages, not cleaved by alkaline hydrolysis thus leading to mixtures of monomeric and ether-linked polymeric fragments, has been recognised.

2. The nature of 12% of the component acids, not previously identified has been elucidated, including a number having a chain of 14 carbon atoms, where only those of 16 were known or suggested. The new acids are the nonhydroxy acids (C_{14}, C_{16} and C_{18} saturated and unsaturated; 0.5%), 6-ketotetrdecanoic acid (0.25%), 6-hydroxytetradecanoic acid (5%), ω-hydroxyhexadecanoic acid (0.5%), ω-hydroxyhexadec-9-enoic acid (1.3%), threo 9,10-dihydroxytetradecanoic acid (3%) and threo 9,10-dihydroxyhexadecanoic acid (1%).

EXPERIMENTAL.

Column adsorption chromatography.

Chromatography was carried out on neutral alumina or silicic acid. The former was prepared[72] by shaking alumina (1 Kg., Peter Spence - grade H) with ethyl acetate (2l.) for two days. The alumina was filtered off, washed with boiling water (5l.), heated at 250°C for two days and allowed to cool in a desiccator.

The silicic acid (Mallinckrodt - 100 mesh) was activated at 120° for 24 hours immediately before use. It was then slurried with petroleum ether (40-60°):diethyl ether (4:1) and packed under nitrogen pressure (up to 8lbs/sq.in.). When chromatographing hydroxy esters, the column was washed with benzene before loading; when chromatographing the methoxy or acetoxy derivatives, it was washed with petroleum ether:benzene (85:15) then petroleum ether alone. Columns were run under pressures of up to 8lb/sq.in. giving flow rates of 1 - 3ml/min. Suction from below gave less reproducable results.

All solvents were carefully purified before use - diethyl ether and benzene being distilled from sodium and kept over sodium wire. Petroleum ether was washed with concentrated sulphuric acid (3 times), water (3 times), dried and distilled through a Vigreux column, the portion boiling between 40°-60° being retained.

Thin layer chromatography.

Thin layers of Kieselgel G (as described by Stahl) were applied to glass plates (20x20cm. or 20x5cm.) in a thickness of

250μ by a commercial applicator. The plates were dried and activated by heating at 120° for half an hour and stored in a desiccator.

5μl. of a chloroform solution (10%) of the esters was applied to the plates with spots 1-2cm. apart on a line 1-2cm. from the bottom of the plate. Plates were developed by ascending elution in sealed tanks, lined with filter paper and containing the eluting solvent (usually pet. ether, ether, methanol mixtures) to a depth of 0.5cm. Spots were made visible by exposure to iodine and chromatograms recorded by photographing.

Plates impregnated with boric acid or silver nitrate[99] were prepared as required by spraying normal plates with saturated solutions of boric acid in methanol or silver nitrate in water and re-activating as before.

Gas liquid chromatography.

Gas chromatography of the methyl esters was carried out on a Pye Argon Chromatograph with a Sr^{90} β-ray ionisation detector. The columns (glass, 4' long) were packed with 2½, 5, 10 or 20% Apiezon L. on alkali-washed celite[123], and 10% QF-1[124] (Dow-Corning, fluoro-silicone) or SE-30[125] (Applied Science Laboratories Inc., silicone gum) on acid-washed celite. The columns were operated at 150-225°C with an argon gas flow of 33.3ml./min. Samples were injected as liquids or in ether solution from 0.025 or 0.1μl. pipettes after stopping the gas flow for a moment. Retention times were measured from the negative air peaks and the results expressed as carbon

numbers ("C.No.")[106], which were found from a straight line plot of log(retention volume) for a series of saturated ester standards against the chain-lengths of the esters.

Acids were converted to methyl esters for G.L.C. examination by refluxing for two hours with a fifty fold excess of dry methanolic hydrogen chloride (2%). At the end of this time, water was added and the solution ether extracted. The ether was washed with 0.5N sodium bicarbonate then water and dried over anhydrous sodium sulphate. Alternatively, with small amounts of esters, boron trifluoride/methanol complex was used[126]. The acids were refluxed with excess of the reagent in methanol (12½%) for two minutes, then poured into water and ether extracted as above. This method, however, has been shown to produce methoxy artefacts from unsaturated acids[127] and to demethylate methoxy acids[128] so care must be taken in using it.

Methylation of hydroxy esters.

Attempted methylation of methyl threo 9,10-dihydroxyoctadecanoate[104].

Methyl threo 9,10-dihydroxyoctadecanoate (219mg.) was dissolved in dimethyl formamide (2.5ml.) and methyl iodide (1ml.) at room temperature, silver oxide (1.0g.) added over half an hour and the mixture shaken for 24 hours. The solution was filtered and the salts washed with dimethyl formamide (10ml.) and added to a solution of potassium cyanide in water (2%, 100ml.). The products (217mg.) were ether extracted. G.L.C., however, showed that reaction was not complete as there were two approximately equal peaks of C.No.s 19.7

and 20.4 (2½% Ap.L.).i.e. for the dimethoxy and methoxy-hydroxy esters. T.L.C. also showed two spots. The pure dimethoxy ester was recovered after chromatography on neutral alumina (40g. alumina; eluting solvent, benzene:ether 3:1) in 52% yield.

In a similar experiment in which the reaction mixture was refluxed, no improvement was detectable.

Methylation of methyl aleuritate. Methyl aleuritate (108mg.) was dissolved in sulpholane (4ml.) and methyl iodide (1ml.) and silver oxide (450mg.) added. The mixture was refluxed (at approx. 45°) for 8 hours. A solution of potassium cyanide in water (50ml.,2%) was added and the solution extracted with ether. G.L.C. showed only one product with some unremoved sulpholane and T.L.C. confirmed this. The pure trimethoxy ester was obtained (85mg., 73.3%) after chromatography on neutral alumina (eluting solvent, benzene:ether 3:1), the sulpholane remaining on the column.

Methylation of other hydroxy esters. The method was repeated on three other hydroxy esters. Dimethyl shellolate (243mg.) gave the required methoxy ester (70mg., 31%), methyl 12-hydroxyoctadecanoate (70mg.) gave the methoxy ester (46mg., 67.5%) and methyl erythro 9,10-dihydroxyoctadecanoate (150mg.) gave the methoxy ester (91mg., 60.5%).

All the methoxy esters were liquid at room temperature. G.L.C. gave the carbon numbers quoted in table 1 (2½% Ap.L., 200°). A vicinal dimethoxy group increases the retention time by 1.7 carbon units, an ω-methoxy group increases it by 2.0 units and a secondary

methoxy group by 1.1 units.

Hydroxy ester (methyl).	C.No.s. Before - .	After -.
Threo 9,10-dihydroxystearate.	21.3.	19.7.
Erythro " .	21.3.	19.7.
Shellolate.	- .	19.1.
Aleuritate.	- .	19.7.
12-hydroxystearate.	19.6.	19.1.

Table 1.

Acetylation of hydroxy esters.[84]

Methyl 16-hydroxypalmitate (103mg.) was dissolved in acetic anhydride (2ml.) and kept at 100° for 4 hours. The acetic anhydride was removed using a rotary film evaporator to yield the required acetoxy ester (116mg., 98.3%). G.L.C. and T.L.C. confirmed its purity.

Methyl 12-acetoxystearate, 9,10,16-triacetoxypalmitate, 9,10-diacetoxystearate and diacetoxy-shellolate were prepared similarly, in equally good yield, from the corresponding hydroxy esters. In each case, G.L.C. and T.L.C. showed that reaction was complete. All are liquid except the last which was a crystalline solid (recrystallised from benzene/pet. ether, m.p. 123-124°. lit[43] 126-127.5°). Carbon numbers were obtained on 10% SE-30 at 225° (table 2).

A vicinal diacetoxy group increases the retention time by 4.2 carbon units, an ω-acetoxy group by 3.8 units and a secondary acetoxy by 2.6 units.

Acetoxy ester (methyl).	C.No.
16-acetoxypalmitate.	19.8.
12-acetoxystearate.	20.6.
9,10,16-triacetoxypalmitate.	23.8.
9,10-diacetoxystearate.	22.2.
diacetoxy-shellolate.	21.6.

Table 2.

Solvent extraction of shellac.

Angelo Super Blonde shellac (commercially dewaxed) was used throughout all this investigation.

Finely ground shellac (100.2g.) was packed into a soxhlet extractor and extracted with pet. ether (40-50°) for 24 hours and then with diethyl ether. After a few hours with the latter solvent, the soxhlet gummed up so further extraction was impossible. The pet. ether extract (0.427g., 0.43%) was retained.

Similarly, when shellac was stirred continuously with ether in the cold using a Hershberg stirrer, the mixture went solid.

Finely ground shellac (100.2g.) was, accordingly, mixed with Hyflo Supercel (72g.) and placed in thimbles in a soxhlet extractor. The mixture was extracted for 24 hours with pet. ether as before, then with ether. In this case, it was found necessary to remove the shellac from the thimbles every 8 hours, vacuum off the solvent and regrind the mixture in a homogeniser. After 24 hours extraction, the ether was replaced by chloroform and the process repeated. Extraction with ethyl acetate, acetone and methanol followed in the same manner.

The extracts obtained in this way were -

Solvent.	Weight (g.≡%).	Description.
Pet. ether.	0.2	White waxy solid.
ether.	18.0	viscous yellow oil.
chloroform.	23.2	light yellow solid.
ethyl acetate.	14.0	yellow glassy solid.
acetone.	7.1	dark yellow glassy solid.
methanol.	4.8	brown glassy solid.

Table 3.

67.3% (67.3g.) of the shellac was recovered in this way though the residue, mixed with supercel, was retained for further examination if necessary.

Petroleum ether extract.

The first pet. ether extract (0.427g.) was hydrolysed with aqueous alcoholic sodium hydroxide (1N, 5ml.) at room temperature overnight and the free acids liberated by passing them down an ion-exchange column (Zeo-Karb 225). The acids (0.377g.) were obtained on removal of the solvent in a rotary film evaporator at a temperature not greater than 45°. The methyl esters (0.366g.) were prepared by refluxing with methanolic hydrogen chloride. G.L.C. examination showed that the main acid present was a hexadecenoic acid (C.No. 15.8., 10% Ap.L.) with a small amount of myristic acid (C.No. 14.0) and minor amounts of palmitic (C.No. 16.0) and tetra-decenoic (C.No. 13.8) acids. T.L.C. confirmed that no oxygenated acids were present.

The pet. ether extract from the second abortive extraction (0.33g.) was divided into acidic and neutral fractions by dissolving it in ether and extracting with aqueous alkali. The acid fraction (0.176g., 70% of the recovered material) was methylated. The neutral fraction (0.076g., 30%) was hydrolysed and the methyl esters prepared. G.L.C. indicated little difference between the two, the same acids being present in approximately the same proportions as before.

Most of the acids must, therefore be present in the extract as the free acids, though a little, the neutral portion, may be some residual wax.

The ether extract.

A portion of the extract (13.27g.) was hydrolysed and the free acids obtained as before (12.95g.). The methyl esters (12.86g.) were obtained by refluxing with methanolic hydrogen chloride. T.L.C. showed that the mixture was extremely complicated and seemed to include mono, di and tri-hydroxy esters. The major component observed by G.L.C. was of C.No. 16.2 (2½% Ap.L.) with small amounts of esters of C.No.s 14.3, 18.6, 18.8, 18.0 and 17.8 (in approx. order of decreasing magnitude), though most of the esters were not eluted.

Methylation of the ether extract methyl esters. The methyl esters of the ether extract (209mg.) were methylated using methyl iodide and silver oxide in sulpholane as described previously. The crude products (185mg.) were chromatographed on neutral alumina (40g.) and

two fractions obtained. The first (87mg.; eluting solvent, benzene: ether 3:1) was mainly an ester of C.No. 15.4 (10% Ap.L.) with slightly less of esters of C.No.s 17.8 and 17.9 and minor amounts of esters of C.No.s 16.0, 17.2 and 19.1. The second (41mg.; eluting solvent, benzene: ether 1:1) was mainly trimethoxy palmitate (C.No. 19.8) with some methoxy shellolate (C.No. 19.1).

Chromatography of the ether extract methyl esters. The methyl esters (7.17g.) were chromatographed on neutral alumina (225g.) eluting with 1 litre lots of benzene/ether and ether/methanol mixtures. The results are summarised in table 4. The esters recovered (5.35g., 74.6%) were examined by G.L.C. and T.L.C.

No.	Solvent*	Weight (g.)	% of load.
1	B	0.132	1.9
2	BE(25)	0.117	1.6
3	BE(50)	0.017	0.2
4	BE(75)	0.538	7.5
5	E	1.818	25.3
6	EM(2)	1.323	18.5
7	EM(5)	0.707	9.9
8	EM(10)	0.439	6.1
9	M	0.258	3.6

Table 4.

* B=benzene, E=ether, M=methanol; the figure in parentheses indicates the percentage by volume of the second component in the solvent mixture.

Of the nine fractions obtained, only the first four could be examined properly by G.L.C., the rest being too polar, and it was necessary to methylate them for satisfactory G.L.C. examination. The fractions were also run on T.L.C. against a series of hydroxy ester standards and fair separation according to polarity was observed.

As later work lessens the value of much of this, the results obtained for each fraction are only discussed briefly. No attempt was made at accurate quantitisation by G.L.C., because of the uncertain response of the detector towards oxygenated esters and since conversion factors cannot be applied to unknown mixtures

Fraction 1. G.L.C. and T.L.C. indicate that this contains additional non-hydroxy acids. It is mainly palmitic (C.No. 16.0, 10% Ap.L) and myristic (14.0) acids with a small amount of corresponding mono-unsaturated acids (15.8 and 13.8) and a small amount of similar C_{18} acids (C.No.s 17.8 and 18.0). on hydrogenation, the only acids apparent are myristic, palmitic and a little stearic.

Fraction 2. G.L.C. (2½% Ap.L.) showed a complex mixture of esters with principal peaks at C.No.s 16.7, 18.8 and 19.1, smaller peaks at 17.9, 17.5 and 18.0, and a large number of minor peaks. T.L.C. gave a large single spot running between non-hydroxy and mono-hydroxy standards. On hydrogenation, large peaks appeared at C.No.s 18.0 and 15.2, while those at 18.8, 19.1 and 16.7 disappeared.

Fraction 3. G.L.C. showed major peaks (2½% Ap.L.) of C.No.s 16.2 and 20.1 with a large number of minor peaks including those at 16.6, 16.9, 18.8, 19.1, 18.3, 17.8 and 18.0. T.L.C. showed two spots - one just above a mid-hydroxy standard and the other just below it.

Fraction 4. G.L.C. showed one large peak at C.No. 16.2 and minor peaks at 18.5 & 18.8. T.L.C. gave 2 spots, one running just above and the other just below methyl 12-hydroxystearate (standard). The acid giving the peak at 16.2 was subsequently shown to be 6-hydroxy-tetradecanoic acid by various degradative and synthetic studies detailed later. Later work also indicates that the other two peaks are 16-hydroxyhexadec-9-enoic acid and the corresponding saturated acid.

Fraction 5. G.L.C. showed peaks at C.No.s 16.1, 18.5 and 18.8 mainly with a minor peak at 17.7, but much of the material is not eluted. T.L.C. showed a complex range of spots. Attempts to improve the separation by chromatographing on neutral alumina, using benzene/chloroform mixtures as the eluting solvent, was not particularly successful, some separation being achieved but not sufficient to warrant persuance of the method. With preparative scale T.L.C., the yields and separations were also disappointing.

A portion of the fraction was methylated using the sulpholane procedure and the products purified by chromatography on neutral alumina. The main fraction (77.6% of the product) was a fairly complex mixture exhibiting peaks on G.L.C. of C.No.s -

$$15.3 > 18.0,\ 17.8 > 16.0 > 17.2,\ 19.1$$

- while the remaining major fraction (14.5%) has -

$$17.15 > 17.8,\ 18.0 > 19.3 > 19.7 > 16.15 > 15.3$$

The peaks of C.No. 19.1 and 19.7 are possibly from methoxy shellolate and methoxy aleuritate respectively. Those of C.No. 15.3, 18.0 and 17.8 are probably the mono- methoxy derivatives of the

hydroxy esters found in fraction 4. On hydrogenation of the methoxy compounds, the peak at 17.8 disappeared and that at 18.0 increased.

Iodination - deiodination[107] (detailed method given later) showed that C_{16} and C_{14} acids were present in approximately equal amounts.

Fraction 6. The esters were too polar for direct examination by G.L.C. but T.L.C. showed 3 spots - one running just above dimethyl shellolate, one corresponding to it and one to methyl aleuritate. A portion was methylated by the usual procedure and the products chromatographed on neutral alumina. The first major fraction (41.4%) had peaks at

17.2, 16.0 > 18.2, 18.7 > 19.1, 17.8 > 15.2

The next fraction (40%) consisted of material of C.No. 19.1, presumably dimethyl shellolate (methoxy derivative).

Fractions 7,8 and 9. T.L.C. of these fractions showed spots for all running with methyl aleuritate and also more polar esters. The fractions were combined and rechromatographed on neutral alumina with a more gradual change of solvent system, but no real improvement in separation was evident.

The other extracts.

The chloroform extract (15.12g.) was hydrolysed, the acids (14.96g.) liberated and the methyl esters (13.79g.) prepared as before. A portion of the esters (6.15g.) was chromatographed on neutral alumina (225g.) using the same solvent system as with the ether extract esters, so a total of 9 fractions (5.96g., 77.6%) was

collected.

The ethyl acetate extract (8.05g.) was hydrolysed, the acids (7.64g.) liberated and the methyl esters (6.72g.) prepared as before. A portion of these (6.41g.) was chromatographed as above and nine fractions recovered (5.98g., 93.2%).

The acetone extract (5.63g.) was hydrolysed, the acids (5.61g.) liberated and the methyl esters (5.56g.) prepared. A portion of these (5.18g.) was chromatographed as above and nine fractions (4.74g., 91.3%) recovered.

The methanol extract (4.15g.) was hydrolysed, the acids (4.14g) liberated and the methyl esters (3.99g.) prepared. A portion of these (3.94g.) was chromatographed as before and nine fractions (3.58g., 91.1%) recovered.

No.	% of load.			
	chloroform	ethyl acetate	acetone	methanol
1	0.6	0.3	0.1	0.1
2	0.7	0.7	0.2	0.2
3	6.7	11.6	2.2	0.1
4	27.1	33.4	24.2	20.6
5	8.4	15.3	9.5	11.3
6	19.0	17.5	28.2	23.1
7	7.2	5.6	10.9	19.1
8	4.4	4.5	8.1	8.1
9	3.5	4.3	7.9	8.5

Table 5.

The fractions were examined by T.L.C. and by G.L.C. where possible. No real difference in the types of acid in corresponding fractions was evident. The proportion of more polar material increased in the later extracts as can be seen from the summary of the results in table 5.

6-hydroxytetradecanoic acid.

The ether extract methyl esters - fraction 4 was a white waxy low melting solid, which consisted largely of a single ester (C.No.s 16.2, 2½% Ap.L.; 15.8, 10% Ap.L.; 18.0, QF-1.). The fraction (0.486g) was rechromatographed on neutral alumina (40g.) with the hope of removing most of the impurities, eluting with benzene/ether mixtures (100ml. lots) and collecting six fractions (0.449g., 92.5%) as detailed in table 6.

No.	Solvent.	Weight (g.)	% of load.
1	BE(50)	0.010	2.2
2	BE(60)	0.004	0.7
3	BE(65)	0.070	14.4
4	BE(70)	0.333	68.5
5	BE(75)	0.029	6.0
6	E	0.003	0.7

Table 6.

G.L.C. and T.L.C. showed that the fourth and largest fraction was almost entirely the single acid required and the degradative procedures necessary to establish its structure were carried out on

this material.

Chain-length determination (Iodination - deiodination)[107].

The ester (52mg.), iodine (270mg.) and red phosphorus (620mg.) were heated together at 100° for an hour after which the remaining iodine was removed under reduced pressure. The residue, dissolved in ether, was washed successively with water, sodium bisulphite solution and water. The iodinated ester (67mg.), remaining after removal of the solvent, was refluxed for four hours with methanolic hydrogen chloride (5%, 5mls.) and activated zinc (approx. 200mg.). This was prepared as required by refluxing zinc dust (1g.) with methanol (5ml.) and hydrogen iodide (0.1ml.) for 5 minutes, decanting the solvent and washing thoroughly with methanol. The product (34mg), recovered by ether extraction, was indistinguishable from methyl tetradecanoate in its chromatographic behaviour.

The position of the hydroxyl group.

The ester (50mg.) was dissolved in acetic acid (1ml.) and oxidised at room temperature by chromic anhydride (50mg.) in acetic acid solution (1ml.). After one hour, water (10ml.) was added, excess oxidant was destroyed by sulphur dioxide, and the keto ester (45mg., C.No. 15.4, Ap.L.) was recovered. Dissolved in 80 %ethanol (1ml.), this was refluxed with solutions of hydroxylamine hydrochloride (85mg.) and fused sodium acetate (82mg.) in 80 % ethanol (2ml.). Thereafter, the oximes (46mg.) were recovered and heated to 100° with concentrated sulphuric acid (0.2ml.) for one hour to effect Beckmann rearrangement. After cooling, water (0.4ml.) was added and the mixture boiled for three hours to hydrolyse the amides. The

resulting monobasic acid was extracted with light petroleum and the dibasic acid subsequently with ether. After methylation, these were examined by G.L.C. and shown to be methyl nonanoate (C.No. 9.0, Ap.L. and QF-1) and methyl adipate (C.No.s 9.0, Ap.L.; 11.5, QF-1).

Characterisation of the acid.

By hydrolysis of the hydroxy ester, followed by recrystallisation from pet. ether, a sample of the acid m.p. 58-58.5° was obtained. The corresponding keto acid was also obtained by oxidation of the hydroxy ester as described above followed by hydrolysis and recrystallisation from pet. ether, m.p.70-71°. The semicarbazone of the 6-keto acid was also prepared[58]. The keto acid (177mg.) was dissolved in the minimum quantity of alcohol and a solution of semicarbazide hydrochloride (184mg.) and sodium acetate (166mg.) in water (3ml.) added. The mixture was heated for 5 minutes and allowed to stand overnight. The crude semicarbazone (113mg.) which separated out was filtered off and recrystallised from ethanol/water to yield the pure compound (53mg., 24.3%), m.p. 129-131°. (Found: C, 60.40; H, 9.81; N, 13.97. $C_{15}H_{29}O_3N_3$ requires C, 60.16; H, 9.77; N, 14.04.).

Optical activity.

The optical activity of the ester was determined as $(\alpha)_D^{19°}$ -0.9°, $(M)_D^{19°}$ -2.3° (ethanol solution, c 6, l 2dm.).

The racemic hydroxy ester was also prepared. The keto ester (145mg., prepared as above) was dissolved in methanol (90%, 25ml.) and excess sodium borohydride added over half an hour. The solution

was then added to a large volume of water and ether extracted. The ether was removed to yield the hydroxy ester (0.130g., 88.9%). G.L.C. showed a single product of C.No. 16.2 (2½% Ap.L.). The ester was hydrolysed to yield the crude acid (125mg.) which was recrystallised from light petroleum to give the racemic 6-hydroxytetradecanoic acid (84mg., 76.7%) m.p. 61.5-63°.

Synthesis of 6-ketotetradecanoic acid and (±)-6-hydroxytetradecanoic acid.

Nonanoyl chloride (6.67g.) in dry chloroform (20ml.) was added over 15 mins. to a stirred solution of 1-morpholinocyclopent-1-ene [129] (5.10g.), triethylamine (4.02g., distilled from sodium) and dry chloroform (40ml.) at 35°. After a further 15 mins., 6N hydrochloric acid (20ml.) was added and the two phase mixture stirred vigorously for 30 mins. at 35°. The chloroform layer was washed with water until the washings had pH 5-6 and the water layer, together with the aqueous washings were neutralised with dilute sodium hydroxide to pH 5-6 and then extracted with chloroform. The combined chloroform extracts yielded 2-nonanoylcyclopentanone (6.35g., 74%, b.p. 116-120°/0.4mm), which gave a deep wine red colour with a methanolic solution of ferric chloride.

The diketone was refluxed with 5% aqueous potassium carbonate solution (250ml.) for 2½ hours and, after removal of neutral material by ether extraction, the aqueous layer was acidified hydrochloric acid) and 6-ketotetradecanoic acid (4.98g.,73%, m.p. 66-69°) recovered. After recrystallisation from petroleum ether,

this acid (3.84g., 41% based on the enamine) melted at 71-71.5° (lit[114]. 71.5°) alone or when mixed with the keto acid derived from the natural acid, (Found: C, 68.9; H, 10.7. Calc. for $C_{14}H_{26}O_3$: C, 69.3; H, 10.8). Semicarbazone, m.p. 128-130° (lit[114] 130°) alone or when mixed with a sample derived from the natural acid, (Found: C, 60.4; H, 9.4; N,14.1. Calc. for $C_{15}H_{29}O_3N_3$: C, 60.2; H, 9.8; N, 14.0). The methyl ester of the keto acid had C.No.s 15.4 (Ap.L.) and 18.8 (QF-1).

Excess of sodium borohydride (2.11g.) was added to the keto ester (1.0g.) in methanol (50ml.) over ½ hour. Diluted with water and extracted with ether, the reaction mixture furnished the hydroxy ester (0.98g., 98%) of C.No. 15.8 (10% Ap.L.) and 18.0 (QF-1). Hydrolysis gave the (±)-hydroxy acid (78%), m.p. 65-66° (from light petroleum) alone and 63-65° when mixed with the (±)-hydroxy acid derived from (-)-6-hydroxytetradecanoic acid, (Found: C, 69.2; H, 11.6; Calc. for $C_{14}H_{28}O_3$: C, 68.8; H, 11.6).

Chromatography of other fractions.

The chloroform extract - fraction 4 (0.64g.) was rechromatographed on silicic acid (35g.), loading in benzene and eluting with various benzene/ether mixtures using suction from below to increase the flow rate. 11 fractions (0.544g., 85.1%) were collected. Preliminary examination by T.L.C. showed that a reasonable degree of separation had been achieved. Similar quantities of esters from the ethyl acetate extract methyl esters - fractions 3,4 and from the chloroform extract - fraction 3 were chromatographed in the same

way and corresponding fractions combined. For the four columns, from the esters loaded (2.560g.), 11 combined fractions (2.352g., 91.8%) were recovered as detailed in table 7.

No.	Solvent.	Weight (g.)	% of load
1	B	0.007	0.3
2	BE(10)	0.013	0.5
3	BE(20)	0.272	10.6
4	BE(30)	0.297	11.6
5	BE(40)	0.321	12.5
6	BE(50)	0.304	11.9
7	BE(60)	0.330	12.9
8	BE(70)	0.356	13.9
9	BE(80)	0.206	8.0
10	BE(90)	0.133	5.2
11	E	0.113	4.4

Table 7.

On examination by G.L.C. and T.L.C., the first three fractions were found to contain esters already examined. The fourth fraction showed one prominent peak (C.No.s 19.4, 2½% Ap.L.; 22.8, QF-1) and a number of minor peaks. Later fractions appeared more complex. The 4^{th} and 5^{th} fractions (0.383g.) were rechromatographed on silicic acid in the hope of improving the separation. Eight fractions (0.341g., 88.9%) were collected as detailed in table 8.

Fraction 4-4 gave two spots on T.L.C. though on G.L.C. only one peak was evident. (C.No. 22.8., QF-1). This was, therefore,

examined further

No.	Solvent	Weight (g.)	% of load
4-1	B	0.002	0.4
-2	BE(10)	0.003	0.7
-3	BE(20)	0.029	7.5
-4	BE(25)	0.138	36.0
-5	BE(30)	0.084	22.0
-6	BE(35)	0.047	12.4
-7	BE(40)	0.029	7.6
-8	BE(50)	0.009	2.3

Table 8.

Chain-length determination. The esters (27mg.) were subjected to iodination - deiodination as described earlier to yield the saturated non-hydroxy esters (18mg.). C_{14} and C_{16} were found in the approximate ratio 1:3.

Oxidation[70]. To the methyl esters (24mg.) in acetone (2ml.) was added dropwise a solution of chromic oxide (25mg.) in water (1.5ml.) and concentrated sulphuric acid (0.4ml.). After shaking for half an hour, the mixture was poured into dilute alkali and ether extracted for the neutral material (2mg.). Acidification and re-extraction gave the acidic material (20mg.) from which the methyl esters were prepared. G.L.C. of these showed a number of products of low C.No. (6.0-12.0, Ap.L.) and one other peak (C.No.s 18.2, Ap.L.; 24.9, QF-1).

It seemed likely that two acids were present, a mid.ω-di-

Mean?

hydroxy C_{14} acid (showing up on G.L.C.) and a vic.dihydroxy C_{16} acid not eluted. By extrapolation from known compounds, the C.No. of the former would be 19.0 (Ap.L) or 22.8 (QF-1) and the keto-dibasic acid formed on oxidation would have a C.No. of 18.3 (Ap.L.) or 24.4 (QF-1) as can be seen from table 9.

Dihydroxy ester.

	C.No.			Phase.
Chain	ω-OH	Mid-OH	Total	
14	2.8	2.2	19.0	Ap.L.
14	4.8	4.0	22.8	QF-1.

Keto-dibasic ester.

Chain	ω-ester	Keto	Total	Phase
14	2.9	1.4	18.3	Ap.L.
14	5.6	4.8	24.4	QF-1.

Table 9.

When the esters were chromatographed on normal T.L.C. plates and on plates sprayed with boric acid[99], one spot ran beside methyl threo 9,10-dihydroxyoctadecanoate on both. Attempts to use this on a large scale to separate the esters by impregnating silicic acid with sodium borate, as has been reported for silver nitrate[115], were unsuccessful.

More of the esters were obtained similarly by repeated chromatography of the ethyl acetate extract - fraction 4 and the acetone extract - fractions 3 and 4.

Position of the vic.dihydroxy group[130]. The acids (73mg.), prepared

by hydrolysis of the esters, were dissolved in a solution of potassium carbonate (87mg.) in water (66ml.) and potassium periodate (180mg.) and potassium permanganate (2mg.) in water (32ml.) added. After shaking for 24 hours, the solution was acidified and thoroughly extracted with ether. The acids (88mg.) were methylated for G.L.C. examination. Two fragments were evident of C.No.s 7.0 and 11.9 (Ap.L.) and 7.0 and 14.6 (QF-1). These correspond to a C_7 monobasic acid and a C_9 dibasic acid, suggesting that the original acid was threo 9,10-dihydroxyhexadecanoic acid.

Isopropylidene compounds[23]. The esters (125mg.) were dissolved in dry acetone (3ml.) and anhydrous copper sulphate (approx. 0.2g.) added. After 24 hours, the solution was filtered, the copper salts washed with dry ether and the combined solutions evaporated to yield the isopropylidene compounds (0.118g.). After chromatography on neutral alumina, the pure isopropylidene compounds (26mg., 22% eluting solvent - benzene:ether 3:1) were recovered. G.L.C. showed a major peak of C.No. 18.4 (QF-1) as expected with a smaller one (<10%) at 16.4, presumably a similar C_{14} acid. The ester of C.No. 22.8 (QF-1) was also recovered (0.073g. , 61.5%).

Oxidation of the mid.ω-dihydroxy acid. The ethyl ester of the acid was prepared and oxidised by Matic's procedure[70]. The ester (188mg.) was dissolved in acetic acid (1ml.) and shaken with a slight excess of chromic oxide (approx. 18mg.). After 24 hours, the mixture was added to water (50ml.), excess oxidant destroyed with sulphur dioxide and the solution ether extracted. The acids were methylated

with diazomethane[131] to avoid interesterification and the products examined by G.L.C.

The results proved difficult to interpret, being more complex than expected. However, the major peak occurs at C.No. 12.0 (Ap.L.), 14.6 (QF-1), i.e. equivalent to a C_9 dibasic acid, so it seems likely that the acid is principally 6,14-dihydroxytetradecanoic acid, though a mixture of isomers may be present. More information is necessary.

Chromatography of the total lac esters.

Shellac (104.8g.) was hydrolysed with aqueous alcoholic potassium hydroxide (1N, 250ml.) at room temperature overnight and the free acids (102.6g.) liberated on an ion-exchange column (Zeo-Karb 225) and recovered after removal of the solvent in a rotary film evaporator ($<45^{\circ}$). The methyl esters (99.7g.) were prepared by refluxing with methanolic hydrogen chloride.

Two approximately equal batches of the esters were chromatographed on neutral alumina[72] (700g. each), loading in benzene and eluting with 21. lots of solvent. Corresponding fractions from the two columns were combined, 6 fractions (88.17g., 88.6 % being recovered as detailed in table 10. T.L.C. showed that a reasonable separation had been achieved.

Fraction 1. was rechromatographed on neutral alumina (500g.) to recover the non-hydroxy esters (eluted with 2l. benzene). Only two fractions (5.248g., 78%) were collected (table 11).

No.	Solvent*	Weight (g.)	% of load.
1	B	6.98	7.0
2	BE(50)	5.85	5.9
3	E	11.22	11.3
4	EM(5)	51.94	52.1
5	M	10.93	11.0
6	MW(10)	1.25	1.3

Table 10. *W = Water

No.	Solvent	Weight (g.)	% of load.
1A	B	0.482	6.9
1B	EM(5)	4.946	71.1

Table 11.

T.L.C. and G.L.C. confirmed that the desired separation had been achieved.

The non-hydroxy esters.

G.L.C. showed that the usual pattern of saturated and unsaturated esters had been recovered. A portion of these (0.174g.) was chromatographed on silicic acid impregnated with silver nitrate[115] (30g.) and eluted with 100ml. lots of light petroleum/ether mixtures, in order to separate the saturated from the unsaturated esters. Five fractions (0.156g., 89.6%) were recovered as detailed in table 12. G.L.C. showed that fraction 4 contained only the C_{14}, C_{16} and C_{18} mono-unsaturated esters, the saturated esters being in the earlier fractions.

No.	Solvent*	Weight (g.)	% of load.
1	P	0.017	9.8
2	PE(1)	0.078	44.8
3	PE(2)	0.017	9.8
4	PE(3)	0.043	24.7
5	PE(5)	0.001	0.5

Table 12. *P = light petroleum

The unsaturated ester mixture was separated into the individual esters by preparative G.L.C. (Perkin Elmer Fractometer - Model 451) on a 20% Ap.L. column at 225°, fractions being collected in glass traps filled with cotton wool soaked in methanol and cooled in a solid carbon dioxide bath. The recovery was very poor but sufficient of each of the esters, the C_{14} (4mg.), the C_{16} (9mg.) and the C_{18} (11mg.), were recovered for the position of the double bond in each to be determined.

Position of the double bonds[116]. The C_{18} ester (11mg.) was hydrolysed to the acid (10mg.). This was dissolved in a solution of potassium carbonate (180mg.) in water (5ml.) and a solution of potassium permanganate (1mg.) and potassium periodate (70mg.) in water (20ml.) added. After 24 hours, the excess oxidant was destroyed with sulphur dioxide, the solution was neutralised and the volume reduced by a half on a rotary film evaporator. The acidified solution was saturated with sodium chloride and extracted with ether (5 x 30ml.). The acids (13mg.), thus obtained, were methylated and examined by G.L.C. Two major fragments were found of

C.No. 9.0 and 11.9 (Ap.L.) and 9.0 and 14.6 (QF-1), corresponding to a C_9 monobasic and a C_9 dibasic acid, showing the original acid to be octadec-9-enoic acid. A small amount of decanoic acid was also evident so a trace of the isomer with the double bond in the 8-position must also be present.

Similarly, the C_{16} acid was shown to be hexadec-9-enoic acid with just a trace of the Δ^8 isomer.

The C_{14} acid, however, had only 50% of tetradec-9-enoic acid, with significant amounts of the Δ^5 and Δ^6 isomers and minor amounts of the Δ^7 and Δ^8 isomers.

Unsat. acid	Monobasic acid(s)	Dibasic acids	Pos^n of Unsat.
C_{18}	$C_9(C_{10})$	C_9	$\Delta^9(\Delta^8)$
C_{16}	$C_7(C_8)$	C_9	$\Delta^9(\Delta^8)$
C_{14}	$C_5(C_6,C_7,C_8,C_9)$	C_9	$\Delta^9(\Delta^8,\Delta^7,\Delta^6,\Delta^5)$

Minor products shown in parentheses.

Urea fractionation.

Fractions 1B, 2 and 3 were combined (21.97g.) and acetylated in acetic anhydride (275ml.) at 100° for four hours. The acetic anhydride was removed in a rotary film evaporator at 70° to yield the acetoxy esters (24.85g.). These were taken up in methanol (200ml.), urea (35g.) added and the mixture warmed to give a solution. After 24 hours, the solid was collected, decomposed by water and ether extracted to yield the adducted esters (fraction U-1, 2.27g.). G.L.C. examination showed two major peaks of C.No. 19.2 and 19.5 (Ap.L.) in the approximate proportions 4:1, with a number

of minor peaks.

The fractionation was repeated on the non-adducted esters so so that all the ω- acetoxy esters might be removed. A little more adduct was obtained (50mg.) and the non-adducted acetoxy esters (15.48g.) were also recovered.

To obtain better samples of the esters, the urea fractionation was repeated on fraction U-1 and the adduct (0.710g., U-2) and non-adduct (1.21g., U-3) recovered. G.L.C. examination of fraction U-2 gave two major peaks on an Ap.L. column (C.No. 19.2 and 19.5) and a single one on an SE-30 column (C.No. 19.8). There were also two minor peaks of C.No. 17.7 and 15.8 on the Ap.L. column. The hydroxy esters, prepared by deacetylation of the acetoxy esters[132], had C.Nos. of 18.4 and 18.7 (Ap.L.).

Hydrogenation. The acetoxy esters of fraction U-3 (117mg.) in methanol (20ml.) were hydrogenated by shaking in an atmosphere of hydrogen for 24 hours using 10% palladium/charcoal (20mg.) as catalyst. The catalyst was filtered off and the filtrate evaporated to dryness, affording the saturated esters (96mg.). G.L.C. showed that the peak at 19.2 (Ap.L.) had disappeared, while that at 19.5 was considerably enlarged.

Separation of the acids. The acetoxy esters of fraction U-2 (0.688g.) were chromatographed on silicic acid impregnated with silver nitrate[115] (30g.), eluting with 100ml. portions of various petrol/ether mixtures. Nine fractions (0.611g., 88.8%) were obtained as detailed in table 13.

No.	Solvent	Weight (g.)	% of load.
1	PE(5)	0.009	1.3
2	PE(10)	0.127	18.5
3	PE(10)	0.019	2.7
4	PE(10)	0.008	1.2
5	PE(15)	0.060	8.7
6	PE(15)	0.087	12.6
7	PE(15)	0.053	7.7
8	PE(20)	0.028	4.1
9	E	0.220	32.0

Table 13.

G.L.C. showed that the desired separation had been achieved. The second fraction was a waxy white solid containing the saturated ester, with two minor components (approx. 2%; C.No.s 17.7 and 18.6, Ap.L.). Fractions 5 to 8 contained the unsaturated ester only and were, therefore, combined.

The saturated hydroxy ester.

The acetoxy ester (50mg.) was deacetylated[132] by dissolving in dry methanol (5 ml.) and adding a catalytic amount of sodium methoxide in dry methanol (prepared immediately before by dissolving a small piece of fresh sodium in dry methanol). The solution was refluxed for 5 mins., then added to water (50ml.) and ether extracted to yield the hydroxy ester (43mg.).

Chain-length determination[107]. The hydroxy ester (20mg.) was subjected to iodination - deiodination. The product was

indistinguishable from methyl palmitate in its chromatographic behaviour (G.L.C.). A small amount of C_{14} (approx. 2%) was also detected.

Oxidation. The ester (16mg.) was oxidised by Matic's procedure[70] (chromic oxide in aqueous acetone with sulphuric acid). The product (17mg.) was acidic, so was methylated for G.L.C. examination. One major peak was evident (18.8, Ap.L.; 21.7, QF-1) with two minor ones (approx. 2%), one of C.No. 16.9 (Ap.L.) and 19.8 (QF-1) and the other of C.No. 17.8 (Ap.L.) and 20.8 (QF-1). The diester, recrystallised from aqueous methanol, had m.p. 51-52°. This would suggest, therefore, that the oxidation product was methyl hexadecane-dioate (lit[133] m.p. 53°) and that the original hydroxy acid is 16-hydroxyhexadecanoic acid. A trace of a similar C_{14} acid may also be present.

Characterisation of the saturated acid. The hydroxy acid was obtained by hydrolysis of the acetoxy ester and recrystallised from light petroleum m.p. 90-91° (lit[117]. 91-93°). (Found: C, 70.87; H, 11.82. Calc. for $C_{16}H_{32}O_3$: C, 70.54; H, 11.84). The methyl ester, prepared by deacetylation[132] of the acetoxy ester and recrystallised from light petroleum, had m.p. 50-52° (lit[117]. 55°). (Found: C, 69.77; H, 11.50. Calc for $C_{17}H_{34}O_3$: C, 71.28; H, 11.96).

Position of the double bond in the unsaturated ester. The acetoxy ester (73mg.) was deacetylated[132] and the hydroxy ester (65mg.) so obtained, methylated by the sulpholane procedure. The methoxy ester (71mg.) gave a single peak on G.L.C. (C.No. 17.7, Ap.L.; 19.0, QF-1).

The methoxy ester was hydrolysed to the acid (32mg.) and subjected to a von Rudloff oxidation[116]. The products were methylated with diazomethane so that there was no hydrolysis of the ether linkage and examined by G.L.C. This showed two major fragments of C.No. 9.1 and 11.8 (Ap.L.) and 10.5 and 14.6 (QF-1). These correspond to an ω-methoxy C_7 acid and a C_9 dibasic acid. The original acid must, therefore, be 16-hydroxyhexadec-9-enoic acid.

A large number of minor products were also observed, which may be from position isomers of the above acid or from some oxidation at the carbon atom bearing the methoxyl group.

Characterisation of the unsaturated acid. The acid obtained by hydrolysis of the acetoxy ester was a waxy low melting solid m.p. 17-19°. (Found: C, 70.42; H, 10.77. $C_{16}H_{30}O_3$ requires C, 71.07; H, 11.18). No prominent peak could be observed in the infra red spectrum of the acid at 10.3μ, suggesting that the double bond has a cis configuration.

Chromatography of the non-adducted esters.

The non-adducted acetoxy esters (15.48g.) were hydrolysed with aqueous alcoholic potassium hydroxide (0.5N) and the acids (13.65g.) converted to the methyl esters (12.41g., designated fraction 3'). These (8.537g.) were chromatographed in approximately 1g. lots on silicic acid (30g.), eluting with 100ml. portions of various benzene/ether mixtures. Corresponding fractions from eight columns were combined and 11 fractions (7.996g., 93.7%) recovered in all as

detailed in table 14.

No.	Solvent	Weight (g.)	% of load.
3'-1	B	0.019	0.2
-2	BE(10)	0.279	3.3
-3	BE(20)	2.591	30.3
-4	BE(30)	1.038	12.2
-5	BE(40)	1.384	16.2
-6	BE(50)	1.056	12.4
-7	BE(60)	0.534	6.3
-8	BE(70)	0.329	3.9
-9	BE(80)	0.227	2.6
-10	BE(90)	0.172	2.0
-11	E	0.367	4.3

Table 14.

Fractions were monitored by T.L.C. and by G.L.C. where possible. It was necessary to methylate the hydroxy esters for complete examination. In the light of later evidence, the value of this work is reduced so the results are reported briefly.

Fraction 1. A minor fraction. G.L.C. shows a complex range of peaks.

Fraction 2. G.L.C. showed this to be largely a single ester of C.No. 15.4 (Ap.L.). It was subsequently shown to be methyl 6-keto-tetradecanoate as will be described.

Fraction 3. G.L.C. of the hydroxy esters gave methyl 6-hydroxy-tetradecanoate as the major component. On methylation, however, two

further peaks became evident of C.No. 16.7 and 17.7 (Ap.L.). The latter is probably some residual ω-hydroxy ester. The other is probably a mid-hydroxy C_{16} ester, as on oxidation of the hydroxy esters by Matic's procedure[70], followed by borohydride reduction of the neutral material and methylation of the products, the peak at 16.7 (approx. 5%) remains with the 6-methoxy C_{14} ester. Chain-length determination on this material gave largely C_{14} with some C_{16} (approx. 5%). It was hoped to obtain the ester in a pure state by preparative G.L.C. of the methoxy derivatives, but the instrument proved unsuitable and the esters were not eluted.

Fraction 4. G.L.C. of the methoxy esters showed three main peaks of C.No. 17.4, 16.8 and 15.1 (Ap.L.) with a large number of minor components. A chain-length determination gave C_{14} and C_{16} esters in approximately equal amounts. The methoxy esters were chromatographed on silicic acid and the fractions monitored by G.L.C. An encouraging separation was achieved though none of the fractions contained single esters and there was insufficient material to make rechromat- of any of the fractions feasible.

Fraction 5. G.L.C. of the methoxy esters showed this to be a complex fraction with three main components of C.No. 15.2, 16.0 and 17.6 (Ap.L.)

Fraction 6. G.L.C. of the methoxy esters again showed this to be a fairly complex fraction.

Fractions 7-11. These were not examined since on G.L.C. examination of the previous two fractions, it was necessary to load the columns

heavily to obtain recognisable peaks. It seemed likely, therefore, that some components were present which were not eluted.

6-Ketotetradecanoic acid.

G.L.C. examination of fraction 3'-2 showed a major component of C.No. 15.4 (Ap.L.) or 18.9 (QF-1) with a number of minor components. Its infra red spectrum showed a marked "hump" on the ester peak indicating the presence of a further carbonyl group. The C.No. remained unaltered after methylation by the sulpholane procedure. Its chromatographic behaviour was very similar to that of methyl 6-ketotetradecanoate, synthesised earlier in this study.

The esters (0.210g.) were rechromatographed on silicic acid (30g.), eluting with 100ml. portions of various benzene/ether mixtures. 4 fractions (0.198g., 94.2%) were collected (table 15).

No.	Solvent.	Weight (g.)	% of load
1	B	0.001	0.5
2	BE(5)	0.112	53.5
3	BE(10)	0.032	15.2
4	E	0.053	25.2

Table 15.

G.L.C. and T.L.C. showed that the second fraction was almost entirely the required ester.

Borohydride reduction. The ester (21mg.) was reduced using sodium borohydride in aqueous methanol. The resulting hydroxy ester (19mg.) had C.No. 15.8 (Ap.L.) and 18.0 (QF-1) on examination by G.L.C. The

infra red spectrum showed hydroxyl adsorption but no additional carbonyl "hump".

Chain-length. The hydroxy ester (10mg.) was subjected to iodination-deiodination[107]. The product (5mg.), identified by G.L.C., was almost entirely methyl tetradecanoate.

Methylation. The hydroxy ester (7mg.) was methylated by the sulpholane procedure. The resulting methoxy ester (7mg.) had C.No. 15.1 (Ap.L.).

Position of the keto-group. From the keto ester (33mg.), the oximes (35mg.) were prepared and subjected to a Beckmann rearrangement followed by hydrolysis. The acid products (18mg.) were methylated for examination by G.L.C. Two fragments were found corresponding to nonanoic acid (C.No. 9.0, Ap.L. or QF-1) and adipic acid (C.No. 9.0, Ap.L.; 11.5, QF-1), indicating that the original acid must have been 6-ketotetradecanoic acid.

Characterisation. The acid, obtained by hydrolysis of the ester and recrystallisation from petroleum ether, had m.p. 68-69° alone or when mixed with the synthetic acid. The semicarbazone was also prepared and had m.p. 129-131° alone and when mixed with the similar derivative of the synthetic acid.

Chromatography of fraction 4.

G.L.C. examination of the methoxy esters (0.265g.) prepared from some of the fraction 4 esters (0.253g.) showed the fraction to be extremely complex. Major peaks were evident at 19.7 (probably

tri-O-methyl aleuritate), 15.7, 17.7, 15.1 and 16.4 (Ap.L.) with numerous minor peaks. The esters (57.1g.) were chromatographed in two approximately equal lots on neutral alumina (700g., each), eluting with 21. lots of various ether/methanol mixtures. Corresponding fractions were combined, three fractions (49.5g., 86.7%) in all being collected (table 16).

No.	Solvent	Weight (g.)	% of load
4A	EM(2)	8.0	14.0
B	EM(5)	25.9	45.4
C	M	15.6	27.3

Table 16.

The first of these fractions was rechromatographed on silicic acid (30g.) in approximately 1g. portions, eluting with various benzene/ether/methanol mixtures. 7 columns in all were run (7.996g.) and corresponding fractions combined. Four fractions (7.855g., 98.2%) were collected (table 17).

No.	Solvent	Weight (g.)	% of load
4A-1	BE(30)	0.778	9.7
-2	BE(50)	3.345	41.8
-3	BE(80)	2.357	29.5
-4	EM(5)	1.375	17.2

Table 17.

Fraction 4A-1.

The esters (0.747g.) were acetylated with acetic anhydride[84].

The acetoxy compounds (0.829g.) gave two major peaks of C.No. 16.5 and 19.7 (the mono-acetoxy esters) and two lesser peaks of C.No. 18.2 and 19.1 (SE-30). The acetoxy esters were chromatographed on silicic acid (30g.) and eluted with 100ml. lots of various petrol/ether mixtures. Ten fractions (0.756g., 91.1%) were collected as detailed in table 18.

No.	Solvent	Weight (g.)	% of load
1	PE(10)	0.022	2.6
2	PE(15)	0.116	14.0
3	PE(20)	0.041	4.9
4	PE(25)	0.073	8,8
5	PE(30)	0.107	12,9
6	PE(35)	0.098	11.8
7	PE(40)	0.101	12.2
8	PE(45)	0.068	8,2
9	PE(50)	0.052	6,3
10	PE(80)	0.078	9,4

Table 18.

Fraction 8 consisted largely of a single ester of C.No. 18,2 (SE-30) or 21.7 (QF-1). Fraction 9 was mainly an ester of C.No. 19.1 (SE-30) or 23.5 (QF-1). Attempts to obtain purer samples by rechromatography were unsuccessful.

Hydrolysis. The esters of both fractions were hydrolysed to the acids and remethylated with boron trifluoride/methanol. From both acids, a mixture of products of similar composition was obtained,

methyl aleuritate being a major component. Recrystallisation of the products (aqueous ethanol) from the acetoxy ester of C.No. 19.1 gave a sample of methyl aleuritate m.p. 67-69° (lit[17]. 69-70°). (Found: C, 63.96; H, 10.40. Calc. for $C_{17}H_{34}O_5$: C, 64.10, H, 10.76). A similar sample was obtained from the other ester.

Fraction 4A-2.

The hydroxy esters (3.345g.) were acetylated and the products (3.845g.) gave two major peaks of C.No. 23.8 and 18.45 and minor ones of 20.2, 21.0 and 21.4 (SE-30) on G.L.C. examination. Some of these (1.014g.) were chromatographed on silicic acid (30g.) and eluted with 100ml. lots of various petrol/ether mixtures. 9 Fractions (1.012g., 99.8%) were recovered (table 19).

No.	Solvent	Weight (g.)	% of load
1	PE(30)	0.012	1.2
2	PE(40)	0.034	3.3
3	PE(50)	0.359	35.4
4	PE(60)	0.165	16.3
5	PE(70)	0.078	7.7
6	PE(80)	0.107	10.6
7	PE(90)	0.114	11.2
8	E	0.058	5.7
9	EM(5)	0.085	8.4

Table 19.

T.L.C. examination showed that a reasonable separation was

achieved. The third fraction was a single spot on T.L.C. but on G.L.C. examination under the usual conditions (SE-30, 225°; QF-1, 200°), nothing was eluted and the same result was found when the temperature was raised to 260° and the flow rate doubled.

Chain-length. The acetoxy ester (47mg.) was hydrolysed to the acid (34mg.) and subjected to iodination - deiodination[107]. On examination by G.L.C., the product (17mg.) was indistinguishable from methyl palmitate. (A little C_{14} was also detected).

Hydrolysis. The acetoxy esters (209mg.) were hydrolysed with potassium hydroxide (0.5N) to the acid (187mg.). T.L.C. showed a single product of similar polarity to a dihydroxy acid. This was methylated using boron trifluoride/methanol complex. The methyl ester (145mg.) was found to be a complex mixture of products by T.L.C., including one corresponding to methyl aleuritate. Some of the esters were re-acetylated and the acetoxy esters (59mg.) examined by G.L.C. (SE-30). The major product had C.No. 23.7 (corresponding to tri-O-acetyl aleuritate). Two other large peaks had C.No. 18.5 and 20.3 which would be expected of a mid-acetoxy C_{16} ester and a vic.diacetoxy C_{16} ester. Oxidation of some of the hydroxy esters (20mg.) with chromic oxide in acetic acid gave mainly C_7 and C_9 dibasic acids (23mg.; C.No. 9.9 and 11.9, Ap.L., or 12.6 and 14.6, QF-1) with a number of minor fragments of low C.No. Treatment of part of fraction 4A-2(3) with boron trifluoride/ methanol complex alone or with methanolic hydrogen chloride gave similar results. The acetoxy fraction (23mg.) with sodium methoxide

in dry methanol gave a single product (22mg.) on T.L.C. of similar polarity to a dihydroxy ester.

Isopropylidene derivative. The hydroxy ester (22mg.) was treated with acetone and anhydrous copper sulphate[23]. T.L.C. examination of the product (21mg.) indicated that no reaction had occurred.

Oxidation. The acid (36mg.) prepared by alkaline hydrolysis of the original acetoxy ester was oxidised using potassium permanganate and potassium periodate[116] and the product (32mg.) methylated with diazomethane. On G.L.C. examination of this, nothing was eluted.

Molecular Weight. The molecular weight was determined using a "Mechrolab Vapour Pressure Osmometer" with benzene as solvent and found to be 625 ± 15, for the hydroxy ester prepared by deacetylation of the acetoxy polymer with sodium methoxide in methanol. The M.W. expected for two methyl aleuritate molecules joined by one ether linkage is 618.

Chromatography of the polymer-free mixed esters.

Preparation of the acetoxy esters. Shellac (22.1g.) was hydrolysed as before and the acids (23.0g.) in methanol, methylated with diazomethane. The methyl esters (23.9g.) were acetylated with acetic anhydride to yield the acetoxy esters (30.7g.). On taking these up in ether to transfer to a flask for distillation, a brown solid (0.451g.) separated out.

The brown solid. This was soluble in methanol and water but not in

ether or benzene. It melted over the range 210-230° before and after recrystallisation from methanol/ether. Its infra-red spectrum had a band near 3μ presumably due to hydroxyl absorption but no carbonyl or acetate absorption was evident. On standing in air, it became darker in colour and appeared to be deliquescent.

The distillation. The acetoxy esters (30.13g.) were distilled at 140° and 10^{-3}mm. pressure in a "Quickfit" molecular still. (The conditions were determined after a trial experiment with tri-O-acetyl aleuritate). The mixture was fractionated four times until no more distilled and the distillates combined. The monomer was a light yellow mobile liquid while the polymer was a deep red viscous liquid (Total recovery 26.18g., 86.9%; Table 20).

Fraction.	Weight (g.)	% of load.
Monomer	17.52	58.2
Polymer	8.66	28.7

Table 20.

G.L.C. examination of the monomer gave peaks at C.No. 24.0 (tri-O-acetyl aleuritate), 16.4 and 19.6 (the mono-acetoxy esters) with unknown peaks at 18.55, 19.25, 20.35, 22.0 and 23.15 on an SE-30 column at 225°. T.L.C. showed a more discreet series of spots with less "tailing" than had previously been observed.

Chromatography of the monomer.

The monomer acetoxy esters (17.278g.) were chromatographed on silicic acid (80g.) in five lots and eluted with various petrol/

ether mixtures (250ml. portions). Corresponding fractions were combined and six fractions (16.905g., 97.8%) in all collected (table 21) and monitored by G.L.C. and T.L.C.

No.	Solvent	Weight (g.)	% of load.
1	P	0.051	0.3
2	PE(20)	1.692	9.8
3	PE(40)	6.344	36.7
4	PE(60)	5.315	30.8
5	PE(80)	2.808	16.2
6	E	0.695	4.0

Table 21.

Fraction 1. The non-hydroxy esters.

Fraction 2. The mono-acetoxy esters.

Fraction 3. T.L.C. showed one large spot corresponding to tri-O-acetyl aleuritate with some less polar esters. G.L.C. showed approx. 50% tri-O-acetyl aleuritate (C.No. 24.0, SE-30) with other major peaks at 18.6, 19.4 and 20.4 and minor peaks at 21.5, 22.4 and 23.2.

Fraction 4. T.L.C. indicated tri-O-acetyl aleuritate and di-O-acetyl shellolate. G.L.C. confirmed this with peaks at 24.0 and 21.6. There were also significant peaks at 21.1 and 22.1 with a minor one at 22.7.

Fraction 5. T.L.C. gave two spots, both more polar than di-O-acetyl shellolate. G.L.C. gave two peaks at 21.3 and 21.9 with a smaller one at 19.45 and a number of minor ones.

Fraction 6. T.L.C. showed that the esters were fairly polar. G.L.C.

gave major peaks at 20.6, 20.1 and 21.45.

Chromatography of fraction 3.

The acetoxy esters were chromatographed in two lots on silicic acid (80g.) and eluted with various petrol/ether mixtures (200ml. portions). Four fractions (6.166g., 97.9%) were collected (table 22) and monitored by G.L.C.

No.	Solvent.	Weight (g.)	% of load.
3-1	PE(20)	0.025	0.4
-2	PE(30)	0.799	12.7
-3	PE(35)	1.410	22.4
-4	E	3.932	62.4

Table 22.

Fraction 3-1. Further mono-acetoxy esters.

Fraction 3-2. Two peaks of C.No. 18.6 and 20.3 (SE-30).

Fraction 3-3. Three major peaks of C.No. 18.6, 19.2 and 22.2. with a number of minor ones.

Fraction 3-4. Largely tri-O-acetyl aleuritate.

Fraction 3-2 (0.635g.) was rechromatographed on silicic acid (30g.) and eluted with 100ml. portions of various petrol/ether mixtures. Five fractions (0.622g., 98%) were collected (table 23).

Fractions 2 and 3 of these contained only the ester of C.No. 20.3 (SE-30) and were combined and used to determine the structure of the acid.

No.	Solvent	Weight (g.)	% of load.
1	PE(15)	0.009	1.4
2	PE(20)	0.080	12.6
3	PE(20)	0.090	14.2
4	PE(20)	0.041	6.5
5	PE(40)	0.402	63.3

Table 23.

The fourth and fifth of these fractions were rechromatographed on silicic acid (30g.) and eluted with 100ml. portions of various petrol/ether mixtures. Six fractions (0.392g., 89%) were collected (table 24).

No.	Solvent	Weight (g.)	% of load.
1	PE(15)	0.008	1.8
2	PE(20)	0.004	0.9
3	PE(20)	0.034	7.7
4	PE(20)	0.095	21.5
5	PE(25)	0.211	48.0
6	PE(30)	0.040	9.1

Table 24.

Fraction 5 contained only the ester of C.No. 18.6 (SE-30) and was, therefore, used to determine the structure of the acid.

Threo 9,10-dihydroxytetradecanoic acid.

The acetoxy ester of C.No. 18.6 (SE-30) was shown to be derived from the above acid in the following experiments.

Chain-length. The acetoxy ester (59mg.) was de-acetylated with sodium methoxide in methanol[132] to yield the hydroxy ester (43mg.). Some of this (12mg.) was subjected to iodination - deiodination[107]. The product (8mg.), on examination by G.L.C., was indistinguishable from methyl tetradecanoate. (Trace amounts of C_{16} and C_{12} esters were also detected.).

Oxidation. The hydroxy ester (21mg.) was oxidised with chromic oxide in acetone with concentrated sulphuric acid[70]. The product (19mg.) was acidic so was methylated and the methyl esters (20mg.) examined by G.L.C. The major product was nonanedioic acid (C.No. 11.9, Ap.L.; 14.6, QF-1).

Isopropylidene derivative. Fractions 1,2 and 3 (table 24, 46mg.), containing mainly the ester of C.No. 18.6 with some of that of C.No. 20.3 (SE-30), were de-acetylated as before and the hydroxy esters (36mg.) allowed to react with dry acetone and anhydrous copper sulphate[23]. G.L.C. examination of the product (41mg.) gave two peaks of C.No. 16.5 and 18.5 (SE-30). The isopropylidene derivative of synthetic 9,10-dihydroxystearate had C.No. 20.5 i.e. an increase in apparent chain-length of 2.5 units.

Position of the glycol group. The acetoxy ester (143mg.) was hydrolysed to the acid (110mg.) with aqueous alcoholic potassium hydroxide and a portion (11mg.) subjected to von Rudloff oxidation[116]. The product was methylated with boron trifluoride/methanol complex for G.L.C. examination. One major peak, corresponding to nonanedioic acid, was found (C.No. 11.8, Ap.L; 14.6, QF-1).

Optical activity. No measurable optical rotations could be detected, the deviation of the dispersion curves from the base line indicating a molecular rotation less than 1° even at 250mμ.

Characterisation. The hydroxy acid, obtained by hydrolysis of the acetoxy ester and recrystallise from petrol/ethyl acetate, had m.p. 78.5-79.5° (lit[121] 80°). (Found: C, 64.49; H, 10.92. Calc. for $C_{14}H_{28}O_4$: C, 64.58; H, 10.84). The methyl ester of the hydroxy acid, obtained by de-acetylation of the acetoxy ester and recrystallisation from petrol, had m.p. 58-60°. (Found: C, 65.41; H, 10.85. Calc. for $C_{15}H_{30}O_4$: C, 65.66; H, 11.02). The hydroxy ester behaved similarly to methyl threo 9,10-dihydroxystearate on a T.L.C. plate impregnated with boric acid[99].

Threo 9,10-dihydroxyhexadecanoic acid.

The acetoxy ester of C.No. 20.3 (SE-30) was shown to be derived from the above acid in the following experiments.

Chain-length. The acetoxy ester (34mg.) was de-acetylated as before to yield the hydroxy ester (28mg.). Some of this (18mg.) was subjected to iodination - deiodination[107]. The product (9mg.) was indistinguishable in its behaviour on G.L.C. from methyl palmitate. (A trace of C_{14} was also detected).

Position of the glycol group. The acetoxy ester (132mg.) was hydrolysed to the acid (109mg.), a portion of which (26mg.) was subjected to von Rudloff oxidation[116]. The product (26mg.) was acidic so was methylated with boron trifluoride/methanol complex for G.L.C.

examination. Two products, methyl heptanoate (C.No.7.0, Ap.L. or QF-1) and dimethyl nonanedioate (C.No. 11.8, Ap.L.; 14.6, QF-1), were found - indicating that the original glycol group was in the 9,10-position.

Optical activity. No measurable optical rotations could be detected, the deviation of the dispersion curves from the base line indicating a molecular rotation less than 1° even at 250mμ.

Characterisation. The hydroxy acid, obtained by hydrolysis of the acetoxy ester and recrystallisation from ethyl acetate/petroleum ether, had m.p. 93-94° (lit[122] 87°). (Found: C, 66.83; H, 10.97. Calc. for $C_{16}H_{32}O_4$: C, 66.63; H, 11.18). The methyl ester, obtained by de-acetylation of the acetoxy ester and recrystallisation from petroleum ether, had m.p. 62-64° (lit[122] 65°). (Found: C, 68.62; H, 11.31. Calc. for $C_{17}H_{34}O_4$: C, 67.51; H, 11.33). The hydroxy ester behaved similarly to methyl threo 9,10-dihydroxystearate on a T.L.C. plate impregnated with boric acid[99].

Fraction 3-3.

The acetoxy esters (1.394g.) were chromatographed on silicic acid (30g.) and eluted with 100ml. portions of various petrol/ether mixtures. Four fractions (1.275g., 91.4%) were collected (table 25).

G.L.C. examination showed that all the fractions were complex and contained a large number of unknown esters. Fraction 3-3(3), which contained approx. 50% of an ester of C.No. 22.4 (SE-30) was examined in a little more detail.

No.	Solvent	Weight (g.)	% of load.
1	PE(10)	0.007	0.5
2	PE(20)	0.021	1.5
3	PE(30)	0.500	35.9
4	E	0.747	53.6

Table 25.

Chain-length. The acetoxy esters (60mg.) were de-acetylated with sodium methoxide in methanol[132] to the hydroxy esters (46mg.). Some of these (19mg.) were subjected to iodination - deiodination. The products (10mg.) were methyl palmitate and methyl myristate in the approximate ratio 2:1 (by G.L.C.).

Isopropylidene derivatives. (Attempted). The hydroxy esters (21mg.) were allowed to react with dry acetone and anhydrous copper sulphate. Comparison of the products (20mg.) and the original esters on G.L.C. indicated that no reaction had occurred.

Further chromatography of this fraction on silicic acid did not improve the separation.

Fraction 4.

The acetoxy esters (5.275g.) were chromatographed in two lots on silicic acid (80g.) and eluted with various petrol/ether mixtures (250ml. portions), combining corresponding fractions. Four fractions (5.013g., 95%) were collected as detailed in table 26 and monitored by G.L.C. and T.L.C.

No.	Solvent	Weight (g.)	% of load.
4-1	PE(30)	0.014	0.2
-2	PE(50)	0.991	18.8
-3	PE(50)	1.919	36.4
-4	E	2.089	39.6

Table 26.

Fraction 4-1. A minor fraction with a large number of peaks on G.L.C.

Fraction 4-2. Almost entirely tri-O-acetyl aleuritate.

Fraction 4-3. Largely tri-O-acetyl aleuritate (approx. 70%) with a large number of other components giving broad "humps" at C.No. 21 to 22 (SE-30).

Fraction 4-4. A complex mixture giving two major peaks of C.No. 21.25 and 21.9 (SE-30) with possibly another component which may be di-O-acetyl shellolate (C.No. 21.6) between them.

Attempted chain-length determinations.

Fraction 4-4. The acetoxy esters (57mg.) were de-acetylated with sodium methoxide in dry methanol giving the hydroxy esters (42mg.). Some of these (28mg.) were subjected to iodination - deiodination. T.L.C. showed that the products (25mg.) were highly polar and, in fact, they were not eluted from an SE-30 column.

Fraction 5. The hydroxy esters (65mg.) were prepared by de-acetylation of the acetoxy esters (101mg.). Iodination - deiodination of some of these (33mg.) gave a product (17mg.) which was of high

polarity on T.L.C. and was not eluted on G.L.C. from an SE-30 column.

Fraction 6. The hydroxy esters (29mg.) were prepared by de-acetylation of the acetoxy esters (57mg.). Iodination - de-iodination of some of these (22mg.) gave a product (18mg.) which was of high polarity on T.L.C. and was not eluted on G.L.C. from an SE-30 column.

Carbon Numbers.

Ester (methyl)	Liquid Phase		
	Ap.L.*	QF-1	SE-30
Palmitate	16.0	16.0	16.0
nonanedioate	11.8	14.6	
6-ketotetradecanoate	15.4	18.8	
6-hydroxytetradecanoate	15.8	18.0	
(O-methyl derivative)	15.1	15.8	
(O-acetyl derivative)			16.4
16-hydroxyhexadecanoate	18.8	20.8	
(O-methyl derivative)	17.9	19.0	
(O-acetyl derivative)	19.5(5)		19.8
9,10-dihydroxytetradecanoate		20.8	
(di-O-acetyl derivative)			18.6
9,10-dihydroxyhexadecanoate	19.4(2½)		
(isopropylidene derivative)	18.2	18.4	18.5
(di-O-methyl derivative)	17.7		
(di-O-acetyl derivative)			20.3
aleuritate (isopropylidene deriv.)	20.3(2½)		
(tri-O-methyl derivative)	19.7(2½)		
(tri-O-acetyl derivative)			23.8

*10% or the figure in parentheses.

Mono-unsaturated esters have a C.No. 0.2 units less than the corresponding saturated esters on Ap.L. columns. On QF-1 and SE-30 columns, the C.No.s are the same.

Index to General Methods.

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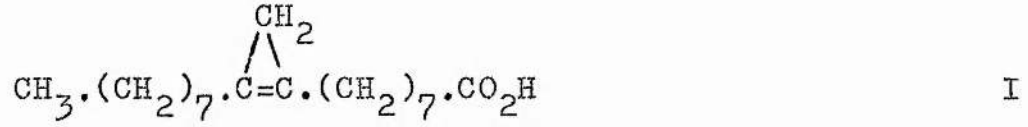
APPENDIX.

LIPOLYTIC STUDIES OF SOME SEED OILS CONTAINING STERCULIC ACID.

INTRODUCTION.

The kernel of the seeds of Sterculia foetida gives a yellow oil which polymerises readily. Early attempts to isolate and identify the acid responsible for this property were unsuccessful[1,2] until sufficiently mild conditions were used. Nunn[3], however, was able to isolate the acid in a pure form, by saponification of the oil in the cold followed by fractional crystallisation of the urea complexes. Degradative studies showed the acid to be 2-octyl-1-cyclopropene-1-octanoic acid (I).

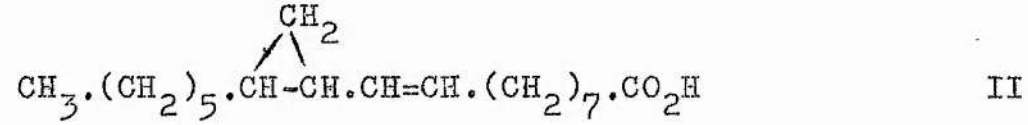


I

The acid (m.p. 18°) polymerises slowly at 0° and fairly rapidly at room temperature.

A precedent existed for assigning the unusual cyclopropene group to the acid as Hofmann and Lucas[4] had isolated a saturated C_{19} acid from the lipids of Lactobacillus arabinosus for which they proposed a cyclopropane structure.

Some confusion was caused when the alternative structure 2-hexyl-1-cyclopropanedec-9-enoic acid (II) was proposed[5-9].



II

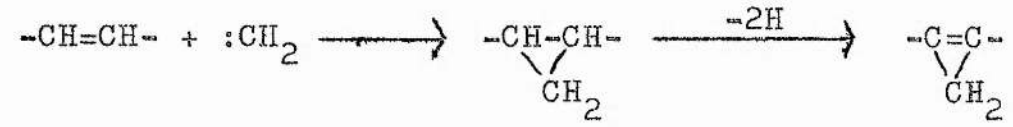
A large weight of additional evidence, however, lead to the acceptance of Nunn's structure for sterculic acid. Infra-red measurements and the Halphen test gave the first confirmation[10-13],

then the ozonolysis product of sterculic acid, 9,11-dioxononadecanoic acid, was unequivocally synthesised[14-17]. Further synthetic evidence was obtained when DL-cis-9,10-methylene-octadecanoic acid (dihydrosterculic acid) was synthesised[18,19] and shown to be identical to the acid obtained from the natural source. Later, a synthesis of sterculic acid, itself, was reported[21]. Further degradative studies confirmed the structure of dihydrosterculic acid[22,23] as did crystallography studies[24]. Nuclear magnetic resonance[25,26], while not positively proving Nunn's structure, was able to show that the one proposed by Varma was impossible. Finally, Brooke and Smith[20] were able to discredit Varma's experimental evidence and so end the controversy.

Meanwhile, "lactobacillic acid" was shown to be 11,12-methylene-octadecanoic acid by chemical means[27] and this was confirmed by X-ray crystallography[24,28,29]. Lactobacillic and related acids have since been shown to be present in the lipids of a large number of bacteria[30-40]. One other important cyclopropene acid has been positively identified, 2-octyl-1-cyclopropene-1-heptanoic acid ("malvalic acid") from Malva seed oils[12,41]. Other cyclopropene acids have been reported from kapok seed oil[10] ("bombacic acid") and from Paquira aquatica[42] but no structures are reported.

With the application of gas-liquid chromatography to the analysis of oils containing sterculic acid, it became evident that malvalic and dihydrosterculic acids may also be present in seed oils to varying degrees, and the occurrence of these homologues,

differing in chain length by a single carbon atom, presents an interesting biogenetic problem. Microbacterial synthesis of dihydrosterculic acid from oleic acid has been reported[33] and it has been suggested[43] that a possible scheme for the biosynthesis of cyclopropenoid acids is -



i.e. one carbon addition across the double bond followed by dehydrogenation of the cyclopropane ring. Such a scheme would require a C_{17} mono-enoic acid precursor for malvalic acid and in fact measurable quantities of this have been detected in seed oils[43,44].

Sterculic and malvalic acids have been reported from the seed oils of Sterculia foetida, Hibiscus syriacus and Lavatera trimestris[43,44] from three species each of Malvaceae and Sterculiaceae[45] and from Bombacopsis glabra (Bombax oleagineum)[46].

The nature of the polymerisation of sterculic acid has been studied[47-49] and appears to proceed via the opening of the cyclopropene ring by a carboxylate ion. Catalytic hydrogenation under mild conditions[3,43] gives largely the dihydro-compound but also significant amounts of 9(10)-methyloctadecanoic acid; under more vigorous conditions, large amounts of the straight-chain C_{19} acid also result[40,43]. Hydrogen halide adds readily across the double bond[50] and this property is used in the titrimetric estimation of cyclopropenoid acids[51,52]. The preparation of the hydrogen chloride

addition compound followed by chlorine analysis has also been suggested[53] as has spectrophotometric determination using a modified Halphen test,[54] though the latter appears to have been superceeded by direct titration methods.

Oils containing sterculic acid are found to be physiologically active when incorporated into the diet of laying hens. This is considered to be because of the addition of sulphur compounds e.g. cysteine across the double bond[55].

DISCUSSION.

One Bombax (Bombacopsis glabra)and two Sterculia (Sterculia parviflora and S. macrophylla) seed oils were available, the first as fresh seeds and the others as comparitively older oils. The object of this study was to determine the distribution of any cyclopropenoid acids, which might be found in these, between the 1 and 2 positions in the triglycerides.

Most workers stress that cyclopropene acids polymerise readily and that the mildest possible conditions must be used in handling them. Where seeds were available, therefore, the oil was obtained by repeated extraction with light petroleum at room temperature. The triglycerides were obtained by chromatography of the oil on silicic acid (fraction eluted with benzene) and the methyl esters prepared from these by transesterification with sodium methoxide in boiling methanol for 5 min. In the short time at this temperature, no significant polymerisation was found to occur.

As has been reported previously[43,44,46], considerable decomposition of the cyclopropene esters occurred on G.L.C. examination and direct quantitative analysis of the esters was impossible. It was necessary to hydrogenate the esters to obtain accurate estimates of the percentages of esters of various chain lengths and to combine this with data from the non-hydrogenated esters on different liquid phases to obtain the total composition of the oil. It was assumed that the response of the detector to

straight-chain, branched-chain and cyclopropane esters was uniform. Room temperature catalytic hydrogenation of cyclopropene esters with palladium/charcoal (5%) as catalyst and methanol as solvent gave largely the cyclopropane ester with some (approx. 15%) branched-chain ester but no straight-chain ester. The retention times, recorded as carbon numbers (C.No.s)[57], were similar to those quoted by other workers[43,44,58] (table 1.).

Ester (methyl)	C.No.	
	5% Ap.L.	20% P.E.G.A.
Sterculate	18.6	20.0
dihydrosterculate	18.8	19.4
9(10)-methylstearate	18.3	18.3
malvalate	17.6	18.9
dihydromalvalate	17.8	18.4
8(9)-methylheptadecanoate	17.3	17.4

Table 1.

Titration with hydrogen bromide in acetic acid[51,52] proved a useful check on results.

The experimental procedures available for examining the glyceride structures of fats and oils have recently been reviewed[59]. In this study, pancreatic lipase hydrolysis of the triglycerides was used[60]. The triglycerides were hydrolysed with pancreatic lipase at constant temperature and pH, the mixture of glycerides was extracted with ether and the free acids removed on a column of amberlite resin. The monoglycerides were obtained by chromatography

on silicic acid (eluted with ether) after the removal of the tri- and di-glycerides (benzene:ether 9:1). The methyl esters of the mono-glycerides were prepared by transesterification with sodium methoxide in dry methanol and examined on G.L.C. before and after hydrogenation.

Bombacopsis glabra.

The oil (54%) from the seed kernels (Northern Rhodesian) gave a strong positive Halphen reaction[11] and an infra-red absorption band at 9.92 μ (cyclopropene). The methyl esters of the triglycerides (74% of the oil) were prepared and examined by G.L.C. qualitatively and quantitatively before and after hydrogenation. Table 2 illustrates the molar percentages of the various esters as calculated from analysis of the mixed esters alone, and when combined with the analysis after hydrogenation (a trace of C_{17} was also detected).

Ester (methyl).	%	
	Before hydrog.	After hydrog.
16:0	60.0	53.3
malvalate	0.8	1.6
18:0	3.8	2.7
18:1	9.3	7.6
18:2	5.1	4.2
dihydrosterculate	3.9	3.2
sterculate	17.1	27.4

Table 2.

i.e decomposition of the cyclopropene esters resulted in apparently higher percentages of the normal acids. This composition is rather different from that already reported for the total oil[46]. The HBr titration was in reasonably close agreement giving a value of 30.4% cyclopropene acids by weight.

Some of the triglycerides were subjected to lipolysis and the methyl esters from the mono-glyceride fraction examined qualitatively and quantitatively by G.L.C. before and after hydrogenation. This gives the component acids attached to the C_2 position (β position), from which the composition of the acids attached to the C_1 and C_3 (α') positions can be calculated

$$\alpha' = \frac{(\text{mixed acids} \times 3) - \beta}{2}$$

The results are summarised in table 3.

Ester (methyl)	% β	% α'	E.F.*
16:0	5.4	77.3	0.1
malvalate	1.3	1.7	0.8
18:0	trace	4.0	0.0
18:1	16.2	3.3	2.1
18:2	10.7	0.9	2.7
dihydrosterculate	4.3	2.7	1.3
sterculate	62.1	10.1	2.4

Table 3.

*E.F. = enrichment factor = $\frac{\%\text{ester in monoglycerides}}{\%\text{ ester in triglycerides.}}$ [61]

Sterculia parviflora.

The seed oil gave a positive Halphen test and an infra-red absorption band at 9.92μ. The methyl esters of the triglyceride fraction (66.8% of the oil) were prepared and examined qualitatively and quantitatively by G.L.C. before and after hydrogenation (table 4).

Ester (methyl)	%.
16:0	11.9
16:1	0.4
unknown*	0.3
17:0	0.6
17:1	0.6
malvalate	19.3
18:0	5.8
18:1	10.0
dihydrosterculate	5.8
sterculate	45.3

Table 4.

*unknown ester of C.No. 16.8 (Ap.L.) after hydrogenation - possibly a C_{17} cyclopropene ester.

The HBr titration gave a value of 60.7% by weight of cyclopropene acids.

After lipolysis and analysis of the methyl esters of the monoglyceride fraction, the following results were obtained (table 5).

Ester (methyl)	%β	%α'	E.F.
16:0	8.5	13.8	0.7
16:1	0.5	0.4	1.3
unknown	0.4	0.2	1.3
17:0	0.1	0.9	0.2
17:1	0.5	0.6	0.8
malvalate	10.2	23.8	0.5
18:0	3.6	6.9	0.6
18:1	16.7	6.6	1.7
dihydrosterculate	4.1	6.6	0.7
sterculate	55.4	40.2	1.2

Table 5.

This oil had been examined previously by other workers[62] but prior to the isolation and identification of sterculic acid.

Sterculia macrophylla.

The seed oil gave a positive Halphen test and an infra-red absorption band at 9.92μ. The methyl esters of the triglyceride fraction (60.4% of the oil) were prepared and estimated as before (table 6). The HBr titration gave 67.5% sterculate by weight.

Attempts to obtain a pure sample of methyl sterculate by chromatography of the mixed esters on silicic acid impregnated with silver nitrate[63] was unsuccessful because of decomposition of the cyclopropene acids on the column. Similar behaviour has been found by other workers.[64].

Ester (methyl)	%
16:0	17.5
16:1	0.4
malvalate	4.4
18:0	4.1
18:1	6.6
dihydrosterculate	3.6
sterculate	63.4

Table 6.

After lipolysis and analysis of the methyl esters of the monoglyceride fraction, the following results were obtained (table 7).

Ester (methyl)	%β	%α'	E.F.
16:0	14.9	18.8	0.9
16:1	0.6	0.3	1.5
malvalate	1.0	6.1	0.2
18:0	3.4	4.5	0.8
18:1	12.0	3.9	1.8
dihydrosterculate	3.0	3.9	0.8
sterculate	65.1	62.5	1.0

Table 7.

The oil has not previously been examined.

Conclusions

In vegetable fats, the constituent acids are not distributed

at random in the triglycerides but unsaturated C_{18} acids are usually more concentrated in position 2. This pattern is quite clearly evident in the oil from Bombacopsis glabra, sterculic acid behaving as a normal unsaturated acid in this respect. With the other two oils, a similar effect is apparent though less marked. This may be because the greater age of these oils has allowed some deterioration to occur.

EXPERIMENTAL.

Gas liquid chromatography was carried out on a Pye Argon Chromatograph (Sr^{90} β-ray detector) on a glass column (4') packed with 5% Apiezon L on alkali-washed celite. Areas of peaks were determined directly by a Pye Integram fitted to the machine. In addition, a Perkin Elmer Fractometer - Model 451 (flame ionisation detector), with a stainless steel column (2m.) packed with 20% polyethylene glycol adipate (P.E.G.A.) on fire brick, was used. Peak areas in this case were measured by triangulation. With the β-ray detector, the area of a peak is proportional to the molar concentration of the component; with the flame ionisation detector, the area or a peak is proportional to the weight concentration of the component.

Bombacopsis glabra.

Extraction of seeds. The seeds were shelled and the kernels ground in a mill to a fine powder (18.9g.) which was shaken for 24 hours with light petroleum (40-60°) to extract the oil. The suspension was filtered periodically and the solid particles dried, reground and extracted with fresh solvent. The oil (10.0g., 53%) was obtained on removal of the solvent from the combined filtrates in a rotary film evaporator at 30°.

Halphen test[11]. The oil (50mg.) was dissolved in freshly distilled carbon disulphide (1ml.) at room temperature. After two days, the

solution became orange in colour and after a few weeks, this deepened to a bright orange red.

The oil gave an absorption peak in the infra-red spectrum at 9.92μ.

The triglycerides. The oil (2.643g.) was chromatographed on silicic acid (80g.) and eluted with benzene (200ml.), which removed the triglycerides (1.951g., 73.8%) and then with ether (500ml.) which removed the remaining material (0.686g., 26.0%), the solvent being taken off on a rotary film evaporator. T.L.C. confirmed that the desired separation had been achieved.

Esterification[56]. Some of the triglycerides (0.187g.) were dissolved in dry methanol (3ml.) with sufficient dry benzene to effect solution and a catalytic amount of sodium methoxide in methanol (prepared immediately before by dissolving a piece of fresh sodium in dry methanol) added. The solution was refluxed for 5min. then poured into water and ether extracted to yield the methyl esters (0.188g.), which were examined qualitatively and quantitatively by G.L.C.

Hydrogenation. The esters (41mg.) were hydrogenated by shaking in an atmosphere of hydrogen for ½ hour using 5% palladium/charcoal (10mg.) as catalyst. The catalyst was filtered off and the filtrate evaporated to dryness affording the saturated esters (41mg.), which were estimated by G.L.C.

Hydrogen bromide estimation [51,52]. Hydrogen bromide in acetic acid

reagent was standardised to approx. 0.1N against sodium carbonate standard using crystal violet as indicator, a correction for a blank titration being applied[65]. The triglycerides (approx.1.5g.) were transferred to a flask fitted with a magnetic stirrer and sealed leaving only a small hole for the burette. The oil was dissolved in benzene/acetic acid and titrated with hydrogen bromide/acetic acid at 3°, with crystal violet as indicator, until the blue-green end-point was reached and persisted for ½min. The solution was warmed to 55° and retitrated, a correction for a blank titration again being applied. The triglycerides were found to contain no epoxides and 30.4% sterculic acid.

Pancreatic lipase hydrolysis[60,66]. The triglycerides (0.595g.) were transferred to a jacketted vessel maintained at 40°. An ammonium chloride/ammonia buffer solution (30ml., 1.2M., pH 8.5) was added together with a solution of calcium chloride (2ml., 22%) and a solution of a bile salt (0.1ml., 25%). When the contents of the vessel reached 40°, pancreatic lipase (100mg.) was added and the vessel closed with a cover carrying glass and calomel electrodes and a stirrer. The contents of the vessel were maintained at pH 8.5 by the addition of ammonia (0.88.S.G.) from a micropipette.

After 10-15min., the contents were brought to pH 1 by the addition of hydrochloric acid (4N), the reaction mixture thoroughly extracted with ether and the combined ethereal extracts passed through a column of ion-exchange resin (30g., Amberlite IRA 400) to remove the liberated fatty acids.

The neutral glyceride mixture (216mg.) was chromatographed on silicic acid (30g.) and eluted with benzene/10% ether (200ml.) to obtain the tri- and di-glycerides (35mg., 16.3%) and with ether (200ml.) to obtain the monoglycerides (170mg., 78.8%). T.L.C. confirmed that the desired separation had been achieved.

The monoglycerides were converted to the methyl esters (137mg) using sodium methoxide in dry methanol, for estimation by G.L.C. A portion (34mg.) was hydrogenated and the resulting esters (31mg.) similarly examined.

Sterculia parviflora.

Preliminary tests. The oil gave a positive Halphen test and an infra-red absorption peak at 9.92μ.

Triglycerides. The oil (2.843g.) was chromatographed on silicic acid (80g.), eluting with benzene (200ml.) which removed the triglycerides (1.899g., 66.8%) and with ether (500ml.) to obtain the rest (0.935g.,32.9%. T.L.C. confirmed the separation. Some of the triglycerides (108mg.) were transesterified with sodium methoxide in dry methanol and the methyl esters (102mg.) estimated by G.L.C. Some of these (43mg.) were hydrogenated and the saturated esters (41mg.) examined similarly.

Titration with hydrogen bromide/acetic acid indicated that there were no epoxy acids in the triglyceride fraction but 60.7% by weight of cyclopropene acids (calculated as sterculic acid).

Lipolysis. The oil (0.611g.) was hydrolysed with pancreatic lipase and the glyceride fraction (0.315g.) chromatographed on silicic acid (30g.). Benzene/10% ether (200ml.) removed the tri- and di-glycerides (0.169g., 53.7%) and with ether (200ml.) to obtain the monoglycerides (0.126g., 40%). T.L.C. confirmed the separation.

The monoglyceride fraction was methylated with sodium methoxide in dry methanol and the esters (0.088g.) estimated by G.L.C. A portion (24mg.) was hydrogenated and the saturated esters (23mg.) examined similarly.

Sterculia macrophylla.

Preliminary tests. The oil gave a positive Halphen test and an infra-red absorption peak at 9.92μ.

Triglycerides. The oil (3.121g.) was chromatographed on silicic acid (80g.) eluting with benzene (200ml.) which removed the triglycerides (1.885g., 60.4%) and with ether (500ml.) to obtain the rest (1.192g., 38.2%). T.L.C. confirmed the separation. Some of the triglycerides (119mg.) were transesterified with sodium methoxide in dry methanol and the methyl esters (112mg.) estimated by G.L.C. Some of these (32mg.) were hydrogenated and the saturated esters (31mg.) examined similarly.

Titration with hydrogen bromide/acetic acid indicated that there were no epoxides in the triglyceride fraction and 67.5% by weight of cyclopropene acids (calculated as sterculic acid).

Lipolysis. The oil (0.601g.) was hydrolysed with pancreatic lipase

and the glyceride fraction (0.144g.) chromatographed on silicic acid (30g.). Benzene/10% ether (200ml.) removed the tri- and di-glycerides (24mg., 16.6%) and ether (200ml.) removed the mono-glycerides (106mg., 73.5%). T.L.C. confirmed the separation.

The monoglyceride fraction was methylated with sodium methoxide in dry methanol and the esters (95mg.) estimated by G.L.C. A portion (30mg.) was hydrogenated and the saturated esters (30mg.) examined similarly.

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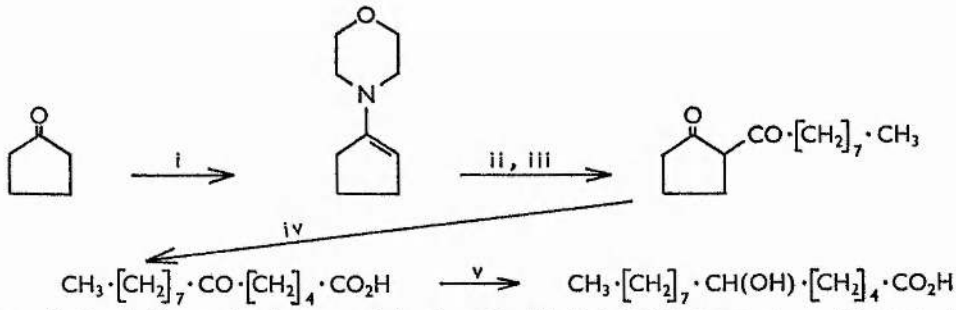
1100. *Shellac. Part I. The Structure of Butolic Acid.*

By W. W. CHRISTIE, F. D. GUNSTONE, and H. G. PRENTICE.

Hydrolysis of lac resin furnishes a mixture of hydroxy-acids from which 6-hydroxytetradecanoic acid has been isolated in 5—8% yield. This is considered to be identical with butolic acid, previously described as 6-hydroxypentadecanoic acid.

CRUDE lac consists of a resin accompanied by two colouring matters, an odoriferous principle, and a wax. The resin is probably a mixture of cross-linked polyesters largely derived from hydroxy-acids. The acids present in lac hydrolysate have been the subject of many investigations during the last forty years but little progress has been made in identifying them. The major component (~30%) is aleuritic acid (9,10,16-trihydroxyhexadecanoic acid) and this aliphatic compound is accompanied by an unsaturated, tricyclic, dibasic C_{15} dihydroxy-acid, shellolic acid. The structure of this acid has recently been elucidated,[1] but there is still uncertainty about the amount of it in lac resin and, indeed, whether it is a primary hydrolysis product. No other component has been adequately identified though there are reports of mono-,[2] di-,[2,3] and tetra-hydroxyhexadecanoic acids,[4] of a monohydroxypentadecanoic acid (butolic),[5] of acids closely related to shellolic acid,[1,6] and of carbonyl-containing acids.[7] Estimates of the composition of lac hydrolysate suggest that it contains aleuritic and isomeric acids (30—35%), aldehydic acids (20—25%), shellolic and related water-soluble acids (20—25%), and butolic acid (~2%), with the remainder undesignated;[8] the structures of aleuritic and shellolic acid only have been known hitherto with certainty.

We hydrolysed lac resin to its component acids and submitted the derived methyl esters to a number of separation procedures, mainly chromatographic. By these means a fraction was obtained (~7%) which was shown, by thin-layer chromatography and by gas–liquid chromatography, to be almost entirely a single component. Its chromatographic behaviour, and the fact that it gave methyl tetradecanoate by iodination-deiodination,[9] showed it to be a monohydroxytetradecanoate. The hydroxy-ester was oxidised to a keto-ester, the oximes of which, when submitted to Beckmann rearrangement, furnished amides; these were hydrolysed and two of the four possible products, nonanoic and adipic acid, were identified.[10] These must have come from 6-hydroxytetradecanoic acid.



Reagents: (i) Morpholine and toluene-*p*-sulphonic acid; (ii) $CH_3{\cdot}[CH_2]_7{\cdot}COCl$–$NEt_3$; (iii) HCl; (iv) K_2CO_3; (v) MeOH–HCl; $NaBH_4$; KOH.

6-Oxo- and (±)-6-hydroxytetradecanoic acid were synthesised and shown to be identical with similar products derived from the natural acid. This synthesis was achieved from cyclopentanone and nonanoic acid by adaptation of the enamine procedure described

by Hünig and Lendle.[11] The 6-oxo-acid has been synthesised previously by another method,[12] but neither the (±)- nor the (−)-hydroxy-acid has been reported before.

The natural acid was similar in many respects to butolic acid, considered by Sen Gupta and Bose [5,8] to be 6-hydroxypentadecanoic acid. It seemed likely that these might be the same and a re-investigation, by our chromatographic procedures, of a sample of butolic acid isolated by Sen Gupta and supplied by him, showed it to be a C_{14} acid with the hydroxyl group attached to C-6.

This acid, comprising 5—8% of the hydrolysate of the lac used in this investigation, can now be included with aleuritic and shellolic acid among the constituents of lac resin of known structure.

Experimental

Light petroleum is the fraction of boiling range 40—60°. Gas–liquid chromatography was carried out at 150° or 200° with a Pye Argon chromatograph and 4 ft. columns packed with Apiezon L (10%) or the fluorinated silicone, QF-1, (10%) on Celite. Relative retention times are reported as " carbon numbers." [13] In thin-layer chromatography the adsorbent was silica gel G (Merck) and the developing solvent a mixture of ether, light petroleum, and methanol (50 : 48 : 2); the separated components were detected by exposure of the plate to iodine vapour.

Isolation of Butolic Acid.—Butolic acid has been isolated from lac esters by a number of chromatographic procedures, one of which is described here. Its methyl ester is eluted from neutral alumina with ether, immediately after a small amount of non-hydroxy-esters, and from silica by 7 : 3 benzene–ether.

" Super Blonde Shellac " (200 g.) was hydrolysed by aqueous-alcoholic N-sodium hydroxide (1·1 l.) during 16 hr. The solution was not heated and care was taken to see that the temperature did not exceed 30° at any time. The free acids were liberated by passing the alkaline solution through a column of ion-exchange resin (Zeo-Karb 225) and were recovered by removal of solvent in a rotary-film evaporator at $\gtrsim$40°. Methylation was effected by boiling methanolic hydrogen chloride.

The mixed esters (116 g.) were placed on a column of alumina (750 g.; previously treated with ethyl acetate [14]). Ether (4·5 l.) eluted half of the esters and these were re-chromatographed on a similar column; successive 1-l. portions of ether eluted 1·2, 7·2, 2·5, 3·2, and 3·6% of the original esters. The second fraction was almost pure methyl butolate, a little of which was also present in the third fraction.

Methyl butolate, $[\alpha]_D^{19}$ −0·9° (*c* 6 in EtOH, *l* 2 dm.), had " carbon numbers " of 15·8 (Apiezon L) and 18·0 (QF-1). On a column containing only 2½% of Apiezon L the carbon number was 16·2. The methoxy-ester had a " carbon number " of 15·1 on Apiezon L. Hydrolysis of methyl butolate gave (−)-*butolic acid*, m. p. 58—58·5° (from light petroleum) (Found: C, 68·4; H, 11·4. $C_{14}H_{28}O_3$ requires C, 68·8; H, 11·6%).

6-Oxotetradecanoic acid was obtained by oxidation of this acid with chromic anhydride in acetic acid. After crystallisation from light petroleum it melted at 70—71° (lit., 71·5° [12]) (Found: C, 68·9; H, 10·8. Calc. for $C_{14}H_{26}O_3$: C, 69·3; H, 10·8). The semicarbazone had m. p. 129—131° (lit.,[12] 130°) (Found: C, 60·4; H, 9·8; N, 14·0. Calc. for $C_{15}H_{29}N_3O_3$: C, 60·2; H, 9·8; N, 14·0%). Methyl 6-oxotetradecanoate showed a " carbon number " of 15·4 (Apiezon L).

(±)-Butolic acid, m. p. 61·5—63° (from light petroleum), was obtained from the oxo-ester by reduction with sodium borohydride and subsequent hydrolysis.

Conversion of Methyl Butolate into Methyl Tetradecanoate.—Methyl butolate (52 mg.) was heated at 100° with iodine (270 mg.) and red phosphorus (620 mg.) for 1 hr. The excess of iodine was removed under reduced pressure and the residue, dissolved in ether, was washed successively with water, sodium hydrogen sulphite solution, and water. The iodinated ester (67 mg.) remaining after removal of the solvent was refluxed for 4 hr. with methanolic 5% hydrogen chloride (5 ml.) and activated zinc (~200 mg.). This metal was prepared as required by refluxing zinc dust (1 g.) with methanol (5 ml.) and hydrogen iodide (0·1 ml.) for 5 min., decanting the solvent, and washing the residue thoroughly with methanol. The organic product, recovered by ether-extraction, was undistinguishable from methyl tetradecanoate in its chromatographic behaviour (Apiezon L).

Position of the Hydroxyl Group.—Methyl butolate (50 mg.) was dissolved in acetic acid (1 ml.) and oxidised at room temperature by chromic anhydride (50 mg.) in acetic acid (1 ml.). After 1 hr., water (10 ml.) was added, the excess of oxidant was destroyed by sulphur dioxide, and the keto-ester (45 mg.) was recovered. This was refluxed for 2 hr. with hydroxylamine hydrochloride (85 mg.) and fused sodium acetate (82 mg.) in 80% ethanol (2·0 ml.). Thereafter the oximes (46 mg.) were recovered and heated to 100° with concentrated sulphuric acid (0·2 ml.) for 1 hr. to effect Beckmann rearrangement. After cooling, water (0·4 ml.) was added, and the mixture boiled to hydrolyse the amides. The resulting monobasic acid was extracted with light petroleum, and the dibasic acid subsequently with ether. After methylation, these were examined by gas–liquid chromatography and shown to be methyl nonanoate (Apiezon L and QF-1) and methyl adipate with " carbon numbers " of 9·0 (Apiezon L) and 11·5 (QF-1).

Synthesis of 6-Oxotetradecanoic Acid and (±)-6-Hydroxytetradecanoic Acid.—Nonanoyl chloride (6·67 g.) in dry chloroform (20 ml.) was added in 15 min. to a stirred solution of 4-cyclopent-1′-enylmorpholine [15] (5·10 g.), triethylamine (4·02 g.; distilled from sodium), and dry chloroform (40 ml.) at 35°. After a further 15 min., 6N-hydrochloric acid (20 ml.) was added and the two-phase mixture stirred vigorously for 30 min. at 35°. The chloroform layer was washed with water until the washings had pH 5—6 and the water layer, together with the aqueous washings, was neutralised with dilute sodium hydroxide solution to pH 5—6 and then extracted with chloroform. The combined chloroform extracts yielded 2-nonanoylcyclopentanone (6·35 g., 74%), b. p. 116—120°/0·4 mm., which gave a deep wine-red colour with methanolic ferric chloride.

The diketone was refluxed with 5% aqueous potassium carbonate (250 ml.) for 2·5 hr. and, after removal of neutral material by ether, the aqueous layer was acidified with hydrochloric acid; 6-oxotetradecanoic acid (4·98 g., 73%), m. p. 66—69°, was recovered. After crystallisation from light petroleum this acid (3·84 g., 41% based on the enamine) melted at 71—71·5° (lit.,[12] 71·5°) alone or mixed with oxo-acid derived from butolic acid (Found: C, 68·9; H, 10·7. Calc. for $C_{14}H_{26}O_3$: C, 69·3; H, 10·8%); its semicarbazone had m. p. 128—130° (lit.,[12] 130°) alone or mixed with a sample derived from butolic acid (Found: C, 60·4; H, 9·4; N, 14·1. Calc. for $C_{15}H_{29}N_3O_3$: C, 60·2; H, 9·8; N, 14·0). The methyl keto-ester had a " carbon number " of 15·4 (Apiezon L).

An excess of sodium borohydride (2·11 g.) was added to the keto-ester (1·0 g.) in methanol (50 ml.) in 30 min. Diluted with water and extracted with ether, the mixture furnished the hydroxy-ester (0·98 g., 98%) of " carbon number " 15·8 (Apiezon L) and 18·0 (QF-1). Hydrolysis gave the *(±)-hydroxy-acid* (78%), m. p. 65—66° (from light petroleum) alone and 63—65° when mixed with the (±)-acid derived from (−)-butolic acid (Found: C, 69·2; H, 11·6. $C_{14}H_{28}O_3$ requires C, 68·8; H, 11·6%).

Examination of a Sample of Butolic Acid Supplied by Mr. Sen Gupta.—A sample of butolic acid (70 mg.), isolated by the method of Sen Gupta and Bose,[5] was supplied by Mr. Sen Gupta. It melted at 50—55° alone and when mixed with our natural 6-hydroxytetradecanoic acid. Gas–liquid chromatography of the methyl esters showed only a peak of " carbon number " 16·2 (Apiezon L, 2½%), but thin-layer chromatography showed that the major spot due to a monohydroxy-ester was accompanied by minor spots indicating the presence of non-hydroxy- and dihydroxy-esters. This compound, degraded in the same way as our acid, gave nonanoic and adipic acid as the major mono- and di-basic acids.

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