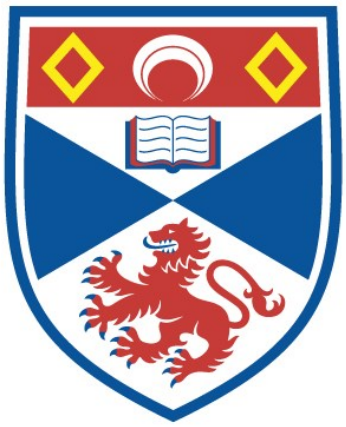
STUDIES OF SOME ALIPHATIC CONSTITUENTS OF SHELLAC

Hugh Graham Prentice

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

HUGH GRAHAM PRENTICE, A.R.C.S.T., A.R.I.C.

to the

UNIVERSITY OF ST. ANDREWS

in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY

AUGUST, 1962.

D E C L A R A T I O N

I hereby declare that this Thesis is a record of the results of my own experiments, that the Thesis is my own composition and that it has not previously been presented in application 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., A.R.I.C.

C E R T I F I C A T E

I hereby certify that Mr. Hugh Graham Prentice has spent eleven terms at research work under my supervision, has fulfilled the conditions of Ordnance No.16 (St. Andrews), and is qualified to submit the accompanying Thesis in application for the Degree of Doctor of Philosophy.

Research Supervisor.

ACADEMIC CAREER

I entered the Royal College of Science and Technology, Glasgow in September 1956, pursued a recognised course for graduation in Chemistry and graduated A.R.C.S.T. with First Class Honours in Chemistry in 1959.

I was admitted as a Research Student to the United College of St. Salvator and St. Leonard, University of St. Andrews in August, 1959 and was awarded a grant from the Indian Lac Exporter's Association. In October, 1961 I was elected an Associate of the Royal Institute of Chemistry.

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

I also wish to thank Professor J. Read, F.R.S., for the provision of research facilities.

I thank also my parents, for financial assistance during my undergraduate studies and without whose help I should not have been able to pursue my work to this stage.

I am grateful to the Indian Lac Exporter's Association for financial assistance throughout this work and for their gift of Super Blonde Shellac which was used throughout my research.

Finally, I thank Mr. R. Morris, Mr. T. Norris, and other members of the St.Andrews University, Chemistry Department for their help from time to time.

SUMMARY

Hydrolysis of lac resin yields a mixture of acids among which only two - aleuritic (up to 40%) and shellolic (up to 5%) - have been adequately characterised and examined by several investigators. The work embodied in this thesis represents some studies of the aliphatic acids in lac hydrolysate. The presence of a range of non-hydroxy acids (1.1%), 6-hydroxytetradecanoic acid (not less than 8%), 16-hydroxyhexadecanoic acid, and another monohydroxyhexadecanoic acid has been demonstrated. The 6-hydroxytetradecanoic acid has been shown to be identical with butolic acid recently isolated by chemists of the Indian Lac Research Institute but considered by them to be a C_{15} hydroxy acid. The other acids have not previously been reported as constituents of lac resin.

Reversed-phase chromatography was found to be unsuitable for the examination of lac acids, probably due to their highly hydroxylated nature.

Dehydroxylation experiments on lac acids by iodination-deiodination and by bromination-debromination revealed the presenc of hydroxylated tetradecanoic and hexadecanoic acids; bromination debromination reactions also showed the presence of small amounts

of vicinal dihydroxy acids of these two series.

Partition of lac acids between petroleum ether and 80% aqueous methanol and subsequent examination of the petroleum ether-soluble material (1.1%) revealed the presence of dodecanoic (trace), tetradec-9-enoic and tetradecanoic acids (25%), hexadec-9-enoic acid (13%), hexadecanoic acid (53%), octadec-9-enoic acid (7%) and octadecanoic acid (2%).

Examination of lac acids by adsorption, gas-liquid and thin layer chromatography showed the presence of 6-hydroxy-tetradecanoic acid (8% or more of lac acids), 16-hydroxy-hexadecanoic acid and another monohydroxyhexadecanoic acid.

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INTRODUCTION

I. THE FORMATION OF LAC

Lac resin is a secretion product of the lac insect which is parasitic on certain species of trees in India and some of the south east Asian countries.

The official name of the lac insect is laccifer lacca. The family is widespread geographically but the economic production of lac is confined to certain localities in India, Burma and Thailand where climatic conditions appear to be favourable to the insect. The insect is a natural parasite on such trees as the Palas(syn.Frondosa), Ber (syn.Jujuba) and Kusum(syn.Trijuga) in India.

When the larvae emerge from the mature female cell, they crawl along the branches, find a suitable spot to settle, and push their proboscis into the phloem tissues to reach the sap juices. The secretion of lac, which is produced by glands in the insect body, begins very quickly and, as the insect bodies touch and coalesce they form a continuous encrustation round the twig.

The lac is "cultivated" by the simple process of transferring the lac infection to fresh trees with young shoots. By treating trees in rotation and transferring, a few days before the emergence of the new generation of insect

is due, some encrusted twigs from the infected tree to a tree with fresh growth, a high proportion of the insects survive and produce a larger yield of lac.

The collection of lac consists in cutting off all the lac-encrusted branches of the tree. As far as possible the lac is then separated from the twigs by scraping or by twisting the twig to detach the encrustation, but the thinner twigs are simply chopped up into small pieces, and the aggregate of lac cells and wood fragments is known as sticklac.

Refining is carried out in two stages. The sticklac is ground to break open the cells and washed to remove sticks and the red dye (lac dye), after which it is known as seedlac. The seedlac is then melted and strained and either poured in thin films over cylinders or plates and, after cooling, scaled off in thin flakes, known as shellac, or it is poured into moulds to form "button" or garnet lac. This is the orange shellac of commerce. White or blonde shellac is produced by decolourising orange shellac with activated charcoal in alcoholic solution.

Shellac is used extensively in the production of paints and varnishes as a coating for wood and metal and in dielectric compositions. It is also an important ingredient in a number

of anti-fouling paints, helping to regulate the decomposition of the paint film, which makes fresh toxic material available at the coating surface.

II. CHEMICAL CONSTITUTION OF SHELLAC

A Resin Structural and Hydrolysis

Young[1] has described the shellac of commerce as, "a hard amorphous material consisting of a solid solution of several complex resinous condensates, associated with small and varying amounts of other products of the lac insect, in particular a red dye (laccaic acid), a yellow dye (erythrolaccin), an odoriferous principle, a wax and a resin", the latter being the portion of interest in the work embodied in this thesis.

A small portion (3%) of the lac resin is soluble in petroleum ether. Of the remainder about 25% is soluble in ether; this is a soft resin of acid value 100, molecular weight ca. 550, and behaving as a monobasic acid. The ether-insoluble fraction is much harder, has an acid value of 55, molecular weight ca.2000 and behaves as a dibasic acid.[1,2]

The resin structure and hydrolysis of lac was investigat by Knott[3], who found that after addition of alkali to a neutralized alcoholic solution of lac, the acid value of the lac rose rapidly from 66 to 125 units. The hydrolysis then slowed down and finally ceased at a point corresponding to an acid value of 136. This premature cessation of hydrolysis was

explained on the grounds that the presence in the solution of so many foreign ions (with consequent partial adsorption thereof) so increases the stability of the micelles that at a certain point, after the smaller and less stable micelles have been destroyed, resistance by the larger aggregates brings the hydrolysis to a standstill. Hydrolysis only recommences on warming the solution, thermal agitation thereby resulting in a further breakdown of micelles with a consequent exposure to attack of more hydrolysable groups. A similar effect was also noted by Whitmore et al[4] during the hydrolysis of lac with boiling 2N alkali. They found the rate of hydrolysis was much slower using 2N alkali than was the case with N alkali.

Knott[3] studied lac hydrolysis from the point of view of micelle structure and its influence on hydrolysis velocities but Whitmore, Weinberger and Gardner[4] made a detailed examination of the method of determining the saponification number of shellac. They explained their results on the basis that saponification proceeds in three distinct stages: (a) immediate neutralisation of free acid groups, (b) hydrolytic cleavage of easily saponifiable ester bonds such as occur in lactide or anhydride linkages and (c) saponification of remaining ester bonds.

Chemical studies of the nature of shellac to be detailed later have shown lac resin to consist of a mixture of hydroxy

acids. Some of these acids are monobasic and others are dibasic; most of them contain more than one hydroxyl group. These are the functional groups necessary for the formation of polyesters capable of intra-esterification[5]. It has been known for some time[6] that hydroxy acids having the hydroxyl carbon atom separated from the carboxyl group by four or more carbon atoms do not form cyclic esters (lactones), but instead esterify intermolecularly with the formation of polymeric esters. The way in which the constituent acids of shellac are linked in the molecule is still uncertain.[6,7]

Gardner et al.[4] are of the opinion, however, that since shellac is a natural product, it contains lactones, lactides and anhydrides, as well as inter-esters, but the evidence presented is too fragmentary to be conclusive.

It seems reasonable to suppose that the esterification process has already taken place to some extent in the sticklac and that the subsequent heat treatment[8] to produce shellac continues the reaction to a greater degree. Hence, aleuritic acid and any other straight chain hydroxy acids present in shellac would form linear polyesters, and, since dibasic acids are present, ester linkages are established between chains, and an infusible three-dimensional structure is eventually built up

B Constituent Acids of Shellac

Shellac contains colouring matter, a wax and the lac resin, which is of particular interest in the present investigations. This resin apparently consists of polyesters produced from several hydroxy-acids containing fifteen or sixteen carbon atoms. Hydrolysis of lac resin yields a complex mixture of acids which has been studied by several investigators. There is general agreement over a limited amount of information, but for the greater part there is disagreement amongst several investigators.

The constituent acids of shellac reported thus far, their isolation and structure, are discussed below.

1. Aleuritic Acid.

This is the most easily isolated acid from the resin hydrolysate, advantage being taken of the low solubility of its sodium salt. The yields of aleuritic acid are generally about 30%,[1,9,10] though Gidvani[11] claims 43%.

Aleuritic acid was first isolated by Tschirch and Farner[12] in 1899 and examined more fully by Harries and Nagel[10] (1922), who showed it to be a trihydroxy palmitic acid (m.p. 100-101°, methyl ester, m.p. 69-70°). Five years later Nagel[13] showed it to be 9, 10, 16 trihydroxy palmitic acid:

$HOCH_2(CH_2)_5CH(OH)CH(OH)(CH_2)_7COOH$

He oxidised the acid with ozone or with 3% alkaline potassium permanganate and obtained azelaic acid, $HOOC(CH_2)_7COOH$ and a syrupy C_9 hydroxy-acid which, on oxidation with hot potassium permanganate gave pimelic acid, $HOOC(CH_2)_5COOH$.

Raudnitz, Schindler and Petrů[14] confirmed the presence of the two adjacent hydroxyl groups by oxidation of aleuritic acid with lead tetra-acetate in acetic acid and obtained 8-formyloctanoic acid; $OHC(CH_2)_7COOH$.

Nagel and Mertens[15] further established that two of the hydroxyl groups were adjacent by their reaction with acetone in the presence of a dehydrating agent; oxidation of the iso-propylidene derivative (ketal) gave a dicarboxylic acid, hydrolysed by mineral acid to 9,10-dihydroxy hexadecanedioic acid (m.p. 125-127°).

Later, Nagel and Mertens[16] converted aleuritic acid into hexadec-9-enoic acid, which on oxidation with potassium permanganate gave threo-9,10-dihydroxy palmitic acid (m.p. 89-9[illegible]). The solid olefinic acid was apparently accompanied by a non-crystalline acid which was oxidised to erythro-9,10-dihydroxy palmitic acid (m.p. 125°). This might indicate that the aleuri[illegible] acid used by Nagel and Martens contained some erythro-9,10,16-trihydroxy palmitic acid in addition to the threo-isomer.

The first complete synthesis of aleuritic acid was by Baudart[17] using the following scheme of reactions:

$$CH_3O(CH_2)_6COOCH_3 \quad + \quad CH_2 = CH(CH_2)_7COOCH_3$$

$$\downarrow \text{Na, xylene}$$

$$CH_3O(CH_2)_6COCH(OH)(CH_2)_7CH = CH_2$$

$$\downarrow (Pr^1O)_3Al$$

$$CH_3O(CH_2)_6CH(OH)CH(OH)(CH_2)_7CH = CH_2$$

$$\downarrow \underset{AcOI}{Ac_2O,\ ZnCl_2}$$

$$AcO(CH_2)_6CH(OAc)CH(OAc)(CH_2)_7CH = CH_2$$

$$\downarrow O_3, KMnO_4$$

$$AcO(CH_2)_6CH(OAc)CH(OAc)(CH_2)_7COOH$$

$$\downarrow \text{Hydrolysis}$$

$$HO(CH_2)_6CH(OH)CH(OH)(CH_2)_7COOH$$

By this method, the two forms (m.p. 131-132° and 102-104°) of aleuritic acid have been prepared. The lower melting form was shown to be identical with the natural product which suggests that the glycol system in natural aleuritic acid has the threo-configuration. This has been confirmed by Hunsdiecker,[18] but it is still uncertain whether or not the acid is optically active.[8]

A second synthesis of the erythro- isomer of aleuritic acid has been reported by Hitter et al.[19,20,21]

The availability of aleuritic acid and its polyfunctional nature have made it a useful starting material for the preparation of other compounds of interest including civetone,[18,23,25] epiambrettolic acid[24] and 8-octadecenedioic acid.[25]

2. Isomer of Aleuritic Acid.

Schaeffer and Gardner[26] isolated what may possibly have been the methyl ester (m.p. 63-64°) of an isomer of aleuritic acid; they based their tentative conclusions on elementary analysis and Rast molecular weight determinations. The acid, prepared from the methyl ester in the usual manner had m.p. 97-97.5°.

Barnes[27] also claimed to have separated another isomer of aleuritic acid, m.p. 89-90°. This product was analysed and the number of hydroxyl groups was determined by preparing the acetate ester and estimating its ester number. The acid gave a viscous ethyl ester which was converted to a soft, semisolid, colourless hydrazide.

3. Shellolic Acid.

Shellolic acid (m.p. 199-201°, decomp.,) differs from aleuritic acid in being a cyclic compound. The acid has attract a great deal of attention partly because several investigators

have assumed that its presence in lac may have biochemical significance[28,29] and partly from the great difficulty of obtaining definite ideas relating to its structure.

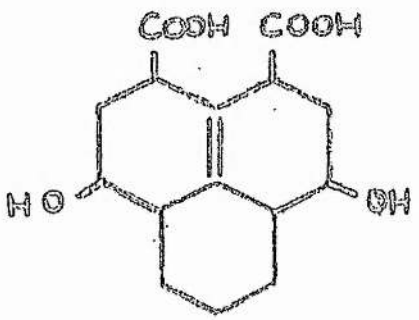
The acid was first isolated by Harries and Nagel[30] in 1922 from the hard portion of the lac resin. First they completely removed aleuritic acid from lac hydrolysate; the remaining acids were then converted to their methyl esters, and dimethyl shellolate was separated by crystallisation from a diethyl ether solution.

An improved separation of shellolic acid was claimed by Gidvani,[11] based on the low solubility of zinc shellolate in hot water, and this separation is the one most commonly used for its isolation.[34,36]

Other investigators[8,9,26,31] were unable to repeat the isolation of Harries and Nagel and so other methods for separating the acids have been developed, but which have yielded products of entirely different properties from those of shellolic acid. No one has yet succeeded in separating shellolic acid directly from lac hydrolysate and some authors were of the opinion that shellolic acid is not a primary product of hydrolysis,[11,27,28,30,32] but Gardner et al.,[35] consider that shellolic acid is one of the constituents of the inter-est

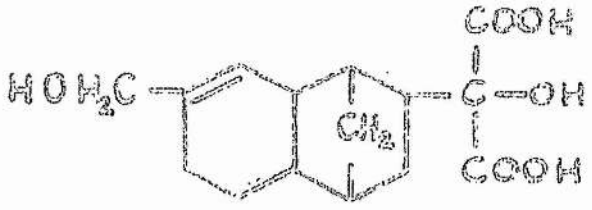
composing lac resin. These workers also found that the greatest quantity of shellolic acid obtainable from several different lacs in any one experiment was 3.6% of the lac resin; this result is in close agreement with the findings of Nagel and Mertens.[28]

The evidence presented for the structure of shellolic acid has, until recently, been very fragmentary and inconclusive Harries and Nagel,[30] largely on the basis of the empirical formula $C_{18}H_{20}O_6$ and the fact that the substance gave two colour reactions, namely the Liebermann cholesterol test and the Salkowsky-Hesse test, assigned a hydroaromatic structure (I) to shellolic acid.



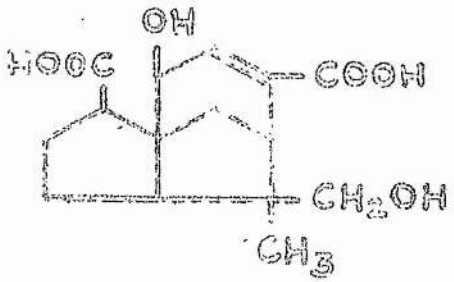
I.

Later, Nagel[28] changed this formula to a bridged naphthalene structure (II), but presented no chemical evidence for his conclusions.

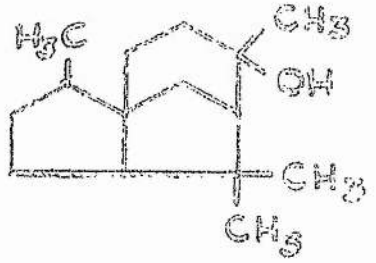


II.

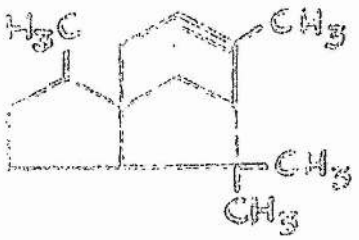
In fact, at that time and until very recently, the only facts known about shellolic acid were that it was an unsaturated, dihydroxy, dibasic acid of molecular formula $C_{15}H_{20}O_6$ which, on hydrogenation, gave dihydroshellolic acid (m.p. 158°). However, Yates and Field,[34] two American workers at Harvard, finally proved by a series of degradation reactions that shellolic acid is a sesquiterpene of the following structure (III) with the rare cedrene skeleton[35] (V):



III.



IV.



V.

The only other naturally occurring compound hitherto reported to possess this skeleton is cedrol (XV), an intimate relative of cedrene (V).

More recently, Carruthers, Cook, Glen and Gunstone[56] have described a series of experiments which support the formulation of Yates and Field.

4. Acids Related to Shellolic Acid.

Kirk, Spoerri and Gardner[53] isolated limited amounts, too small for complete purification, of five different crystalline products. Four of the products consisted entirely of hexagonal crystals like dihydroshellolic acid, and only the crystals of the fifth were cubic like shellolic acid. From elementary analysis and the crystalline form they suggest these products may have been the following: Two isomeric homologues of dihydroshellolic acid (m.p. 166 and 266°), an isomer of shellolic acid (m.p. 238°) and two isomers of dihydroshellolic acid (m.p. 226 and 245°). These tentative conclusions must be regarded as very doubtful until some positive proof of the structure of these compounds is produced.

Bhattacharya[8] and Gardner[9] in attempts to isolate shellolic acid obtained acids m.p. 91°, which Barnes[27] considered

to be the same material. The acid, designated lacolic lactone by Gardner, was amorphous and no satisfactory elementary analysis was obtained. This supported Barnes[9] idea that the substance may be a polyester of shellolic acid. Kirk[38] later showed that lacolic lactone was a mixture of dibasic acids.

5. Other Acids.

In addition to the compounds reported above, there are several other acids which have been reported at one time or another, but none of which has been adequately confirmed. These are mainly hydroxy acids though recent studies have also suggested the presence of an aldehydic acid.

Tschirch and Ludy[37] claimed to have been able to break down the ether-soluble portion of lac into monohydroxy palmitic acid; they also reported the possible existence of dihydroxy palmitic acid, but Gupta[30] was unable to find these substances.

Gardner and his colleagues[9,26] have isolated two samples of a tetrahydroxy palmitic acid (m.p. 132°, 135°) which they named kerrolic acid. On acetylation with acetyl chloride the product had an ester number of 500 for a tetraacetyl derivative of empirical formula $C_{16}H_{32}O_6$. Both samples of acid gave hydrazides whose melting points, molecular weights and composition were in fairly close agreement. A mixture of both hydrazides showed no depression in melting point, but when

attempts were made to prepare other derivatives of this acid, the substance was very susceptible to the effect of heat and alcoholic solutions of the acid turned yellow below the boiling point, indicating either decomposition or polymerisation. In fact, when such solutions were evaporated to dryness, a clear yellow resin was obtained giving further evidence of polymerisation. No degradation reactions have been described for this compound.

In 1952, Sen Gupta and Bose,[39] by fractional precipitation of the heavy metal salts of lac acids, claimed to have obtained a monohydroxy monobasic acid, m.p. 54-55°, which they name butolic acid. This acid, which appeared to have the molecular formula $C_{15}H_{30}O_3$, was reduced to pentadecanoic acid and was considered by Sen Gupta and Bose to be monohydroxy pentadecanoic acid different from the known 11-hydroxy pentadecanoic acid (m.p. 63.5-64°). No further attempt has been made to locate the hydroxyl group and fuller and independent proof of the structure is desirable.

The presence of an aldehydic acid was recently claimed by Kamath[40] (m.p. of 2,4-dinitrophenylhydrazone 232°), which he named Jalaric acid and is reported present to the extent

of 50% in Jalari seedlac. According to Kamath the empirical formula of this acid is $C_{16}H_{32}O_5$ and the acid contains one carboxyl, one carbonyl in the form of an aldehyde, and two hydroxyl groups.

Sen Gupta *et al.*,[40,41] using Kamath's procedure report the presence of an aldehydic acid both in kusmi seedlac (25%) and in shellac (10%), but none of these reported aldehydic constituents has been adequately confirmed nor any evidence presented for their possible structure.

Conclusions.

Aleuritic (up to 40%) and Shellolic acids (up to 5%) are the only constituents of shellac whose structure has definitely been established, indicating the presence of two types of acids in shellac.

The greater part of the composition of lac is still unknown and the various other acids reported from time to time have not been adequately confirmed.

The work embodied in this thesis is mainly concerned with the aliphatic components of lac.

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PART I

An attempted separation by Reversed-Phase Chromatography and Solvent Fractionation of Lac Acids.

DISCUSSION

The purpose of this investigation was to isolate and identify acids present in lac hydrolysate other than those (aleuritic and shellolic) which have already been identified.

Most separations of lac acids have been based hitherto on the differing solubilities of various metallic salts. In general these methods are unsatisfactory; yields are poor, the techniques chemically vigorous, and separations incomplete. Clearly, more efficient techniques are desirable and reversed-phase chromatography was first examined. At the same time a sample of lac acids was separated into several fractions by solvent fractionation.

In column partition chromatography a mobile phase is percolated through a column containing the stationary phase held on an inert support. For satisfactory chromatography the stationary phase should be the solvent in which the compounds to be separated have greater solubility; they are then eluted by the greater volume of mobile phase in which they are less soluble.

Partition chromatography has been applied to the separation of long chain fatty acids by making the less polar solvent the stationary phase. This modification is known as reversed-phase chromatography.

This procedure was first proposed by Howard and Martin,[1]

who separated mixtures of C_{12} to C_{18} monobasic acids using liquid paraffin and a range of aqueous acetones of increasing acetone content as stationary and mobile phases respectively. The paraffin was held on Kieselguhr made non-wetting by treatment with dichlorodimethylsilane. The scope of this method has been extended by other investigators[2,3,4,5,6,7] to the examination of fatty acids of varying chain length.

Of greater interest in the present connection are reports of the separation of hydroxy acids by this method. Savary and Desnuelle[8] separated di- and tetrahydroxy C_{18} acids using castor oil and Desnuelle and Burnet,[8,9] using a powdered rubber support, extended this method to a range of hydroxy acids. Matic[10] used this technique to separate the mono-, di- and trihydroxy acids of plant cuticles whilst Gunstone and Sykes[11] have successfully separated mono- and dihydroxy acids. It therefore seemed appropriate to try and separate the lac acids in this way.

In the experiments made, Kieselguhr made non-wetting by treatment with dichlorodimethylsilane was used as the inert support and the mobile phase consisted of a range of aqueous acetones of increasing acetone content. Castor oil and acetylated castor oil were employed as stationary phases.

The chromatographic behaviour of aleuritic acid, shellolic

acid and lac acids was examined but in all cases unsatisfactory results were obtained. Recoveries were low and the elution curves as determined by titration of 2 ml. eluates with standard alkali indicated that the acids were not being eluted in a sharp band. The partition coefficients of aleuritic and shellolic acid in various aqueous acetones were measured in order to determine the optimum eluting solvent[6] but indefinite results were obtained.

The difficulties encountered with these lac acids are probably related to the more hydrophilic nature of aleuritic acid (a trihydroxy palmitic acid) and shellolic acid (a dihydroxy dibasic acid). It was reasonable, therefore to overcome this difficulty by reducing the hydrophilic nature of these acids and in this connection several preparations of acetylated aleuritic acid were examined. The chromatographic results were slightly more promising with the acetylated acids but the acetylation procedures examined were not satisfactory. These experiments were therefore abandoned and attempts were made to examine the acids after removal of the hydroxyl groups (see Part II).

At the same time as the reversed-phase chromatography experiments were being made the lac acids were fractionated by a range of solvents. The lac acids which had been isolated from

the lac with particular care to avoid high temperatures or vigorous conditions were extracted successively with water, petroleum ether (40-60°), ether and ethyl acetate. The solvent was removed from each extract at a temperature not exceeding 40°. These fractions were used in subsequent studies.

The results indicated a possible method for the preliminary fractionation of lac acids. Shellolic acids and other acids of this type are probably concentrated in the water extract and the petroleum ether, ether and ethyl acetate extracts should contain acids of increasing hydrophilic character. This type of separation is, of course, far from perfect but differences in the extracts are indicated later (Part II).

EXPERIMENTAL

The chromatographic procedures used in the present work were based on the directions of Howard and Martin,[1] Silk and Hahn[3] and Gunstone and Sykes.[6,11]

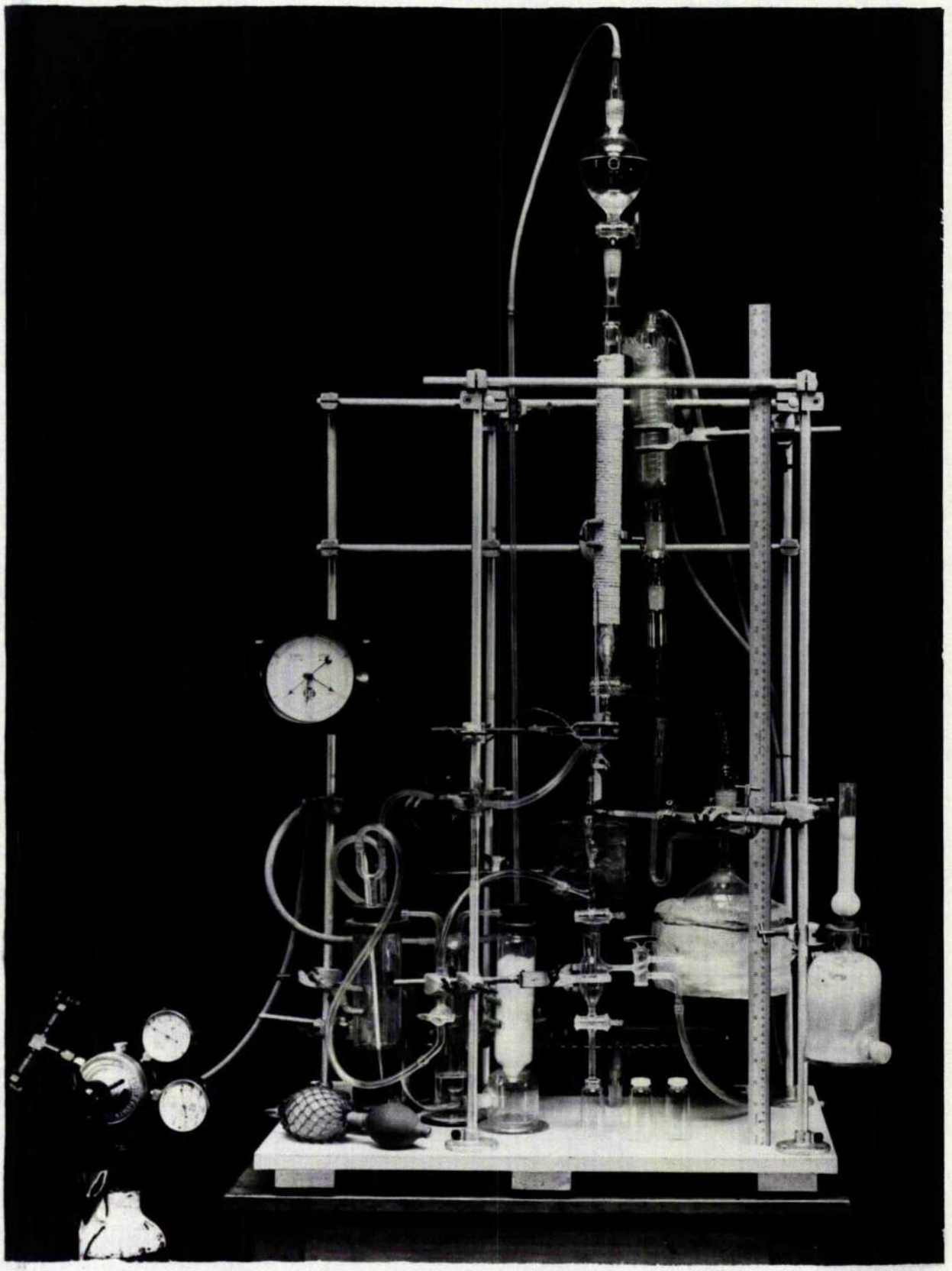
Materials.

Non-wetting kieselguhr was prepared by exposing Hyflo-supercel to the vapours of dimethyldichlorosilane in a partially evacuated desiccator. The siliconised material was washed free of acid with methanol and dried at 110°. Medicinal castor oil was shaken with petroleum ether and the petrol extracts rejected, as described by Achaya and Saletore.[12] The solvent was removed from the residual oil and the product neutralised by passage through an alumina column.

Acetylated cator oil was obtained by acetylation (acetic anhydride) of the above product.

The mobile phase used for all the chromatograms consisted of a range of aqueous acetones.

The mull used for packing the columns was made up in batches, the ratio of siliconised kieselguhr to stationary phase being 1.4 for paraffin and 1.3 for castor oil or acetylated castor oil.



Apparatus.

A photograph of the apparatus is shown opposite.

After loading the sample on top of the column, elution was effected from a solvent reservoir. The eluate collects in a small siphon of 2ml. capacity (Fig. 1), and nitrogen gas protects it from atmospheric carbon dioxide.

From the siphon, the eluate passes into a specially constructured cell (Fig. 2) where it is titrated under a stream of nitrogen with 0.01N methanolic potassium hydroxide, using an Agla-micrometer syringe as a microburette; the indicator solution used was bromothymol blue. The cell was lit with a background of white light.

At the end of a titration the liquid was drained from the cell through a capillary tube by means of the apparatus shown in Fig. 3.

Experimental Technique.

Castor oil or acetylated castor oil Kieselguhr mull was poured into the column as a slurry with 60% aqueous acetone and the packing was compressed at an excess pressure of 5 cm. mercury.

The mixture of acids to be separated was added to the column in the requisite mull.

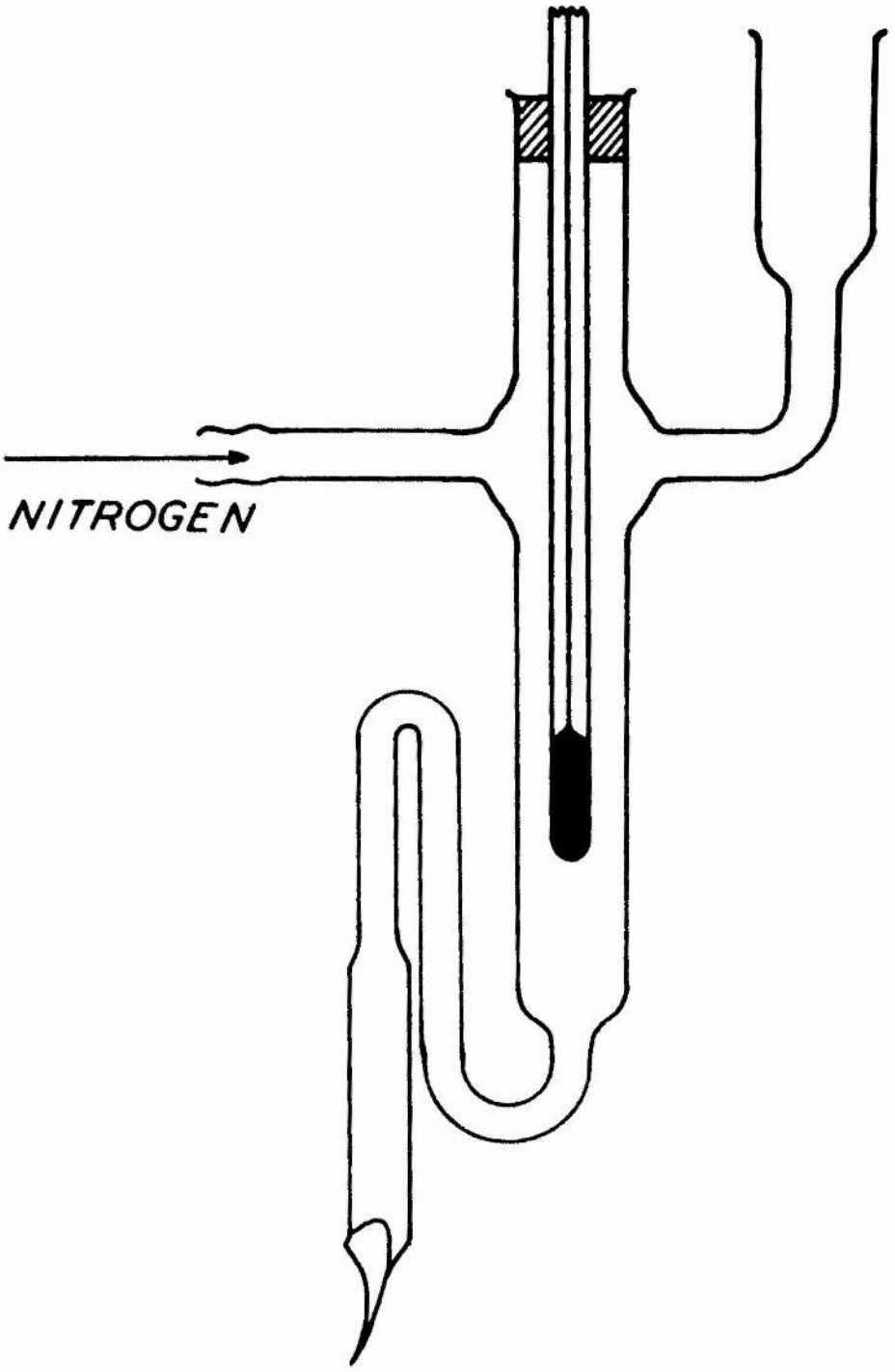


FIG 1. THE SIPHON.

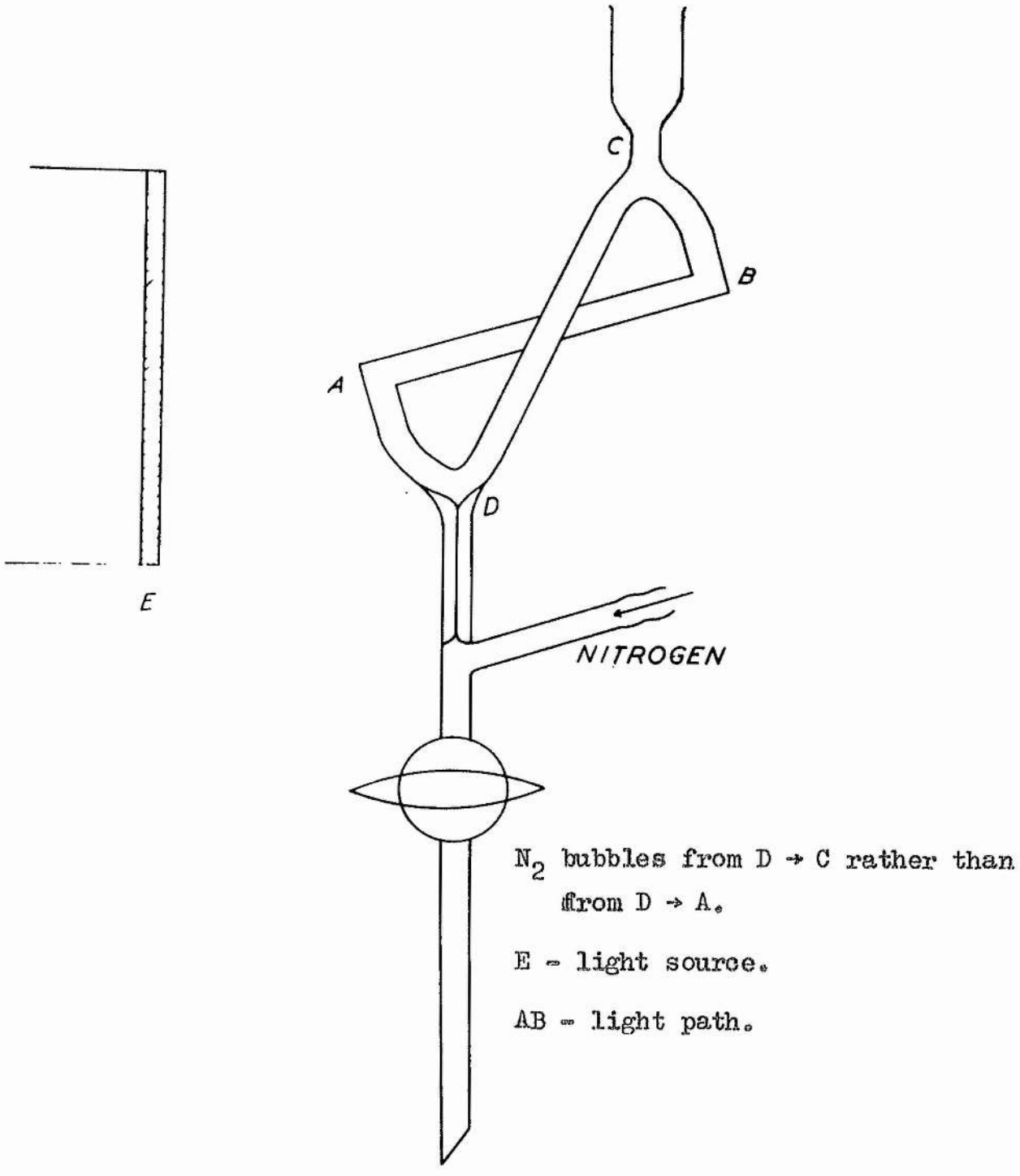


FIG 2. THE TITRATION CELL.

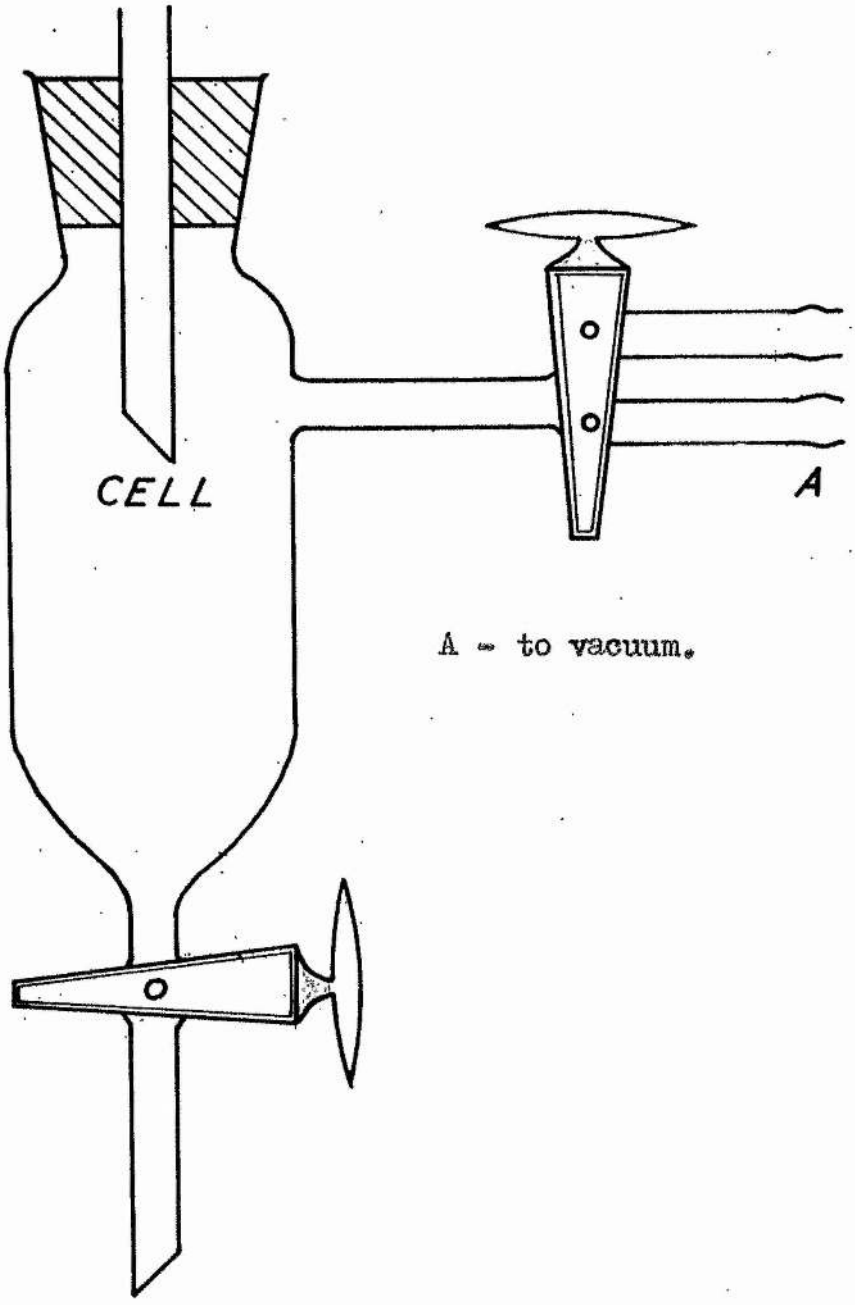


FIG 3. VACUUM DRAIN.

Values of the partition coefficient (K) were measured under conditions which resembled those operating during column elution by the method of Gumstone and Sykes.[6]

Aleuritic Acid.[13]

Super Blonde Shellac (200 g.) was dissolved in water (200 ml.) containing sodium hydroxide (40 g.) by warming on a water bath. The hydrolysis was allowed to proceed at room temperature for ten days. The mixture was then diluted with sodium hydroxide (5N: 250 ml.) and the liquid filtered through kieselguhr. The insoluble sodium salt retained on the filter was dissolved in the minimum quantity of hot water and the hot solution filtered. The sodium salt crystallised as a brown powder (24 g.) and a further crop of the sodium salt (6 g.) was obtained from the concentrated mother liquors. Acidification of the sodium salts gave aleuritic acid (26 g.) which was recrystallised from ethanol:water (1:1) to give the pure acid (24 g., m.p. 99-100°, Lit.[13] 100-101°).

Shellolic Acid.

Dimethyl shellolate (1 g.)[14] was refluxed with a solution of sodium hydroxide (0.5 g., 100% excess) in water (15 ml.) for 3 hr. The mixture was cooled and the acid liberated with the

required amount of Zeo-Karb 225 ion-exchange resin. Using a rotary film evaporator, water was then distilled off under reduced pressure until an oil separated. Crystallisation was induced by scratching the inside walls of the boiling tube with a glass rod to give impure shellolic acid (0.534 g.). The acid was recrystallised from water (0.28 g., m.p. 212-213°, Lit.[15] 202-203°).

Acetylation of Aleuritic Acid.

Method 1.

Aleuritic acid (3.14 g.) was refluxed with acetic anhydride (6.6 ml.) for 3 hr. Water (5 ml.) was added and the mixture boiled for a further 30 min. The product was extracted with ether and the ether extracts washed with water (10 x 100 ml.); the ether was then removed under reduced pressure and the product evacuated to constant weight (3.81 g.; 88.6%). The product had an equivalent weight of 519 (Theoretical value = 430). This value was unchanged by washing with water but was reduced to 474 by boiling with water to destroy any anhydride.

Method 2.[16]

A mixture of finely powdered aleuritic acid (1.52 g.), ether (8 ml.), acetyl chloride (5.5 ml.) and sufficient acetone to make the mixture homogeneous was refluxed for 2 hr. The solvent was then removed and the product (1.75 g., 81%) extracted

with ether in the usual manner. The equivalent (860) was reduced to 422 after boiling with water.

Method 3.

A mixture of finely powdered aleuritic acid (1.52 g.), ethyl acetate (50 ml.) and acetyl chloride (5.5 ml.) was allowed to stand overnight. One portion was evaporated to constant weight (0.79 g., equivalent weight 545); the remainder was treated with water and extracted with ether (0.85 g., equivalent weight 471).

Method 4.[17]

A hydroxyl containing compound (4 mmole) and acetic anhydride (2M, 5 ml.) in ethyl acetate containing perchloric acid (or p-toluenesulphonic acid) as catalyst were allowed to react for 5 min. at room temperature. Water (2 ml.) was then added followed by pyridine/water solution (3:1, 10 ml.) and the mixture allowed to stand for 10 min. The amount of hydroxyl compound present was calculated from the difference between titrations of the blank and sample with sodium hydroxide using cresol red/thymol blue indicator. A summary of the results obtained for control compounds and for aleuritic acid are given below. The results are expressed as the number of hydroxyl groups acetylated.

Catalyst	Cyclohexanol	Dihydroxy Stearic Acid	Aleuritic Acid.
Perchloric Acid	0.96 0.92	1.87 1.85	2.55 2.62
p-Toluene sulphonic Acid	0.85 0.88	1.59 1.58	2.50 2.63

The acetylation of aleuritic acid was further examined using various reaction times. The results are tabulated below

Reaction Time (min.)	No. of OH groups acetylated	Acetylation %
10	2.72	90.5
20	2.94	98.1
30	2.92	97.4
60	3.10	103.4

On the basis of the above results aleuritic acid triacetate was prepared as follows:

Aleuritic acid (0.3 g.) was shaken with the perchloric acid acetic anhydride reagent (5 ml.) until dissolved and allowed to react at room temperature for 30 min. Water (2 ml.) was added, followed by pyridine/water mixture (3:1, 10 ml.) and the mixture allowed to stand for 10 min. The product was then extracted with ether and worked up in the usual manner to give the triacetate

(0.39 g., 91.2%, equivalent weight 440).

Lac Mixed Acids.

Super Blonde Shellac (20 g.) was hydrolysed with N. aqueous alcoholic sodium hydroxide (110 ml.) without external heating for 16 hr., care being taken that the temperature did not exceed 30°. The free acids (19.2 g.) which were liberated by passage through a column of Zeo-Karb 225 ion-exchange resin, remained after complete evaporation of the solvent at a temperature not exceeding 40°.

Reversed Phase Chromatography Experiments.

The following experiments were carried out.

1 and 2.

Using an acetylated castor oil column neither aleuritic acid nor shellolic acid could be eluted.

3 and 4.

Using a castor oil column aleuritic acid was not eluted and lac mixed acids were removed only in low yield (40%) with no well defined peaks.

5 to 8.

The acetylated aleuritic acid (method 1 and 4) was chromatographed on a castor oil and an acetylated castor oil column. Reasonable elution curves were obtained but the

recovery in all cases was low (55-65%).

Solvent fractionation of Lac mixed acids.

A portion of the mixed acids (15.2 g.) was extracted with a range of solvents by shaking, rotating or boiling under the conditions set out in the table below. Soluble material was recovered by evaporation of the solvent at temperatures not exceeding 45°C.

Solvent	Vol.(ml.).	Temp.	Extract (g., %)
Water	2 x 300	15-20°	4.40 (28.1) 0.19 (1.2)[a]
Petroleum Ether (40-44°)	300	40-44°	0.17 (1.1)
Ether	3 x 300	35°	6.99 (44.6)
Ethyl Acetate	300	15-20°	1.76 (11.2) 1.14 (7.3)[b]
Residue			0.34 (2.2)
Losses			0.67 (4.3)

(a) A white crystalline solid which separated during extraction, m.p. 93-95°, mixed m.p. with aleuritic acid (m.p. 99-100°) 93.5-95.5°, analysed satisfactorily for $C_{16}H_{32}O_5$ (required: C,63.1 H,10.60%; Found: C,63.38; H,10.55%).

A comparison of the infrared absorption spectrum of the material with that of aleuritic acid confirmed its identity.

(b) A pale brown crystalline solid (m.p. 81-84°) which separated during extraction. Experiments regarding the nature of this material are given in Part II.

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P A R T II

Examination of Lac acids after Dehydroxylation

DISCUSSION

In the examination of natural hydroxy acids, useful results have followed from the examination of dehydroxylated compounds. In his study of cutin from plants, Matic[4] reduced the ether-soluble fraction of cutin to the parent acids by an iodination - deiodination procedure. Downing, Kranz and Murray,[10] in an examination of the aliphatic constituents of hydrolysed wool wax used a similar procedure to convert hydroxy acids to the corresponding iodides, followed by further reactions to the parent hydrocarbons; these were than identified by gas-liquid chromatography.

Since the failure of Reversed-Phase Chromatography may be attributed to the highly hydroxylated nature of lac acids, it was considered that dehydroxylation would give more illuminating results. At about this time also, we obtained facilities for gas-liquid chromatography (G.L.C.).

Iodination - Deiodination by Matic's procedure.

Matic's procedure[4] involving the reaction of a hydroxy acid with hydrogen iodide and phosphorus followed by zinc, methanol and concentrated hydrochloric acid, has been applied to (i) aleuritic acid, (ii) 9,10-dihydroxyoctadecanoic acid, (iii) shellolic acid, (iv) ether-soluble lac acids, (v) ethyl acetate-soluble lac acids, (vi) lac mixed acids and (vii) ethyl

acetate - insoluble lac acids.

(i) Aleuritic Acid

Aleuritic acid gave methyl hexadecanoate as expected. At first the yield was low (40%) because methylation was incomplete but when the product was re-esterified this rose to 80%. Following Matic, the product was examinated only after percolation through alumina; it was later discovered that other products also formed were removed by this means (p.38).

(ii) 9,10-Dihydroxyoctadecanoic acid.

A similar reaction with 9,10-dihydroxyoctadecanoic acid yielded the expected methyl octadecanoate.

(iii) Shellolic acid.

With dimethyl shellolate, no definite results were obtained.

(iv) The ether-soluble lac acids (see p. 32)

When this fraction of lac acids was dehydroxylated, G.L.C. showed the product to be very complex. Many peaks (16-25) have been observed and it has not been possible to designate the majority of these.

Examination of the dehydroxylated product by urea adduct formation or hydrogenation or R.P.C. gave little useful information. The major components had carbon numbers of

14.0, 16.0, 17.5, 18.0, 18.5 and 20.0 and an attempt has been made to desginate only these.

Carbon number 14.0.

This is probably methyl tetradecanoate because of its behaviour on G.L.C. and also on R.P.C. where it is eluted from paraffin columns with 62% acetone.

Carbon number 16.0.

This is eluted from a paraffin column (R.P.C.) with 67% acetone and has been isolated. The melting point of the acid and the ester and its chromatographic behaviour show it to be methyl hexadecanoate.

The remaining peaks are designated with less certainty:

Carbon Number	Eluting solvent (% acetone) on R.P.C. column	Possible identification
17.5	40)	Dihydroxy
20.0	40)	compounds
18.0	53)	Monohydroxy
18.5	62)	compounds

These must result from incomplete reaction during iodination-deiodination.

Hexadecanoic acid would result from any aleuritic acid present in this fraction and also from any other hydroxylated C_{16} acids. The formation of tetradecanoic acid indicates the

presence of one or more hydroxylated C_{14} acids. Acids of other chain length either do not occur or are present only in minor amounts in the ether-soluble lac acids. Later experiments indicate that the ester of carbon number 18.5 which occurs frequently throughout this work may be methyl-16-hydroxyhexadecanoate present as such in the lac acids and/or as aleuritic acid or other poly-hydroxylated compound.

(v),(vi) The ethyl acetate-soluble lac acids and and total lac acids.

Similar examinations of the dehydroxylated products from the ethyl acetate-soluble and the total lac acids (p.32) revealed much the same results as in (iv) with the main components having carbon numbers of 14.0, 16.0 and 18.5.

(vii) Ethyl acetate-insoluble lac acids.

The recrystallised ethyl acetate insoluble material, (p.32) when submitted to dehydroxylation also produced carbon numbers of 14.0, 16.0 and 18.5 when examined by G.L.C. The material was therefore concluded to be a mixture of C_{14} and C_{16} acids.

An improved iodination-deiodination procedure.

In the initial dehydroxylation study of aleuritic acid the product was examined by G.L.C. after treatment with alumina. Subsequent examination of another portion of dehydroxylated

aleuritic acid by G.L.C. without alumina treatment showed a more complicated chromatogram and attempts were therefore made to improve the dehydroxylation procedure. In this connection, three portions of aleuritic acid were submitted to different dehydroxylation procedures and gave the results set out below.

PROCEDURE	G.L.C. Carbon Nos.
1. (i) HI/P (Ref.4) (ii) activated Zn/aq.HCl/CH_3OH (iii) methylation	16.0 v. minor 17.3
2.A. (i) I_2/P (Ref.10) (ii) activated Zn/aq.HCl/CH_3OH (iii) methylation	16.0
B. (i) I_2/P (ii) activated Zn, anhydrous HCl, methanol	16.0

The chromatogram of product 2B was the most satisfactory and since this procedure involved reduction of one reaction stage, it was decided to adopt this method in future

dehydroxylation reactions.

The ether-soluble lac acids were examined by the improved procedure but the dehydroxylated material again gave the recurring carbon numbers of 14.0, 16.0 and 18.6, probably corresponding to tetradecanoic, hexadecanoic and 16-hydroxyhexadecanoic acids. Separation of these three products by distillation was unsuccessful.

Lac mixed acids were examined in the above manner and the dehydroxylated product gave the following carbon numbers when examined by G.L.C.:

13.8, 14.0, 15.0, 16.0, 18.4, 18.6.

The main peaks are underlined.

The dehydroxylated esters were hydrolysed and the acids examined by R.P.C., but they could not be cleanly resolved by this method, probably due to overloading of the columns.

Examination of dimethyl shellolate by the improved procedure again gave no clear results.

General conclusion from iodination-deiodination procedures.

The ether-soluble and ethyl acetate-soluble portions of lac acids and the lac mixed acids, when submitted to iodination-deiodination to reduce OH groups, give complex mixtures in which tetradecanoic and hexadecanoic acids have

been recognised as major components. Aleuritic acid would give rise to the C_{16} acid though other hydroxy acids of this chain length may be present. One or more hydroxy C_{14} acids must also be present. Acids of other chain length seem to be only minor components.

Later studies showed that tetradecanoic and hexadecanoic acids were themselves present among lac acids but these concentrate in the petroleum ether-soluble fraction and though small amounts were later shown to occur in the ether-soluble fraction it is not considered that these were sufficient to account for all the C_{14} and C_{16} acids obtained after dehydroxylation.

Bromination - Debromination.

The dehydroxylation studies already discussed were based on an iodination-deiodination procedure in which all hydroxyl groups were replaced by hydrogen.

An improved procedure involves (a) bromination[11] of all hydroxyl groups, (b) a debromination reaction in which all vicinal dibromides yield olefinic compounds and isolated bromo-substituents are unaffected (later experiments showed that this was not entirely true), (c) removal of isolated bromo-substituents by iodination-deiodination.[12] The reaction is illustrated for aleuritic acid:

$HO(CH_2)_8.CH(OH)CH(OH).(CH_2)_7.COOH$

Stage 1 ↓ (i) HBr, AcOH, $C.H_2SO_4$; (ii) CH_3OH, HCl

$Br(CH_2)_8\ CHBr.CHBr(CH_2)_7\ COOCH_3$

Stage 2 ↓ Zn, CH_3OH

$Br(CH_2)_8\ CH = CH\ (CH_2)_7\ COOCH_3$

Stage 3 ↓ (i) NaI, acetone; (ii) Zn, CH_3OH, HCl

$CH_3(CH_2)_8CH = CH(CH_2)_7COOCH_3$

This procedure shows the chain length of the original hydroxy acid and also indicates the presence of α-diol groups, the actual position of which could be determined by oxidation experiments.

(i) 9,10-Dihydroxyoctadecanoic acid.

This compound gave methyl octadec-9-enoate (carbon number 17.8), with none of the corresponding saturated ester.

(ii) Ether-soluble lac acids.

This technique was applied to the ether-soluble lac acids and the products examined by G.L.C. After bromination-debromination, chromatography showed a large peak of carbon numbe

19.5 (Methyl 16-bromohexadec-9-enoate), smaller peaks of carbon numbers 13.8 (tetradecenoate) and 15.8 (hexadecenoate) and still smaller peaks of carbon numbers 14.0 (tetradecanoate) and 16.0 (hexadecanoate).

Several interesting conclusions drawn from these results are set out in the next paragraph. They are, however, subject to the following assumptions, and experiments were carried out (see later) to test these (i) that the reactions proceed as set out above.for aleuritic acid without any by-products, (ii). That all the tetradecenoate and hexadecenoate found after the reaction is not present in the lac fraction used in this investigation (this is not likely for the reasons already outlined in the iodination-deiodination discussion).

The bromohexadecenoate at the end of stages 1 and 2 which subsequently affords additional hexadecenoate is the product expected from aleuritic acid. This is, however, accompanied by very small amounts of saturated C_{14} and C_{16} esters which must be present in the original acids, and by larger amounts of unsaturated C_{14} and C_{16} acids probably derived from vicinal dihydroxy acids of these chain lengths.

A portion of the esters from the ether-soluble fraction

of lac acids after stage 3 was hydrolysed and the acids examined by R.P.C. Separation of the constituents was unsuccessful, probably due to overloading of the columns. During hydrolysis of these esters the material of carbon number 18.5 (16-hydroxyhexadecanoic ester) appeared to alter to 17.8. Methyl 16-methoxyhexadec-9-enoate was later found to have a carbon number of 17.8, but it seems unlikely that the conversion of $OH \Rightarrow OCH_3$ would take place either during hydrolysis with alcoholic potassium hydroxide or during subsequent esterification with methanolic hydrogen chloride.

(iii) Aleuritic acid.

Since it is important that the assumptions made above be examined rigorously the bromination-debromination of aleuritic acid was studied. During the course of these experiments the carbon numbers of several possible products were deduced. They are summarised below:

	Hexadec-9-enoic acid	Hexadecanoic acid
16-OH	18.3	18.5
16-Br	19.5	19.8
16-I	20.7	-
16-MeO	17.8	-
16-Acetoxy	18.2	-

Bromination-debromination of aleuritic acid gave, in addition to the expected 16-bromohexadec-9-enoic acid, smaller amounts of 16-bromohexadecanoic, 16-hydroxy-hexadec-9-enoic, hexadec-9-enoic and hexadecanoic acid. Hydrolysis with aqueous potassium hydroxide then furnished 16-hydroxy-hexadecanoic acid (along with smaller amounts of hexadec-9-enoic and hexadecanoic acids) and the same product was obtained by an alternative sequence in which the bromo acid was converted to the hydroxy acid via the iodo and acetoxy compounds. 16-Methoxyhexadecanoic acid was prepared from the 16-hydroxy acid.

The hexadec-9-enoic and hexadecanoic acids accompanying the major products could arise from impurities in the original aleuritic acid or as products of side reactions and in this connection it was shown that bromination-debromination of pure methyl 16-hydroxyhexadecanoate gave the 16-bromo acid accompanied by a little hexadecanoic acid. It is apparent that the isolated bromine atom is partially reduced during reaction.

Conclusion.

These experiments have revealed the presence of hydroxylated C_{14} and C_{16} acids in lac hydrolysate including the possibility of some vicinal dihydroxy compounds (give rise to tetradecanoic and hexadecanoic acids in the bromination-debromination reaction). They have also shown several limitations of this approach:

(i) The products obtained and recognised give very little information about the structure of the acids from which they are derived and may result from several different compounds.

(ii) The complexity of the product which arises from the complex nature of the starting material and also from the presence of side reactions accompanying the main reaction, makes interpretation of the results rather difficult.

EXPERIMENTAL

Gas-liquid chromatography (G.L.C.) was used to examine the reaction products and the retention volume (V_R) relative to methyl tetradecanoate or some other standard was compared with the values for known acids. All the chromatograms were run on a Pye Argon Chromatograph with a ^{90}Sr β-ray ionisation detector. The columns used were either 5, 10 or 20% apiezon 'L' grease on alkali-washed celite prepared according to the method of Farquhar et al.[1] and were used at 150° or 200° with an argon gas flow of ca. 35 ml. minute. Samples, either as liquids or as ether solutions, were injected by stopping the gas flow, removing the gas lead, discharging the sample on to the top of the column from a 0.025, 0.05 or 0.1 μl. pipette, replacing the gas lead and restoring the argon flow. Retention times were measured from the negative air peak and the results expressed as carbon numbers,[2,3] which were found from a straight line plot of log V_R for a range of suitable standards against chain length of the saturated acids.

Acidic products were run as methyl esters prepared by methylation with a 5% solution of anhydrous hydrogen chloride in methanol either for 2 hr., under reflux or at room temperature overnight, or by esterification with

diazomethane[5] in ethereal solution.

Reversed-phase chromatography was carried out in essentially the same manner as described in Part I, except that liquid paraffin columns were operated at 35° by jacketing the columns in the vapour of 2-chloropropane (b.p.34.8°).

The iodination-deiodination and bromination-debromination procedures employed in this section are described initially with respect to one particular compound; the method of dehydroxylation for subsequent materials is essentially the same unless otherwise stated.

The alumina used was Peter Spence (Grade 'H').

Iodination - Deiodination.

By Matic's[4] procedure.

Aleuritic Acid.

Aleuritic acid (1 g.), hydrogen iodide (s.g. 1.7; 15 ml.) and red phosphorus (0.15 g.) were refluxed for 8 hr. with continuous stirring. The product was poured into water, extracted with ether and the ethereal extracts washed with water (5 x 200 ml.), sodium bisulphite (5%; 3 x 50 ml.) and water (5 x 200 ml.) and dried over anhydrous sodium sulphate. The ether was distilled off under reduced pressure to give a dark-brown oil (1.53 g.), which was dissolved in methanol

(15 ml.) and refluxed for 4 hr. in the presence of zinc (1 g.) and concentrated hydrochloric acid (7.5 ml.). The product was diluted with water and worked up in ether to give a yellow-orange oil (1 g.).

A portion (0.45 g.) of this product was chromatographed on alumina (20 g.) but a low recovery (40%) was obtained; the remainder of the reduced oil was therefore treated as follows:

The oil (0.55 g.) was dissolved in ether (300 ml.) and extracted with dilute sodium hydroxide (2N; 50 ml.) and the alkaline portion acidified with dilute hydrochloric acid (2N; 60 ml.). The acidic material was then extracted with ether and gave a colourless oil (0.48 g.), which was methylated with methanolic hydrogen chloride (5%; 30 ml.) under reflux for 2 hr. The product was poured into water and extracted with ether (2 x 100 ml.) and worked up in the usual manner to give a yellow oil (0.49 g.), which was chromatographed on alumina (20 g.). The recovery in this case was 80%; both the petroleum ether (0.20 g.) and the ether eluates (0.18 g.) were shown to be methyl hexadecanoate by G.L.C. and by melting point (29.5-30°) and mixed melting point (29.5-30°) with an authentic sample of methyl hexadecanoate (29.5-30°).

9,10-Dihydroxyoctadecanoic acid.

9,10-Dihydroxyoctadecanoic acid (0.50 g.), hydrogen iodide (s.g.1.7; 7.5 ml.) and red phosphorus (0.075 g.) were refluxed for 8 hr. and gave iodinated material (0.61 g.).

This product was deiodinated/methylated in one stage as follows:

The iodinated compound (0.61 g.) was heated under reflux for 4 hr. with methanolic hydrogen chloride (15%; 30 ml.) and zinc (0.50 g.). The product was poured into water and extracted with ether to give a white crystalline solid (0.49 g.) which was chromatographed on alumina (20 g.). The petroleum ether (0.19 g.) and ether eluates (0.29 g.) were shown to be methyl octadecanoate by G.L.C. and by melting point (36.5-37°) and mixed melting point (36.5-37°) with an authentic specimen of methyl octadecanoate (m.p. 36.5-37°).

Shellolic acid.

Dimethyl shellolate (0.50 g.), hydrogen iodide (s.g.1.7; 7.5 ml.) and red phosphorus (0.075 g.) were heated under reflux for 8 hr. and the resulting iodinated compound (0.23 g.) was dissolved in methanol (7.5 ml.) and treated with zinc (0.50 g.) and concentrated hydrochloric acid (3.75 ml.). The product (0.19 g.) was esterified with

methanolic hydrogen chloride (5%, 15 ml.) and the esters (0.18 g.) were chromatographed on alumina (15 g.). Three fractions were removed with petroleum ether (250 ml.; 0.02 g.) petroleum ether/ether (1:1, 250 ml.; 0.07 g.) and ether (250 ml.; 0.02 g.) and these were examined by G.L.C. without any clear results.

Ether-soluble lac acids. (see Part I, p.32)

A portion (0.26 g.) of the ether-soluble fraction of lac acids, hydrogen iodide (s.g. 1.7; 4 ml.) and red phosphorus (0.04 g.) were heated under reflux for 8 hr. and gave iodinated product (0.33 g.). This material (0.33 g.) was deiodinated/ /methylated in one stage by heating under reflux with methanolic hydrogen chloride (15%; 20 ml.) in the presence of zinc (0.35 g.) to give a yellow-orange oil (0.23 g.).

This product was examined by G.L.C. and the chromatogram showed 25 peaks with the following carbon numbers (the main components are underlined): 8.5, 10.1, 11.5, 12.0, 12.7, 14.0, 14.5, 14.7, 15.0, 15.3, 15.5, 16.0, 16.3, 17.0, 17.1, 17.2 17.5, 18.0, 18.2, 18.5, 18.8, 19.9, 20.2, 20.8, 21.0.

A repeat dehydroxylation of the ether-soluble lac acids (2.13 g.) gave dehydroxylated product (1.89 g.), which was examined by G.L.C. The chromatogram showed 16 peaks, viz: 9.3, 10.1, 11.1, 12.0, 13.0, 14.0, 14.8, 15.1, 15.6, 16.0, 16.3, 17.1, 17.5, 18.0, 18.5, 19.5, 20.0.

Further investigation of the dehydroxylated product.

(1) Urea adduct formation.[9]

A portion (0.097 g.) of the dehydroxylated material was dissolved in methanol (3 ml.) containing enough urea (0.50 g.) to saturate the solution at room temperature, and the solution allowed to cool slowly. The two crops of urea-adduct obtained were decomposed by dilute hydrochloric acid/water (1:1, 50 ml.) and the liberated esters were extracted with ether to give an oil (0.03 g.) The non-adduct forming material (0.04 g.) was recovered from the filtrates by extraction with ether.

Both products were examined by G.L.C. and the carbon numbers obtained are given in the next table (p.54). There is little evidence of any useful separation.

(2) Urea adduct formation.

The remainder of the dehydroxylated methyl esters (1.75 g.) was added to the material recovered from the urea fractionation, and the combined esters (1.8 g.) dissolved in methanol (54 ml.) containing urea (9 g.). The solution was heated to boiling and allowed to cool; three crops of crystals were removed and each adduct fraction was then decomposed.

Fraction	Wt. of inclusion compound (g).	Wt of recovered ester (g).	%
1	3.42	0.76	42
2	14.37	0.03	1.6
3	28.99	0.009	0.5
Filtrate		0.79	50

From the sharp fall in weight of material removed by urea it was apparent that any separation was complete after three crystallisations.

The urea adducted and non-adducted materials were examined by G.L.C. and the results are given in the table below with the G.L.C. results of the first and second urea fractionations, along with the carbon numbers of the dehydroxylated esters. Again, there is little evidence of any useful separation.

	1		2	
Dehydroxylated product (2nd Expt.)	Material Recovered from adduct	Material Recovered from filtrate	Material Recovered from adduct	Material Recovered from filtrate
9.2				
10.1		10.1		10.1
11.1		11.1		11.1
12.0	12.0	12.0	12.0	12.1
13.0	13.0	13.0	13.0	13.0
14.0	14.0	14.0	14.0	14.0
14.8		14.8		14.8
15.1		15.1		15.1
15.6	15.6	15.6	15.6	15.6
16.0	16.0	16.0	16.0	16.0
16.3	16.3	16.3	16.3	16.3
17.1		17.1		17.1
17.5	17.5	17.5	17.5	17.5
18.0	18.0	18.0	18.0	18.0
18.5	18.5	18.5	18.5	18.5
19.5		19.5		19.5
20.0	20.0	20.0	20.0	20.0

(3) Hydrogenation.

This experiment was undertaken to determine whether there was any significant change in carbon number for any of the components.

A portion of the methyl esters (0.04 g.) which formed a urea adduct (second urea fractionation) was dissolved in ethanol (20 ml.), added to Pd/C catalyst (20%, 0.04 g.) and the mixture shaken in an atmosphere of hydrogen for 20 hr. The catalyst was filtered off and the filtrate evaporated to dryness to give the product (0.04 g.).

A portion of the methyl esters (0.05 g.) which did not form an adduct was hydrogenated in the same manner.

The results obtained after examination of the above products by G.L.C. are set out below, along with the carbon numbers obtained previously for the total dehydroxylated product and for the adduct forming and non-adduct forming fraction. Some minor peaks have been omitted from this table.

	Material forming a urea adduct.		Material not forming a urea adduct.	
Dehydroxylated material	Before Hydrogn.	After Hydrogn.	Bdfore Hydrogn.	After Hydrogn.
14.0	14.0	14.0	14.0	14.0
14.8	-	-	14.8	14.8
15.1	-	-	15.1	15.1
15.6	15.6	15.6	15.6	15.6
16.0	16.0	16.0	16.0	16.0
16.3	16.3	16.3	16.3	16.3
17.5	17.5	17.5	17.5	17.5
18.0	18.0	18.0	18.0	18.0
18.5	18.5	18.5	18.5	18.5
19.5	-	-	19.5	19.5
20.0	20.0	20.0	-	-

From a semi-quantitative examination of the chromatograms it was concluded that esters of carbon numbers 14.0, 15.6, 16.0, 16.3, 17.5, 18.0, 18.5 and 20.0 form urea-adducts, and those of carbon numbers 14.8,15.1 and 19.5 do not form urea adducts

(4) Examination of the urea adduct-forming material by R.P.C.

Urea adduct-forming esters (0.50 g.) were hydrolysed by treatment with boiling alcoholic potassium hydroxide (1N; 7.5 ml

for 1 hr. The reaction mixture was poured into water, extracted with ether and the ether layer washed with water. The aqueous extracts were acidified (dilute sulphuric acid) and the liberated acids (0.44 g.) extracted with ether.

Portions of these were examined by R.P.C., the course of the separation being followed by titration with alkali. Appropriate fractions were combined and examined by G.L.C. after esterification.

Fraction	Eluate (% acetone)	1st Expt.(a)	2nd Expt.(a)	Carbon Nos.(G.L.C.)
1	35	22		15.6, 17.5
			13	19.5 20.0
2	44	8		
3	53	20	34	14.0, 16.3, 18.0
4	62	26	27	14.0, 18.0, 18.5
5	67	24	26	16.0

(a) These were carried out using 50 and 106 mg. respectively. The figures given indicate the percentage of material removed by each eluate.

Fraction 5 was shown to be hexadecanoic acid by m.p. and mixed m.p. of the acid (61-61.5°) and methyl ester (29°). The ester of carbon number 14.0 was shown to be methyl tetradecanoate since, in the acid form it is eluted with 62% acetone (cf. Gunstone and Sykes[7,8]).

Ethyl acetate-soluble lac acids. (see Part I, p.32)

A portion (0.23 g.) of the ethyl acetate-soluble fraction of lac acids, hydrogen iodide (s.g.1.7; 4 ml.) and red phosphorus (0.04 g.) were heated under reflux for 8 hr. and gave iodinated material (0.26 g.). This product (0.28 g.) was deiodinated/ /methylated by heating under reflux for 4 hr. with methanolic hydrogen chloride (15%; 20 ml.) in the presence of zinc (0.70 g.) to give a yellow-orange oil (0.2 g.).

This product was examined by G.L.C. before and after alumina treatment. In the alumina fractionation, a portion of the dehydroxylated esters (69 mg.) was adsorbed on alumina (10 g.) a three fractions eluted with petroleum ether (150 ml.; 0.001 g.), petroleum ether/ether (1:1, 150 ml.; 0.009 g.) and ether (150 ml.; 0.005 g.); the G.L.C. results are set out below.

The results are very similar to those obtained from the ether-soluble lac acids with the main peaks at 14.0, 16.0 and 18.5. The saturated acids appear to form a regular series C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{16}, C_{18}, but apart from the C_{14} and C_{16} acids, are present in small amounts.

Dehydroxylated Product	Petroleum Ether Fraction	Petroleum Ether Ether Fraction	Ether Fraction
10.0	10.0	10.0	10.0
11.0	11.0	11.0	11.0
12.0	12.0	12.0	
13.0	13.0	13.0	
14.0	14.0		
14.8		14.8	14.8
15.1		15.1	
15.3		15.3	
15.6		15.6	
16.0		16.0	16.0
16.3			
17.1			17.1
17.4			17.4
18.0		18.0	18.0
18.5		18.5	18.5
19.5			19.5
20.1			
20.8			

The main peaks are underlined.

Lac mixed acids. (see Part I, p.32)

Lac mixed acids (0.54 g.), hydrogen iodide (s.g. 1.7; 8 ml.) and red phosphorus (0.08 g.) were heated under reflux for 8 hr. and gave iodinated material (0.65 g.). The product (0.65 g.) was heated under reflux for 4 hr. with methanolic hydrogen chloride (15%; 30 ml.) in the presence of zinc (1 g.) and gave deiodinated methyl esters (0.44 g.) which were adsorbed on alumina (20 g.). Three fractions were removed with petroleum ether (400 ml.; 0.02 g.), petroleum ether/ether (1:1, 250 ml.; 0.18 g.) and ether (400 ml.; 0.08 g.) and these were examined by G.L.C.

Petroleum Ether Fraction	Petroleum Ether Ether Fraction	Ether Fraction
Chromatogram	14.0	10.2
showed no	14.5	12.2
peaks	16.0	13.6
	18.5	14.9
		15.6
		16.0
		17.0
		17.7
		18.2
		20.6

The main peaks are underlined.

Ethyl acetate-insoluble lac acids. (see Part I, p.32).

A portion of the brown solid (0.45 g.; m.p. 81-84°) was dissolved in boiling ethanol, decolourised with animal charcoal and allowed to crystallise from aqueous ethanol to give white plates (m.p. 86-87°). Two analyses of this material gave the following results:

1.		C	H	O (by difference).
	1.	63.5	10.7	25.8
	2.	62.6	10.6	26.8
$C_{16}H_{32}O_5$ requires		63.1	10.6	26.3
$C_{18}H_{36}O_5$ requires		62.0	10.4	27.6.

Catalytic acetylation[9] of the recrystallised material gave a value of 4.5 for the number of hydroxyl groups acetylated.

A portion of the recrystallised material (0.019 g.), hydrogen iodide (s.g.1.7; 1 ml.) and red phosphorus (0.01 g.) were heated under reflux for 8 hr. and gave iodinated material (0.02 g.). This product was deiodinated/methylated under reflux with methanolic hydrogen chloride (15%; 10 ml.) in the presence of zinc (0.25 g.) and gave the dehydroxylated product (0.019 g.).

Examination of this product by G.L.C. gave a complicated chromatogram with the main peaks at 14.0, 16.0 and 18.6, and was therefore concluded to be a mixture of acids of varying chain length and was not further examined.

Second dehydroxylation of Aleuritic Acid.

This experiment was effected to determine whether the dehydroxylation process could be improved.

Aleuritic acid (0.95 g.), hydrogen iodide (s.g. 1.7; 15 ml.) and red phosphorus (0.15 g.) were heated under reflux for 8 hr. and deiodinated as before to give the product (0.89 g.).

Examination of this material by G.L.C. before and after alumina treatment showed a complicated chromatogram and it was therefore decided to attempt improvement of this dehydroxylation procedure.

Three different dehydroxylation procedures were examined.

Portions of zinc dust were activated as required as follows:

To zinc dust (1 g.) was added methanol (5 ml.) and hydrogen iodide (s.g. 1.7; 0.10 ml.) and the mixture boiled for 5 min. The solvent was decanted, the zinc washed with methanol and dried in an oven at 110°.

1. (Ref. 4 using activated zinc). Aleuritic acid (1 g.), hydrogen iodide (s.g. 1.94, 15 ml.) and red phosphorus (0.15 g.) were heated under reflux for 8 hr., and gave iodinated product (1.36 g.).

This material (1.36 g.) was dissolved in methanol (15 ml.) and heated under reflux for 4 hr. with activated zinc (1 g.) and concentrated hydrochloric acid (7.5 ml.) The yellow crystalline product (0.64 g.) obtained after ether extraction was methylated under reflux with methanolic hydrogen chloride (5%, 50 ml.) for 2 hr. and gave the esters (0.63 g.). Examination of this product by G.L.C. gave the expected carbon number of 16.0 for methyl hexadecanoate with a very minor component of carbon number 17.3.

2. (Ref.10). Aleuritic acid (1 g.), iodine (5 g.) and red phosphorus (1.22 g.) were heated at 100° on a boiling water bath for 1 hr. using an air condenser. After 1 hr. the remaining iodine was evaporated at 100° under reduced pressure.

The residue was extracted with ether, washed with water (3 x 200 ml.), sodium bisulphite (5%, 3 x 50 ml.) and water (10 x 100 ml.) and the ethereal extracts dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to give the product (1.25 g.).

This material was divided into two portions and deiodinated methylated by two different methods:

A. A portion of the product (0.61 g.) was dissolved in methanol (10 ml.) and refluxed for 4 hr. in the presence of activated zinc (0.50 g.) and concentrated hydrochloric acid (4 ml.). The product (0.31 g.) recovered by ether extraction was methylated by heating under reflux for 2 hr. with methanolic hydrogen chloride (5%, 25 ml.) and gave the ester (0.30 g.).

Examination of this product by G.L.C. gave only the expected carbon number of 16.0 for methyl hexadecanoate.

B. The other portion of iodinated material (0.6 g.) was heated under reflux for 4 hr. with methanolic hydrogen chloride (15%, 30 ml.) in the presence of activated zinc (1 g.) and the ester (0.36 g.) isolated with ether.

Examination of this product by G.L.C. gave only the expected carbon number of 16.0 for methyl hexadecanoate.

Since procedure 2B involved one less reaction step and gave a homogeneous product, it was decided to adopt this method in future iodination-deiodination reactions.

<u>An improved iodination-deiodination procedure.</u>

<u>Ether-soluble lac acids.</u>

A portion of the ether-soluble lac acids (1 g.), iodine (5 g.) and red phosphorus (1.22 g.) were heated at 100° for 1 hr. and gave iodinated product (1.05 g.), which was heated under reflux for 4 hr. with methanolic hydrogen chloride (15%,

60 ml.) in the presence of activated zinc (2 g.). The resulting methyl esters (0.62 g.) were examined by G.L.C. and gave carbon numbers of 14.0, 16.0, and 18.6, probably corresponding to tetradecanoic, hexadecanoic and a C_{16} acid, which could be 16-hydroxyhexadecanoic acid.

In an attempt to separate this mixture the esters (0.6 g.) were distilled under reduced pressure (0.25 mm.) on an oil bath at 185-220°; a little less than half the esters distilled at 120-140°. When examined by G.L.C. the distillate showed carbon numbers of 14.0, 16.0 and 18.6, and the residue carbon numbers of 16.0 and 18.6. It was concluded that no satisfactory separation was possible in this way.

Lac mixed acids.

Lac mixed acids (0.51 g.), iodine (2.5 g.) and red phosphorus (0.61 g.) were heated at 100° for 1 hr. and gave iodinated material (0.35 g.).

This product (0.35 g.) was heated under reflux for 4 hr. with methanolic hydrogen chloride (15%; 30 ml.) in the presence of activated zinc (1 g.) and gave the esters (0.20 g.).

Examination of this material by G.L.C. gave the following carbon numbers:

13.8, <u>14.0</u>, 15.0, <u>16.0</u>, 16.4, <u>18.6</u>.

The main peaks are underlined.

The esters (0.20 g.) were hydrolysed by treatment with boiling alcoholic potassium hydroxide (1N; 4 ml.) for 1 hr. The reaction mixture was poured into water, extracted with ether and the ether layer washed with water. The aqueous extracts were acidified (dilute sulphuric acid) and the liberated acids (155 mg.) extracted with ether.

The acids (154 mg.) were examined by R.P.C. using a paraffin column and a range of aqueous acetones as eluting solvents. Fractions (24 x 20 ml.) were collected but the elution curve obtained from a plot of fraction number against ml. alkali per fraction showed the peaks to be poorly defined, probably due to overloading of the column.

Shellolic Acid.

Dimethyl shellolate (0.20 g.), iodine (1 g.) and red phosphorus (0.25 g.) were heated at 100° for 1 hr. and gave a product (0.03 g.). This yield is much lower than expected and indicates either incomplete reaction or marked solubility of the product in water.

The above material (0.03 g.) was heated under reflux for 4 hr. with methanolic hydrogen chloride (15%; 10 ml.) in the presence of activated zinc (0.20 g.) and gave a product (0.02 g.).

Examination of this product by G.L.C. gave no clear results.

Bromination - Debromination.[11]

9,10-Dihydroxyoctadecanoic acid.

To 9,10-dihydroxyoctadecanoic acid (0.5 g.) was added hydrogen bromide/acetic acid (d.1.25; 5 ml.) followed by concentrated sulphuric acid (0.5 ml.) with shaking and cooling The solid had completely dissolved in a few hours, but after 16 hr. a small layer of oil separated; the mixture was then heated at 100° for 8 hr. (a further 0.5 ml. of the hydrogen bromide/acetic acid was added after 4 hr.). Water was then added and the pale red oil isolated with light petroleum (60-80°). The extract was washed with water, dried over anhydrous sodium sulphate and the solvent removed under reduced pressure to give a yellow oil (0.69 g.).

The oil (0.69 g.) was methylated with methanolic hydrogen chloride (5%; 40 ml.) under reflux for 2 hr., to give the ester (0.65 g.). This product was debrominated by refluxing for 1 hr. with methanol (30 ml.) and activated zinc (3 g.). The unsaturated ester (0.41 g.) was isolated with ether.

Examination of the product by G.L.C. gave only one peak (carbon number 17.8) corresponding to methyl octadec-9-enoate. No methyl octadecanoate was produced in the reaction.

Ether-soluble lac acids.

To the ether-soluble lac acids (2.72 g.) was added hydrogen bromide/acetic acid (d.1.25; 37.5 ml.) followed by concentrated sulphuric acid (3.75 ml.) in small portions with shaking and cooling. The mixture was heated at 100° for 8 hr. (a further 3.75 ml. of the hydrogen bromide/acetic acid was added after 4 hr.). The product (4.42 g.) was methylated with methanolic hydrogen chloride (5%; 150 ml.) under reflux for 2 hr. and the esters (4 g.) recovered in the usual manner.

The esters (4 g.) were debrominated by refluxing for 1 hr. with methanol (56 ml.) and activated zinc (11.2 g.) and the product (3.40 g.) examined by G.L.C.

Carbon Number	Probable Structure.
13.8	Tetradecenoate
14.0	Tetradecanoate
15.8	Hexadecenoate
16.0	Hexadecanoate
18.5	Hydroxy C_{16} ester
19.5	Bromo C_{16} ester

A portion of the debrominated product (0.9 g.) was iodinated[12] by heating under reflux for 4 hr. in dry acetone (45 ml.) in the presence of sodium iodide (2 g.). Most of the acetone was distilled off, water added, and the esters (0.93 g.)

isolated with ether.

The product (0.93 g.) was deiodinated by heating under reflux for 4 hr. with methanolic hydrogen chloride (5%; 60 ml.) in the presence of activated zinc (2 g.), to give a yellow oil (0.81 g.).

Examination of this product by G.L.C. gave the following results:

Carbon Number	Probable Structure
13.8	Tetradecenoate
14.0	Tetradecanoate
15.8	Hexadecenoate
16.0	Hexadecanoate
18.5	Hydroxy C_{16} ester.

Examination of the deiodinated product by R.P.C.

The dehydroxylated methyl esters (0.35 g.) were hydrolysed with boiling alcoholic potassium hydroxide (1N; 10 ml.) for 1 hr. The reaction mixture was poured into water, extracted with ether and the ether layer washed with water. The aqueous extracts were acidified (dilute sulphuric acid) and the liberated acids (0.24 g.) extracted with ether.

Portions of these were examined by R.P.C. using a paraffin column, the course of the separation being followed by titration with alkali. Appropriate fractions were combined

and after esterification examined by G.L.C.

Fraction	Eluate (% acetone)	1st Expt.[a]	2nd Expt.[a]	Carbon Nos. G.L.C.
1	35	3	10	14.0, 15.8, 16.0
2	53	22	17	17.8
3	62	45	44	13.8, 14.0, 16.0
4	67	30	29	16.0

[a] These were carried out using 122 and 118 mg. respectively. The figures given indicate the percentage of material removed by each eluate.

As may be seen from the results, no useful separation of the components was achieved.

The carbon number of 18.5 appears to have changed to 17.8 on hydrolysis with methanolic potassium hydroxide.

Aleuritic Acid.

To aleuritic acid (0.50 g.) was added hydrogen bromide/acetic acid (d.1.25; 7.5 ml.) followed by concentrated sulphuric acid (0.75 ml.) in small portions with shaking and cooling. The mixture was heated at 100° for 8 hr. (a further 0.75 ml. of the hydrogen bromide reagent was added after 4 hr.). The product (0.84 g.) was methylated with methanolic hydrogen chloride (5%; 40 ml.) under reflux for 2 hr. and the ester

(0.73 g.) recovered in the usual manner.

The product (0.73 g.) was debrominated under reflux for 1 hr. in methanol (30 ml.) in the presence of activated zinc (3 g.). The product (0.47 g.) was isolated with ether and examined by G.L.C.:

Carbon Number	Probable Structure
15.8	Hexadecenoate
16.0	Hexadecanoate
18.5	Hydroxy C_{16} ester
19.5	C_{16} bromo ester (unsaturated)
19.8	C_{16} bromo ester (saturated)

Examination of the debrominated material by R.P.C.

A portion of the methyl esters (0.26 g.) gave the acids (0.2 g.) on hydrolysis with boiling alcoholic potassium hydroxide ($1N_3$ 8 ml.).

Two portions of these acids (33 and 110 mg. respectively) were examined by R.P.C. the course of the separation being followed by titration with alkali; two fairly well defined peaks were obtained in each case.

The two main fractions recovered from the second column were esterified and examined by G.L.C., as was a portion of the original acids:

Debrominated Product	Re-esterified acids after hydrolysis.	Fraction 1	Fraction 2
15.8	15.8		15.8
16.0	16.0		16.0
	17.8	17.8	17.8
18.5			18.5
19.5			
19.8			

It is again apparent that the material of carbon number 18.5 has changed to 17.8 on hydrolysis.

The nature of these two materials and that of carbon number 19.5 was elucidated by the scheme of reactions set out below.

Preparation of 16-Bromohexadec-9-enoic acid from aleuritic acid.[211]

To aleuritic acid (5 g.) was added hydrogen bromide/ acetic acid (d,1.25; 75 ml.) followed by concentrated sulphuric acid (7.5 ml.) in small portions with shaking and cooling. The mixture was heated at 100° for 8 hr. (a further 7.5 ml. of the hydrogen bromide reagent was added after 4 hr.). The product (8.40 g.) was debrominated under reflux for 1 hr. in methanol (100 ml.) in the presence of activated zinc (19.4 g.).

The product was isolated with ether and removal of the solvent under reduced pressure gave white crystalline material (5.10 g.).

The crude acid was recrystallised from methanol to give white plates (4.43 g.; m.p. 42-43°, Lit.[13] 41-42°).

Methylation of 16-Bromohexadec-9-enoic acid.

(a) with diazomethane.

Diazomethane[5] (0.2 g.) in ether (5 ml.) was added to 16-bromohexadec-9-enoic acid (0.20 g.) and the mixture allowed to react at room temperature for five minutes. The ether and excess diazomethane were distilled off under reduced pressure and the product examined by G.L.C.

Carbon Number	Peak size	Structure
15.8	minor	Hexadecenoate
16.0	v.minor	Hexadecanoate
19.5	major	ω -Br Hexadecenoate
19.8	minor	ω -Br Hexadecanoate

(b) with methanolic hydrogen chloride.

16-Bromohexadec-9-enoic acid (0.20 g.) was heated under reflux for 2 hr. with methanolic hydrogen chloride (5%; 10 ml.) and gave the ester (0.19 g.) which was examined by G.L.C.

Carbon Number	Peak size	Structure
15.8	minor	Hexadecenoate
16.0	v.minor	Hexadecanoate
19.5	major	ω -Br Hexadecenoate
19.8	minor	ω -Br Hexadecanoate

Attempted conversion of 16-Bromohexadec-9-enoic acid to 16-Hydroxyhexadec-9-enoic acid.

(a) With aqueous potassium hydroxide

16-Bromohexadec-9-enoic acid (0.20 g.) was refluxed for 4 hr. with aqueous potassium hydroxide (2N; 5 ml.), the reaction mixture poured into water and acidified (dilute hydrochloric acid). The liberated acid was extracted with ether to give a white crystalline solid (0.12 g.; m.p. 39-40°). A small quantity was methylated and examined by G.L.C.

Carbon Number.	Peak Size	Structure
15.8	minor	Hexadecenoate
16.0	v.minor	Hexadecanoate
18.3	major	ω -OH Hexadecenoate

(b) via The acetoxy compound.

16-Bromohexadec-9-enoic acid (200 mg.) was added to dry acetone (10 ml.) containing sodium iodide (0.50 g.) and the mixture refluxed for 4 hr. Most of the acetone was distilled off, water added, and the product extracted with ether to give white crystals (0.15 g.; m.p. 45.5-46.5°).

Carbon Number	Peak size	Structure
15.8	minor	Hexadecenoate
16.0	v.minor	Hexadecanoate
19.5	minor	ω -Br Hexadecanoate
20.7	major	ω -I Hexadecenoate

The above product was heated under reflux for 6 hr. in acetic acid (5 ml.) containing fused potassium acetate (0.50 g.). Most of the acetic acid was distilled off under reduced pressure, water added and the product extracted with ether. The solvent was removed under reduced pressure to give white crystals (0.11 g.) m.p. 27-28.5°).

Carbon Number	Peak size	Structure
15.8	minor	Hexadecenoate
16.0	v.minor	Hexadecanoate
18.2	major	ω - Acetoxy Hexadecenoate

The product (0.11 g.) was heated under reflux for 1hr. with alcoholic potassium hydroxide (0.5N, 5 ml.). The product after acidification (dilute hydrochloric acid), was isolated with ether to give white crystals (0.08 g.) m.p. 39-40°).

Carbon Number	Peak size	Structure
15.8	minor	Hexadecenoate
16.0	v.minor	Hexadecanoate
18.3	major	ω -OH Hexadecenoate

Preparation of 16-hydroxyhexadec-9-enoic acid from 16-bromohexadec-9-enoic acid.

16-Hydroxyhexadec-9-enoic acid (0.78 g.) was prepared according to method (a) from 16-bromohexadec-9-enoic acid (1 g.)

16-Hydroxyhexadec-9-enoic acid (0.45 g.) was dissolved in boiling petroleum ether (40-60°; 50 ml.) and allowed to crystallise at room temperature. White crystals separated and were filtered off and dried in a vacuum desiccator over P_2O_5 (0.3 g.; m.p. 66.5-67.5° Lit.[14,15] 69°). A portion of this product was methylated and examined by G.L.C. and gave only one peak of carbon number 18.3 corresponding to 16-hydroxyhexadec-9-enoic ester.

Preparation of methyl-16-methoxyhexadec-9-enoate from methyl-16-hydroxyhexadec-9-enoate.[16,17]

To a solution of methyl-16-hydroxyhexadec-9-enoate (0.15 g.) in methyl iodide (2ml.) and acetonitrile (2 ml.) was added silver oxide (0.35 g.) and a vigorous reaction ensued which was controlled by cooling. The mixture was heated under reflux for 1 hr. after which most of the acetonitrile and methyl iodide was removed under vacuum. The residue was poured into water, extracted with ether and removal of the solvent under reduced pressure gave a yellow oil (0.1 g.) which was examined by G.L.C. The chromatogram showed only one peak at 17.8

Preparation of 16-hydroxyhexadecanoic acid from 16-hydroxyhexadec-9-enoic acid.

Pure 16-hydroxyhexadec-9-enoic acid (0.12 g.) with Pd/C catalyst (10%, 0.12 g.) in methanol (20 ml.) was shaken overnight at room temperature in an atmosphere of hydrogen. The catalyst was filtered off and washed with methanol (5 x 5 ml.) and the filtrates evaporated to give the hydrogenated acid (0.12 g.; m.p. 93-94°). A small amount of the product was methylated and examined by G.L.C., and gave only one peak of carbon number 18.5.

To determine whether any hexadecanoic acid is produced in the bromination-debromination reaction on compounds which contain an isolated hydroxyl group e.g. 16-hydroxyhexadecanoic acid, the following reactions were carried out:

To pure 16-hydroxyhexadecanoic acid was added hydrogen bromide/acetic acid (d,1.25; 1 ml.) followed by concentrated sulphuric acid (0.1 ml.) with shaking and cooling. The mixture was heated at 100° for 8 hr. (a further 0.1 ml. of hydrogen bromide reagent was added after 4 hr.).

The product was debrominated under reflux for 1 hr. in methanol (2 ml.) in the presence of activated zinc (0.40 g.) and the product (0.07 g.) methylated. Examination of this

material by G.L.C. gave a minor peak of carbon number 16.0, confirming that palmitic acid is produced as a by-product in this reaction. The major peak, caused by 16-bromohexadecanoic ester, had a carbon number of 19.8.

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PART III

Studies of Lac acids by adsorption gas-liquid and thin-layer chromatography

DISCUSSION

1. Separation techniques.

Attention was next turned to possible methods of separating the hydroxy acids of shellac without any modification of their structures, and the behaviour of lac acids on alumina was examined. In this connection the work reported by Meakins and Swindells[1] on certain acids present in olive leaves was of particular interest. These investigators isolated 9,10,18-trihydroxyoctadecanoic acid (the C_{18} analogue of aleuritic acid) and 10,16-dihydroxyhexadecanoic acid after chromatography of the mixed esters on neutral alumina.

Silicic acid was not used as adsorbent because of the slow elution rate and it was decided to examine chromatography on neutral alumina as a means of separating mono-, di-, and trihydroxy acids which may be present in the lac hydrolysate. Preliminary experiments showed that methyl 12-hydroxyoctadecanoate (1:1 benzene, ether), methyl 9,10 dihydroxyhexadecanoate (98:2 ether, methanol) were readily separated, each ester being eluted by the solvent indicated in parenthesis.

Experiments with the esters of the ether-soluble and ethyl acetate-soluble lac acids gave some evidence of separation but were of limited value because there was no adequate means of examining the fractions, each of which still seemed to be a

mixture. This was eventually achieved by gas-liquid chromatography and by thin-layer chromatography and experiments in this connection are described.

Gas-liquid chromatography.

Relatively little has been reported about the use of gas-liquid chromatography (G.L.C.) for the examination of long-chain hydroxy acids probably because of thedr very low volatility. However, several workers[27,28,29,30,31] have examined steroids and sugar mixtures[26] by G.L.C. using silicone grease on celite. The esters of a number of mono-, di-, and trihydroxy acids were available and attempts were made to find suitable means for examining these by G.L.C. by (a) modifying the stationary phase and or (b) modifying the hydroxy ester by chemical reaction.

Using columns containing either 5% or 2½% Apiezon 'L' grease the esters of several mono- and dihydroxy acids were conveniently separated and their 'carbon numbers' are given on p.95. With Edwards silicone high vacuum grease adequate separations were also obtained but the results were not sufficiently different from those on Apiezon 'L' grease to merit further examination.

Several attempts to find a stationary phase suitable for the examination of dimethyl shellolate and methyl aleuritate

by G.L.C. met with only limited success. The most satisfactory column was prepared by[9,10,11,12,13] coating acid-washed celite 545 (B.D.H.) with dimethyldichlorosilane and packing the G.L.C. column with this material; the inside of the glass column was pretreated with a 1% chloroform solution of dimethyldichlorosilane to reduce active sites on the glass surface. Dimethyl shellolate then gave a reasonable elution curve but methyl aleuritate, though eluted, gave a broader curve which was not completely satisfactory.

The possibility of modifying vic dihydroxy compounds by the formation of their isopropylidene derivatives has been examined and some results are given on p. 95. According to Christie,[32] however, more promising results are being obtained by methylation of the hydroxy esters.

Thin-layer chromatography.

The technique of applying a thin layer of adsorbent to one face of a glass strip and using this as an 'open' adsorption column was studied by Kirchner et al[35] and was extended and standardized by Stahl.[16,17,18] This technique, now known as thin-layer chromatography (T.L.C.) was applied to the resolution of many complex lipid mixtures by Mangold and Malins[34,35,36] and to lipid extracts of blood by Weicker.[37] Studies of various epoxy and hydroxy acids and esters by Morris

et al.[38,39,40] have shown that small differences in polarity of some isomeric oxyacids enable the isomers to be resolved on thin-layer chromatograms. For these purposes the layers are about 250-275 μ thick and up to 1 mg. is used for each chromatogram.

A technique of preparative thin-layer chromatography was developed by thickening the layer of silicic acid on glass to 1.6 mm., when 50-100 mg. or more of a mixture could be separated on one plate by running ten chromatograms.

Straight-chain esters containing none, one, two and three hydroxyl groups were separated using an elution solvent of the composition 50% ether, 48% petroleum ether, 2% methanol. This technique was most useful in monitoring fractions from our adsorption experiments on neutral alumina.

2. Separation of some non-hydroxy and monohydroxy acids from mixed lac acids.

In the solvent fractionation of lac acids (see Part I, p.32), a small portion of the acids (1.1%) was soluble in petroleum ether. When this was esterified and examined by G.L.C it appeared to be a mixture of saturated and unsaturated non-hydroxy esters. Subsequently acids of this type were more effectively removed by a partition process.[42]

Lac acids from 200 g. of shellac were partitioned between petroleum ether and 1:4 aqueous methanol. Further examination of the petrol-soluble fraction (2.25 g.; 1.1%) is described later.

Portions of the aqueous methanol-soluble acids, after esterification, were adsorbed on neutral alumina and progressively eluted with solvents of increasing polarity. The eluted esters were examined by G.L.C. and T.L.C. and there was evidence of several monohydroxy acids, of dihydroxy acids (possibly), and of more polar compounds such as aleuritic and shellolic acids.

Further separation was effected by rechromatography with columns of alumina and by preparative T.L.C. using silicic acid. In this way a compound having 'carbon number' 16.2 was isolated in a pure state and two other fractions were obtained enriched in (a) esters of carbon numbers 16.2, 17.6, 18.6 (fraction F) and (b) esters of carbon numbers 19.4, 20.5 (fraction G).

3. Identification of the separated compounds.

The non-hydroxy acids.

Gas-liquid chromatography of the petrol-extract, after esterification, showed seven peaks. This was reduced to four after hydrogenation. Quantitative studies gave the results set out below.

Carbon Numbers		%	Structure
Before Hydrogenation	After Hydrogenation		
12.0	12.0	trace	Dodecanoic
13.8	-	25[E]	Tetradecenoic
14.0	14.0		Tetradecanoic
15.8	-	13	Hexadecenoic
16.0	16.0	53	Hexadecanoic
17.8	-	7	Octadecenoic
18.0	18.0	2	Octadecanoic

[E] Mainly saturated.

The unsaturated acids are considered to be practically entirely of the $\Delta^{9,10}$ class on the basis of the following evidence. The acids were hydroxylated and the dihydroxy acids produced from the unsaturated acids were separated from the unchanged saturated acids and then oxidised. The only dibasic ester recognised among the fission fragments was azelate.

These non-hydroxy acids have not been previously reported present in shellac.

The hydroxy acid of carbon number 16.2

In early experiments this was mistaken for hexadecanoic acid (carbon number 16.0) but when this had been removed it was evident that another acid with similar behaviour on Apiezon

columns was present to an extent of not less than 8%. Various samples were isolated and it was shown to be 6-hydroxytetradecanoic acid on the following evidence:

(i) After iodination-deiodination the ester gave a product which from its chromatographic behaviour must have been methyl tetradecanoate.

(ii) The hydroxy ester was oxidised to a keto ester the oximes of which were submitted to Beckmann rearrangement and then hydrolysed (p. 88). The monobasic acid (nonanoic) and dibasic acid (adipic) were recognised by G.L.C.

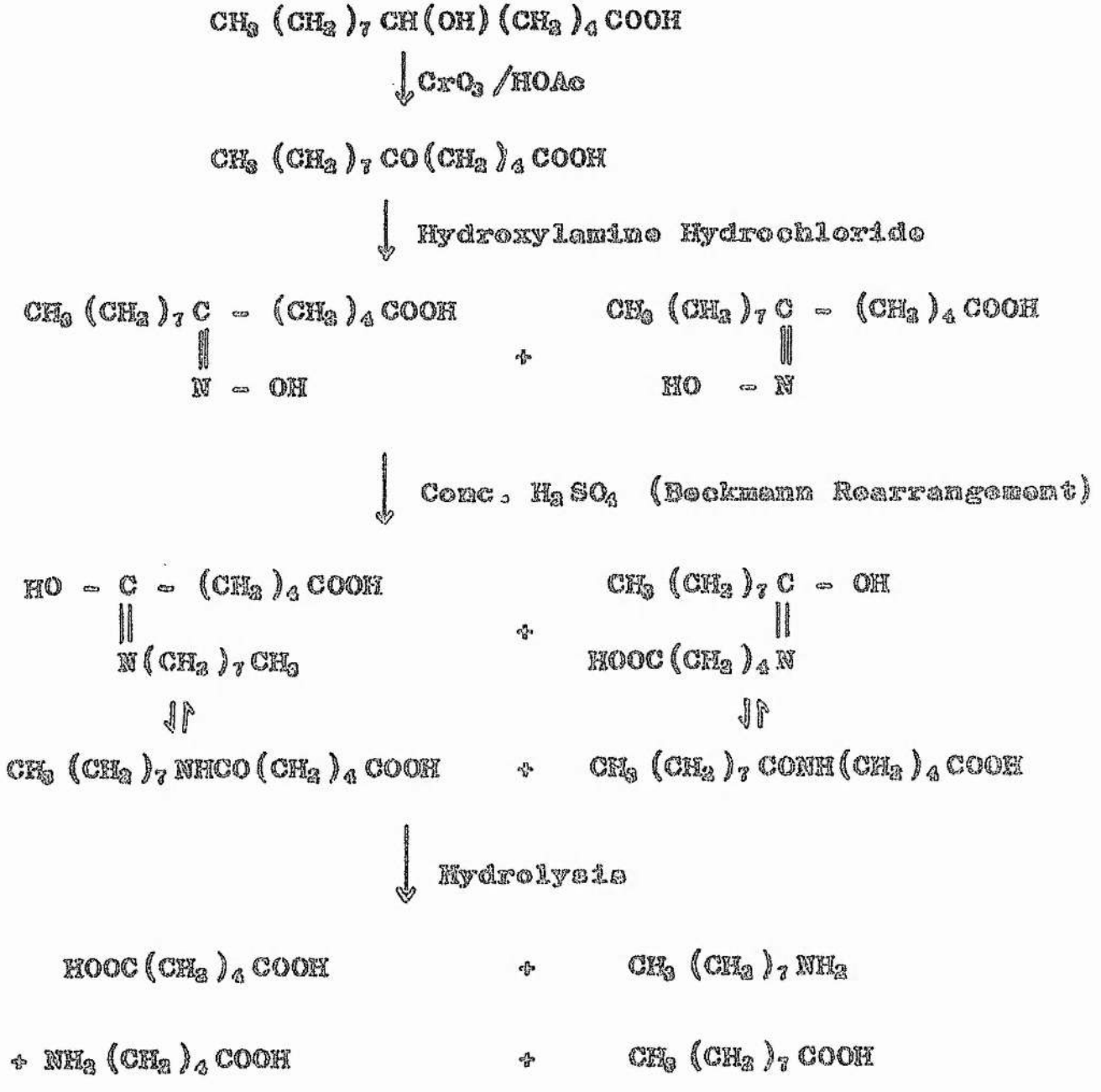
(iii) Samples of the hydroxy acid and its corresponding keto acid gave satisfactory analyses.

This acid, forming not less than 8% of all lac acids, has not been previously recognised from this or any other source, nor has it been synthesised. It is possible however that butolic acid isolated by Sen Gupta[46] and considered to be a monohydroxy pentadecanoic acid may be a mixture of monohydroxy C_{14} and C_{16} acids since this study has provided no evidence for any aliphatic C_{15} acids. The C_{14} acid here described is compared with butolic acid.

Hydroxy Acid	C_{14} (Prentice)	C_{15} (Sen Gupta)
m.p.	58-58.5°	54-55°
%C	68.4	69.5
%H	11.4	11.5
Keto Acid		
m.p.	69.5-70.5°	69.5-70°
%C	68.9	-
%H	10.6	-

Butolic acid was reduced to a non-hydroxy acid considered to be pentadecanoic acid. In view of its low melting point it would be interesting to have chromatographic evidence of its homogeneity.

		Chain length of acid			
	Reduced Butolic Acid	C_{14}	C_{15}	C_{16}	$C_{14} + C_{16}$ (equimolecular wts)
m.p.	48-49°	54.4°	52.3°	62.9°	48.3°
Acid value	230	246	232	219	-
Mol.Wt. (Rast).	245	228	242	256	-


CH3 (CH2)7 CH(OH) (CH2)4 COOH
CrO3 /HOAc
CH3 (CH2)7 CO(CH2)4 COOH
Hydroxylamine Hydrochloride
CH3 (CH2)7 C - (CH2)4 COOH
N - OH
+
CH3 (CH2)7 C - (CH2)4 COOH
HO - N
Conc. H2SO4 (Beckmann Rearrangement)
HO - C - (CH2)4 COOH
N(CH2)7 CH3
+
CH3 (CH2)7 C - OH
HOOC(CH2)4 N
CH3 (CH2)7 NHCO(CH2)4 COOH
+
CH3 (CH2)7 CONH(CH2)4 COOH
Hydrolysis
HOOC(CH2)4 COOH
+
CH3 (CH2)7 NH2
+ NH2 (CH2)4 COOH
+
CH3 (CH2)7 COOH

Other monohydroxy acids.

Evidence is now presented for the presence of some other hydroxy acids though further information must be sought about the amount of these and the detailed structure of some of them.

After extensive chromatography on alumina columns and silicic acid layers fractions were obtained which were still mixtures. One group of samples (F) contained esters of carbon numbers 16.2, 17.6 and 18.6 and another group (G) esters of carbon numbers 19.4 and 20.5. The ester of carbon number 16.2 is the hydroxytetradecanoate already discussed.

The mixture F when iodinated-deiodinated contained a trace of C_{12} ester and roughly equal amounts of C_{14} and C_{16} esters. When oxidised (CrO_3/C. H_2SO_4/acetone) the product had carbon numbers of 15.5, 16.9 and 18.8. Thin-layer chromatography showed the mixture F to contain only monohydroxy esters. It is concluded from these results that 16-hydroxy-hexadecanoate, another hydroxyhexadecanoate and hydroxy-tetradecanoate are present in this mixture.

Structure assigned.	Observed carbon number.		Expected carbon number.	
	Before oxidation.	After oxidation.	Before oxidation.	After oxidation.
6-hydroxytetradecanoate	16.2	15.5	16.2	15.5
?-hydroxyhexadecanoate	17.6	16.9	≡	≡
16-hydroxyhexadecanoate	18.6	18.8	18.6	18.9

≡ These values are not known but it is noted that the change in carbon number through oxidation is the same (-0.7) for the first two esters listed in this table.

The mixture G (carbon numbers 19.4, 20.5) yields C_{12} (trace), C_{14}, C_{16} and C_{18} esters when iodinated-deiodinated. T.L.C. shows it to contain mono- and dihydroxyesters as well as more polar esters. Oxidation gives azelaic acid (possibly from aleuritic acid) and esters of carbon numbers 16.0 and 16.9. No definite conclusions are possible about this mixture which is still apparently complex.

Butolic Acid.

A sample (70 mg.) of butolic acid, kindly sent to us by Mr. S. C. Sen Gupta of the Indian Lac Research Institute, arrived after the completion of this thesis.

It melted at 50.5-55° and at 50-55° when mixed with a sample of the 6-hydroxytetradecanoic acid (m.p. 58-58.5°) obtained in these studies. Chromatography of the ester on an Apiezon 'L' column showed a single peak of carbon number 16.2 but thin-layer chromatography revealed that the monohydroxy ester present as major product was accompanied by smaller amounts of non-hydroxy ester (possibly hexadecanoate) and dihydroxy ester.

The hydroxy ester was oxidised to a keto ester, the oximes of which were submitted to Beckmann Rearrangement and then hydrolysed (experimental details as for 6-hydroxytetradecanoic acid previously isolated, see p.111). The monobasic acid (nonanoic) and dibasic acid (adipic) were recognised by G.L.C. Therefore butolic acid is identical with our 6-hydroxytetradecanoic acid and is not a monohydroxypentadecanoic acid as suggested by Sen Gupta and Bose.[45]

EXPERIMENTAL

Gas-liquid chromatography

G.L.C. was normally effected at 200° using lower percentages of Apiezon 'L' as stationary phase (2½ or 5%) than was normal practice with less polar compounds. The columns were prepared in the usual manner[9] using the appropriate ratio of grease to celite.

Columns coated with Edwards silicone grease were also used. These were prepared as follows:

Pretreatment of Celite[9] and grease.[10]

Celite was treated with alkali to remove silicic acid, thereby decreasing the adsorptive properties of the celite.

Dry, size-graded celite 545 (B.D.H; 50 g.) was suspended in methanolic potassium hydroxide (0.5N; 400 ml.). After the mixture settled it was decanted and washed to neutrality with methanol on a Buchner funnel. The celite was then spread on a porcelain dish and dried in an oven at 100°.

Edwards silicone high vacuum grease (25 g.) was stirred with ethyl acetate (25 ml.), diluted with more ethyl acetate (125 ml.) and ethanol (125 ml.) added. The solvent was decanted and the residual grease washed withethanol. Excess solvent was removed by heating on a steam bath and

and finally by heating in an oven at 100°.

Preparation of column packing (5%).

The silicone grease (1 g.) treated as described was dissolved in enough chloroform to wet the pretreated celite and the mixture thoroughly shaken to effect mixing of the two phases. The solvent was then removed under vacuum on a rotary film evaporator.

The 5% silicone grease celite/column was unsuitable for dimethyl shellolate, methyl aleuritate and other polar compounds. Two other typesof columns were therefore examined.
(i) To reduce active sites on the glass surface the inside of the glass column was treated[11] with a chloroform solution (1%) of dimethyldichlorosilane overnight, then with methanol. The plugs of glass wool at the inlet and outlet of the column were treated in a similar manner. This tube was then packed with celite containing 2% silicone grease. This column gave satisfactory carbon numbers for mono-and dihydroxy esters and isopropylidene compounds but methyl aleuritate and dimethyl shellolate were not eluted from the column at 200°.
(ii) Celite 545 as supplied by B.D.H. was washed first with hydrochloric acid (0.3N), fines being decanted off, and then with water to neutrality.

A silicone coating[12,13] was then applied to the prepared celite by exposure to the vapours of dimethyldichlorosilane in a partially evacuated desiccator for 20 hr. The silicone treated celite was then washed acid-free with methanol according to the method of Howard and Martin[14] and dried at 110°. The inside of the glass column was treated as described above (i) and the column packed with siliconised celite in the normal manner.

Dimethyl shellolate gave a reasonable elution curve when examined with this column but methyl aleuritate, though eluted, gave a broader curve which was not completely satisfactor

Preparation of isopropylidene compounds.[15]

The glycol was taken up in acetone, a few drops of concentrated sulphuric acid and some anhydrous sodium sulphate added, and the mixture allowed to stand at room temperature for 3 hr. The solution was then rendered alkaline (2N sodium hydroxide) and the acetone removed under reduced pressure. Sodium chloride was added and the isopropylidene compound isolated with ether. The ethereal extracts were washed with water and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave the isopropylidene compound.

Using the columns described above the carbon numbers listed below were obtained.

Chain length	OH Groups	Configuration	Columns. 5% and or 2½% Apiezon 'L' grease celite	2% silicone grease celite (pretreated column)
C_{16}	ω -OH	-	18.5	19.0
C_{18}	12-OH	-	19.6	20.1
C_{18}	ω -OH	-	20.5	20.9
C_{16}	9,10 diOH	erythro	19.4(18.2)	20.1(18.7)
$C_{18}(\Delta^9)$	12,13 diOH	threo	20.8	21.1
C_{18}	9,10 diOH	threo	21.3	21.2
C_{18}	9,10 diOH	erythro	21.3(20.2)	21.4(20.7)
C_{16}	9,10,16 tri OH	threo	- (20.3)	- (21.4)
C_{18}	tetra OH	dierythro	- (21.8)	- (-)
Methyl 12-keto-octadecanoate	-	-	19.3	19.3
C_{16} dibasic diester	-	-	17.9	18.0

Figures in parentheses refer to isopropylidene derivatives.

Using the siliconised kieselguhr, dimethyl shellolate had a carbon number of 20.8.

Thin-layer chromatography.

Thin-layer chromatography (T.L.C) was carried out using the apparatus designed by Stahl[16,17,18] and available from Camlab (Cambridge).

A plastic board (22 x 113 cm.) with retaining ledges (1.8 cm. wide) along a short and long side, was placed on a bench so that the long edge faced the operator. The short retaining edge is now on the right side of the board. A glass plate (5 x 20 cm.) and five carefully cleaned square glass plates (20 x 20 cm.) of uniform thickness and another plate (5 x 20 cm.) were placed closely together in a row on the board. The applicator was placed on the small plate in the 'open' position.

A slurry of adsorbent was made by thoroughly mixing silica gel G (30 g.; Merck, a commercial preparation containing calcinised calcium sulphate) with water (60 ml.) in a stoppered round-bottomed flask. The slurry must be of uniform consistency and free of air bubbles; the time required for preparing the slurry should not exceed 1-1½ min. The mixture was then poured into the applicator and the lever turned through 180° to permit the slurry to run out of the slit as the applicator was being moved smoothly across the row of glass plates on the board.

The chromatoplates were then stacked in a rack and dried at 110° for 1 hr., after which they were stored in a desiccator over self-indicating silica gel till required for use. The adsorbent layer produced was about 250-275 μ thick and very uniform in appearance.

Samples were applied in chloroform (5% solution) using a micropipette, as 'spots' (approximately 10 to one plate, up to 1 mg. each) about 2 cm. from the bottom edge of the plate.

The plates were developed by ascending elution in a tank containing the required developing solvent and after development (ca. 30 min.) the separated components of mixtures were detected by exposure of the plate to iodine vapour; the components show up as brown marks on a white background.

For preparative T.L.C. the method was as described above except that the silica gel slurry (30 g. in 60 ml. water) was applied to one plate using a perspex frame 1.6 mm. deep. After drying in an oven at 120° samples (50 mg. or more) were applied in the manner described as a series of spots. After development and detection of the components in iodine vapour, the portion of adsorbent containing separated compounds was scraped off using a razor blade. The adsorbent was heated in an oven at 120° for 1 hr. to remove iodine and the organic matter was then

extracted with boiling chloroform. The silica gel was filtered off and the solvent removed under reduced pressure.

Using a developing system of Ether (50%), petroleum ether (40-60°; 48%) and methanol (2%) it was possible to separate mono-, di- and trihydroxy esters.

Preparation of Shellac mixed acids.

Super Blonde Shellac (200 g.) was hydrolysed with aqueous alcoholic sodium hydroxide (1N; 1 l.) without external heating for 16 hr. care being taken that the temperature did not exceed 30°. The free acids (213.5 g.) were liberated by passage through a column of Zeo-Karb 225 ion-exchange resin, and recovered by complete evaporation of the solvent.

Separation of Hydroxy from non-hydroxy acids.[14]

The mixed acids obtained as above were separated into two fractions by partition between petroleum ether (40-60°) and methanol-water (4:1) which had been previously equilibrated by shaking together.

To two portions of 80% methanol (2 x 1.2 l.) in each of two separatory funnels was added about 107 g. of lac mixed acids; 600 ml. of 80% methanol was placed in each of three further funnels. Petroleum ether (600 ml.) was added to the first funnel and after equilibrium passed to each of the other four funnels in turn. This was followed by more petroleum ether

(11 x 600 ml.) until little material was extracted from the methanol solutions. Acids remaining in the methanol solutions (206 g.) were then recovered using a rotary film evaporator.

The twelve petroleum ether extracts contained 0.64, 0.39, 0.26, 0.12, 0.16, 0.14, 0.14, 0.11, 0.09, 0.06, 0.07, 0.07 g (Total 2.25 g., 1.1%) of non-hydroxy acids.

Preparation of neutral alumina.[1]

Neutral alumina was prepared by stirring Peter Spence material (grade H) with an excess of ethyl acetate (2 l.) at 20° for 2 days. The alumina was filtered off and washed repeatedly with boiling water (5 l.) and heated at 250° for 2 days.

The behaviour of hydroxy acids on alumina.

Aleuritic acid[4] (1 g.) was treated with an excess of diazomethane[2] in ether and gave methyl aleuritate (0.95 g., m.p. 70.5-71.5°, Lit.[3] 70-71°).

The compounds chosen for examination contained one, two and three hydroxyl groups viz., methyl 12-hydroxyoctadecanoate, methyl9,10 dihydroxyhexadecanoate and methyl 9,10,16-trihydroxy-hexadecanoate (methyl aleuritate). These were adsorbed on alumina (i) separately and (ii) as a mixture, and examined to find the optimum eluting solvent for each class of compound.

(i)

Compound	Wt. adsorbed (g)	Optimum eluting solvent	Wt. recovered (g.)	% recovery
12-OH C_{18}	0.12	Benzene-ether (1:1)	0.10	86
9,10 diOH C_{16}	0.10	Ether-methanol (98:2)	0.08	80
9,10,16 triOH C_{16}	0.11	Ether-methanol (9:1)	0.07	64

(ii)

Compound	m.p.	Wt. in mixture (g.)	Eluting solvent	Wt. recvd. (g)	m.p.	% recov
12-OH C_{18}	50-51°	0.053	Benzene-ether (1:1)	0.049	50-51°	93
9,10 diOH C_{16}	94-95°	0.056	Ether-Methanol (98:2)	0.050	94-95°	90
9,10,16 triOH C_{16}	70.5-71.5°	0.069	Ether-methanol (9:1)	0.054	70-71°	78

It was concluded that mono-, di- and trihydroxy compounds can be separated from each other from an alumina column with the above solvents.

Adsorption chromatography using silicic acid.

Silicic acid (Mallinckrodt grade) was treated according to the method of Hirsch and Ahrens.[8,9]

Silicic acid (50 g.) was agitated with methanol (500 ml.) and then with ether (500 ml.) and allowed to settle for 30 min. The suspension was decanted and the remaining silicic acid dried at 50°.

Methyl 12-hydroxyoctadecanoate (0.086 g.) was adsorbed on silicic acid (15 g.) and eluted (0.05 g.) with 64 ml. benzene-ether (75:25) in 4½ hr. Attempts to increase the flow rate[6,7] were made by mixing the treated silicic acid with hyflosupercel (2:1) but a column made in this way from 15 g. of mixed adsorbent had a flow rate of 20 ml./hour for benzene-ether (3:1). This was considered too slow to be practicable.

Chromatographic separation of lac acids soluble in 1:4 aqueous methanol as their methyl esters on alumina columns.

The acids were esterified with methanolic hydrogen chloride and then adsorbed on alumina columns and eluted with a range of solvents. The results are summarised in the following tables and certain additional information is given in footnotes to the tables.

Alumina chromatography of lac esters (A).

Wt. of esters 20.1 g. (Recovery 17.6 g., 85%)

Wt. of alumina 200 g.

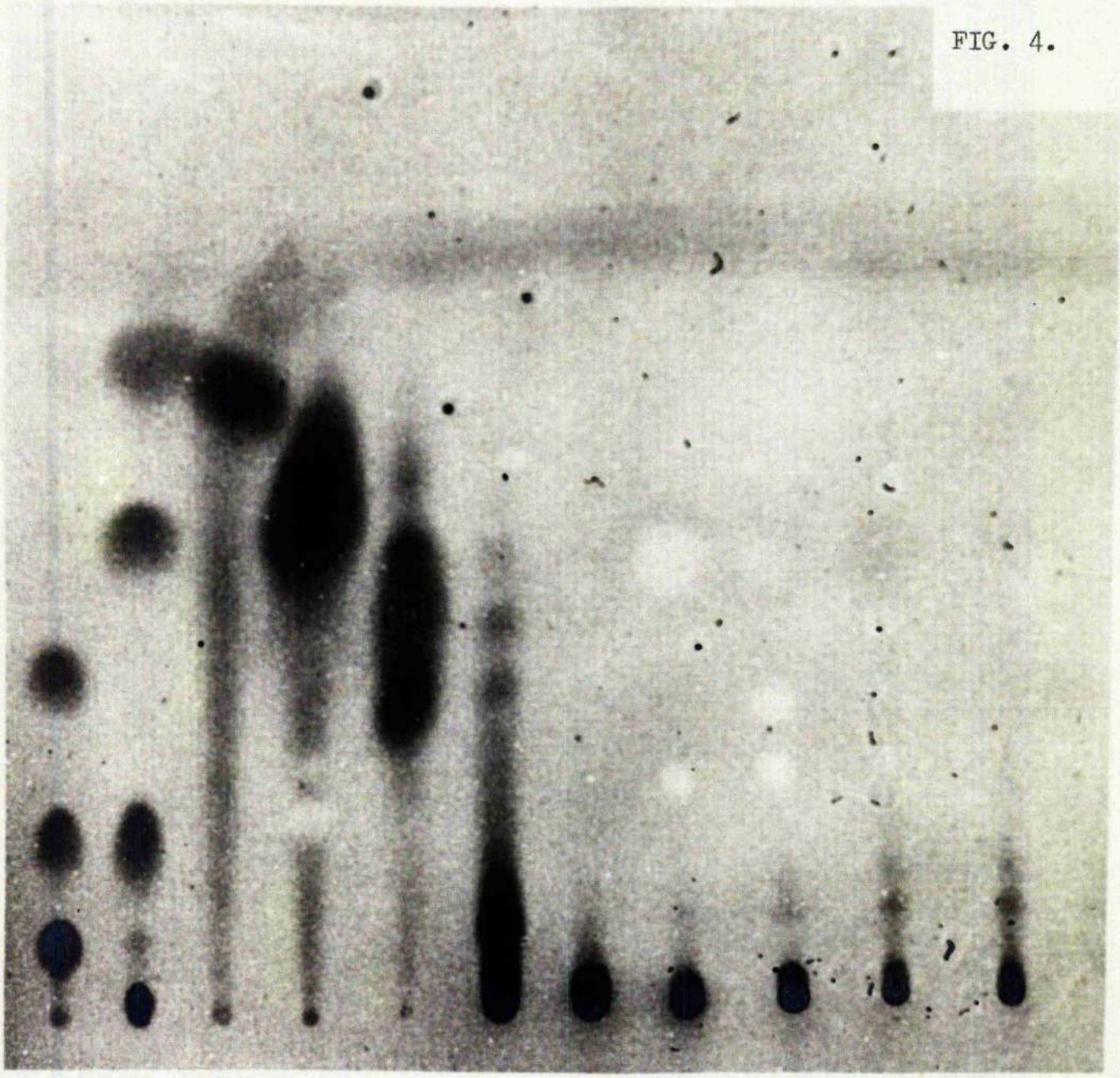
Fraction	A1	A2	A3	A4	A5	A6	A7	A8	A9
Eluting solvent[a]	B	BE(50)	E	EM(2)	EM(5)	EM(10)	EM(50)	M	M
Vol.(l.)	1	1	2	2	2	2	2	1	0.5
Wt.(g.)	0.04	0.13	0.48	9.81	4.04	2.32	0.67	0.06	0.04
%	0.2	0.6	2.3	47.3	19.5	11.2	3.2	0.3	0.2

[a] B = benzene, E = ether, M = methanol; the figure in parenthesis indicates the percentage (by volume) of the second component in the solvent mixture.

Fractions 1 and 2 were examined by G.L.C. before and after hydrogenation. All the chromatograms showed many peaks and these fractions were not examined further.

Fractions 3 to 9 were examined by G.L.C. before and after reaction with acetone and concentrated sulphuric acid but no definite conclusions were obtained.

FIG. 4.



The results obtained by T.L.C. are shown in fig. 4

Developing solvent.

50% Ether
48% Petroleum Ether
2% Methanol

Components detected in iodine vapour.

The key to the plate is as follows (left to right):

18-OH C_{18} methyl hexadecanoate

9,10 diOH C_{18} 12-OH C_{18}

A1 A2 A3 A4 A5 A6 A7 A8

Dimethyl shellolate 9,10 diOH C_{18}

methyl aleuritate

Tetra OH C_{18}

Alumina chromatography of fraction A3.

Wt. of esters 220 mg. (Recovery 180 mg. 81%)
Wt. of alumina 20 g.

Fraction	A3 1	A3 2	A3 3	A3 4	A3 5
Eluting solvent[x]	BE50	BE(75)	E	EM(1)	EM(2)
Vol.(ml.)	100	100	100	100	100
Wt.(mg.)	24	76	70	12	-
%	10.9	34.5	31.8	5.5	-

x B = benzene, E = ether, M = methanol; the figure in parenthesis indicates the percentage (by volume) of the second component in the solvent mixture.

Fractions 2 and 3 were almost a pure substance. They showed one major spot on T.L.C. and one major peak of carbon number 16.2 on G.L.C. The isolation of further quantities of this material is reported on p.105 and experiments to determine its structure on p. 111

Alumina chromatography of lac esters (B)

Wt. of esters 39.4 g.

Wt of alumina 500 g.

Fraction	B1	B2	B3	B4	B5
Eluting solvent	Ether	Ether	Ether	Ether	Ether
Vol. (l)	1	1	1	1	1
Wt.(g.)	0.49	2.67	1.66	3.89	5.11
%	1.2	6.8	4.2	9.9	13.0

The remaining material was stripped off the column with methanol and was not further examined.

Examination of these fractions by G.L.C. (carbon numbers 16.2, 17.6 and 18.6) and T.L.C. indicated the highest concentration of monohydroxy esters other than methyl 6-hydroxy-tetradecanoate in fraction B3.

Alumina chromatography of lac esters (C)

Wt. of esters 115.8 g.
Wt. of alumina 750 g.

Fraction	C1	C2	C3	C4
Eluting solvent[x]	BE(50)	E	E	E
Vol.(l)	1.5	1	1	1
Wt.(g.)	47.47	9.01	1.88	0.60
%	40.99	7.78	1.62	0.52

[x] B = benzene, E = ether; the figure in parenthesis indicates the percentage (by volume) of the second component in the solvent mixture.

The remaining material was stripped off the column with methanol and was not further examined.

Alumina chromatography of fractions C1 to C4.

Wt. of esters 58 g.
Wt. of alumina 750 g.

Fraction	D1	D2	D3	D4	D5
Eluting solvent[x]	Ether	Ether	Ether	Ether	Ether
Vol.(l)	1	1	1	1	1
Wt.(g.)	1.42	8.34	2.91	3.69	4.15
%	1.2	7.2	2.5	3.2	3.6

The remaining material was stripped off the column with methanol and was not further examined.

Examination of these fractions by G.L.C. and T.L.C. indicated that D2 was almost pure methyl 6-hydroxytetradecanoate (Carbon number 16.2) and that fractions D3 and D4 contained the highest concentrations of other monohydroxy esters (Carbon numbers 17.6 and 18.6).

Alumina chromatography of fractions B3, D3 and D4.

Wt. of esters 8 g. (Recovery 6.03 g., 75%)
Wt. of alumina 500 g.

Fraction	Eluting solvent[a]	Vol. (l)	Wt.(g)	%
E1	BE(50)	1	0.01	0.13
E2	BE(75)	1	0.03	0.38
E3	E	0.5	0.01	0.13
E4	E	0.5	0.006	0.08
E5	E	0.5	0.007	0.09
E6	E	0.5	0.018	0.23
E7	E	0.5	0.069	0.86
E8	E	0.5	0.149	1.86
E9	E	0.5	0.249	3.11
E10	E	0.5	0.644	8.05
E11	EM(5)	0.5	1.03	12.88
E12	EM(5)	0.5	3.64	45.50
E13	M	0.5	0.16	2.00
E14	M	0.5	0.007	0.09

[a] B = benzene, E = ether, M = methanol; the figure in parenthesis indicates the percentage (by volume) of the second component in the solvent mixture.

Chromatographic separation of selected ester fractions by preparative T.L.C.

Selected fractions from alumina chromatography (E7-E11), shown by T.L.C. and G.L.C. to be mixtures, were further purified by preparative T.L.C. About 100 mg. were loaded on to a thick plate (1.6 mm.) and developed with 65% ether, 33% petroleum ether and 2% methanol. Exposure to iodine vapour showedthe presence of 4 main bands on each chromatogram. These were scraped off, and the esters recovered and examined by G.L.C. The results are tabulated below. Fractions of similar nature were combined into 2 groups (mixtures F and G) for further examination.

Preparative T.L.C.

Original Fraction	E7	E8	E9	E10	E11
Wt.(mg.)	69	149	250	644	1032
Band 1[x]	5(a,c)	19(a,c)	9(a)	62(a)	17(a)
Band 2[x]	9(a,c)	17(a,b,c)	24(a,c)	40(a,b,c)	51(a,c)
Band 3[x]	8(a,c)	17(d,e)	31(a,b,c)	67(c,e)	49(a,b,c
Band 4[x]	19(e)	9	33(d,e)	-	-

x The figures quoted are mg. recovered from each band; the letters in parenthesis refer to the main G.L.C. peaks of carbon numbers 16.2(a), 17.6(b), 18.6(c), 19.4(d) and 20.5(e).

Fractions E7/3, E8/2, E9/3, E10/2 and E11/3 were combined (mixture F) as were E7/4, E8/3, E9/4 and E10/3 (mixture G).

Identification of separated compounds.

Non-hydroxy compounds.

Methyl esters derived from the petroleum ether soluble acids were examined by G.L.C. which showed peaks of the followin carbon numbers: 12.0, 13.8, 14.0, 15.8, 16.0, 17.8, and 18.0. After hydrogenation only four peaks were present in the chromato gram: 12.0, 14.0, 16.0 and 18.0.

The original esters were rechromatographed quantitative and the results obtained are given on p. 85 .

The structure of the unsaturated acids.[43,44]

The clear solution of sodium salts obtained by warming the acids (0.3 g.) on the water bath with an equal weight of sodium hydroxide dissolved in water (35 ml.) was cooled. After dilution with ice-cold water (240 ml.) the mixture was shaken at 10° while potassium permanganate solution (1%, 24 ml.) was added quickly. After 5 min. the liquid was decolourised with sulphur dioxide and concentrated hydrochloric acid (9 ml.) added.

The white flocculent precipitate of mixed dihydroxy acids was drained for a short time and washed with (a) petroleu

ether (60-80°; 7.5 ml.), (b) warm petroleum ether (60-80°; 20 ml.). The petroleum ether filtrates gave a white crystalline solid (0.16 g.). The insoluble dihydroxy acids (0.03 g.) were dried in a vacuum desiccator over phosphorus pentoxide.

The dihydroxy acids (0.03 g.) were dissolved in a solution of potassium carbonate (0.04 g. in 30 ml. water) and a solution of potassium periodate (0.08 g.) and potassium permanganate (0.0009 g.) in water (14 ml.) added. The mixture was shaken for 24 hr. at room temperature, then acidified (dilute sulphuric acid), saturated with sodium chloride, extracted with petroleum ether (40-60°; 3 x 100 ml.) to remove the monobasic acids and extracted with ether (3 x 100 ml.) to give the dibasic acid. The two acid extracts were washed with water (3 x 100 ml.) and the recovered acids methylated with methanolic hydrogen chloride (5%).

G.L.C. showed the presence of esters of carbon number 7.0 (major), 8.0 (v.minor) and 9.0 (major) for the monobasic acid fraction but only a single peak corresponding to dimethyl azelate was observed with the dibasic acid fraction.

These results indicate that the unsaturated acids are probably entirely of the Δ^9 type.

The monohydroxy acid of carbon number 16.2.

The structure of this acid was determined by iodination-deiodination[41] and by the Beckmann rearrangement of the corresponding keto acid using the small amount of material first isolated in a reasonably pure state (Fractions A3 2 and A3 3). Analytical samples of the hydroxy and keto acids were prepared from the larger amount of the hydroxy ester obtained in later experiments (Fraction D2).

Iodination-deiodination.

A portion of fractions A3/2 and A3/3 (0.03 g.), iodine (0.15 g.) and red phosphorus (0.36 g.) were heated on a boiling water bath for 1 hr. after which the remaining iodine was evaporated under reduced pressure. The product was extracted with ether and the ethereal extracts washed with water (3 x 200 ml.), sodium bisulphite (5%, 2 x 100 ml.) and water (5 x 200 ml.) and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave the iodinated ester (0.035 g.

This material (0.035 g.) was refluxed with methanolic hydrogen chloride (15%, 5 ml.) in the presence of activated zinc (0.2 g.). The product was poured into water, extracted with ether and worked up in the usual manner to give a yellow oil (0.024 g.). Examination of this product by G.L.C. gave one peak with a carbon number of 14.0, proving that the compound contained a straight chain of 14 carbon atoms.

Oxidation of the hydroxy ester[19] to a keto ester and Beckmann rearrangement of its oximes.

A further portion of the esters (40 mg.) was dissolved in glacial acetic acid (1 ml.) and oxidised at room temperature with a similar weight of chromic anhydride in glacial acetic acid solution (1 ml.). As the reaction is exothermic, the flask was cooled under running water. After 1 hr. the reaction mixture was poured into water (10 ml.) and sulphur dioxide passed into the solution. The resultant compound was extracted with ether, washed several times with water and the ethereal extracts dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave the oxidised material which was remethylated with methanolic hydrogen chloride (5%); the keto-ester was extracted with ether to give an oil (0.04 g.) of carbon number 15.5.

The keto-ester in alcohol (80%, 1 ml.) was mixed with solutions of hydroxylamine hydrochloride (0.08 g.) in alcohol (80%, 1 ml.) and of fused sodium acetate (0.08 g.) in alcohol (80%, 0.5 ml.) and refluxed for 2 hr. The reaction mixture was poured into water and the mixed oximes extracted with ether and isolated as an oil (0.05 g.).

The mixture of oximes (0.05 g.) was heated with

concentrated sulphuric acid (0.2 ml.) at 100° for 1 hr. (Beckmann rearrangement). The product was cooled and twice this volume of water added slowly with cooling. The mixture was then boiled for 3 hr. to hydrolyse the amides. Water was added and the solution extracted with petroleum ether (40-60°) to remove the monobasic acid and with ether to remove the dibasic acid. These acids were methylated and examined by G.L.C. Each fraction showed single peaks corresponding to nonanoic ester and adipic ester.

Analytical specimen

A portion of fraction D2 was chromatographed twice more on alumina columns to give material showing only one spot on T.L.C. and one peak of carbon number 16.2 on G.L.C.

This ester (0.26 g.) was hydrolysed by boiling with alcoholic potassium hydroxide (1N; 5 ml.) for 1 hr. The solution was acidified (dilute hydrochloric acid) and the acid isolated with ether, recrystallised from petroleum ether (40-60°) and dried in a vacuum desiccator over phosphorus pentoxide.

This sample melted at 58-58.5° and contained C,68.4; H,11.4. $C_{14}H_{28}O_3$ requires C,68.8; H,11.5%).

Another sample of the hydroxy acid was oxidised as already described and the keto-acid, after recrystallisation from ether/petroleum ether (1:1), melted at 69.5-70.5° and

contained C,68.9; H,10.6. ($C_{24}H_{28}O_3$ requires C,69.3; H,10.8).

Other Hydroxy acids in mixtures F and G.

The mixtures F and G were examined by G.L.C. and T.L.C. Mixture F contained 3 major peaks of carbon numbers 16.2, 17.6 and 18.6 and was shown by T.L.C. to contain only monohydroxy esters. Mixture G contained 2 major peaks of carbon numbers 19.4 and 20.5 and was shown by T.L.C. to contain mono- and dihydroxy esters as well as more polar esters.

Samples of both mixtures were iodinated-deiodinated and oxidised.

Mixture F (0.02 g.), iodine (0.15 g.) and red phosphorus (0.36 g.) were heated at 100° for 1 hr. The iodinated product (0.03 g.) was refluxed for 4 hr. with methanolic hydrogen chloride (15%; 5 ml.) in the presence of activated zinc (0.2 g.). The product was isolated with ether and G.L.C. examination gave carbon numbers of 12.0 (trace), 14.0 (approx. 50%) and 16.0 (approx. 50%).

A similar experiment on mixture G gave carbon numbers of 12.0 (trace), 14.0, 15.8, 16.0 and 18.0.

Portions of both mixtures were oxidised[20] as follows:

Mixture F (0.025 g.) was dissolved in acetone (2 ml.) and a solution of chromic anhydride (20 mg.) was added dropwise followed by water (1.25 ml.) and concentrated sulphuric acid (0.2 ml.). After addition of the reagent, the mixture was stirred for 30 minutes at room temperature, poured into water and extracted with ether. The product, examined by G.L.C. after esterification, showed peaks of carbon numbers 15.5, 16.9 and 18.8.

A sample of synthetic ω -hydroxyhexadecanoic acid (carbon number 18.6) was similarly oxidised and the product had carbon number 18.9.

When synthetic ω -hydroxyhexadecanoic ester was run on G.L.C. with mixture F, no new peak was obtained.

Mixture G was oxidised in a similar way. G.L.C. of the product showed azelaic ester (probably from aleuritic acid) and small peaks of carbon numbers 16.0 and 16.9.

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