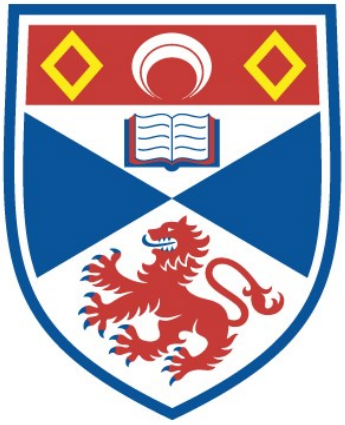
SYNTHETIC, DEGRADATIVE, ANALYTICAL AND CONFIGURATIONAL STUDIES OF LONG CHAIN EPOXY AND HYDROXY ACIDS

Lindsay Johnston Morris

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



1958

SYNTHETIC, DEGRADATIVE, ANALYTICAL AND CONFIGURATIONAL STUDIES OF LONG CHAIN EPOXY AND HYDROXY ACIDS

being a Thesis

presented by

LINDSAY JOHNSTON MORRIS, B.Sc.

to the

UNIVERSITY OF ST. ANDREWS

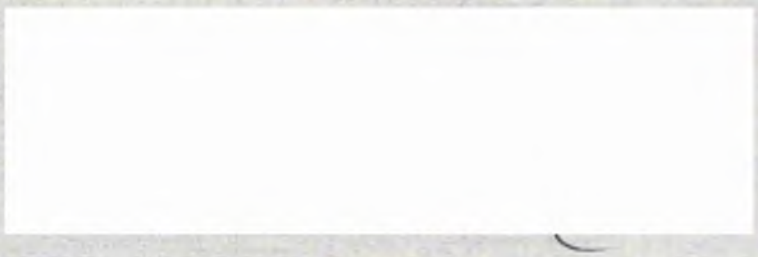
in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY.

September, 1958.

DECLARATION.

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



CERTIFICATE.

I hereby certify that Mr. Lindsay Johnston Morris has spent ten terms at research work under my supervision, has fulfilled the conditions of Ordinance No. 16 (St. Andrews) and is qualified to submit the accompanying Thesis in application for the Degree of Doctor of Philosophy.

Research Supervisor.

UNIVERSITY CAREER.

I entered the United College of St. Salvator and St. Leonard, University of St. Andrews, in October 1951, holding a Taylour - Thompson Entrance Bursary, pursued a recognised couse for graduation in Science and graduated B.Sc. with Second Class Honours in Chemistry in 1955.

I was admitted as a Research Student in September, 1955 and was awarded a grant from Messrs. Thomas Hedley and Co., Ltd., Newcastle, which I held until August 1958.

ACKNOWLEDGMENTS.

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

I also wish to thank Miss D.R.W. Price, B.Sc., of this Department and Drs. D. Chapman and J.F. Nacey, of the Research Department of Unilever Ltd., Port Sunlight for carrying out the infra-red determinations and to Mr. I. Bayne for the reproductions.

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Finally thanks must be expressed to Messrs. Thomas Hedley and Co., Ltd. for financial assistance throughout this work and to the University of St. Andrews for a supplementary grant in 1958.

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Summary

Synthetic, Degradative, Analytical and Configurational Studies of Long Chain Epoxy and Hydroxy Acids.

Part I. Synthetic Studies. cis-Hydroxylation of Long-chain Olefins.

The Woodward cis-hydroxylation procedure, involving the use of iodine and silver acetate in wet acetic acid, has been adapted and applied to the cis-hydroxylation of long-chain olefins. It is a satisfactory method for oxidising these compounds and has some advantages over other methods of cis-hydroxylation.

A further cis-hydroxylation procedure, involving the use of iodine and silver nitrate in acetonitrile has also been developed.

Part II. Degradative Studies. The Epoxy Acid in Cameline Oil.

A hydroxy acid, isolated from Camelina sativa seed oil, has been shown to be threo-15:16-dihydroxyoctadec-cis-9-cis-12-dienoic acid. Infra-red studies indicate that this acid is present in the oil as glycerides of cis-15:16-epoxylinoleic acid and is thus closely related to the only other naturally occurring long-chain epoxy acid viz. cis-12:13-epoxyoleic acid.

Part III. Analytical Studies. The Occurrence of 9-Hydroxy-octadec-cis-12-enoic Acid in Various Strophanthus Oils.

9-Hydroxyoctadec-12-enoic acid has been identified, qualitatively and quantitatively, in ten Strophanthus oils.

In two oils a small amount of optically active erythro-9:10-dihydroxystearic acid accompanies the monohydroxy acid and may occur throughout the genus.

The oils have been analysed by three methods and these are discussed comparatively. The analytical results lead to some generalisations and correlations.

Part IV. Configurational Studies.

9-Hydroxyoctadec-cis-12-enoic acid has been isolated and three of the four isomeric 9:12:13-trihydroxystearic acids prepared from it. The rotations of these and other hydroxy acids have been measured.

It is tentatively suggested that the 9-hydroxy acid has the D-configuration, thus being in the same series as natural ricinoleic acid.

Unsuccessful attempts to synthesise long-chain dihydroxy acids, of known configuration, by extension of the chain of optically active tartaric acid are reported and a more suitable synthetic route is suggested.

Publications

(i) Fatty Acids. Part V.- Applications of the Woodward *cis*-Hydroxylation Procedure to Long-chain Olefinic Compounds. F.D. Gunstone and L.J. Morris, *J.C.S.*, 1957, 487.

(ii) A New Method of *cis*-Hydroxylation of Olefines. L.J. Morris, *Chem. and Ind.*, in the press.

(iii) Fatty Acids. Part VI.- The Epoxy Acid in Cameline Oil. To be published.

(iv) Vegetable Oils. Part VII.- Further Studies of *Strophanthus* Oils. To be published.

Part I.

Synthetic Studies.

cis-Hydroxylation of Long-chain Olefinic Compounds.

Introduction.

The oxidation of olefins to the corresponding glycols is an important and widely used reaction for which several reagents are available[1]. Unsymmetrically substituted olefins when so oxidised are converted into the corresponding threo- and erythro-glycols and if a stereospecific reagent is used, one or other may be isolated. Cis-addition to a cis-double bond or trans-addition to a trans-double bond gives rise to a product with the erythro-spatial arrangement of the added groups, while trans-addition to a cis-double bond or cis-addition to a trans-double bond leads to a product with the threo-configuration. The connotations threo- and erythro- derive from the biose carbohydrates threose and erythrose.

Trans-hydroxylation can be readily effected by organic peracids[2], those most commonly used being perbenzoic, peracetic and performic acids although perphthalic, percamphoric and perfuroic acids have been used. The intermediate epoxide is formed by cis-addition but fission of the epoxide ring occurs with inversion of configuration so that the nett result is trans-addition[3].

Persulphuric acid[4] gives the same stereochemical result and other inorganic peracids used in catalytic amounts with hydrogen peroxide have been shown to give trans-glycols[5]. The most successful of these were pertungstic and permolybdic acids although various other metal oxides in hydrogen peroxide can be used.

Another convenient route to trans-glycols is by reaction

with Prevost reagent[6], namely silver benzoate and either iodine or bromine in anhydrous benzene. The silver iodo- or bromo-benzoate complex first formed adds across the double bond to give an iodo- or bromo-benzoate which further reacts to give the dibenzoate. This may be hydrolysed to the trans-glycol. This reaction will be further discussed below.

Trans-hydroxylation also results from addition of halogens[7] or hypohalous acids[3,8] followed by hydrolysis of the dihalides or halohydrins to the glycols. A modification of this last method involves the use of N-bromosuccinimide in aqueous solution to add the elements of hypobromous acid across the double bond. This has proved successful when applied to olefins resistant to peracid oxidation[9]. N-bromo-acetamide has been used widely in the sterol field to give the same reaction[10].

One further method for producing trans-glycols is of considerable interest[11]. Oxidation is carried out by hydrogen peroxide catalysed by cation exchange resins and is an extension of a similar method of oxidising olefins to epoxides[12]. This method has possibilities of modification to give a continuous trans-hydroxylation process.

Cis-hydroxylation can be effected by permanganate[13] or by various reactions dependent on osmium tetroxide. Osmium tetroxide can be used alone, in calculated amount, in anhydrous ether or dioxane solution[14], the intermediate addition complexes being hydrolised to the cis-diols. Pyridine

is used as an accelerator and since osmium tetroxide reacts with alcohols, phenols and amines, these groups must first be protected.

Osmium tetroxide is also used in catalytic amounts along with metal chlorates[15], this reaction being limited to water soluble olefins, in conjunction with hydrogen peroxide or tert-butyl peroxide[16].

Alkaline potassium manganate[17] has also been used to give cis-hydroxylation.

x x x

The oxidation of long-chain mono-olefinic acids to the corresponding dihydroxy acids is an important method of identifying these compounds and cis- and trans-hydroxylation are best effected by cold, dilute alkaline permanganate[18], and by performic acid[19], respectively. Both of these methods are simple to carry out and give the desired dihydroxy acid in almost quantitative yield. The reaction with permanganate, however, has some disadvantages. Following Lapworth and Mottram's procedure, very dilute solutions must be used(ca. 1 litre per g. of acid) so that the oxidation of large quantities becomes very tedious. (Attempts to reduce the volume have been made by Traynard[20] who succeeded in carrying out the reaction in a dilution of only 37.5 ml. per g. of acid. His yields(70 -75%) however are considerably lowered). In addition this method is less satisfactory when applied to substances which are insoluble

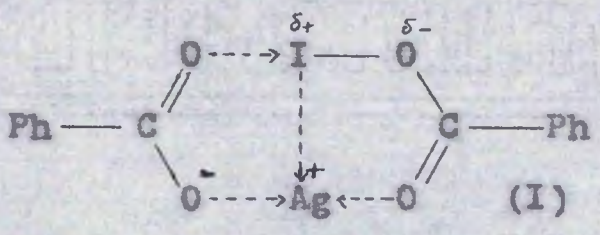
in aqueous alkali, though acetone may then be used as solvent[21].

Of the other methods giving cis-hydroxylation only osmium tetroxide has been widely used, and a method which overcomes the disadvantages of the permanganate method and avoids the use of the expensive and highly dangerous osmium tetroxide (it combines a high toxic effect with an abnormally high vapour pressure) is therefore desirable.

x x x

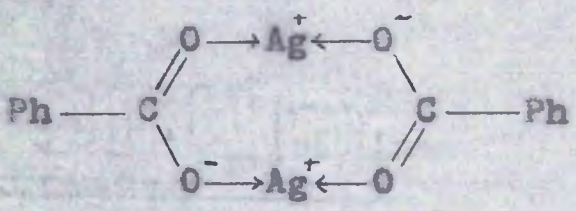
The Prévost reaction depends on work first performed by Simonini[22] and substantiated by other investigators[23] which showed that equivalent quantities of iodine and silver salts of monocarboxylic acids react to give a complex which can be isolated:- $2R.COOAg + I_2 \longrightarrow (R.COO)_2AgI + AgI$. This complex was represented as $[(R.COO)_2Ag^-]I^+$ which, having a positive charge on the iodine atom is a very reactive electrophilic reagent. Birckenbach, Goubeau and Berninger[24], however, represented the complex as a mixture of silver salt, R.COOAg and pseudohalogen, R.COOI and stated that if two equivalents of halogen were used only the pseudohalogen is formed, along with silver iodide. These pseudohalogens or hypohalites have a distribution of charge resulting again in a positive charge on the iodine atom, viz. $R.COO^-I^+$.

A recent suggestion has been made for the structure of the silver iodo-benzoate complex[25] to explain why this substance can cause homolytic phenylation as well as electrophilic halogenation. The suggested structure is shown (I) - p.7.

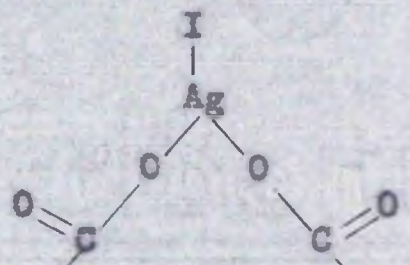


(I)

The dotted lines are weak coordinations and the weak iodine - silver bond is seen as resulting from overlap of equatorial I^+ electrons with vacant Ag^+ orbitals. The relationship to the normal dimeric form of silver benzoate (II) is obvious and it is suggested that reaction is probably via the intermediate (III).



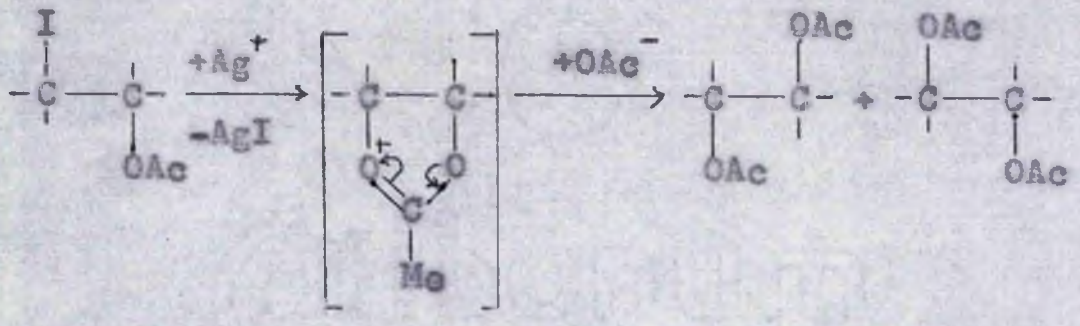
(II)



(III)

Reaction of these complexes with olefins, as mentioned above,(p.4), leads to trans-halo-acyl esters and, if the reaction is carried out in dry carbon tetrachloride, these products can be isolated[26]. Under the conditions for Prévost reaction i.e. benzene solution, the halogen is readily substituted and the trans-dibenzoxy compound is produced, which yields the trans-glycol on hydrolysis.

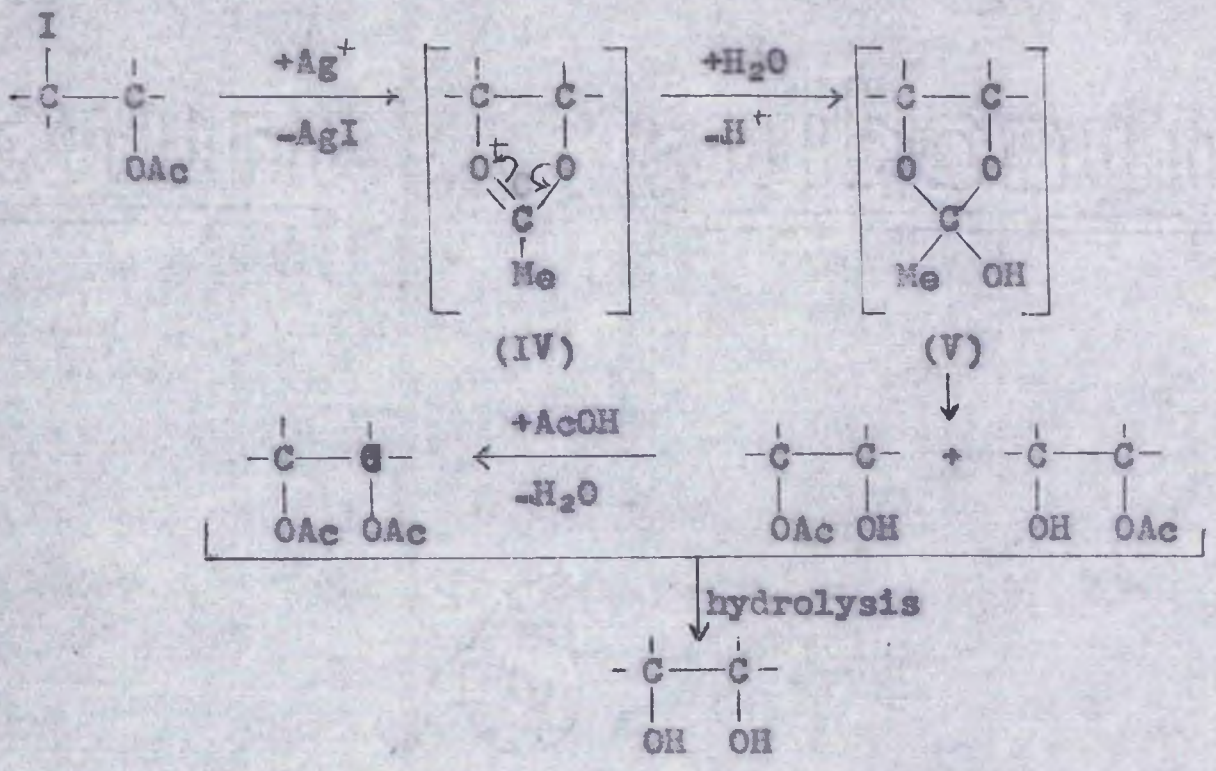
The replacement of the halogen by a second benzoxy group takes place with retention of configuration. Winstein and Buckles[27] have explained the retention of configuration in the replacement of bromine in bromo-acetoxy butanes with a second acetoxy group, in dry acetic acid, by neighbouring group participation.



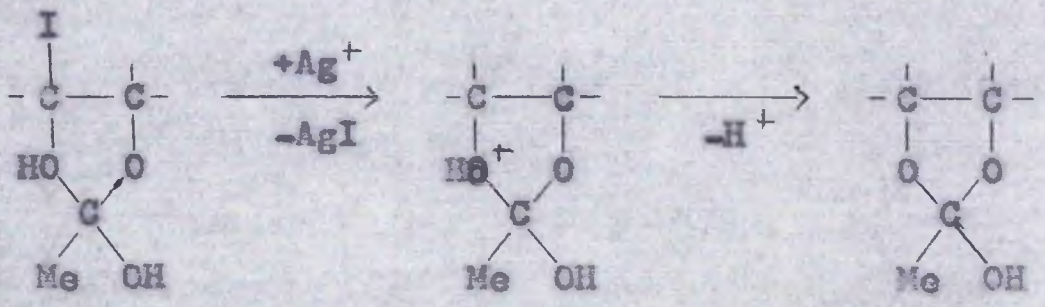
(IV)

This participation by the neighbouring acetoxy group, when the iodine ion is abstracted, prevents "backside" attack by the acetoxy group which would result in inversion. Clearly the benzoxy group in the Prévost reaction must participate in a similar manner to result in retention of configuration during the replacement step.

Winstein and Buckles[28], however, showed that if one equivalent of water be present during this solvolysis of trans-bromo-acetoxy butanes with silver acetate the result is complete inversion of configuration and the product is exclusively the cis-compound. They explained this again by neighbouring group participation, the water, however, converting the cation (IV) exclusively into the orthoacetate (V). The latter easily rearranges to the hydroxy-acetate isomers which may suffer partial esterification to the diacetate. Hydrolysis of this mixed product yields the cis-glycol.



Another possible sequence was suggested[28], involving addition of water to the acetoxy-iodide before displacement of the iodine. This scheme involves the participation of the neighbouring orthoacetate group in the replacement of the iodine atom:-



In the course of the reaction one or other of these two mechanisms may be followed or possibly both, simultaneously.

This highly stereospecific solvolysis has already been used in these laboratories[29] to convert a cis-epoxide into an erythro-glycol via the threo-bromo-acetoxy compound.

x x x

Professor Woodward made use of these two series of reactions; (a) addition to ethylenic bonds of the silver-iodo-acetate complex to give trans-iodo-acetates and (b) stereo-specific acetolysis of these compounds to cis-hydroxy-acetates, to provide a method of obtaining cis-glycols from ethylenic compounds[30].

This method had also been described in two other cases[31] and although all three examples of its use were on high molecular weight, alicyclic compounds, the method promised to be general[32].

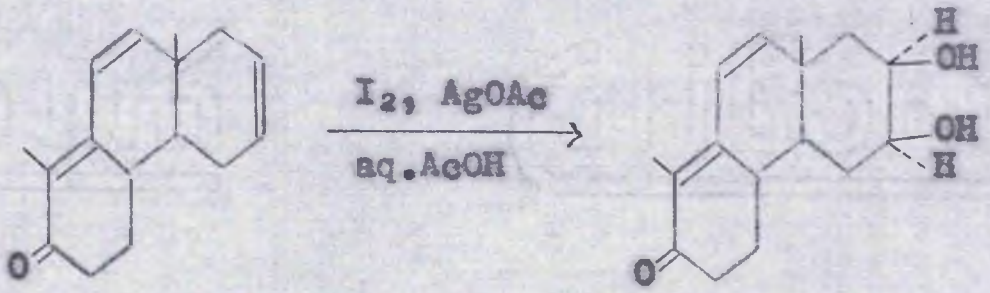
The conditions for the reaction as described by Woodward were:- Powdered iodine (2 equivalents) was added portionwise over half an hour to a mixture of silver acetate (slightly more than two equivalents) and the olefin (1 equivalent) in anhydrous acetic acid, which was stirred vigorously at room temperature. Water (slightly more than 1 equivalent) was added and the mixture heated on a water bath at 95° for three hours. Excess sodium chloride was then added and the precipitated silver salts filtered off and the solvent distilled from the filtrate under reduced pressure. The residue was dissolved in methanol, filtered, neutralised with methanolic potassium hydroxide and hydrolised with excess of the same reagent overnight at room temperature. The hydrolysate was then treated with dilute hydrochloric acid, the methanol removed under vaccuum and the product isolated and purified.

This method resulted in yields of 71, 56 and 67% of the

cis-glycol in the three examples of its use.

Since completion of these experiments this method has been described several more times[33,34], only once, however, in the oxidation of long chain olefinic acids[34].

A distinction between the product formed by Woodward's method and that formed by osmium tetroxide oxidation has been reported in some cases[33(a)(b)(c)], the difference being in the relationship between the cis-glycol group and other groups in the molecule. For example[33(c)] Woodward's method oxidises the 6:7-double bond of dl-anti-trans-4:4a:5:8:8a-hexahydro-1:8a-dimethyl-2(3H)-phenanthrone to give almost exclusively the β-cis-glycol whereas osmium tetroxide gives almost exclusively the α-cis-isomer.



The reason lies in the mechanism of the two reactions. Osmium tetroxide adds on to the least hindered side of the double bond and results in the α-product. Addition by the I^+ ion is also on the least hindered side but, since this addition is trans- the acetate ion is on the more hindered side and the inversion on replacement of the iodine in the second step results in a cis-product on the more hindered side of the molecule. This can lead to a product not otherwise accessible[33(b),(c)].

Discussion.

The aim of this work was to adapt Woodward's procedure to give a general method for the cis-hydroxylation of long-chain olefinic compounds without the disadvantages inherent in the Lapworth and Mottram method and in the use of osmium tetroxide.

Initial attempts to oxidise oleic acid following Woodward's procedure were unsuccessful and an attempted oxidation of methyl hexadecenoate gave a yield of only 10% of pure product.

The conditions of the method were modified one at a time to increase the yield. The time of reaction at room temperature was varied with little improvement, although it was found that addition of iodine portionwise over half an hour was unnecessary. Refluxing the mixture after addition of the water, instead of heating on the steam bath, as recommended by the Americans, led to improved yields and finally the methods of removing silver salts and isolating the crude hydrolysis product were changed. Excess silver acetate and iodine made little difference to the yield.

The method adopted as standard procedure is described fully on page along with a summary of the work leading to the adoption of this method (Table II).

With this modified procedure a variety of olefinic compounds have been oxidised to the corresponding cis-glycols (see Table I). With long chain olefins of high purity the oxidation products are obtained in high yield and are readily purified, whether the starting material be carboxylic acid, ester or alcohol. With less pure starting materials the yield of crude product is

Table I.

Olefin	Crude product		Pure product		M.pt. (literature)	
	yield %	M.pt.	yield %	M.pt.		
Pure olefins:-						
Methyl oleate	99	126-128°	89	130-132°	132°	Hilditch[×], p.498
Methyl elaidate	97	92-93	91	93.5-94.5	95	Hilditch, p.498
Elaidic acid	89	92-94	85	94-94.5	95	Hilditch, p.498
Oleyl alcohol	100	123-125	81	126	126	Hilditch, p.564
Elaidyl alcohol	94	82-84	79	82.5-83.5	82	Hilditch, p.564
cyclo-Hexene	66	-	41	94-97	98	Ref. 35
Acenaphthylene	89	180-204	28	203-208	213	Ref. 36
Crude olefins:-						
Olive oil	87	-	83*	131-132	132	Hilditch, p.498
Olive oil†	94	112-125	97*	125-132	132	Hilditch, p.498
Castor oil	95	-	30*	108-111	112	Ref. 37
			7*	135-137	138	
Methyl undecenoate	49	74-77	42	84-87.5	85-86	Ref. 38
Methyl hexadecenoate	93	-	62	126-128	129	Hilditch, p.516
Methyl linoleate	95	-	14	173	174	Hilditch, p.529
			15	163-165	164	
Oleic acid	95	-	56	123-127	132	Hilditch, p.498

* Based on the assumption that olive oil contains 75% oleic and castor oil 90% ricinoleic acid.

† All oxidations were effected on 0.01 mole of olefin except this case where 0.1 mole was used.

× Hilditch, "The Chemical Constitution of Natural Fats", Chapman and Hall Ltd., London, 1956.

generally high but considerable loss sometimes occurred during purification.

Comments on some oxidations(for detailed accounts see page 18).

(a) Castor oil: The crude oxidation product was separated into the two isomers by the method of Kass and Radlove[37]. Since the crude product contained about 50% of liquid impurities it was thought that some dehydration between the hydroxy groups on the 9 and 12 positions might have occurred in the acid medium, giving furan derivatives. Similar yields, however, resulted from oxidation of acetylated castor oil (28 and 8%). The asymmetric centre at C_{12} must exert considerable directional influence in the addition step since the higher melting and more easily isolated trihydroxy acid is obtained in less than one third of the yield of the low melting isomer. Kass and Radlove obtained yields of 20.0 and 21.7% respectively of the low and high melting isomers by permanganate oxidation.

(b) Methyl undec-10-enoate: The standard procedure gave only 8% yield but this was raised to 42% by prolongation of the reaction times. Monosubstituted olefins are known to be less reactive to electrophilic reagents, a longer time being necessary for performic acid oxidation of this compound to give a yield of 44%[19] while permanganate oxidation gives only 31% yield of the diol[38].

(c) Methyl linoleate: Because this ester contains two double bonds the quantities of reagents were doubled. The crude product was separated into the two isomeric sativic acids by

the method of Reimenschneider et al.[39].

(d) cyclo-Hexene: Only 20% yield of the cis-diol resulted from oxidation by the standard method but this yield was doubled by prolongation of the reaction time. Clarke and Owen[35] obtained yields of 46% with sodium chlorate and osmium tetroxide and 33% with potassium permanganate and magnesium sulphate. These experiments confirm the applicability of this method to the oxidation of alicyclic olefins.

(e) Acenaphthylene and Phenanthrene: With acenaphthylene extension of the reaction time for the first step until the solution had changed from yellow to colourless (indicating complete reaction) raised the yield of final product only from 23 to 28%. Further modification of the conditions for the second step of the reaction and in the isolation and purification of the product would almost certainly result in a much higher yield. Previously this diol had been prepared from acenaphthylene by bromination and subsequent hydrolysis[36], by reduction of the diketo compound[36] and by osmium tetroxide oxidation[14(b)]. The first two of these methods give mixtures of the cis- and trans-diols which can, however, be separated.

Phenanthrene was not oxidised, most of the starting material being recovered unchanged after reaction.

These experiments show that aromatic bonds will also be oxidised, provided they are weak enough in aromatic character as the 1:2 bond of acenaphthylene is known to be. The "weak" 9:10 bond of phenanthrene must therefore, from these results,

have rather more aromatic character.

(f) Tetramethylethylene: This was a test reaction on a sterically hindered double bond. It may be seen from a molecular model that the four methyl groups in this olefin hinder the molecule sterically in reacting with two groups as large as I- and $CH_3.COO$- and no pinacol hydrate could be isolated from the reaction mixture. Even had oxidation succeeded, severe practical difficulties needed to be overcome as was shown by simulating the final stage of the reaction with pure pinacol hydrate, when only 65% of impure pinacol hydrate could be recovered.

(g) Acetylenic compounds:

(i) Methyl ximenynate: It was hoped that it might be possible to convert the trans-ethylenic bond of this compound into the threo-diol leaving the acetylenic bond untouched but this hope was not fulfilled, although the corresponding erythro-diol (M.pt. 88-89°) can be prepared by performic oxidation[40]. Some reaction appears to have taken place since ximenynic acid was not isolated from the product, which gave a small amount of unidentified solid melting at 22-23°.

(ii) Methyl ricinstearolate: The experiments on this compound and on stearolic acid were to determine whether an isolated triple bond is attacked. Methyl ricinstearolate gave ca. 32% yield of a solid (M.pt. 77.5-78, $C_{18}H_{34}O_{4\cdot13}$ - see p.35) which gave a yellow precipitate with 2:4-dinitrophenylhydrazine (M.pt.64-67°). This compound was not identified but it is

possibly 12-hydroxy-9- or-10-ketostearic acid.

Oxidation of methyl ricinstearolate with twice the normal amounts of reagents did not give any diketo compound but only a smaller yield of the product (M.pt. 70-77.5^{0}) obtained before.

(iii) Stearolic acid: Small amounts of two products melting at 50-53 and 59-72^{0} were obtained which were not identified. No stearolic acid was recovered.

(h) Use of iodinium acetate for the first oxidation step: cyclo-Hexene and acenaphthylene were reacted with equi-equivalent amounts of iodine and silver acetate in dry acetic acid (the normal is one to two equivalents respectively). this means that the pseudo-halogen, CH_3COOI is the reacting entity instead of $[(CH_3COO)_2Ag]I$[24]. A further equivalent of silver acetate was added along with the wet acetic acid before reflux and the procedure followed as usual. Lower yields of less pure products resulted in both cases.

(i) Comparison with Woodward's procedure: Oxidations of acenaphthylene and olive oil following Woodward's technique gave slightly lower yields, though these were of the same order and of comparable purity. Thus, although either procedure could be used with similar results, the shorter reaction times of the modified method and its slightly higher yields make it preferable.

(j) The oxidation of olive oil by this method is probably the best and most convenient method for the preparation of

erythro-9:10-dihydroxystearic acid. No purification of starting material is required and the product, obtained in high yield, is readily purified.

Experimental.

Preparation of starting materials:

(i) General:

Olive oil and castor oil were good quality commercial materials; oleic acid, cyclo-hexene, acenaphthylene, phenanthrene and tetramethylethylene were those available from chemical suppliers and methyl undec-10-enoate was prepared from commercial undecenoic acid by refluxing for two hours in methanol solution containing 1% of concentrated sulphuric acid.

Methyl hexadec-9-enoate was derived from a concentrate of unsaturated C_{16} esters isolated from crocodile fat and consisting mainly of this ester[41].

Methyl linoleate had been prepared previously from 9:10:12:13-tetrabromostearic acid[42].

Methyl ricinstearolate was obtained from ricinstearolic acid which had been prepared from ricinoleic acid by bromination and dehydrobromination.

(ii) Methyl oleate:

Olive oil (205g.) was hydrolysed by refluxing for 0.5 hour with 10% aqueous alcoholic potassium hydroxide (1 litre). The resulting soap solution was concentrated and acidified with 25% sulphuric acid and the mixed acids isolated by extraction into ether solution, the extract being washed with water till neutral and dried (Na_2SO_4) before removal of the solvent.

The mixed acids so produced (194g.) were separated into three fractions containing mainly saturated, monoethenoid and polyethenoid acids respectively by fractional crystallisation

from methanol (1940ml.) at -20° and -50°. These fractions were:-

A - insoluble at -20°; 80g. of iodine value - 66.7,

B - insoluble at -50°; 84g. of iodine value - 92.2,

C - soluble at -50°; 24g. of iodine value - 158.8.

The acids contained in Fraction B (80g.) were esterified by standing overnight in methanol (320ml.) containing 1% of dry HCl. The solution was concentrated by distillation, diluted with water and the esters extracted with ether. The extract was washed with alkali and with water till neutral and the esters recovered (80.5g.).

The esters were then fractionally distilled through an electrically heated and packed column (Towers) under a pressure of 0.7mm. of mercury. Four fractions were collected:-

Fraction	Temperature	Weight	Iodine value
1	142°	8.31g.	62.4
2	150	14.04	88.7
3	152	40.39	88.3
4	152	12.40	86.9
Residue	-	2.00	-

Fractions 2,3 and 4 are pure methyl oleate and were combined. Part of this methyl oleate (35g.) was hydrolysed to give pure oleic acid (33.25g.).

(iii) Oleyl alcohol:

Reduction of oleic acid with lithium aluminium hydride afforded the alcohol[43] thus:-

Pure oleic acid (4.25g.) in dry ether solution (100ml.) was added to lithium aluminium hydride (0.8g.) in dry ether (50ml.) which had been refluxed for 1 hr. to effect solution, just fast enough to keep the mixture under reflux. After a further 0.5 hr. period of reflux the mixture was cooled to 0° and 10% sulphuric acid added to decompose the complex. The alcohol was extracted with ether and the extract was washed with 10% sodium carbonate solution and with water till neutral, and the alcohol recovered in 93.3% yield.

(iv) Elaidic acid:

Oleic acid was isomerised by the method of Swern and Scanlan[44] viz.:-

Pure oleic acid (2g.) was heated with selenium (0,09g.) for 1 hr. at 220 - 225° in an atmosphere of nitrogen. The reaction mixture was then cooled to 50°, dissolved in acetone (150ml.) and allowed to stand for 1 hr., after the addition of activated charcoal (0.6g.), before being filtered. The filtrate was then cooled to 0° for several hours and the precipitated acid filtered off and washed with cold acetone (15ml.). The filter cake was dried and consisted of impure elaidic acid (14.4g., M.pt. 41-44°) Concentration of the acetone mother liquors gave a further amount (1.0g., M.pt. 38-41°). Recrystallisation of the main fraction from acetone (70ml.)

at 0° gave pure elaidic acid (13.1g., M.pt. 43-44°).

Although the efficacy of selenium as a catalyst for isomeration under these conditions has been questioned[45], the reaction proceeded satisfactorily.

(v) Methyl elaidate:

Esterification of elaidic acid with methanol and 1% concentrated sulphuric acid as catalyst gave 100% recovery of methyl elaidate.

(vi) Elaidyl alcohol:

Reduction of elaidic acid by lithium aluminium hydride as already described (p.21) gave the corresponding alcohol in 98.7% yield.

(vii) Methyl ximenynate:

Santalum album seeds (300g.) were crushed and extracted with petroleum (B.pt.60-80°) in a Soxhlet extractor. Removal of the solvent gave 133g. (44.4%) of oil.

This fat was hydrolysed by boiling with aqueous ethanolic potassium hydroxide to give, after acidification, 95g. of mixed acids. Ximenynic acid was separated from the mixed acids by crystallisation from 5 volumes of petroleum (B.pt.40 -60°). Two crops of 38.0g. and 28.5g. were taken giving a total of 66.5g. of ximenynic acid (M.pt. 38-39, Lit. 40-41[40]).

Methyl ximenynate (19.7g., 93.8%) was prepared by room temperature reaction of the acid (20g.) with methanol (200ml.) and concentrated sulphuric acid (2ml.).

(iii) Stearolic acid:

This acid was prepared by bromination, dehydrobromination and hydrolysis of ethyl oleate. The method of Adkins and Burkes[46] proved very unsatisfactory and the following modified method was used:-

Bromine, in slight excess, was added in a thin stream to a vigorously stirred solution of ethyl oleate (100g.) in ethanol (50ml.) kept at -30°. Potassium hydroxide (120g.) and water (180ml.) were added and the mixture refluxed for 10 hrs.. The solution was then poured into water (1 litre), acidified with 5N hydrochloric acid and allowed to stand overnight. The water was decanted from the cake which was extracted with ether, the extracts being washed well with water and the crude product recovered as a red oil (99.0g.). Cooling of a solution of this in petroleum (B.pt.40-60°) resulted in the separation of 2.14g. of product (M.pt.82-90°) which is probably threo-dihydroxystearic acid from hydrolysis of the dibromostearic acid[7(b)]. The bulk of crude product was then recovered and crystallised twice from aqueous ethanol to give stearolic acid (9.81g., 10.9%, M.pt.43-46°; Lit. 46-46.5°).

Oxidation experiments:

(i) General procedure:

The following method applies to the hydroxylation of mono-olefinic esters:-

The olefinic compound (0.01mole), silver acetate (0.022mole) and iodine (0.01mole) in glacial acetic acid (65ml.) are shaken (Microid agitator) for 30 minutes at room temperature. Wet acetic acid (10ml. containing 0.2ml., 0.011mole, of water) is then added and the mixture refluxed for 1 hour. After cooling, the precipitated silver salts are filtered off, washed with a little acetic acid, and the solvent then removed from the combined filtrate and washings by distillation under reduced pressure at 100°. The residue is diluted with water, extracted with ether and the extract washed with concentrated ammonia (to remove any remaining silver salts as silver ammino complexes) and then with water. After removal of the solvent the product is hydrolysed by boiling with excess 3N aqueous alcoholic potassium hydroxide for 1 hour. The soap solution is then diluted and acidified with 2N hydrochloric acid and the crude precipitated product filtered, washed well with water, dried in a vaccuum desiccator (containing concentrated sulphuric acid and potassium hydroxide) and crystallised. Crystallisations were from methanol unless otherwise stated.

In the oxidation of olefinic acids the (partially) acetylated glycol cannot be washed with ammonia therefore, after removal of the acetic acid and addition of water and

ether, the mixture is treated with dilute hydrochloric acid and the precipitated silver salts filtered and washed with ether. The ether solutions are combined, washed with water, the solvent removed and the residue hydrolysed.

With neutral products there is no need to acidify the final hydrolysate; the product usually separates when the diluted solution is cooled.

Table II summarises the work leading to the adoption of the above method as standard.

Table II.

No.	Olefin	Reaction periods(minutes)				Product		M.pt. (Lit.)
		Iodine add[n]	+	Further shaking	Heating	Yield(%)	M.pt.	
1	Oleic acid	0	+	30	180(W.B.)	-	-	
2	Oleic acid	0	+	60	180(W.B.)	-	-	
3	Methyl hexadecenoate	0	+	60	180(W.B.)	10.0	124-125°	129°
4	Methyl hexadecenoate	60	+	60	180(W.B.)	24.4	120-123	129
5	Methyl hexadecenoate	60	+	60	120(reflux)	23.0	122-125	129
6	Methyl oleate	30	+	30	120(reflux)	39.0	125-126	132
7	Methyl oleate	30	+	150	120(reflux)	38.2	128-130	132
8	Methyl hexadecenoate	30	+	overnight	120(reflux)	41.7	124-126	129
9	Olive oil	30	+	30	120(reflux)	82.3	128-132	132
10	Olive oil	30	+	30	120(reflux)	74.2	131-132	132
11	Olive oil	0	+	30	60(reflux)	83.2	131-133	132

These percentages were based on the assumption that olive oil contains 75% of oleic acid.

Notes on Table II:- 1. Amounts of reactants were:- olefin(0.61mole), silver acetate(0.024mole) and iodine(0.02mole) except in No.9 where the proportions were 0.01, 0.036 and 0.03 respectively No.10 was a control experiment for No.9 and Nos. 6,7 and 8 were done on half scale.

2. In Nos. 3 and 4 the inital reaction was carried out in wet acetic acid - not successful.
3. In Nos. 4 and 5 silver nitrate was used instead of silver acetate - the role of silver nitrate in these reactions will be discussed in the Appendix to Part I.
4. Removal of silver salts in Nos. 1-10 was effected by filtration coupled with extraction of the filtrate with ether and washing of the extract with water. In No.11 the extract was washed with concentrated ammonia and then washed with water till neutral.
5. In Nos.1-6 the acidified hydrolysis product was extracted with ether to isolate the crude product. In later experiments the precipitated product was filtered.
6. The crude products were purified by crystallisation from methanol.

(ii) erythro-Dihydroxystearic acid:

(a) Pure methyl oleate (2.96g.) gave 3.14g. of crude product (99%, M.pt. 126-128°) which was recrystallised from methanol (50ml.) at 0° to give pure erythro-dihydroxystearic acid (2.81g., 88.9%, M.pt. 130-132°). Concentration of the mother liquors gave an impure second crop (0.13g., 4.1%, M.pt. 105-110°).

(b) Commercial oleic acid (2.82g.) was oxidised by the standard method, the silver salts, however, being recovered by filtration after treatment with dilute hydrochloric acid. The crude hydroxylated product (3.01g., 95%) when recrystallised, gave impure dihydroxy acid (1.76g., 55.7%, M.pt. 123-127°) accompanied by a little higher melting material (0.14g., M.pt. 145-160°) which may have been a mixture of the two racemates of erythro-9:10-erythro-12:13-tetrahydroxystearic acid (M.pts. 174 and 165°)[39] derived from linoleic acid present in the starting material.

(c) Olive oil (2.96g.) gave 2.74g. (87%) of crude product from which pure erythro-9:10-dihydroxystearic acid (1.98g., M.pt. 131-132°) was obtained. Since olive oil contains ca. 75% of oleic acid, this represents an 83% yield. Some higher melting material again separated from the concentrated mother liquor (0.12g., M.pt. 130-160°)

(d) Olive oil (29.5g.) was oxidised with amounts of reagents increased tenfold. The time of reaction at room temperature was extended to 50 minutes and the reflux time to 2 hours to ensure complete reaction, though these changes were probably

unnecessary. The crude product (29.8g., 94%, M.pt.112-125°), crystallised from methanol (200ml.) at 0°, gave 22.0g. of dihydroxy acid (97% - assuming 75% oleic acid in olive oil, M.pt. 125-132°). This contained a very small amount of material which remained solid to ca. 170°.

A second crop (1.94g., M.pt. 80-120°) was obtained from the mother liquors.

(e) Olive oil (2.95g.) was subjected to the reaction conditions described by Woodward (see p.10), the product, however, being worked up as in the standard procedure to give 3.00g. (95%) of crude hydroxylated material. Crystallisation gave the dihydroxystearic acid (2.17g., 91% - if oil content is 75% oleic acid, M.pt. 126-132°).

(iii) threo-Dihydroxystearic acid:

(a) Methyl elaidate (2.96g.) gave 3.05g. (96.5%, M.pt. 92-93°) of crude hydroxylated material and 2.86g. (80.5%, M.pt. 93.5 - 94.5°) of pure threo-dihydroxystearic acid after one crystallisation.

(b) Elaidic acid (2.0g.) was oxidised by the general procedure, silver salts being removed by treatment with dilute hydrochloric acid and filtration. The crude product (2.00g., 89.3%, M.pt. 92-94°) crystallised from methanol to give pure threo-9-10-dihydroxystearic acid (1.90g., 84.8%, M.pt.94-94.5°).

(iv) erythro-Octadecane-1:9:10-triol:

(a) Pure oleyl alcohol (2.05g.) gave 2.31g. (100%, M.pt. 123-125°) of crude product on dilution of the hydrolysate.

Crystallisation from methanol (30ml.) gave pure erythro-Octadecane-1:9:10-triol (1.87g., 81%, M.pt. 126°).

(b) Commercial oleyl alcohol (2.68g.) gave 2.89g. (95.7%) of crude hydroxylated material. This proved difficult to purify and four recrystallisations from ethyl acetate resulted in 1.49g. of triol (49.3%, M.pt. 122-123°).

(v) threo-Octadecane-1:9:10-triol:

Pure elaidyl alcohol (2.0g.) yielded 2.17g. (94.3%, M.pt. 82-84°) which gave the pure threo-triol after one crystallisation (1.82g., 79.1%, M.pt. 82.5-83.5°).

(vi) erythro-Dihydroxypalmitic acid:

Methyl hexadec-9-enoate (2.68g.) was oxidised to 2.68g. (93.1%) of crude material which was purified to give erythro-dihydroxypalmitic acid (1.78g., 61.8%, M.pt. 126-128°).

(vii) 10:11-Dihydroxyundecanoic acid:

(a) Methyl undec-10-enoate (1.84g.) and undec-10-enoic acid (1.70g.) were not oxidised to any appreciable extent by the standard procedure. The crude products did not solidify and were extracted with chloroform as dark coloured oils (0.30g., 0.28g. respectively). Since they were insoluble in light petroleum they are probably oxy-materials but could not be crystallised.

(b) Methyl undec-10-enoate (1.84g.) was therefore oxidised with two modifications to the standard procedure; shaking at room temperature was continued overnight (ca. 15 hrs.) and refluxing was prolonged for 2.5 hrs. A solid product (0.99g., 48.9%, M.pt. 74-77°) after crystallisation from aqueous

ethanol yielded 10:11-dihydroxyundecanoic acid (0.84g., 41.5%, M.pt. 84-87.5°).

(viii) α- and β-erythro-9:10:12-Trihydroxystearic acids:

(a) Castor oil (3.12g.), oxidised by the normal method gave crude hydroxylated product (3.15g., 95%) which was thoroughly extracted with boiling petroleum (B.pt. 40-60°) and then with boiling chloroform. The residue from this extraction, crystallised from (i) ethanol, (ii) aqueous acetic acid and (iii) ethanol gave β-9:10:12-trihydroxystearic acid (0.19g., 7.0% - if ricinoleic acid content of castor oil is 90%, M.pt. 135-137°).

The chloroform soluble fraction was separated by crystallisation at 0°. Recrystallisation, as above, gave α-9:10:12-trihydroxystearic acid (0.88g., 29.5%, M.pt. 108-111°).

A previous oxidation of castor oil gave 12.2 and 26.0% respectively of impure β- and α-isomers (M.pts. 127-138; 100-108°).

(b) Acetylated castor oil (3g.) was prepared by refluxing castor oil for 4 hrs. in acetic anhydride (3 vols.) and then for a further 2 hrs. after addition of water (2 vols.), the product being extracted, washed with water, alkali solution and finally neutral with water before recovery. Oxidation gave a product (2.64g., 94%) from which the β-isomer (0.19g., 7.6%, M.pt. 135-138°) and α-isomer (0.69g., 27.6%, M.pt. 107-112°) were isolated.

(ix) α- and β-Sativic acids - dierythro-9:10:12:13-tetrahydroxy-stearic acids:

Methyl linoleate (2.0g.) was oxidised by the standard procedure, double quantities of reagents being used. The crude product (2.22g., 94.5%, M.pt. 142-167°), crystallised from 30% acetic acid (40mls.) to remove soluble occluded impurities gave 1.98g. of solid which was extracted with two lots of boiling acetone (200ml. and 100ml.) leaving a residue of 0.38g. (M.pt. 171-173°). Recrystallisation of this material from 50% ethanol (76ml.) gave β-sativic acid (0.32g., 13.6%, M.pt. 173°).

The acetone extracts on concentration and cooling gave 0.45g. of product (M.pt. 154-156°) and a viscous liquid residue (1.09g.) on distillation of the mother liquors. The first crystallisation of the solid from 45% ethanol (100ml.) produced 0.40g. (M.pt. 155°). This material is a eutectoid formed by a mixture of the α- and β-sativic acids[47] but another crystallisation from 50% ethanol (80ml.) gave 0.37g. (M.pt. 155-160°) and a final recrystallisation from the same solvent (74ml.) gave pure α-sativic acid (0.35g., 14.9%, M.pt. 163-165°).

The large amount of liquid product (1.09g.) produced during the acetone extraction of what had been a crystalline solid was unexpected and can only be accounted for by chemical change during the extraction. The most likely explanation seemed to be that condension had occurred between acetone and the glycol groups to give isopropylidene derivatives. This

requires acid conditions and traces of hydrochloric and acetic acids from the previous two operations could give the acidity required. Hydrolysis of the residue by warming with dilute hydrochloric acid caused the mass to go solid and extraction with ether, followed by washing with water and distillation gave a further 0,17g. of the eutectoid of ∝- and β-sativic acids (7.2%, M.pt. 154-155°).

This last low recovery could be due to two reasons; (a) ether is not a good solvent for these polyhydroxy acids, so that although a good recovery was possible from the acid hydrolysate, it was not in fact effected and (b) besides the formation of isopropylidene compounds during the acetone extraction the formation of pyran and furan derivatives may have occurred by intramolecular dehydration. This dehydration is also acid catalysed but in this case acid hydrolysis would not regenerate the glycols and may in fact have converted some of the isopropylidene compounds, via the glycols, into these cyclic products.

This aspect was not pursued but it is clear that minimum contact of the product with acids is essential. Substitution of ether for 30% acetic acid in the first purification step and care in the acidification of the alkaline hydrolysate would probably lead to higher yields.

(x) cis-cyclo*Hexane*-1:2-diol:

(a) cyclo-*Hexene* (1.64g., 0.02mole) gave 0.87g. (37.5%) of crude product which was separated from the hydrolysate by

evaporation to dryness and extraction of the residue with chloroform. Recrystallisation from ethyl acetate gave cis-cyclo-hexanediol (0.51g., 22.0%, M.pt. 94-97°).

(b) cyclo-Hexene (0.82g.) was oxidised with one modification to the standard procedure, reaction at room temperature being prolonged to 4.5 hrs. The acidified hydrolysate on evaporation to dryness and extraction with chloroform gave a crude product (0.76g., 65.9%) which was purified by crystallisation from ethyl acetate (0.48g., 41.4%, M.pt.94-97°).

(c) cyclo-Hexene (0.82g.) was shaken with silver acetate (1.9g.) and iodine (2.54g.) in dry acetic acid (65ml.) for 1 hr. - i.e. CH_3COOI is the reacting entity. Wet acetic acid was added and a further 1.9g. of silver acetate and the mixture refluxed for 1.5 hrs. This procedure gave 0.85g. (73.3%) of crude material which gave impure cis-cyclo-hexanediol (0.35g., 31.9%, M.pt. 82-97°) after one crystallisation.

(xi) cis-Acenaphthene-1:2-diol:

(a) Standard oxidation of acenaphthylene (1.28g.) gave a crude hydroxy product (1.27g., 80.9%) which after boiling with charcoal in acetic acid, filtering and crystallising gave the cis-diol (0.35g., 22.3%, M.pt. 205-208°)

The fact that the initial reaction period did not entirely dissipate the yellow colour of acenaphthylene from the solution showed that insufficient time was

allowed for this step.

(b) Extension of the initial reaction to 2 hrs. gave a colourless solution and 1.66g. (89.9%) of crude was obtainedfrom 1.52g. of acenaphthylene. This was purified as above to give 0.61g. (M.pt. 198-207°), further crystallisation giving cis-acenaphthenediol (0.52g., 28.0%, M.pt. 203-208°). Further purification raised the melting point to 204-207.5° after four recrystallisations. (Found:- C,77.0; H,5.5%. Calculated for $C_{12}H_{10}O_2$:- C,77.4; H,5.4%).

(c) Reaction of acenaphthylene with CH_3COOI (see (x)-(c)), the second equivalent of silver acetate being added before reflux, gave 1.48g. (79.5%) of crude material. Purification by vaccuum sublimation (at 180° and 0.7mm.) followed by crystallisation of the sublimate from acetic acid gave the cis-diol (0.19g., 12.5%, M.pt. 201-205°).

(d) Acenaphthylene (1.52g.) was oxidised under Woodward's conditions (seep.10) to give 1.18g. (63.4%) of crude product which was purified by decolourisation and crystallisation (0.39g., 20.9%, M.pt. 202-207°).

(xii) Attempted oxidation of Methyl ximenynate:

Methyl ximenynate (2.92g.) gave a crude liquid product (2.23g., M.pt. ca.10°) when oxidised. Crystallisation from petroleum (B.pt. 60-80°) gave 0.70g. of solid (M.pt. 22-23°) which was not ximenynic acid (M.pt. 40-41°)[40] but which was not identified. The residue (1.53g.) was liquid.

(xiii) Attempted oxidation of Methyl ricinstearolate:

(a) Methyl ricinstearolate (2.0g.) gave 1.65g. of oxidised material, crystallisation of which from petroleum (B.pt. 60-80°) gave a white solid (0.63g., M.pt. 73-77°). This melting point was raised to 77.5-78° by repeated recrystallisation from a mixture of ether and light petroleum. Analysis figures were obtained (Found:- C,68.35; H,10.74%) which correspond to $C_{18}H_{34}O_{4;3}$ and a yellow dinitrophenylhydrazone*(M.pt. 64-7°) was formed. These indicate the formation of a monoketo-compound, possibly either 12-hydroxy-9-ketostearic acid or 12-hydroxy-10-keto stearic acid. These compounds are unknown so that no comparison is possible.

(b) Methyl ricinstearolate was oxidised with double the normal proportions of reagents to give 0.77g. of crude product which gave 0.14g. (M.pt. 70-77.5°) from ether-petroleum mixture. This gave no depression in melting point when mixed with the product described above.

(xiv) Attempted oxidation of Stearolic acid:

Stearolic acid (2.0g.) yielded a crude product (1.96g., M.pt. 32-40°) which on crystallisation from petroleum (B.pt. 40-60°) gave 0.72g. (M.pt. 45-65°). Further crystallisations from ethyl acetate furnished small amounts of two unidentified components:- 0.04g. (M.pt. 50-53°) and 0.02g. (M.pt. 59-72°).

* Found, C, 60.45; H, 7.95; N, 11.52%; $C_{24}H_{38}N_4O_7$ requires C, 58.29; H, 7.95; N, 11.33%.

(xv) Attempted oxidation of Phenanthrene:

The product (1.62g., M.pt. 91-97°) resulting from the oxidation of phenanthrene (1.78g.) is almost entirely starting material (no depression of mixed melting point) but contained a small amount of crystals which melted above 100°. This compound was not separated.

(xvi) Attempted oxidation of Tetramethylethylene:

(a) Tetramethylethylene (0.84g.) was oxidised in the normal way, silver salts being precipitated by addition of salt and filtration. The alcohol in the final hydrolysate was distilled off and the remaining aqueous solution cooled to 0°. No product could be isolated.

(b) Pure pinacol hydrate (2g.) was subjected to the hydrolysis reaction with 3N aqueous alcoholic potassium hydroxide (40ml.) to test the feasibility of isolating pinacol hydrate from this reaction. The alcohol was distilled and the aqueous solution cooled to give only 1.3g. (65%) of impure pinacol hydrate.

All melting points were determined on a Gallenkamp hot-stage microscope. Stem corrections have not been made.

Appendix to Part I.

Oxidation of Olefins with Silver Nitrate and Iodine.

Introduction.

Birckenbach et al[24,48] first showed that when the silver salts of several inorganic and organic acids were treated with iodine in suitable solvents pseudohalogens were formed along with silver iodide:-

$$AgX + I_2 \longrightarrow IX + AgI$$

Other heavy metal salts, notably mercuric salts, were found to give the same reaction.

These pseudohalogens, being polarised in such a way as to give the iodine atom a small positive charge, are strong electrophilic reagents. For example, mercuric nitrate or silver nitrate and iodine in carbon tetrachloride converted olefins to vicinal iodonitrates[24,48]. In this solvent reaction stopped at this point but in ether some substitution occurred to give 1:2-dinitrates.

Uschakow et al[26a] demonstrated that, with silver salts, chlorine and bromine reacted in the same way to give good yields of chloro- and bromo-esters by addition to olefins.

The occurrence of the secondary substitution reaction giving dinitrates is dependent on the solvent used and is to be expected since the metathetical reaction between silver nitrate and alkyl halides is a well known method of preparing nitrate esters[49]. The low yields of dinitrates obtained by Birckenbach et al are due to the extreme

slowness of substitution of halogen next to a nitrate ester group. It has been stated[50] that the reaction of 2-bromo-3-nitroxybutane with silver nitrate is 90% complete only after 45 days. Kornblum[51] has shown that substitution of halogen with nitrate (and nitrite) occurs with complete inversion, being a normal substitution reaction.

The conversion of a nitrate to the corresponding alcohol is also a widely used reaction[49], especially in the field of sugar chemistry[52] and it can be effected in various ways.

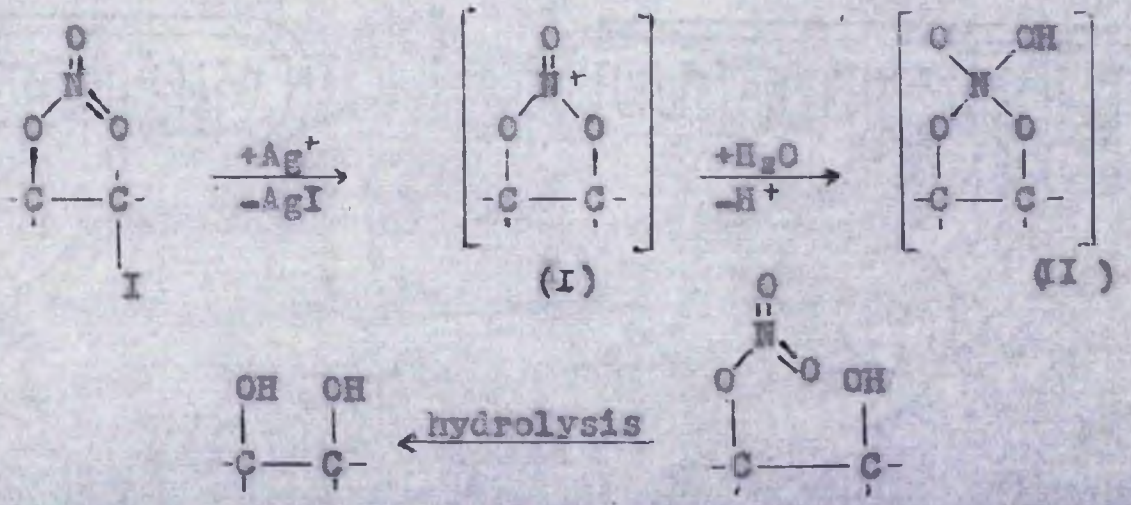
These three sets of reactions; addition, substitution and denitration, do not, however, appear to have been used as a reaction sequence for the conversion of olefins to glycols.

Discussion.

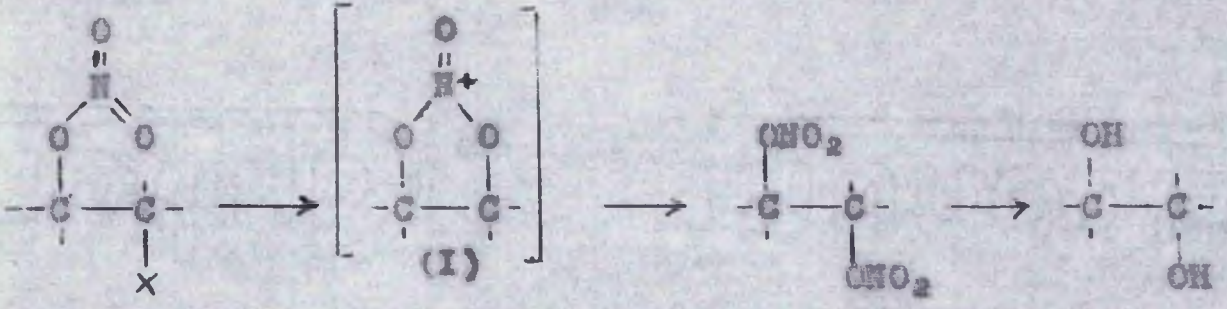
During the preliminary work of adaptation of the Woodward oxidation technique to the hydroxylation of long chain olefins, three experiments were carried out using silver nitrate instead of silver acetate along with iodine in acetic acid (see p.26). Appreciable yields of glycol were obtained after hydrolysis and although the reaction was almost certainly due to iodinium acetate, since the medium was acetic acid, the experiments suggested that silver nitrate might give an analogous reaction to silver acetate if a non-interfering solvent were used.

This was found to be the case and methyl palmitoleate was oxidised in nitromethane, by a similar procedure to the silver acetate/iodine method, to give a low yield (3%) of *erythro*-9:10-dihydroxypalmitic acid.

The formal similarity between the nitrate and acetate ions suggested that the nitrate ester group might exhibit the same sort of neighbouring group participation as the acetoxy group in producing the *cis*-product in the presence of some water[26]:-

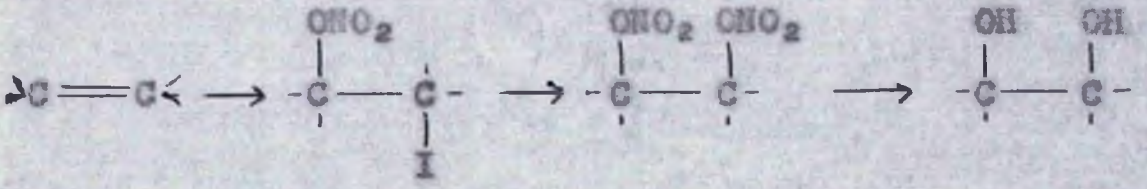


If the two ~~strictly~~ are strictly analogous then in an anhydrous medium the trans-glycol should result, due to protection from "backside" attack given by the cyclic intermediate (I):-



Acetonitrile was chosen as solvent because the high solubility of silver nitrate leads to a homogeneous reaction mixture, the silver iodide precipitating out as formed[53], and two oxidations were carried out. One was kept anhydrous throughout while one equivalent of water was added to the other before reflux. In both cases erythro-9:10-dihydroxystearic acid, the product of cis-addition, was formed from methyl oleate in low yield.

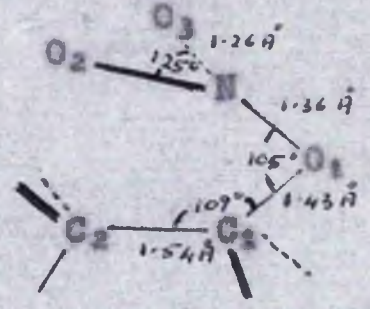
The mechanism of reaction, therefore, cannot be analogous to that of silver acetate as suggested above but must be by trans-addition followed by replacement with inversion:-



A thorough proof that the nitrate ester group fails to exhibit a neighbouring group effect has been given by Fishbein[54]; dl-2:3-dibromobutane with silver nitrate in acetonitrile being shown to give threo-2-bromo-3-nitroxy-

butane and thence meso-2:3-dinitroxybutane, while meso-2:3-dibromobutane gave dl-dinitroxybutane via the erythro-intermediate. The present experiments have therefore confirmed Fishbein's results.

Consideration of the structure of the nitroxy group as determined by electron diffraction studies and Raman spectra for methyl nitrate[55] shows that the minimum distance between the oxygen atoms and the α-carbon atom is far too great to permit interaction to take place. Assuming the nitrate to have the same structure as in methyl nitrate when it attached to a secondary carbon atom, the structure is:-



- where C_2, C_1, O_1 and N are coplanar (in the plane of the paper) and O_1, N, O_2 and O_3 are coplanar at right angles to the C_1-O_1-N axis. By geometry, the distance between O_2 or O_3 and C_2 is 2.66Å which is clearly too great for any possibility of interaction to exist.

x x x

The very low yields of glycol obtained using the standard hydroxylation (p.24) are due to side-reactions predominating in the hydrolysis step. In the hydrolysis

of simple alkyl nitrates with ethanolic potassium hydroxide three reactions occur[49,52,56]:-

$$R.CH_2.O.NO_2 + KOH \xrightarrow{+EtOH} \begin{cases} R.CH_2.O.C_2H_5 + H_2O + KNO_3 & \text{(Ether)} \\ R.CH_2OH + KNO_3 & \text{(Alcohol)} \\ R.CHO + H_2O + KNO_3 & \text{(Carbonyl)} \end{cases}$$

Honeyman and Morgan[52] state that, "When attached to a secondary carbon atom, the nitrate ester may be hydrolysed like a carboxylic ester or like a sulphonate but with additional reaction leading to carbonyl compounds becoming apparent. In compounds having more than one nitrate group, carbonyl compound formation predominates."

Among the methods recommended[49,52] for conversion of nitrate esters to alcohols are high pressure hydrogenation[57], lithium aluminium hydride reduction[58] and reduction with a mixture of zinc and iron powder in acetic acid[59]; only the last mentioned was found to be successful.

Methyl oleate, after a prolonged reaction time (25 days at room temperature and 24 hours reflux) was oxidised by silver nitrate and iodine in anhydrous acetonitrile to the dinitrate; the weight of silver iodide filtered off indicated 99.6% conversion. Reduction of this compound with zinc and iron powder in acetic acid gave methyl-erythro-9:10-dihydroxystearate in 68% yield, hydrolysed to erythro-9:10-dihydroxystearic acid.

Methyl elaidate was similarly oxidised (weight of silver iodide filtered indicated 98.3% conversion to the dinitrate)

to give ca.62% yield of methyl threo-9:10-dihyroxystearate and thence threo-9:10-dihydroxystearic acid.

One further experiment was carried out on methyl oleate with shorter reaction times (89.4% conversion to dinitrate indicated). Reduction was effected with zinc powder and acetic acid and a modified procedure for isolation of the product gave, after recrystallisation, 51.2% of impure erythro-dihydroxystearate. The mother liquors from the crystallisation gave, after hydrolysis, a further 15.8% of impure dihydroxy-acid.

x x x

This method then is yet another capable of cis-hydroxylating double bonds and although the yields are not yet comparable to the standard methods of cis-hydroxylation they are quite high and could doubtless be improved by further modifications of the procedure.

This procedure would be expected to give the same cis-isomer as Woodward's method in cases like those described on p.11 since the mechanism is similar.

Of possible interest is the fact that, if the starting material is an olefinic ester, the dihydroxy ester is obtained directly and this may be of value in some cases.

The main disadvantage of the method is in the long reaction times required but it has the advantages over the Woodward method of milder conditions and no alkaline hydrolysis step.

Experimental.

1. Oxidation of methyl hexadec-9-enoate in nitromethane:

Methyl palmitoleate (2.68g.) was shaken in anhydrous nitromethane (65ml.) with silver nitrate (4.0g.) and iodine (2.54g.) for 3.5 hours, wet nitromethane (10ml., containing 0.2ml. of water) added and the mixture refluxed for 1.5 hours. The standard procedure (see p.24) of hydrolysis and isolation gave 2.60g. of crude product which was partially solid. Extraction with petroleum (B.pt. 40-60°) several times removed 1.90g. of soluble material and the residue crystallised from aqueous ethanol to give erythro-9:10-dihydroxypalmitate (0.08g., 2.8%, M.pt.118-22°) whose melting point was raised (119-27°) in admixture with an authentic sample.

2. Oxidation of methyl oleate in dry and wet acetonitrile:

(a). Pure methyl oleate (1.48g.) was dissolved in anhydrous acetonitrile (35ml.) and treated with silver nitrate (1.87g.) and iodine (1.27g.) and the mixture shaken for 5 days. The mixture was not refluxed but after filtration and removal of the solvent, was hydrolysed with ethanolic potassium hydroxide (15ml. of 10%) for 2 hours and the crude product extracted after dilution and acidification of the soap. This orange oil (1.44g.) was partitioned between 80% methanol and petroleum (see p.84) and the methanol extract (0.64g.) crystallised from ethyl acetate to give impure erythro-9:10-dihydroxystearic acid

(0.05g., 3.2%) whose melting point (120-5°) was raised to 124-8° in admixture with an authentic sample.

(b). Methyl oleate (1.48g.) was similarly oxidised in acetonitrile containing water (0.1ml.), the crude product (1.51g.) being partitioned as above and the methanol extract (0.86g.) crystallised from ethyl acetate to give impure erythro-9:10-dihydroxystearic acid (0.06g., 3.8%, M.pt.123-128°, mixed M.pt. 124-8°).

3. Oxidation of methyl oleate and methyl elaidate:

(a). Pure methyl oleate (1.48g., 5mM) was shaken with silver nitrate (1.87g., 11mM) and iodine (1.27g., 5mM) in anhydrous acetonitrile (35ml.) for 10 days at room temperature. A further portion (1.87g.) of silver nitrate was then added and shaking continued for a further 15 days and the mixture refluxed for 24 hours.

The silver iodide was filtered and washed (2.34g.; 2.35g. is theoretical weight for 100% conversion to dinitrate, thus conversion = 99.6%) and the solvent removed from the filtrate under reduced pressure.

The residue was dissolved in acetic acid (10ml.) and treated with excess of a mixture of zinc and iron powder. A violent reaction ensued which was modified by cooling, and gentle heating was then continued until a spot test with brucine-concentrated H_2SO_4 reagent gave no red colouration[61], indicating that all nitrate had been reduced. The mixture was then diluted with water and extracted several times with ether, the extract being washed with

water, potassium hydroxide solution and again with water till neutral. The product was recovered from the dried extract (1.35g., 82.3%, M.pt 90-100°). Recrystallisation from aqueous methanol gave pure methyl erythro-9:10-dihydroxystearate (1.10g.,67.7%, M.pt.101-2°; Lit. 103°[61]).

Hydrolysis of a portion of this ester (200mg.) with methanolic potassium hydroxide gave the acid (170mg., M.pt. 126-9°) which was recrystallised from methanol to give pure erythro-9:10-dihydroxystearic acid (145mg., M.pt.130-132°; mixed M.pt. 131-2°).

(b). Pure methyl elaidate (1.48g.) was oxidised in the same way to impure methyl threo-9:10-dihydroxystearate (1.03g., M.pt.40-58°), the weight of filtered silver iodide (2.31g.) indicating 98.3% conversion to the dinitrate. The crude product was crystallised from aqueous methanol to give two crops:- 510mg.(31.1%, M.pt.52-60°) and 228mg. (14.0%, M.pt.45-50°) The first crop recrystallised from ethyl acetate to give 163mg.(M.pt.60-60.5°).

The residue from this last crystallisation was hydrolysed to give, without purification, pure threo-dihydroxystearic acid (292mg., M.pt. and mixed M.pt. 92-4°).

The low melting point of the ester product (Lit.[61] 70°) is probably due to partial acetylation, this reaction unlike the previous one having been heated gently overnight with acetic acid and the metal powders and heating for the minimum time necessary for this step would probably prevent this acetylation.

4. Oxidation of methyl oleate with shortened reaction times:

Methyl oleate (1.48g.), silver nitrate (3.74g., i.e. 100% excess) and iodine (1.27g.) were shaken together in anhydrous acetonitrile (35ml.) for 24 hours and refluxed for a further 24 hours. The silver iodide was filtered (2.10g., or 89.4% conversion to dinitrate) and the solvent removed from the filtrate under reduced pressure.

The residue was dissolved in acetic acid (10ml), excess of zinc powder only added and the mixture gently heated until the brucine/H_2SO_4 spot test was negative (ca. 4hr.). The resultant solution was decanted from the solids which were washed with chloroform and the resulting solution evaporated to dryness under reduced pressure. The residue was dissolved in chloroform, dried, filtered and the solvent removed to yield 1.65g.(100%) of crude solid material which crystallised from aqueous methanol to give impure methyl erythro-dihydroxystearate (0.84g., 51.2%, M.pt.95-100°)

The residue from this crystallisation yielded impure erythro-dihydroxy acid (0.25g., 15.8%, M.pt.120-28°), on hydrolysis.

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Part II.

Degradative Studies.

Part IIA.

Introduction: Epoxy and Hydroxy Acids Occurring in Seed Oils.

Epoxy Acids.

12:13-epoxyoctadec-9-enoic acid (Vernolic acid) forms 74% of the component acids in the seed oil of Vernonia anthelmintica. It was first observed by Vidyarthi and Mallya[1], who believed it to be an isomer of ricinoleic acid, 11-hydroxyoctadec-9-enoic acid. Gunstone[2], however, showed that it was an epoxy acid acid of the assigned structure, this being the first report of the natural occurrence of an epoxy acid. Raman[3] later confirmed Gunstone's work.

The same epoxyoleic acid was later shown by Bharucha and Gunstone[4] to be the main component acid (66%) in the seed fat of Cephalcroton cordofanus, which had been reported by Henry and Grindley[5] to contain ricinoleic acid. Other scources of this acid are okra oil (Hibiscus esculentus) in 3 - 4% and Vernonia colorata seed oil, in high proportion[6].

The present work has shown the presence of a small amount (ca.1%) of 15:16-epoxylinoleic acid in the seed oil of Camelina sativa.

These are the only known instances of the occurrence of epoxy acids in nature but in view of the fact that the same epoxyoleic acid has been isolated from species of three different families (Compositae, Euphorbiaceae and Malvaceae) and the epoxylinoleic acid from a species of another family (Cruciferae) it is possible that epoxy acids are more widely distributed than is known at present.

Hydroxy Acids.

With few exceptions long chain hydroxy acids are not major component acids of vegetable seed fats although they occur frequently and sometimes in significant amounts in various waxes, in brain lipids and in cork.

All the acids here described, except the kamlolenic acids have one or more asymmetric centres and are thus capable of existing in an optically form. Several of them have been shown to exhibit optical rotation, although the values obtained are generally very small, and it is probable that all of these asymmetric acids are present in nature in an optically active form.

Ricinoleic acid (12-hydroxyoctdec-9-enoic acid) is the most important of the hydroxy acids and occurs mainly in the seed fats of the Ricinus species. It comprises 90% or more of the mixed acids of castor oil (Ricinus communis) from which it was first isolated by Saalmuller[7]. The assigned structure was first proposed by Goldsobel[8] and confirmed by Walden[9] in the same year and by various workers since then[10].

Ivory wood oil (Agonandra brasiliensis)[11] is reported to contain ricinoleic acid (47%) and it was also stated to be present in Argemone oil (Argemone mexicana)[12], though recent work has shown that this is not the case[13]. According to Margaillan[14] the oil of Wrightia annamensis contains as its chief component a hydroxy monoethenoid acid, probably ricinoleic acid.

9-Hydroxyoctadec-12-enoic acid, an isomer of ricinoleic acid has been shown by Gunstone[15] to occur in the seed fats of four of the Strophanthus species and during the present work has been shown to be present in a further ten species. It comprises between 6 and 16% of the component acids of these fats, though in most cases approximates to 9 or 10%.

Kamlolenic acid (18-hydroxyoctadec-9:11:13-trienic acid). Gupta, Sharma and Aggarwal[16] isolated a solid unsaturated acid (M.pt.77-8°) from the seed fat of Mallotus philippinensis which gave, on irradiation with ultra-violet light in the presence of iodine, an isomeric acid (M.pt. 88-9°). The two acids were named α- and β-kamlolenic acids. Calderwood and Gunstone[17] proved that it was 18-hydroxy-octadec-9:11:13-trienoic acid and Ahlers and Gunstone[18], from infra-red measurements, showed α-kamlolenic acid to have one cis- and two trans-double bonds (probably cis-9, trans-11, trans-13) whereas the β-acid was the all-trans isomer. These findings have been confirmed by von Mikusch[19], by Crombie and Tayler[20] and by the Indian workers[16]. Recently kamlolenic acid has been isolated from Mallotus japonicus seed oil[21].

Isanolic acid (8-hydroxyoctadec-17-en-9:11-diynoic acid) is a major constituent (44%) of boleka or isano oil, the seed fat of Onguekoa Gore. Riley[22] has established the position of the hydroxyl group at C_8 and from the similarity of its ultra-violet spectrum to that of isanic acid, Kaufmann and his co-workers[23] consider it to have the

above structure. It has recently been suggested[24], however, that the structure is 8-hydroxyoctadec-14-en-10:12-diynoic acid.

8-Hydroxyoctadec-11-en-9-ynoic acid. This acid, related to ximenynic acid (octadec-11-en-9-ynoic acid), is present (3-4%) in the seed fat of Ximenia caffra.[25]

erythro-9:10-Dihydroxystearic acid. An optically active dihydroxystearic acid (M.pt.141°) occurs in minor amounts (0.5-1.0%) in the mixed acids of castor oil[26]. The hydroxy groups were shown by Toyama and Ishikawa[27] to be on C_9 and C_{10} and this was confirmed by King[28] who further proved the acid to be one of the optically active forms of the racemic-9:10-dihydroxystearic acid of M.pt.132°.

The same acid has been isolated in the present work from the seed oils of Strophanthus verrucosus and Strophanthus sarmentosus and may be present throughout the genus.

Hirai and Toyama[29] have obtained a small amount of a 9:10-dihydroxystearic acid from the acids of Lycopodium oil.

Impurolic acid (3:11-dihydroxymyristic acid). Power and Rogerson[30] isolated a small amount of a dihydroxytetradecanoic acid from the seed fat of Ipomea purpurea (South African morning glory) and named it impurolic acid. The same acid was later isolated[31] from the seed oil of Pharbitis nil Choisy (Japanese morning glory). From oxidative studies Asahina and Shimidzu[32] proved the acid

to be 3:11-dihydroxymyristic acid and this work was confirmed by Asahina and Wakanishi[33].

9:14-Dihydroxyoctadec-10:12-dienoic acid was isolated in very small amounts from tung oil[34] but its structure suggests that it is probably a product of autoxidation rather than a true component of the oil.

Part IIB.

The Epoxy Acid in Camaline Oil.

Introduction.

Camelina oil is obtained from the seeds of Camelina sativa (L.),Fr., (or Myagrum sativum, Crantz.) belonging to the family Cruciferae. The oil is otherwise known as Dodder oil, German sesame oil, Leindotterol and Dullol.

The plant was cultivated extensively over Europe several centuries ago and at present is harvested in some parts of south Germany, Holland, Belgium, Hungary, the Balkan States and south Russia and is widely dispersed over Europe as a weed.

The seeds are about 1.0mm. long by 0.5mm. diameter and weigh, on average, about 0.73mg. They have a bitter taste and yield 30-40% of a golden yellow oil. The oil is used in making soft soap, paint and artist's oil colours and the cold drawn oil is sometimes used for edible purposes. The seed cake is used, along with other seed cakes, as cattle feed and at one time exported from Odessa to Liverpool for this purpose.

The first analysis of this oil was made by de Negri and Fabris[35] who stated that the glycerides were compounded from linoleic, oleic and palmitic acids and, in smaller amount, erucic acid. The characteristics of the oil quoted by these authors were:- iodine value, 135; saponification equivalent, 188; specific gravity, 0.9228 to 0.9329 and melting point of mixed acids 18-20°.

Boekenoogen[36] however reported an iodine value of 150

and Heller[37] stated that the oil content of cameline seeds and the iodine value of the extracted oil depended greatly on the habitat of the plants, Russian oils having iodine values as high as 154.

Holmberg and Bellman[38] are the last of a long line of investigators who report erucic acid as a component of the oil, though they consider it to be present to only 3.2%. They determined the component acids of a Swedish grown oil to be:- palmitic - 5.2, stearic - 1.8, arachidic - 1.2, behenic - 0.6; hexadecenoic - 2.4, oleic - 23.9; linoleic - 14.5, linolenic - 33.4, eicosenoic - 13.8; and erucic - 3.2%.

At the same time, von Mikusch[39] published the results of an analysis of a German produced oil and stated that little if any erucic acid was present in the mixed acids. Otherwise his results tally closely with those of the Swedish workers, being:- saturated acids - 8.9; mono-unsaturated acids, 42.8; linoleic acid, 12.1 and linolenic acid, 36.2%. He showed conclusively[40] that eicosenoic acid was one of the component acids and pointed out that the oil was exceptional with regard to the observation made by Hilditch[41] that all Cruciferae seed oils previously investigated, with the exception of Hesperis matronalis, contain 40-50% of erucic acid.

One other feature reported by von Mikusch was the occurrence of 1% of hydroxy acid. The presence of this

acid was shown by a positive hydroxyl value given by the oil and its mixed acids. By the A.O.C.S. method, Cd4-40[42] values of 14.7 and 15.8 respectively were obtained. The presence of an oxy-acid was also shown by the difference between the saponification value of the oil (177.8) and the acid value of the mixed acids (195.7). This is characteristic of ricinoleic acid prepared in the same way and results from the formation of lactones by heating with mineral acids. By distillation, careful neutralisation of the distillate and extraction of the soap he isolated as lactones, with iodine value 94.8 and acid value 5.8, about 10% of the soap. He considered these to be from a mono-unsaturated, monohydroxy acid similar to ricinoleic acid.

Discussion.

I. Isolation of the hydroxy acid.

The hydroxy acid of camaline oil was isolated from the mixed acids by the following series of operations:-

Figure I.

%DiOH acid	%Wt.			%Wt.	%DiOH acid
		Acetylated mixed acids (47lg.)			
		↓ Urea complex form.	→	Acids from urea	79.7
	20.3	Mother liquors			
		↓ Partition	→	Petroleum extract	17.6
	2.7	MeOH extract			
		↓ Hydrolysis	→	Non-sap.	0.4
55	2.3	OH acid concentrate			
		↓ Partition	→	Petroleum extract	0.4
	1.9	MeOH extract			
		↓ Chromatography	→	Fraction A	0.34 0
				Fraction B	0.38 24
				Fraction D	0.33 94
64	0.85	Fraction C			
		↓ Chromatography	→	Fraction C_1	0.48 48
				Fraction C_4	0.03 -
74	0.17	Fraction C_2			
76	0.17	Fraction C_3			
	0.34%				99.66%

Notes on Figure I:-

(a). The fact that urea forms inclusion complexes with compounds of suitable structure has been used extensively

in fatty acid chemistry[43] for the separation of more saturated acids, which form complexes, from less saturated acids, which do not. The presence of a side chain in the molecule is likely to prevent urea complex formation and the hydroxy acids in the mixed acids were acetylated before crystallisation with urea to produce a bulky side chain and thus bring about their concentration in the mother liquors. This preliminary concentration was carried out to reduce the amount of material to be partitioned and thus to reduce to reasonable amounts the volumes of solvents required for the partition.

(b). Ultra-violet absorption before and after isomerisation:

Indication of the presence of two double bonds in the acid from hydrogenation experiments (see (c) below) suggested the determination of ultra-violet absorption before and after isomerisation at 234 and 268mμ corresponding to conjugated diene and triene respectively[44]. The values obtained are shown below (values at 268mμ are negligible):-

Fraction	%DiOH acid	$E^{1\%}_{1cm}$ at 234mμ		
		Before	After	Difference
"OH acid concentrate"	55	139	473	334
B	24	94	247	153
C_1	48	93	368	275
C_2	74	62	489	427
C_3	76	53	482	429
D	94	35	121	86

Fractions C_2 and C_3, from glycol values and $E^{1\%}_{1cm}$ values are considered to be nearly identical

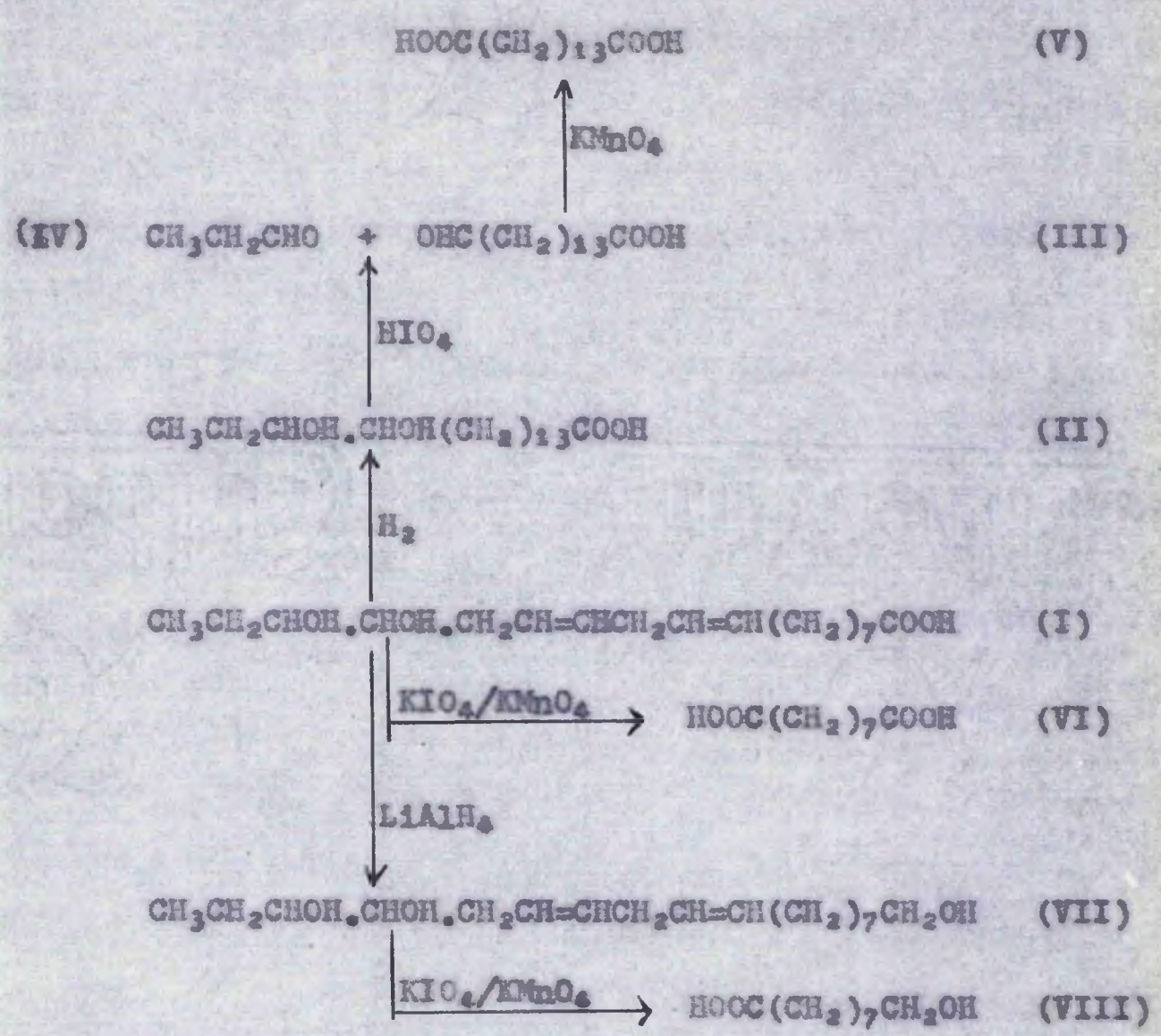
(c). Hydrogenation experiments were carried out on the hydroxy acid concentrate and on Fraction C_2 and gave the same product (15:16-dihydroxystearic acid) in yields proportional to the glycol value. Fraction D, however, on hydrogenation gave a sticky product which could not be crystallised. This result and the low ultra-violet absorption after isomerisation showed that Fraction D was a different acid from the dihydroxy acid in Fractions B, C_1, C_2 and C_3 whose $E^{1\%}_{1cm}$ 'differences' are proportional to their glycol values (see also Figure III, p.80) and it is considered that Fraction D is polymeric material possibly derived from the dihydroxy acid (see also p.78).

(d). Fraction A was completely insoluble in petroleum indicating that it is a hydroxy acid and since it was eluted from the chromatographic column in advance of the dihydroxy acid it is probably a monohydroxy, possibly the monohydroxyoctadecenoic acid described by von Mikusch[39]. The non-glycol portion of Fraction B is probably the same acid. The structure of this monohydroxy acid was not determined.

II. Constitution of the hydroxy acid.

1. General:

The structure of the hydroxy acid was determined by hydrogenation and oxidation studies, the reactions involved being summarised below:-



An authentic specimen of pentadecadioic acid (V) was prepared by Arndt-Eistert bishomologation of tridecadioic acid, obtained by permanganate oxidation of erucic acid isolated from rape oil and a specimen of 9-hydroxynonanoic acid (VIII) by periodate-permanganate oxidation of oleyl alcohol.

2. Hydrogenation:

When the "hydroxy acid concentrate" (see Figure I) was hydrogenated, hydrogen equivalent to two double bonds was taken up and a crystalline solid (42%) was obtained

which was purified to a melting point of 96-7°, which was depressed on admixture with threo-9:10-dihydroxystearic acid (M.pt. 94-4.5°) and with threo-12:13-dihydroxystearic acid (M.pt. 96-7.5°).

Hydrogenation of Fraction C_2 (see Figure I) again resulted in an uptake of hydrogen equivalent to two double bonds and furnished the same saturated acid (58%).

The acid value (320) and the saponification equivalent (312, 319) were determined and, being equal, show the absence of a lactone grouping. The average value (316) is the same as the molecular weight of a dihydroxystearic acid.

The methyl ester (M.pt. 89.5-90.5°) and the ethyl ester (M.pt. 74-74.5°) were prepared and analysed satisfactorily for derivatives of a dihydroxystearic acid.

It is noteworthy that the lowering of melting point on going from acid to methyl ester (6.5°) and from acid to ethyl ester (22.5°) are much less than the corresponding differences between threo-9:10-dihydroxystearic acid and its esters (24 and 35°, respectively)and between threo-12:13-dihydroxystearic acid and its esters (27 and 33°, respectively). This is presumably due to a different crystal structure dependent on the position of the hydroxyl groups in the chain.

3. Position of hydroxyl groups:

The pure saturated acid gave a glycol value[45] of 96.5% showing that the hydroxyl groups are on adjacent carbon

atoms. Steam distillation of the solution resulting from periodate cleavage gave propionaldehyde (IV) which was characterised as its dinitrophenylhydrazone by melting point and mixed melting point determinations and by comparison of its paper chromatographic R_F values with those of the dinitrophenylhydrazones of other short chain aldehydes.

The residual solution from the steam distillation, on cooling, precipitated 14-formyltetradecanoic acid (III) which was oxidised with dilute permanganate and characterised as pentadecadioic acid (V) by mixed melting point determination with an authentic sample of that dibasic acid.

The isolation of these two cleavage fragments shows (a) that the hydroxyl groups are on the 15 and 16 positions and (b) that there is no branching in the chain, since all 18 carbon atoms are accounted for in straight chain products. The saturated acid is thus shown to be 15:16-dihydroxystearic acid probably of the threo- configuration by comparison of its melting point with those of the 6:7- through 12:13-dihydroxystearic acids.[46]

4. Double bonds:

The hydrogenation studies indicated the presence of two double bonds in the molecule and the ultra-violet absorption values at 234mμ after isomerisation (7.5% KOH/ ethylene glycol; 180°/60mins.) shows that at least two

double bonds are present and these are "skipped", i.e. the grouping $-CH=CH.CH_2.CH=CH-$ occurs in the chain.

5. Oxidation of the acid:

Periodate-permanganate oxidation of a portion of Fraction C_2 (see Figure I) by the method of von Rudloff[47] gave azelaic acid (VI) as the dibasic acid fragment, the monobasic fragment being too volatile to be isolated. This indicates the presence of the grouping $>C.(CH_2)_7.C<$ in the molecule so that there can be only two possible structures:-

(A) $CH_3CH_2CHOH.CHOH.CH_2CH=CHCH_2CH=CH(CH_2)_7COOH$

and (B) $CH_3CH_2CHOH.CHOH.CH_2CH=CH(CH_2)_7CH=CHCH_2COOH$

Although the latter acid (B) would be isomerised by heating with potassium hydroxide/ethylene glycol reagent to give an α-ethylenic acid, this product would give an ultra-violet absorption peak in the region 208-210mμ instead of at the observed wavelength of 234mμ[44]. It was therefore considered that (A) was the more likely structure.

The decision between the two structures can be made by "labelling" the carboxyl end of the acid before oxidative cleavage. If the resulting dibasic fragment retains the "label" then structure (A) represents the true constitution of the acid. If free azelaic acid is again produced the structure corresponds to (B). "Labelling" is generally effected in one of two ways; (a) by forming the ester and oxidising under non-hydrolytic conditions[48] and (b) by chain extension by the Arndt-Eistert synthesis[49].

The mild nature of the periodate-permanganate method,

where permanganate is present only in catalytic amounts, suggested that if the carboxyl group were "labelled" by reduction to a primary alcohol group this alcohol group would remain intact. Trial experiments using this method to oxidise oleyl alcohol were successful, giving high yields of 9-hydroxynonanoic acid and nonanoic acid and only trace amounts of azelaic acid showing that the extent of reoxidation of the primary alcohol group was negligible. This method was therefore used to differentiate between structures (A) and (B).

6. Oxidation of the alcohol:

The alcohol (VII) was prepared by lithium aluminium hydride reduction of a portion of Fraction C_3 (see Figure I). Oxidation of this alcohol with periodate-permanganate gave 9-hydroxynonanoic acid (VIII), characterised by its melting point and mixed melting point with an authentic sample and by the melting point and mixed melting point of its p-bromophenacyl ester.

The structure of the hydroxy acid is therefore 15:16-dihydroxyoctadec-9:12-dienic acid with the hydroxyl groups probably in the threo-configuration.

7. Configuration of the double bonds:

Trans-double bonds give a characteristic infra-red absorption peak in the region of $10\mu^{50}$ and the intensity of this peak has been used as a quantitative measure of the extent of trans-unsaturation.

The infra-red spectrum of a film of Fraction C_1 (see Figure I) was obtained and showed no peaks in this region. The double bonds in the hydroxy acid must therefore have the cis-configuration.

There is confirmatory evidence for this in the fact that Fractions C_2 and C_3, having 75% of the acid, did not crystallise even at 0°. Linelaidic acid has a melting point of $28\text{-}9^\circ$ (Ref. 41,p.528) and a dihydroxylinelaidic acid would be expected to have a fairly high melting point.

The structure of the acid has thus been shown to be threo-15:16-dihydroxyoctadec-cis-9-cis-12-dienoic acid, the only doubt being in the configuration assigned to the hydroxyl groups.

8. Possibility of the acid being an artefact:

The dihydroxy acid comprises only ca.1% of the mixed acids of camoline oil and it was possible that it might be merely an artefact of oxidation.

It is considered not to be an artefact because (a) it shows optical activity and (b) the configuration of the hydroxy groups is threo.

(a). Both the 15:16-dihydroxystearic acid obtained after hydrogenation and Fraction C_2 of the unsaturated acid showed optical activity, their specific rotations in ethanol solution being $+3.3^\circ$ and $+6.1^\circ$ respectively. Autoxidation cannot produce optical asymmetry where this was originally absent and linolenic acid would be the

precursor of this compound.

(b). Gold[51] has shown that autoxidation of cis-olefins with gaseous oxygen gives rise to cis- and trans-epoxides which in turn give threo- and erythro-glycols, while trans-olefins give rise to trans-epoxides and erythro-glycols only. If these generalisations are true for the less drastic oxidation of oils occurring during storage, then the production of a threo-glycol unmixed with an erythro-glycol, which would be the more readily isolated product, cannot occur and autoxidation cannot have produced this acid.

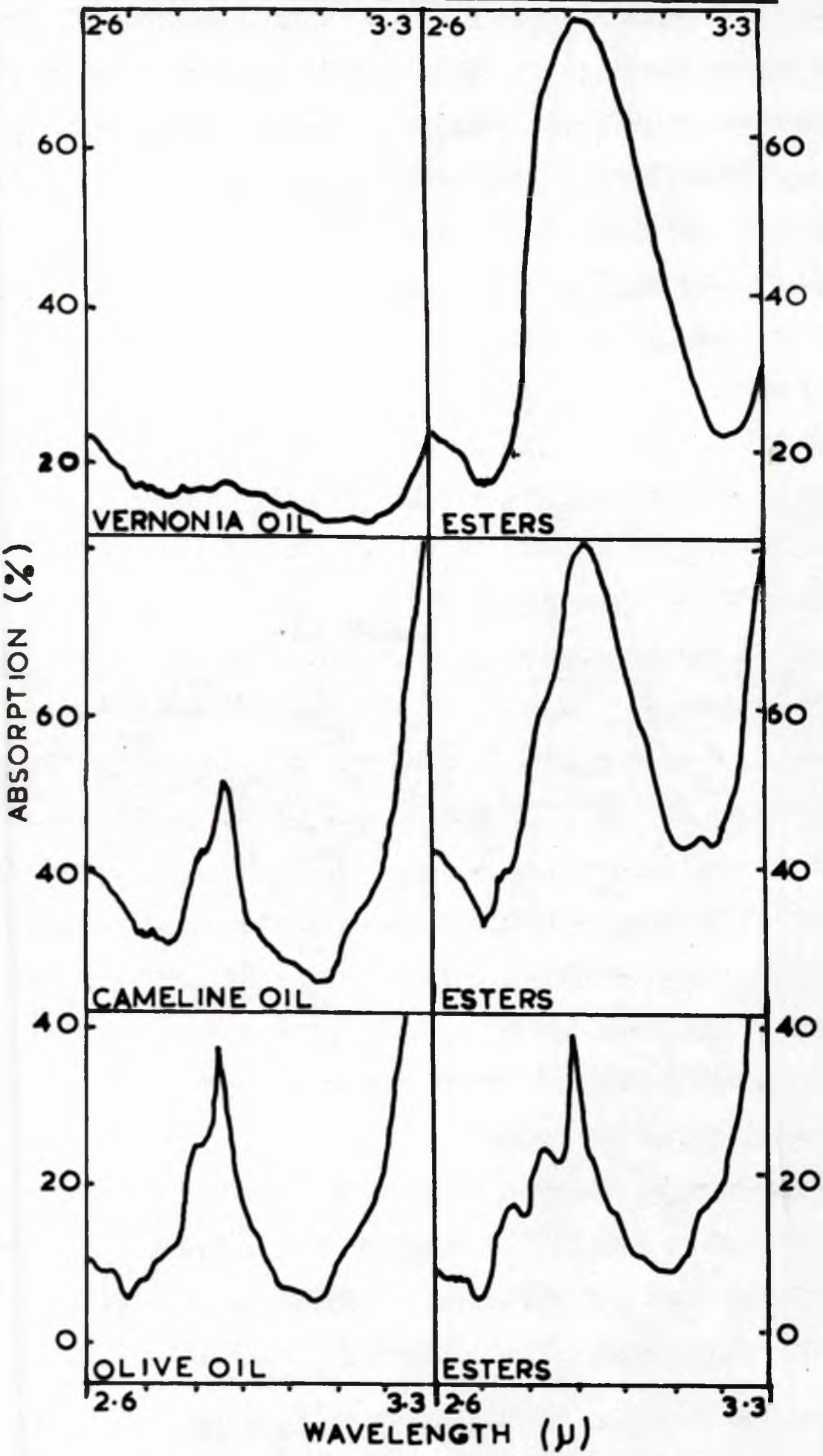
III. Constitution of the acid in the oil:

The acid isolated from the oil as threo-15:16-dihydroxy-linoleic acid may have been derived from 15:16-epoxylinoleic acid, by ring opening with inversion during the isolation, in the same way that 12:13-epoxyoleic acid from a number of sources (see p. 57) is isolated as the glycol, with the threo configuration.

Since the acid comprises only about 1% of the mixed acids the usual methods of epoxide determination[52] are likely to prove inconclusive. It is known that epoxides give rise to infra-red absorption bands in the regions of 8, 11 and 12μ[53] although this work has shown that back-ground absorption by seed oils in these regions is so high as to swamp any small peaks; vernonia oil, having 72% of epoxy acid showed only small bands at 11.82 and 12.15μ

Figure II.

EPOXIDE DETERMINATION BY I.-R. SPECTRA.



because of this. However, free hydroxyl groups give rise to a strong band in the 2.8μ region attributed to the O-H stretching vibration[54a] and if it can be shown that an oil, having little or no absorption in this region, when treated with acid and with alkali, and then esterified gives increased absorption at this wavelength then it is probable that an epoxy group was originally present in the oil.

Camaline oil was investigated in this way and the spectra of vernonia oil and olive oil and their esters also measured as controls. The oils were first neutralised and the spectra of the oils and esters, in the region 2.6 - 3.3μ determined for films of "neat" material are shown on Figure II (opposite). Film thicknesses were 0.1mm. except for vernonia oil and esters where the thicknesses were unknown but similar. Olive oil and esters show small peaks of the same intensity in the 2.9μ region demonstrating (a) that the oil, which was old, contained an appreciable amount of hydroxy artefacts from autoxidation and (b) that the acetolysis, hydrolysis and esterification procedures do not produce additional hydroxyl absorption. Vernonia oil has very small peaks in this region but the esters show a very strong peak at 2.9μ.

Camaline oil shows a small band at 2,89μ and a much higher band is shown by the esters at 2.93μ. It is concluded, therefore, that the dihydroxy acid isolated is present in the oil as cis-epoxy-15:16-octadec-9:12-dienoic acid.

Note:- The position of the bands at 2.9μ instead of at the

free O-H stretching frequency of 2.8μ is due to hydrogen bonding of the hydroxy groups[54b].

IV. Constitution of Fraction D (see Figure I):

Fraction D has a glycol of 94% (calculated as dihydroxy-octadecenoic acid) indicating the presence of vicinal dihydroxy groups. The $E^{1\%}_{1cm}$ value at 234mμ after isomerisation is 87 and this demonstrates the absence of a "skipped" diene system.

Hydrogenation led to an uptake of hydrogen equivalent to rather more than one double bond but the product was a tacky mass which could not be crystallised.

Periodate-permanganate oxidation gave azelaic acid (44%) in one experiment. In another experiment there resulted a dibasic acid (104%, calculated as azelaic acid) which had an equivalent weight of 169 and which furnished the di-p-bromophenacylester of azelaic acid (31%).

The fraction had a specific optical rotation in ethanol of $+2.5^{0}$ and it possible that it is an artefact of the 15:16-dihydroxylinoleic acid produced by polymerisation between double bonds or an oxidation artefact of 15•16-dihydroxylinoleic acid or linoleic acid containing more than one glycol group or, most likely, a mixture of these polymers with other artefacts. The high glycol value is misleading since a high proportion of the acids in this fraction may have more than one glycol group per 18 carbon atoms.

V. Conclusions:

(a). cis-15:16-epoxyoctadec-cis-9-cis-12-dienoic acid is present in the glycerides of Camelina sativa seed oil to about 1% and it is not a product of autoxidation

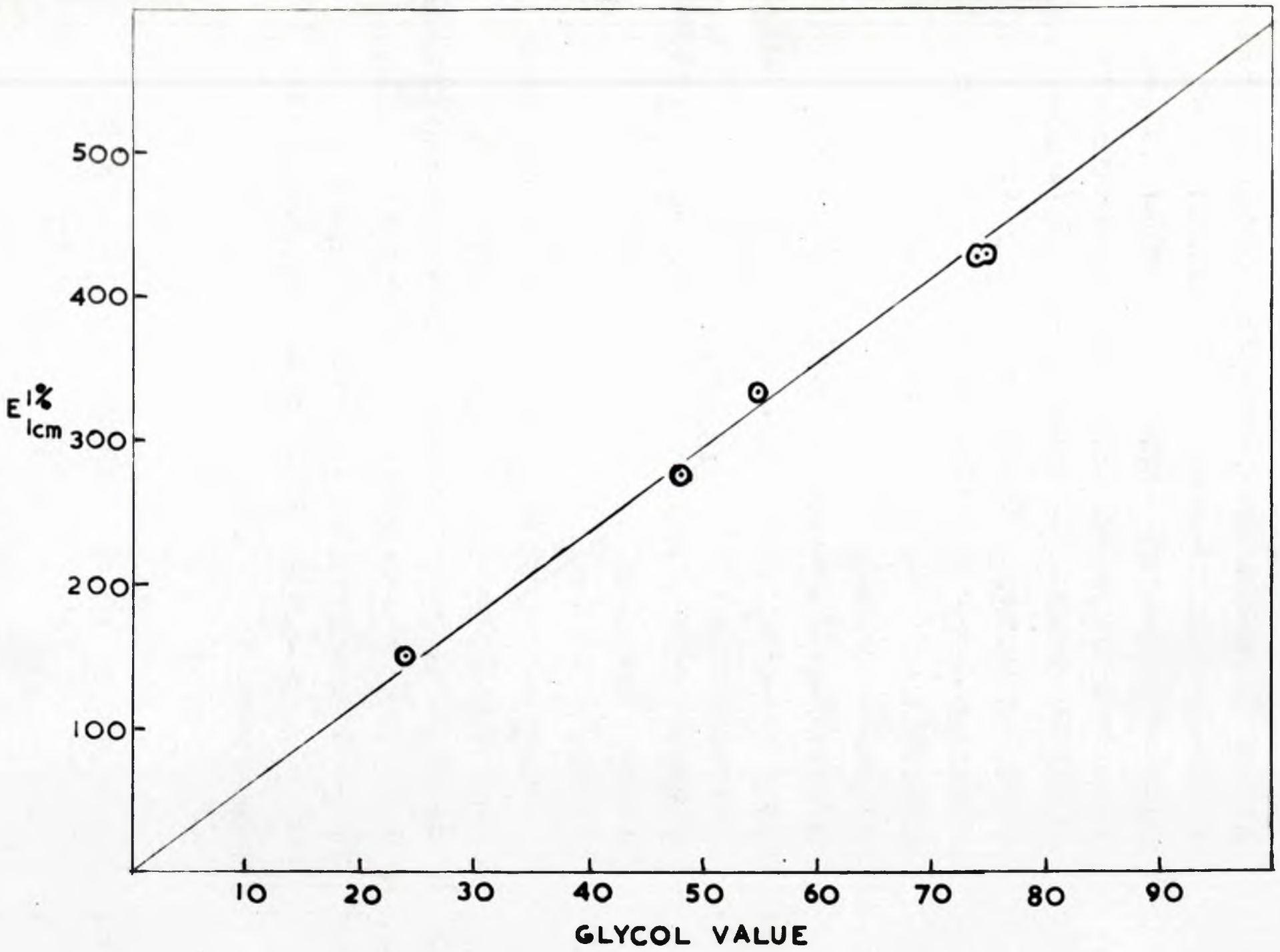
(b). The three vicinal dihydroxy acids which have been isolated from seed oils (9:10-dihydroxystearic, 12:13-dihydroxyoleic and 15:16-dihydroxylinoleic) may have a biosynthetic significance, in that they or the parent epoxides of the unsaturated acids may be the precursors (or vice versa) of oleic, linoleic and linolenic acids respectively. Whereas the last two are isolated as threo-dihydroxy acids, arising from cis-epoxides, the 9:10-dihydroxy acid is isolated as the erythro-isomer and it has been shown[55] that it exists in castor oil as such and not as an epoxide.

If there is a biogenetic relationship between these acids and the corresponding unsaturated acids as suggested above, a different mechanism would thus appear to be operative in the interconversion of 9:10-dihydroxystearic acid and oleic acid than in the interconversion of the epoxy acids and the corresponding olefinic acids.

(c). Although the ultra-violet absorption at 234mμ of the dihydroxylinoleic acid after isomerisation is much lower than that which would result from molecular weight proportionation of the value for pure linoleic acid a

Figure III.

GRAPH OF $E^{1\%}_{1cm}$ AGAINST GLYCOL VALUES.



graph of $E^{1\%}_{1cm}$ against glycol values (Figure III, opposite) is a straight line and this, when extrapolated to 100% glycol value, gives $E^{1\%}_{1cm}$ = 590 for pure 15:16-dihydroxy-linoleic acid. A possible explanation of the lower value is that the glycol group activates the diene system so that maximum conjugation is built up much earlier during isomerisation than in the case of linoleic acid so that a substantial decay has set in before isomerisation was stopped (60 minutes).

(d). The method developed for "labelling" the carboxyl group, by conversion to a primary alcohol before periodate-permanganate oxidation, is a much more convenient procedure than "labelling" by chain extension and is less likely to regenerate the acid than is the procedure of "labelling" by esters.

There was some small oxidation of the terminal hydroxy group since a very small proportion of azelaic acid, as its di-p-bromophenacyl ester, was separated in each case but the oxidation times used were probably far in excess of those required and using the minimum times necessary for complete cleavage this oxidation would probably be almost zero.

Experimental.

I. Separation and Purification of hydroxy acid.

1. Preparation of mixed acids:

The seeds of Camelina sativa were crushed and extracted with petroleum (B.pt. 40-60°) to yield an oil amounting to 32.5% of the seeds.

The oil (500g.) was refluxed with glacial acetic acid (2 litres) for 8 hours, to convert any epoxy acids to the corresponding acetoxy-hydroxy acids, and the acetic acid distilled off under reduced pressure at 100°.

The acetylated oil was hydrolysed by refluxing for 2 hours with aqueous alcoholic potassium hydroxide (3.5 litres of 10%). After removal of about half the alcohol the soap solution was diluted with water, acidified with ice-cold 25% sulphuric acid and the liberated acids extracted with ether. The extract was thoroughly washed with water, dried over sodium sulphate and the ether distilled to give the mixed acids (471g.). Non-saponifiable matter was not removed at this stage.

2. Preliminary concentration of hydroxy acids:

The mixed acids (471g.) were boiled with acetic anhydride (2 litres) for 3 hours, to acetylate hydroxy acids, after which water was added and boiling continued for a further 2 hours. The reaction mixture was then diluted further with water, extracted with ether and the extract washed with water until free from acetic acid. After drying, the

ether was distilled off to give the acetylated mixed acids (471g.).

These acids, in methanol (500ml.), were added over half an hour to a solution of urea (1400g.) in hot methanol (1800ml.). The mixture was left overnight at room temperature, filtered and the filter cake washed well with a cold saturated solution of urea in methanol.

The filtrate was concentrated, diluted with water and extracted with ether to yield the non-adduct-forming acids (95.5g., 20.3%) - Fraction I. The filter cake when treated with water and extracted gave the adduct-forming acids (351g.) - Fraction II. (The loss in weight of 24g. was due to difficulty in handling the large volumes used in the isolation of Fraction II.)

On a similar separation carried out on one fifth of this scale (by Miss M.S. Stott in this Department) the following results were obtained:-

Fraction	% Mixed acids	S.E. OAc-esters	S.E. acids *
I'	17.5	253.5	286.0 (300)
II'	82.5	297.2	284.1 (298.1)

* Figures in parentheses are the calculated equivalents of the esters.

These figures show complete elimination of hydroxy acid from Fraction II' and a concentration of 12.0% of dihydroxy acid (calculated as dihydroxyoctadecdienoic acid)

in Fraction I'. Since this fraction will include unsaponifiable matter which may contain hydroxyl groups, this value may be rather high.

These results can be assumed to be fairly near to those which would have resulted from similar determinations on Fractions I and II. Thus Fraction I will have a dihydroxy acid content of about 12% , indicating 2% of dihydroxy or 4% of monohydroxy acid in the mixed acids.

3. Partition, Hydrolysis and Removal of unsaponifiable matter:

Fraction I (95.5g.) was dissolved in light petroleum, previously equilibrated with 80% methanol, and half the solution put in each of two separatory funnels (900ml. in each), 500ml. of the same solvent being added to each of another two funnels. Four lots of 80% methanol (900ml.) were then passed through this system of four funnels, equilibrating at each stage. The methanol fractions were combined to give 12.64g. of concentrate and the petroleum fractions gave non-hydroxy acids (82.91g.).

The methanol soluble fraction was hydrolysed in the usual way, to remove acetyl groups, and the unsaponifiable material was recovered by extracting the aqueous soap solution four times with ether. The ether extracts were thoroughly washed with alkali and water, the washings combined with the soap solution and the acids recovered by ether extraction after acidification (10.69g.). This "hydroxy acid concentrate" had a glycol value of 54.7% (see p. 66) and this indicates a dihydroxy acid content

of ca.1% in the mixed acids and, if the remainder is monohydroxy acid (see p. 68), ca.2% of monohydroxy acid in the mixed acids.

4. Final purification of hydroxy acids:

The "hydroxy acid concentrate" (5.14g.) was dissolved in 80% methanol (100ml.) and extracted 10 times with light petroleum in 75ml. portions to give petroleum soluble acids (0.90g.) and methanol soluble acids (4.17g.).

The methanol extract (4.17g.) was dissolved in a little warm benzene and applied to the top of a chromatographic column of activated silica gel (300g., 65 x 2.5cm.). The silica was activated by heating to 200° at a pressure of 5mm. of mercury with agitation until no further loss of adsorbed volatile material was apparent (ca.1 hour). The column was prepared by pouring a slurry of silica in benzene into a tilted tube and stirring with a wire to remove air bubbles.

Elution was commenced with pure benzene and the polarity of the solvent increased by the addition of increasing proportions of ether and methanol as shown overleaf:-

Fraction	Solvent	Vol.(ml.)	Wt.(mg.)	G.V.(%) *	$E^{1\%}_{1cm}$ †
A	benzene	800 900 1000	695 33 12		
			740	0	-
B	25% ether/ benzene	200 400 950 1000	345 67 208 217		
			837	24	153
C	75% ether/ benzene	1000 1000 1000 1000 1000 2000	709 464 305 174 98 101		
			1851	64	-
D	10% MeOH/ ether	2000 1000 1000	554 118 50		
			722	94	86

* Control determination on 12:13-dihydroxyoleic acid = 104%

† For values before and after isomerisation see p. 67.

Fraction C was rechromatographed through a column of 100g. activated silica gel (25 x 2.5cm.) as shown overleaf:-

Fraction	Solvent	Vol.(ml.)	Wt.(mg.)	O.V.(%)*	$E^{1\%}_{1cm}$ †
C_1	25%ether/ benzene	1000	989	48	275
C_2	75%ether/ benzene	1000	345	74	427
C_3	10% MeOH/ ether	1000	345	76	429
C_4	30% MeOH/ ether	700	63	?	?

* Control determination on 12:13-dihydroxyoleic acid = 106%.

† For values before and after isomerisation see p. 67.

II. The Saturated Acid.

I. Hydrogenation:

(a). The "hydroxy acid concentrate" (see Figure I and p. 84) (2.55g.) was hydrogenated at room temperature over 5% palladium on charcoal catalyst (1g.) at a pressure of slightly more than atmospheric. The hydrogen uptake at S.T.P. was 387ml. which is the theoretical uptake for two double bonds.

The catalyst was filtered off and the solvent stripped to give a crude product (2.43g.). Crystallisation of this from ethyl acetate, and petroleum (B.pt. 60-80°) extraction of the residue with subsequent crystallisation of the petroleum insoluble material gave a white solid (1.02g., 41.6%) which after several recrystallisations had a final melting point of 96-7°. Mixed melting points with threo-9:10-dihydroxystearic acid (M.pt. 94-4.5°) and with

threo-12:13-dihydroxystearic acid (M.pt. 96-7.5°) were 84-93° and 85-93° respectively. Analysis results were:- Found: C, 68.10; H, 11.52%; Calc. for $C_{18}H_{36}O_4$: C, 68.37; H, 11.40%.

(b). A portion of Fraction C_2 (130mg.) was hydrogenated in the same way giving an uptake of 18.0ml. of hydrogen (two double bonds require 18.7ml.) and a yield of solid crude product (131mg.) which crystallised from ethyl acetate to give the same acid (74.8mg., 57.5%, M.pt. 92-5°, mixed M.pt. 94-6°). This represents a yield of 77% calculated from the glycol value and compares favourably with a yield of 83% of threo-12:13-dihydroxystearic acid obtained from a control hydrogenation of threo-12:13-dihydroxyoleic acid.

2. Preparation of derivatives:

(a). Methyl ester:-

The saturated acid (50mg.) was dissolved in methanol (2.5ml. containing ca.1% of dry hydrogen chloride) and the solution allowed to stand at room temperature overnight. The product was separated by dilution with water and filtration (51.2mg.= 98.1%, M.pt.82-9°). This was purified by washing an ethereal solution with 10% sodium carbonate and crystallising the recovered ester from ethyl acetate several times. The pure ester had M.pt. 89.5-90.5°; Found: C, 69.04; H, 11.49%; $C_{19}H_{38}O_4$ requires: C, 69.88; H. 11.55%.

(b). Ethyl ester:-

This was prepared in the same way, the crude ester (51.1mg., 93.9%, M.pt. 69-72°) being purified to a melting

point of 74-4.5°. Found: C, 69.47; H, 11.48%; $C_{20}H_{40}O_4$ requires: C, 69.72; H, 11.70%.

3. Determination of equivalent weight:

The equivalent was determined by direct titration of the acid against standard alkali and by back titration with standard acid after addition of a known excess of alkali and also separately by normal saponification equivalent determination:-

(a). 100mg. af the saturated acid was weighed and titrated cold with N/10 alcoholic potassium hydroxide giving an equivalent weight of 320.

The solution was then evaporated to dryness, 1.50ml. of N/2 alcoholic potassium hydroxide added and, after refluxing for 0.5hr., the excess alkali was titrated against N/5 hydrochloric acid and the saponification equivalent calculated to be 319.

(b). 100mg. of the acid was weighed and the saponification equivalent determined by addition of 1.50ml. N/2 alcoholic potassium hydroxide and, following 0.5hr. reflux, titration with N/5 hydrochloric acid. Three blanks were done and the saponification equivalent found to be 312.

It will be seen that the acid equivalent and the sap. equivalent in (a) are identical and the average value of the three determinations is 316.

Since these determinations had to be carried out on the semi-micro scale the methods were first perfected on

9:10-dihydroxystearic acid and on the non-hydroxy ester fraction of Strophanthus verrucosus seed oil (see p. 153). Method (a) on the former gave an average equivalent weight of 319 which is close to the correct value of 316.5. Method (b) on the latter gave an average equivalent weight of 292 compared with the previously determined value of 289 (macro scale).

III. Position of hydroxyl groups:

1. Determination of glycol value:

70-80mg. of the acid was dissolved in 10ml. of a 2:1 mixture of acetic acid and chloroform in a stoppered glass bottle and 50ml. of periodic acid solution (1.4g. potassium periodate in 200ml. water and 800ml. acetic acid, filtered immediately before use) was added from an automatic pipette. After standing in the dark for 0.5hr., 10% potassium iodide solution (15ml.) and water (40ml.) were added and the liberated iodine titrated against N/10 sodium thiosulphate using starch solution as indicator. Two blank determinations were carried out and the percentage of glycol present (96.5%) was calculated from the equation:-

$$\% \text{ glycol} = \frac{N(V_b - V_s)M}{2W}$$

where M = molecular weight of the acid,

V_b = blank titre of thiosulphate,

V_s = titre of thiosulphate,

N = normality of thiosulphate

and W = weight of sample.

Trial determinations on pure 9:10-dihydroxystearic acid gave values of 100.1 and 99.7% showing the method to be accurate for saturated glycols.

Note:- The glycol values of the chromatographically separated fractions (pp. 66, 86 & 87) were determined on samples of ca.20mg. and the accuracy of these is probably ca.±5%. Test determinations on 20mg. samples of 12:13-dihydroxyoleic acid gave values which were generally rather more than 100%.

2. Determination of position of hydroxyl groups:

(a). Aldehyde-acid fragment:

The titrated periodate oxidation solution was diluted with water and a white solid precipitated which was filtered off (M.pt. 70-90°). A few milligrams in methanol solution gave an orange-yellow precipitate with Brady's reagent (M.pt.80-85°).

The remainder of the filtered solid was dissolved in aqueous acetone and oxidised with excess potassium permanganate. The oxidised solution was decolourised with sulphur dioxide gas, diluted and the product filtered and dried (17.2mg., M.pt.96-109°). Crystallisation from ethyl acetate and from nitromethane raised the melting point to 107-111° which showed no depression when mixed with synthetic pentadecadioic acid (M.pt. 111-113°), the mixed M.pt. being 107-112° (Lit. 114.6-114.8°)[56]. When mixed with tridecadioic acid (M.pt. 105-114°, Lit.113.5°[56]) the melting point was depressed (92-102°).

(b). Aldehyde fragment:

After filtration of the aldehydo-acid the solution was steam distilled, 50ml. of distillate being collected in a flask containing 20ml. of M/4 dinitrophenylhydrazine reagent (Brady's reagent). An orange precipitate separated during the distillation and the mixture was allowed to stand overnight to ensure complete reaction.

The mixture was then thoroughly extracted with chloroform the extract being washed 3 times with water, and concentrated to 25ml. This solution was added to a column of 7.5g. of a 4:1 mixture of bentonite and keiselguhr, the column having been packed wet in a slurry with chloroform. The dinitro-phenylhydrazone was eluted out with chloroform, unchanged reagent remaining at the top of the column[57]. The orange band of derivative amounted to 44.8mg. (87.7%, M.pt. 125-139°) and was preceded by 15.4mg. of an orange oil which had not shown up as a band on the column. Several crystallisations from ethanol raised the melting point to 154-155° which was undepressed in admixture with authentic propionaldehyde dinitrophenylhydrazone.

The dinitrophenylhydrazone was further proved to the derivative of propionaldehyde by paper chromatographic means. Two different solvent systems were used on Whatmann No. 1 paper and the R_F values measured. (The R_F value is the ratio of distance travelled by the spot to distance travelled by the solvent front.)

(i). 5% ether in petroleum (B.pt. 60-100°) was used as eluent[58] in descending irrigation and the spots were detected by ultra-violet light. Three spots were run side by side on each strip of paper:-

D.N.P.	R_F	D.N.P.	R_F
Propionaldehyde	0.81	n-Butyraldehyde	0.85
Unknown aldehyde	0.81	Unknown aldehyde	0.82
Acetaldehyde	0.75	iso-butyraldehyde	0.85

(ii). The paper was first immersed in 20% NN-dimethylformamide solution in acetone and dried for 5 minutes in warm air. n-Hexane saturated with NN-dimethylformamide was the eluent in downward irrigation[59]. Three spots were added, side by side, to each strip and the paper was conditioned in an atmosphere of the solvent before elution was started:-

D.N.P.	R_F	D.N.P.	R_F
n-Butyraldehyde	0.54	Acetaldehyde	0.29
Unknown aldehyde	0.44	Unknown aldehyde	0.45
Propionaldehyde	0.44	Propionaldehyde	0.45

(iii). Conditions and solvents were as in (ii) but each spot was a mixture of the derivatives of the unknown aldehyde and one of the controls:-

D.N.P. mixture	Result	R_F
Acetaldehyde + Unknown	Separation to 2 spots	0.18+0.29
Propionaldehyde + Unknown	No separation	0.29
n-Butyraldehyde + Unknown	Separation to 2 spots	0.29+0.37

IV. Determination of positions of double bonds.

1. Oxidation of acid:

The stock oxidant solution consisted of potassium periodate (22.43g., 97.5mM) and potassium permanganate (0.395g., 2.5mM) in 1 litre of aqueous solution. This had to be heated shortly before use to effect complete solution of periodate.

A portion of Fraction C_2 (78mg.) was oxidised in 300ml. solution containing tert.-butanol (90ml.), stock oxidant solution (60ml.) and enough potassium carbonate to give a pH of 8 - 9. Oxidation was stopped after 8hr. by the addition of hydrochloric acid and enough sodium metabisulphite to convert all periodate, iodate and iodine to iodide. The decolourised solution was made alkaline and the butanol distilled off under reduced pressure. The remaining solution was acidified, continuously extracted with ether for 15hr. and the extract dried and distilled. The residue was triturated with petroleum (B.pt. 40-60°) to give a soluble fraction (7.1mg.) which, however, did not smell acidic and gave no p-bromophenacyl derivative, and an insoluble residue (54.2mg.) which was crystallised from water to give azelaic acid (31.1mg., 62%, M.pt. 100-4°) whose melting point was raised (102-6°) on admixture with authentic azelaic acid (M.pt.105-7).

A control oxidation on 12:13-dihydroxyoleic acid (78.5mg) using two thirds of the proportions of reagents quoted above furnished a petroleum soluble fraction (38.8mg.) which gave

crude p-bromophenacyl ester of hexanoic acid (84.3mg., M.pt.70-95°) contaminated with excess reagent, andan insoluble residue (54.3mg.) which yielded azelaic acid (27.8mg., 60%, M.pt. 103-6°).

2. Preparation of the alcohol:

A portion of Fraction C_3 (224mg.) in anhydrous ether (10ml.) was added to lithium aluminium hydride (100mg.) in anhydrous ether (which had been refluxed together overnight) at such a rate as to give steady reflux. The reaction mixture was allowed to stand at room temperature for 48hr., the complex decomposed with 10% sulphuric acid and the product extracted with ether, washed with carbonate solution and neutral with water, dried and recovered (195.1mg., 91%).

3. Oxidation of the dihydroxy alcohol:

The alcohol (75mg.) was oxidised by the method described above and the crude product (50.7mg.) triturated with ice-cold petroleum to give a soluble fraction which appeared to be mainly stopcock grease and gave no derivative, and an insoluble fraction (35.6mg.) which crystallised from ethyl acetate-petroleum mixture to give 9-hydroxynonanoic acid (15.2mg., 35%, M.pt. 40-6°). Further crystallisation raised the melting point to 46-9° (Lit. 53-4°)[60] which was unchanged on admixture with an authentic sample and which proved to have an equivalent weight of 186 ($C_9H_{18}O_3$ = 174) when titrated against N/100 alcoholic potassium hydroxide.

The mother liquors from the crystallisations yielded

the p-bromophenacyl ester (15.2mg., M.pt.73-5°) which was purified from aqueous ethanol (M.pt. 75-8°) which melting point was raised to 77-9° when mixed with an authentic derivative (M.pt. 78-9°).

The authentic samples were obtained by oxidising oleyl alcohol as above. From 268mg. was obtained 147.1mg. (93%) of nonanoic acid characterised as its p-bromophenacyl ester (M.pt. 58-65°, Lit. 68.5°) and 157.0mg. (94.3%, M.pt.35-46) of 9-hydroxynonanoic acid, which was crystallised once from ether-petroleum mixture to M.pt. 45-51° and which gave a p-bromophenacyl ester (M.pt. 78-9°). A bad analysis:- Found: C, 55.71; H, 7.10; Br, 18.00%; $C_{17}H_{23}BrO_4$ requires: C, 54.99; H, 6.24;, Br, 21.53%, resulted from insufficient drying due to an undetected fault in the drying pistol vacuum line.

V. Infra-red spectra examinations.

1. Configuration of double bonds:

The infra-red spectrum of Fraction C_1, both as a thin film of "neat" material and as a solution in carbon tetrachloride was obtained on a Grubb-Parsons GS2A Double Beam Grating instrument. In neither case was there any appreciable evidence of a peak in the trans-double bond region of 10.3μ^{50}.

2. Determination of epoxide:

(a). The oils:- Olive oil, vernonia oil and esmeline oil were neutralised by passing a chloroform solution of each

oil through an alumina column. The oils, free of carboxyl OH groups, were recovered by distillation of the chloroform under reduced pressure.

(b). The esters:- Portions of these neutralised oils were boiled with acetic acid (5vol.) for 2hr. and the acetylated oils recovered by ether extraction and then hydrolysed by boiling (1hr.) with excess 10% potassium hydroxide solution, the mixed acids being extracted from the acidified hydrolysate. The mixed acids were esterified with methanolic hydrogen chloride and obtained by dilution with water and ether extraction. The extract was washed with alkali and then with water till neutral and thoroughly dried (Na_2SO_4) before distillation of the ether.

(c). Measurements:- The spectra of the three oils and their esters were determined on thin films of "neat" material. Film thicknesses of the vernonia oil and esters were unknown, but similar, the film being produced between two blocks of sodium chloride. The film thicknesses of the olive and cameline oils and esters were 0.1mm., a sodium chloride cell of that pathlength being used.

The spectra in the region of the O-H stretching vibration (2.6 - 3.3μ) are reproduced on p.76 (Figure II). Apart from the differences shown in this region and the epoxide bands shown by the vernonia oil at ca.12μ the spectra were very similar showing only minor variations, as would be expected.

VI. Fraction D.

1. Hydrogenation:

Fraction D (100mg.) was hydrogenated for 12hr. at room temperature and atmospheric pressure over palladium on charcoal catalyst (100mg.) in ethanol solution. The product (91mg.) was obtained by removal of the solvent after filtration and proved to be a very viscous, tacky substance which would not crystallise from ethyl acetate or ethyl acetate-petroleum mixtures.

2. Oxidation:

(a). A portion of Fraction D (78mg.) was oxidised by the periodate-permanganate method (p. 94) to give 60.5mg. of product which was made up of a petroleum soluble fraction (6.1mg.) which gave a trace of p-bromophenacyl derivative (M.pt. 58-61°; derivative of propionic acid has M.pt. 63°), and a petroleum insoluble residue (54.2mg., M.pt. 86-100°) which crystallised from ethyl acetate-petroleum mixture to give azelaic acid (20.7mg., 44%, M.pt. 102-4°) whose melting point was raised (103-6°) when mixed with authentic azelaic acid (M.pt. 105-7°).

(b). A further portion (86.7mg.) was oxidised in the same way to give 13.5mg. of petroleum soluble and 48.7mg. of insoluble acids. The equivalents of these fractions were determined by titration against 0.0813N. alkali and were 32.6 (titre, 0.51ml.) and 168.7 (titre, 3.55ml.) respectively. Propionic acid has an equivalent of 74 (acetic, 60; formic, 46) and the first fraction has probably not been freed

from hydrogen chloride. The high equivalent of the second fraction (azelaic acid, 94) seems to indicate the presence of polymeric material. This fraction was recovered and treated with p-bromophenacyl bromide to give the di-p-bromophenacyl ester of azelaic acid (32.0mg., 31%, M.pt. 124-9°) which, when mixed with an authentic sample (M.pt. 128-30°) melted at 126-31°.

VII. Preparation of tridecа- and pentadecadioic acids.

1. Erucic acid from Rape seed oil[61]:

Rape oil (80g.) in ethanol (100ml.) was refluxed for 2hr. with potassium hydroxide (20g.) in water (20ml.), the bulk of the alcohol distilled and the residue diluted with water, acidified and extracted with ether to give the mixed acids (95.7g.). These were dissolved in ethanol (200ml.) and water (70ml.) and the filtered solution cooled to 0°. The precipitated acid was filtered and recrystallised from ethanol to give a fraction of pure erucic acid (9.31g., M.pt. 31-4°; Lit. 33°) with iodine value 73.3 (theory, 75).

2. Oxidation of erucic acid to tridecadioic acid:

Erucic acid (6g.) was dissolved in AR acetic acid (45ml.) and powdered potassium permanganate (18g.) added in small portions to the stirred solution which was maintained at 40-50° for 1.5hr. and stood overnight at room temperature. The acetic acid was distilled under reduced pressure, the residue diluted with water, decolourised with sulphur dioxide gas and steam distilled to remove the monobasic acid. The residual solution on cooling precipitated tridecadioic acid

(4.33g., M.pt. 105-14°; Lit. 113-3.2°[56]) which was recrystallised from ethyl acetate (M.pt. 106-11°)

3. Preparation of the acid chloride:

Tridecadioic acid (2.5g.) was refluxed with pure (see p. 200) thionyl chloride (10ml.) for 2hr. and the excess thionyl chloride and other volatile material removed under reduced pressure from the water bath to give the acid chloride (2.85g., 100%) which was used without further purification.

4. Preparation of 1:11-bis-diazoacetylundecane:

The acid chloride (2.85g.) in pure ether (20ml.) was added to a solution of excess diazomethane (see p. 200) in ether over a period of 5min. and the solution stood overnight. The excess diazomethane was distilled off with the ether and the solution concentrated to 20ml., cooled and the diazoketone filtered (1.43g.).

5. Rearrangement to pentadecadioic acid by Newman's method:[62]

The diazoketone (1.43g.), without purification, was dissolved in anhydrous methanol (20ml.) in a conical flask fitted with a dropping funnel and connected to a gas measuring burette. A 10% solution of silver benzoate in triethylamine (5ml.) (see pp.200,204) was added a few drops at a time over 0.5hr. and the mixture stirred for a further 1hr. by magnetic stirrer. Nitrogen evolution was 157ml. at S.T.P. corresponding to 90% reaction.

The solution was boiled to convert silver salts to silver and decolouring charcoal added to the hot solution before

filtering. The solvents were removed from the filtrate and the residue dissolved in ether, washed with alkali and water and recovered as crude dimethylpentadecadioate (2.16g. 32%, overall yield, M.pt. ca.40°; Lit. 43°[56]).

The dimethyl ester was hydrolysed with aqueous alcoholic potassium hydroxide and the crude pentadecadioic acid recovered (1.80g.) which when crystallised from ethyl acetate had M.pt. 100-9° (Lit. 114.6-114.8°[56]). This was purified by vaccuum sublimation at 200° and two recrystallisations from ethyl acetate finally raised the melting point to 111-3°.

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Part III.

Analytical Studies.

The Occurrence of 9-Hydroxyoctadec-12-enoic Acid in Various Strophanthus Oils.

Introduction.

I. Strophanthus seed oils.

The genus Strophanthus comprises[1] about forty five species of which approximately thirty five grow in Africa, ten in Asia and one or two in Madagascar. Most species are climbers of the tropical forests but some are shrubs of the savannah and one is a succulent. Some of those which are lianas reach to a height of over 30 metres and have a trunk diameter of up to 12cm.[2].

Many show very beautiful flowers distinguished by ribbon or thread-like prolongations of the corolla lobes. The fruits, which often take as long as a year to ripen, consist of two long carpels whose thickness varies from species to species and these contain many spindle shaped, rather flat seeds (1 - 2cm. long and ca.5mm. broad) which have featherlike awns. The embryo forms the main part of the seed, the shell and endosperm being very thin. The species is extremely difficult to identify from the seed, although this can be achieved by treatment of cross-sections under the microscope with 80% sulphuric acid, when characteristic colours are produced[3]. Recognition, however, is easy when the plant is blooming or fruiting.

Livingstone[4] first reported that natives in several parts of Africa used preparations from the seeds as arrow poisons. Not all species provide these heart poisons; S. gratus, S. hispidus and S. sarmentosus give the most powerful

preparations and are used in the order of preference given[2]. Since about 1865, Strophanthus preparations have played an important role in medicine for their cardiotonic properties. Following the discovery of the glycoside sarmentocymarin and its aglycone, sarmentogenin, which is oxygenated at the C_{11} position, the latter was investigated as a possible starting material in a partial synthesis of cortisone which has extraordinary therapeutic effects in the treatment of rheumatoid athritis[5].

The seed oils were not examined in great detail prior to 1952 and only very brief reports are available:-

Investigator	Mjoen[6]	Matthes & Rath[7]	van Itallie[8]	Kuhn[9]	Tocco & Senna[10]
Species	S.hispidus	not stated	two oils - not stated	not stated	S.Kombé S.hispidus S.gratus
Acid value	-	17.61		15.16	Characts. of
Sap. value	187.9	189.99		188.8	oils from
Iodine value	73.02	94.97		90.3	diff.parts
Acetyl value	0.00	-			of seeds given.
Palmitic	+	14.7			
Stearic	-	6.3	25.2 26.6		
Oleic	+	58.4	44.3 48.1		
Linoleic	-	14.6	30.5 25.3		

The first detailed analyses of Strophanthus oils were of S. sarmentosus (forest and savannah forms), S. hispidus and S. Courmontii by Gunstone[11] who reported the presence of an unusual acid. This was shown by degradative and other studies to be 9-hydroxyoctadec-12-enoic acid.

The component acids reported are as follows (% weight):-

	S.s.(S.)	S.s.(F.)	S.h.	S.Cour.
Myristic	0.2	0.2	0.1	0.1
Palmitic	11.9	12.2	11.9	13.4
Stearic	9.2	8.1	7.0	4.5
Arachidic	4.0	3.1	2.0	2.8
Oleic	37.7	43.5	35.5	38.6
Linoleic	29.7	26.4	30.0	30.4
Hydroxyoctadecenoic	7.3	6.5	13.5	10.2

Abbreviations:-

S.s.(S.) : Strophanthus sarmentosus DC. (Savannah form),

S.s.(F.) : Strophanthus sarmentosus DC. (Forest form),

S.h. : Strophanthus hispidus DC.,

S.Cour. : Strophanthus Courmontii Sacleux.

A further series of analyses of S. hispidus seed oil was carried out by Bharucha and Gunstone[12] in attempts to devise a simpler analytical procedure for oils containing oxygenated acids. Four procedures were used (see paper) and the results obtained were:-

	(i)	(ii)	(iii)	(iv)
Saturated	55.0	21.4	19.8	21.2
Oleic		34.1	36.6	35.1
Linoleic	29.7	28.3	27.4	28.5
Hydroxyoctadecenoic	15.3	16.2	16.2	15.2

II. The analysis of seed oils containing hydroxy acids.

The quantitative determination of the component acids of a fat[13] usually involves the separation of the mixed acids by low temperature crystallisation or lead or lithium salt separation into fractions differing in degree of unsaturation. These fractions are then separately esterified and fractionally distilled and each of the resulting simple fractions is characterised by determination of mean unsaturation, mean equivalent and ultra-violet absorption after alkali isomerisation.

The above procedure, though satisfactory for most seed oils,is unsatisfactory for oils containing oxygenated acids. Castor oil is the most important of these oils and several methods of analysis for it have been described.

The most complete study of castor oil was carried out by Gupta, Hilditch and Riley[14] who found low temperature crystallisation unsuitable for mixtures containing ricinoleic acid. Determination of ricinoleic acid as hydroxystearic acid after hydrogenation was also unsatisfactory and the procedure ultimately adopted avoided both low temperature crystallisation and fractional distillation. The ricinoleic acid content was calculated from the acetyl value determined by Riley's method[15] after making allowance for the dihydroxystearic acid present which was weighed after crystallisation. The content of linoleic acid was determined from the ultra-violet absorption after alkali isomerisation and oleic acid

was calculated from the iodine value after making allowance for the linoleic and ricinoleic acids. The saturated acids were determined as a group by difference. The calculation of the content of oleic and saturated acids is dependent on an accurate determination of iodine value.

Vidyarthi and Mallya[16] however reported unsatisfactory iodine values of Vernonia anthelmintica seed oil which they considered to contain 11-hydroxyoctadec-9-enoic acid, later shown to be 12:13-epoxyoleic acid[17]. This tendency for hydroxy groups to interfere in iodine value determinations had been noted before by various American workers[18] who were trying to modify the Wijs and Hanus methods by the addition of mercuric acetate solution to get a more rapid procedure. Whereas in the absence of mercuric acetate values for castor oil and ricinoleic acid were normal, the rapid method gave consistently high values for these compounds. Blocking of the hydroxyl group by sulphonation or propionylation resulted in normal values showing that the free hydroxyl was interacting. Gunstone, in his work on S. sarmentosus seed oil[11] found that the iodine values were always high and were not concordant. Acetylation of the hydroxy groups, however, appeared to eliminate this effect[11,16].

Gunstone[11] found that low temperature crystallisation was straightforward (probably due to the low content of hydroxy acid) and the method adopted by him was the standard procedure of low temperature separation of the acids

followed by fractional distillation of the esters, fractions containing hydroxy ester being acetylated before distillation and the proportion of methyl acetoxyoctadecenoate computed solely from the saponification equivalents. This method is dependent on the thermal stability of the acetoxy ester and it is perhaps on account of this factor that Gupta, Hilditch and Riley[14] did not distil their esters.

Another method of analysing mixtures containing ricinoleic acid was suggested by Achaya and Saletore[19] who separated saturated acids via their lead salts and then separated the remaining acids into two fractions by a technique in which oleic and a little linoleic acid fromed urea complexes while ricinoleic and most of the linoleic did not. This last fraction was subsequently esterified, acetylated and distilled. This again depends on the thermal stability of the acetoxy ester. A better separation would have resulted had the unsaturated fraction been acetylated before the fractionation by urea complexes.

Sreenivasan et al.[20] effected a quantitative separation of hydroxy and non-hydroxy esters by heating the mixed esters with succinic anhydride in toluene and separating the hydroxy ester as its hydrogen succinate by extraction with alkali. In a study of castor oil, ricinoleic acid was determined from the weight of the acidic fraction, linoleic acid from the ultra-violet absorption after isomerisation, oleic acid from the iodine value, dihydroxystearic acid

from periodate oxidation and saturated acids by difference. Surprisingly the non-hydroxy fraction showed conjugated unsaturation which may result from the long exposure (48hr.) to high temperature (111°) required for reaction with succinic anhydride.

Bharucha and Gunstone[12] described a method of analysing oils containing epoxy or hydroxy acids which avoided distillation and the determination of iodine value on fractions rich in hydroxy acids. Hydroxy and non-hydroxy acids were separated by partition between petroleum and 80% methanol and the non-hydroxy acids were further separated into saturated and unsaturated fractions by low temperature crystallisation. The hydroxy acid content of the methanol extract was determined solely from saponification equivalents before and after acetylation and the small proportion of non-hydroxy acids present was assumed to have the same composition as in the petroleum extract. The other fractions were characterised in the usual way. This method was found to be satisfactory in spite of the approximation involved.

Some physical methods have been described for the examination of mixtures containing hydroxy acids or esters. The optical activity of ricinoleic acid and its derivatives has been utilised to estimate the proportion of this acid in castor oil[21]. Partition chromatography[22], reversed phase partition chromatography[23] and countercurrent liquid-liquid

distribution[24] have all been studied in the search for a simpler and more reliable analytical procedure for studies of castor oil. A spectrophotometric method for determination of hydroxy ester content was developed by Riley[25] and consists of reacting the hydroxyl groups with a strong chromophoric substance (β-2:4-dinitrophenylpropionyl chloride) and measuring the resultant extinction coefficient. Low values for castor oil were obtained (5.4% low) and the method has not been further applied to the analysis of hydroxy-containing oils.

Various physical methods have been used in autoxidation studies including chromatographic analysis[26], displacement chromatographic analysis[27], countercurrent distribution methods[28] and infra-red spectrometry[29] and these could probably be adapted to give analytical procedures for hydroxy-containing oils.

Paper chromatography and vapour phase chromatography do not appear to have been applied to mixtures containing hydroxy acids.

Further work seems likely to produce a reliable physical procedure of analysis which will replace the chemical methods at present used.

Experimental.

I. Methods of analysis.

The mixed acids, free from unsaponifiable material, were obtained by the usual method - saponification, ether extraction of the soap solution and recovery of the acids by extraction after acidification. The mixed acids were submitted to one or more of the following three analytical procedures. The choice of method is dependent on the amount of material to be analysed. Method A is the preferred procedure and requires 15g. or more of the oil, although the use of micro or semi-micro methods for determination of iodine values and saponification equivalents would reduce this requirement to 5g. or possibly less. Method B is a fuller procedure which needs upwards of 80g. and Method C is an abbreviated method requiring only 3-4g.

The seed oils analysed are listed below together with the abbreviations used to denote them, the amounts which were available and the methods used.

Seed oil	Abbreviation*	Amount available	Method used.
S. verrucosus Stapf	S.v.	300g.	A, B & C
S. congoensis French.	S.cong.	125	B & C
S. intermedius Pax	S.i.	80	B & C
S. gratus French.	S.g.	53	A & C
S. Welwitschii K.Schum.	S.W.	51	A & C
S. Emini Aschers. & Pax	S.E.	47	A & C

continued overleaf:-

continued,

S. Nicholsonii Holmes	S.N.	44	A & C
S. amboensis Engl. & Pax	S.a.	46	A & C
S. Schuchardtii Pax	S.Sch.	37	A & C
S. Thollonii Franch.	S.T.	22	A & C.

See also p. 111.

1. Method A:

The mixed acids were esterified with methanolic hydrogen chloride and subsequently acetylated. The content of hydroxyoctadecenoic acid was computed from the saponification equivalent of the esters, determined in quadruplicate, before and after acetylation. The mixed acids were isomerised with ethylene glycol-potassium hydroxide reagent (180°/60min) and the absorption ($E^{1\%}_{1cm}$) at 234mμ measured, whereby the content of linoleic acid was obtained. $E^{1\%}_{1cm}$ at 268mμ was measured in each case and found to be negligible. Had a positive value resulted, indicating the presence of linolenic acid, the isomerisation would have been repeated at 170° for 15min. before measuring this value. The saturated ester content was determined by oxidation of the mixed esters, the acidic scission products being separated by adsorption on activated alumina[30]. Oleic acid was then calculated by difference.

As a check on this method the iodine value of the mixed acetylated esters was determined, whence, making allowance for linoleic and hydroxyoctadecenoic acids, the oleic acid

content could be calculated and the saturated acids determined by difference. The agreement was generally good.

2. Method B:

The mixed acids were partitioned between 80% methanol and petroleum (B.pt. 40-60°), the acids being first dissolved in the latter solvent. The methanol extract was esterified and acetylated and, from the saponification equivalent of the esters before and after acetylation, the content of hydroxyoctadecenoic acid in this fraction was calculated. The small amount of non-hydroxy acids in this fraction was assumed to have the same composition as the petroleum soluble fraction. The small amount of hydroxy acid remaining in the petroleum extract was determined from the saponification equivalents of the esters before and after acetylation. The linoleic acid was obtained from the absorption ($E^{1\%}_{1cm}$) at 234mμ after alkaline isomerisation and determination of the iodine value of this fraction enabled the oleic acid content to be calculated, allowing for linoleic and the small amount of hydroxyoctadecenoic acid. It was assumed that this small amount of hydroxy acid had no serious effect on the iodine values. The saturated acids in this fraction were obtained by difference.

The linoleic acid content can be checked by determination of the $E^{1\%}_{1cm}$ at 234mμ of the mixed acids, after isomerisation.

3. Method C:

A portion of the mixed acids was esterified with

methanolic hydrogen chloride and the hydroxy ester content determined by infra-red spectrometry. The amounts of linoleic and saturated acids were determined (as in Method A) by absorption at 234mμ after isomerisation and by oxidation, respectively and the oleic acid determined by difference. Direct calculation of the oleic acid content from the iodine value of the acetylated esters again provided a check on the accuracy.

II. Methods of calculation.

The symbols C_{18}, C_{18} and OHC_{18} represent oleic acid, linoleic acid and 9-hydroxyoctadec-12-enoic acid respectively. Methyl esters will be termed, for example, C_{18} ester.

The methods of calculation used in the three analytical procedures will be shown by examples:-

1. Method A: (S.verrucosus)

Absorption at 234mμ ($E^{1\%}_{1cm}$) of mixed acids..... 235.9

Sap. equiv. of mixed esters (E)..... 294.1

Sap. equiv. of acetylated mixed esters (D)..... 280.3

Iodine value of acetylated esters..... 87.4

Saturated esters (% wt.) in mixed esters..... 23.2%

Molecular wt. of hydroxyoctadecenoic acid (M)..... 298.5

(1). The % of hydroxy acid in the mixed acids is calculated by the expression (p. 160)

$$OH.C_{18}\ (wt.\%) = \frac{100.M.(E-D)}{(D-42)(E-14)}$$

Thus $OH.C_{18} = \frac{100 \times 298.5 \times 13.8}{238.3 \times 280.1}$ 6.2%

(ii). The percentage of linoleic acid:-

$$\frac{E^{1\%}_{1cm} \times 100}{\text{Empirical value for pure } C_{18}''} \equiv \frac{235.9 \times 100}{906} \quad \ldots\ldots\ldots\ldots\ldots \quad 26.0\%$$

(iii). Saturated ester content(directly) 23.2%

(iv). Oleic acid (by difference) 44.6%

Note:- It is recognised that the content of saturated material as % acids may not be the same as % esters but the difference is very small and results are to be given finally to the nearest 0.5 unit %.

(v). The alternative to (iii) and (iv) is to calculate the C_{18}' content from the I.V. of the acetoxy esters:-

To convert the I.V. of the acetoxy esters into that of the mixed acids the mean molecular weight of the acetoxy esters must be calcd. This can be done by the expression(see p.161):-

$$\text{Mean M.Wt.} = \frac{D(E - 42)}{(D - 42)}$$

Thus: $$\text{Mean M.Wt.} = \frac{280.3 \times (294.1 - 42)}{(280.3 - 42)} \quad \ldots\ldots\ldots \quad 296.5$$

Hence, $$\text{I.V. of mixed acids} = \frac{\text{I.V. acetd. esters} \times \text{M.M.Wt.}}{\text{S.E. mixed acids}}$$

$$= \frac{87.4 \times 296.5}{280.1} \quad \ldots\ldots\ldots\ldots\ldots \quad 92.6$$

Since the I.V. of pure C_{18}'' and $OH.C_{18}'$ are 180.9 and 85.0 resp., the contribution of these two acids to the above I.V. can be calculated:-

Contribution of $C_{18}"$ = $\frac{Wt.\% \times 180.9}{100}$ = $\frac{26.0 \times 180.9}{100}$ ... 47.0

Contribution of $OH.C_{18}'$ = $\frac{6.2 \times 85}{100}$ 5.3

I.V. due to C_{18}', thus = 92.6 - (47.0 + 5.3)..... 40.3

C_{18}' content, therefore = $\frac{40.3 \times 100}{I.V.\text{pure } C_{18}'}$ = $\frac{40.3 \times 100}{89.8}$..... 44.8%

and Saturated acids = 100 - (6.2 + 26.0 + 44.8) 23.0%

Summary of results:-

	Satd.	C_{18}'	$C_{18}"$	$OH.C_{18}'$
Satd. by oxidn.	23.2	(44.6)	26.0	6.2
C_{18}' by I.V.	(23.0)	44.8		

2. Method B: (S. varneosus)

$E^{1\%}_{1cm}$ at 234mμ of mixed acids, after isomerisation..... 236.3

Partition between 80% MeOH and petroleum gave:-

Methanol extract..... 5.51g.

Petroleum extract 86.68g.

Methanol extract:

Sap. equiv. of acids (E') 294.6

thus Sap. equiv. of esters (E) = E' + 14 ..... 308.6

Sap.equiv. of esters after acetylation (D) 184.3

Petroleum extract:

Sap.equiv. of esters (E_1) 288.6

Sap.equiv. of acetylated esters (D_1) 283.1

$E^{1\%}_{1cm}$ at 234mμ of acids after isomerisation..... 247.1

I.V. of acids 93.6

(i). MeOH extract comprises - $\frac{5.51 \times 100}{5.51 + 86.68}$ 5.98%

The $OH.C_{18}'$ content is calcd. by the same expression as in Method A:-

$OH.C_{18}' = \frac{100.M(E - D)}{(D-42)(E-14)} = \frac{100 \times 298.5 \times 124.3}{142.3 \times 194.6}$..... 88.5%

(ii). Petroleum extract = 100-5.98 of mixed acids..... 94.02%

The small residual amount of $OH.C_{18}'$:-

$OH.C_{18}' = \frac{100M(E_1-D_1)}{(D_1-42)(E_1-14)} = \frac{100 \times 298.5 \times 5.5}{241.1 \times 274.6}$..... 2.5%

As before, $C_{18}'' = \frac{E\frac{1\%}{cm} \times 100}{906} = \frac{247.1 \times 100}{906}$..... 27.3%

Contributions to I.V. of C_{18}'' and $OH.C_{18}'$ are determined as in Method A:-

Contribution of $C_{18}'' = \frac{27.3 \times 180.9}{100}$ 49.3

Contribution of $OH.C_{18}' = \frac{2.48 \times 85}{100}$ 2.1

Contribution due to C_{18}', thus = 93.6-(49.3+2.1). 42.2

Thus $C_{18}' = \frac{42.2 \times 100}{89.8}$ 47.0%

The balance is made up of saturated acids 23.2%

The various components are then converted to percentages of the total mixed acids by multiplication by the factor $\frac{94.02}{100}$

and the $OH.C_{18}'$ content of the MeOH extract is similarly converted by the factor $\frac{5.98}{100}$:-

	Satd.	C_{18}'	C_{18}''	$OH.C_{18}'$	Total
Petroleum extract	21.86	44.19	25.64	2.33	94.02
Methanol extract				5.29	5.98

The remainder of the methanol extract i.e. 5.98-5.29=0.69% is then calculated in the proportions of Satd., C_{18}' and C_{18}'' of the petroleum extract, e.g. Satd.= $\frac{0.69 \times 21.86}{(21.86+44.19+25.64)}$ =0.17%

The increments thus obtained are:-

Satd., 0.17; C_{18}', 0.33; and C_{18}'', 0.19.

The total composition is as follows:-

Satd.	C_{18}'	C_{18}''	$OH.C_{18}'$	Total
22.0	44.5	25.9	7.6	100

(iii) The C_{18}'' content of the mixed acids obtained from the absorption at 234mμ after isomerisation gives a check on one of the components:- $C_{18}'' = \frac{236.3 \times 100}{906}$ 26.1%

3. Method C: (S. verrucosus)

$OH.C_{18}'$ester, by infra-red spectrometry..... 7.00%

All other characteristics are the same as given for Method A.

(i). The $OH.C_{18}'$ester content is converted to acid content (wt.%) as follows:-

	$OH.C_{18}'$	Total
esters(wt.%)	7.00	100
S.E. of esters	312.5	294.1
esters(equivs.)	$\frac{7.00}{312.5}$	$\frac{100}{294.1}$ .....(α)

continued, $OH.C_{18}'$ Total

(α) x S.E.of acids $\frac{7.00 \times 298.5}{312.5} = 6.69$; $\frac{100 \times 280.1}{294.1} = 95.24$.

Therefore $OH.C_{18}'$ acid (wt.%) = $\frac{6.69 \times 100}{95.24}$ 7.02%

(ii). C_{18}'' = $\frac{235.9 \times 100}{906}$ 26.0%

(iii). Satd. esters = Satd. acids 23.2%

(iv). Therefore C_{18}' = 100-(7.02+26.0+23.2) 43.8%

(v). Mean M.Wt. of acetylated esters,..... 296.5
as in Method A

Whence, I.V. of mixed acids = $\frac{87.4 \times 296.5}{280.1}$ 92.5

Contribution of C_{18}'' = $\frac{26.0 \times 180.9}{100}$ 47.0

Contribution of $OH.C_{18}'$ = $\frac{7.02 \times 85}{100}$ 6.0

$\therefore$ Contribution of C_{18}' = 92.5-(47.0+6.0) 39.5

Whence C_{18}' = $\frac{39.5 \times 100}{89.8}$ 44.0%

and Satd. acids = 100-(7.0+26.0+44.0) 23.0%

Summary of results:

	Satd.	C_{18}'	C_{18}''	$OH.C_{18}'$
Satd. by oxidn.	23.2	(43.8)	26.0	7.0
C_{18}' by I.V.	(23.0)	44.0		

III. Identification of hydroxy acids.

1. 9-Hydroxyoctadec-12-enoic acid:

This was identified as the higher melting erythro-9:12:13-trihydroxystearic acid resulting from permanganate oxidation according to the Lapworth and Mottram procedure[31]. From three oils (S. verrucosus, S. congoensis and S. intermedius) the hydroxy acid concentrates were separately oxidised and the product was identical to that previously obtained by Gunstone[11]. From the other seven oils (S. gratus, S. Welwitschii, S. Basinii, S. mahoensis, S. Nicholsonii, S. Schuchardtii and S. Tholonii) the mixed acids were combined, the hydroxy acids concentrated by partition and then oxidised. The same erythro-trihydroxystearic acid was so readily isolated in good yield that it is unlikely that any other monohydroxyoctadecenoic acid is present. (Details are given on p. 186).

2. (-)-erythro-9:10-Dihydroxystearic acid:

During the purification of 9-hydroxyoctadec-12-enoic acid (see p. 184) from the hydroxy acid concentrate of S. verrucosus seed oil ca.65mg. (0.08% of total mixed acids) of an acid (M.pt. 138.5-140°) separated from acetone at 0°. This was shown (see p. 158) to be (-)-erythro-9:10-dihydroxystearic acid identical with the dihydroxy acid known to accompany ricinoleic acid in castor oil[30]. The discovery of this acid occurred after all the analyses had been carried out and most of the hydroxy concentrates

had been used in other studies. A concentrate of hydroxy acid from S. sarmentosus seed oil was available, however, and this yielded the same acid (ca.0.07% of total mixed acids, M.pt. 139-141.5°).

IV. Results.

Tables III and IV show the characteristics of the oils and the analytical results respectively. The characteristics of those oils examined by Gunstone are included in Table III for comparison:-

Table III.

Characteristics of Strophanthus Oils.

	Oil				Mixed acids ex Non-sap.			Acetylated esters	
	I.V.	S.E.	Free acid	n /T	N.S.	I.V.	S.E.	I.V.	S.E.
S.s.(S) [a]	93	292.2	1.2	1.4720/17	2.2	98 [b]	280.5 [b]		
S.s.(F) [a]	87	300.8	1.4	1.4682/17	0.9	95 [b]	284.3 [b]		
S.h. [a]	98	293.7	5.3	1.4655/17	1.3	105 [b]	284.7 [b]		
S.Cour.	95	294.5	1.2	1.4694/17	1.2	103	284.3		
S.Cour.			1.2		1.2		274.8 [c]	89.9	
S.v.(B)	92	298.1	1.1	1.4744/13	2.2	94	279.5 [c]		
S.v.(A)		299.1			2.5	95	280.1	87.4	280.3
S.cong.	85	300.3	4.9	1.4740/13	1.3	94	279.0		
S.i.	91	294.1	2.0	1.4746/13	1.8	95	278.9		
S.g.	100	295.6	2.2	1.4750/13	1.5	95	280.9 [c]	89.9	272.6
S.V.	86	289.5	5.5	1.4760/13	4.4	89	273.7 [c]	81.6	267.7
S.E.	87	298.0	5.2	1.4745/13	1.7	93	281.4 [c]	88.3	265.2
S.N.	101	298.1	3.0	1.4749/13	1.9	105	280.5 [c]	97.0	274.9
S.a.	90	298.5	2.6	1.4738/13	2.6	94	280.5 [c]	84.8	274.9
S.Sch.	90	297.5	1.4	1.4738/13	2.0	92	281.9 [c]	82.1	270.4
S.T.	97	297.1	5.4	1.4735/13	1.7	98	280.6	90.1	272.8

[a] Ref. 11. [b] These figures include unsaponifiable. [c] Calc. from S.E. of esters.

Table IV - Analytical Results for Strophanthus Oils.

	Method A				Method B				Method C			
	Satd.	C18'	C18"	OHC18'	Satd.	C18'	C18"	OHC18'	Satd.	C18'	C18"	OHC18'
S.S.	22.0 (25.0)	(36.0) 33.0	31.5	10.5					22.0 (25.0)	(36.5) 33.5	31.5	10.0
S.W.	24.5 (30.0)	(39.5) 34.0	26.5	9.5					24.5 (29.5)	(38.0) 33.0	26.5	11.0
S.Cour.	21.0 (26.0)	(36.0) 31.0	33.0	10.0[a]								
S.E.	26.5 (26.5)	(27.0) 27.0	32.0	14.5					26.5 (26.0)	(26.0) 26.5	32.0	15.5
S.H.	25.0 (22.0)	(29.5) 32.5	36.5	9.0					25.0 (22.0)	(29.5 32.5	36.5	9.0
S.a.	24.0 (23.5)	(42.0) 43.5	24.0	9.0					24.0 (23.5)	(42.0) 42.5	24.0	10.0
S.Seh.	23.5 (23.5)	(43.0) 43.0	21.5	12.0					23.5 (23.5)	(44.0) 44.0	21.0	11.5
S.T.	25.5 (21.5)	(36.0) 40.0	28.5	10.0					25.5 (21.5)	(39.0) 43.0	28.5	7.0
S.v.	23.5 (23.0)	(44.5) 45.0	26.0	6.0	(22.0)	44.5	26.0 26.0[b]	7.5	23.0 (23.0)	(44.0) 44.0	26.0	7.0
S.cong.					(23.5)	39.0	27.5 28.0[b]	10.0	(24.0)	39.5	27.5	9.0 11.5[c]
S.i.					(24.0)	38.0	28.0 29.0[b]	10.0	(24.5)	39.5	28.5	10.0 9.5[c]

[a] OHC18' taken from previous work; [b] Derived from $E^{1\%}_{1cm}$ of total mixed acids; [c] Infra-red determination on total mixed esters; Figures in brackets are derived by difference.

Discussion.

I. Discussion of results.

The following generalisations and correlations are apparent from this and previous studies covering fourteen species of the *Strophanthus* genus:-

1. 9-Hydroxyoctadec-12-enoic acid is present in each of the fourteen species examined, in small amount (6 to 15%), and it is reasonable to suppose that this acid is distributed throughout the genus.

The optical enantiomorph of *erythro*-9:10-dihydroxystearic acid which is present in *S. verrucosus* and *S. sarmentosus* may occur throughout the genus in amounts of 0.1% or less. This is the first record of its occurrence outside the *Ricinus* genus.

2. The iodine values of the oils studied lie within the range 86 - 101* and when listed in order of decreasing proportion of linoleic acid (see Table V overleaf) it is seen that:-

(a). Despite the variation in iodine value the content of saturated acids is fairly constant (24±3%) most of the oils containing 24 or 25% of saturated acids.

(b). The amount of hydroxy acid present varies from 6 - 15% but there seems to be little relationship between this and the iodine value.

* These iodine values are unreliable and probably high due to the interference of hydroxy acids (see p. 113).

(c). The changes in iodine value arise mainly from varying amounts of linoleic and oleic acid. The values in Table V show clearly that as the content of linoleic acid falls from 37 to 21% that of oleic acid rises from 29 to 43%.

Table V.

Oil	I.V.	I.V. of acids (calc.)	C_{18}''	C_{18}'	Satd.	$OH.C_{18}'$	$C_{18}'' + OH.C_{18}'$	Group
S.N.	101	97	37	29	25	9	46	2
S.E.	87	90	32	27	27	14	46	2
S.g.	100	88	32	36	22	10	42	1
S.h.	98	-	30	34	21	15	45	2
S.Cour.	95	90	30	39	21	10	40	3
S.g.(S)	93	-	30	38	25	7	37	?
S.T.	97	90	28	37	25	10	38	3
S.i.	91	-	28	38	24	10	38	3
S.cong.	88	82	27	39	24	10	37	3
S.W.	86	87	27	39	24	10	37	3
S.g.(F)	87	-	26	43	24	7	33	?
S.V.	92	87	26	45	24	6	32	?
S.a.	90	85	24	43	24	9	33	3
S.Sch.	90	82	21	43	24	12	33	3

3. There is some correlation in the locality of growth and the order shown in Table V above. Those at the top of the table grow mainly in the regions of drier climate - Senegal, the French Sudan and Northern Nigeria, whereas those towards

the bottom of the table are found in the more tropical conditions of French Guinea, Western Ghana, French Cameroons etc.

This variation of linoleic and oleic acids, the former decreasing and the latter increasing as the locality of growth approaches the equator is similar, though not so pronounced, as the variation in linoleic and oleic acid content of sunflower seed oil with temperature of growth[33]. The saturated acids, as in the different varieties of sunflower seed oil tend to remain constant in spite of the variations in unsaturated acids. It would be interesting to grow some varieties in this country and note any variations in the resultant oils.

4. The various species of Strophanthus have been classified with respect to their aglycone content[34] and on this basis have been divided into five groups. These were described as follows:-

Group 1	Group 2	Group 3	Group 4	Group 5
S.gratus	S.Emini	S.amboensis	2 listed	1 listed
	S.hispidus	S.congoensis		
	S.Nicholsonii	S.Courmontii		
	+	S.intermedius		
	9 others listed	S.sarmentosus		
		S.Thollonii		
		S.Schuchardtii		
		S.Welwitschii		
		+ 7 others		

Group 1. The "Sarverogenin producing variety" which contain from 0.05 - 0.2% of sarveroside and panstroside. The locality of growth is eastern part of Ivory Coast, Southern Nigeria, the Cameroons and the Belgian Congo.

Group 2. The "Sarmentogenin producing variety" which contain 0.1 - 0.9% of sarmentocymarin and 0.1 - 0.4% of sarnovid and grow in Senegal, French Sudan and Northern Nigeria, bounded by the sea in the west, the desert in the north and the 13th. parallel in the south.

Group 3. The "Glycoside-poor variety" which contain no, or less than 0.01% of sarveroside and 0.02% of sarmentocymarin and grow in French Guinea and the Western part of Ghana. In this group S. sarmentosus has a special position (see p. 134).

Group 4. The "Sarmutogenin producing variety" consisting of two species, and

Group 5. S. Vanderijstii which forms a section of its own with unique glycosides.

The scources of the seed oils now examined are divided into Groups 1, 2 and 3 and it is of interest that when listed in order of decreasing linoleic acid content (Table V) these groups of seeds fall together. The three members of Group 2 are in the top four listed, along with the sole representative of Group 1 while the members of

Group 3 lie together at the foot of the table. If the oils are listed in decreasing order of linoleic plus hydroxy acid content the placings of S. gratus and S. hispidus are inverted and the separation into groups is complete. If the hydroxy acid is a precursor of linoleic acid (or vice versa), as seems possible, this method of arrangement is perhaps preferable.

S. sarmentosus is a polymorphic species with variants ranging from extreme "Forest Form" to extreme "Savannah Form"[5]. These correspond to a sarverogenin extreme and a sarmentogenin extreme, the intermediate forms containing mixtures of these aglycones and belonging to Group 3.

Of the two S. sarmentosus oils examined by Gunstone, that designated Forest form is not extreme Forest form and the other is probably also an intermediate variant and both therefore belong to Group 3.

S. verracosus has not been analysed for aglycone content but, from the fatty acid composition, it fits into Group 3 and thus should contain both sarmentogenin and sarverogenin. It will be interesting to see whether this is so when the glycosides of this species are examined.

II. Discussion of methods.

1. Method A:

This method gives satisfactory results. Of the alternative procedures (determination of saturated acids directly by oxidation and determination of oleic acid from the iodine value) the former is preferred because it is less open to error. Errors in OH.C_{18}', C_{18}" and in I.V. would all be incorporated in the value calculated for C_{18}'. It follows of course that the oleic acid figures obtained by difference may not be as accurate as the rest. The two procedures check roughly being within 3% in most cases and in no case more than 5%.

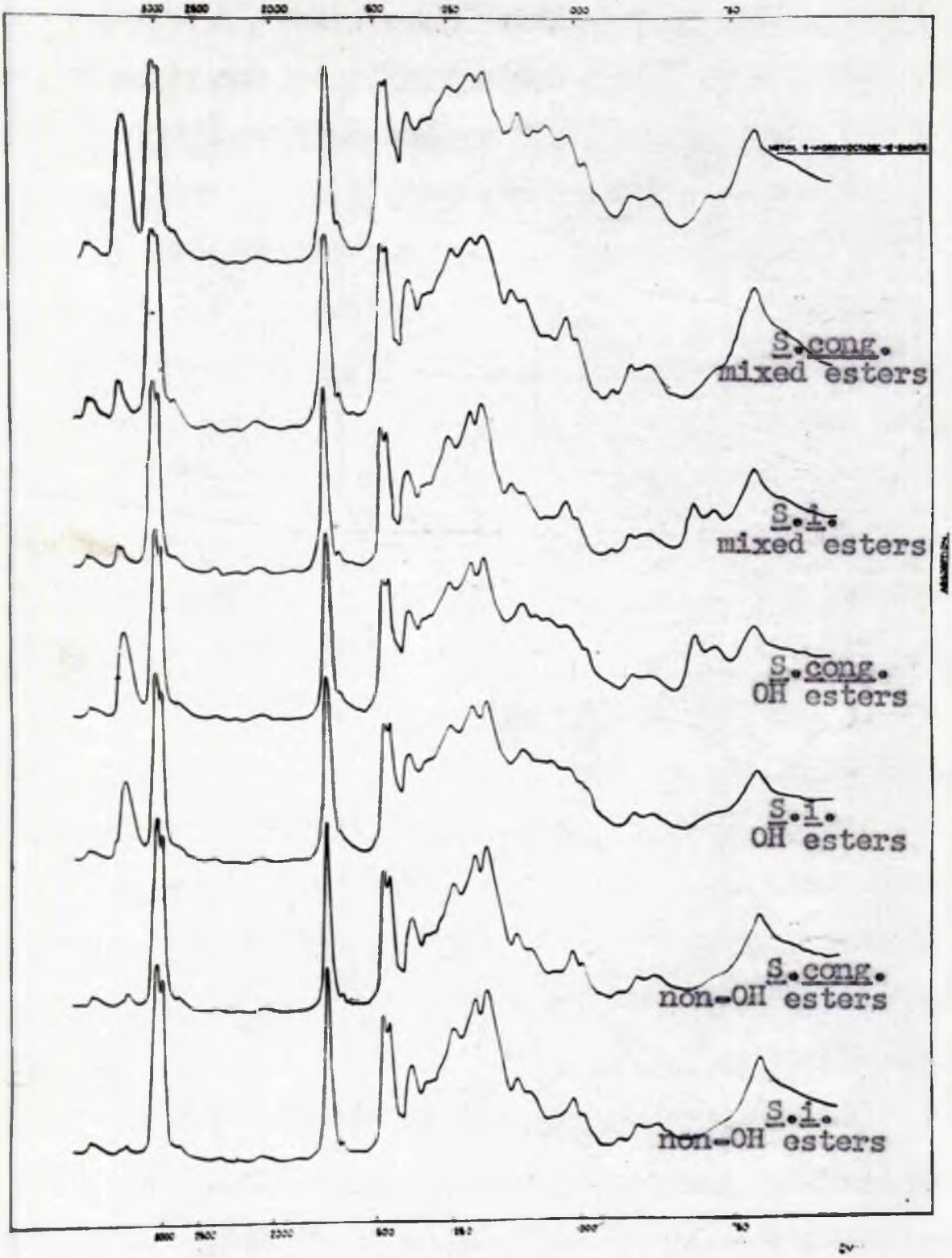
2. Method B:

Two disadvantages are inherent in this procedure though neither is very serious:-

(a). The assumption made that the non-hydroxy acids in the methanol extract have the same composition as the petroleum extract is not entirely valid since this component will be enriched in unsaturated acids. This results in a high value for saturated acids and low values for oleic and, particularly, linoleic acids; cf. C_{18}" determined from the total mixed acids is higher in each case than the calculated value. The results indicate that better values would be obtained in these oils if the non-hydroxy components of the methanol fraction were assumed to be made up of oleic and linoleic acids in the ratio 3:4.

Figure IV.

Infra-red Curves of Strophanthus Ester Fractions.



The C_{18}" content of the methanol fraction could be determined directly from the absorption at 234mμ after isomerisation thus removing uncertainty. A further check is possible by direct determination of the saturated acids in the petroleum fraction by the procedure used in Method A.

(b). The partition procedure does not entirely separate the hydroxy acid and the small amounts (1 - 2% indicated) remaining in the petroleum fraction become important since this fraction is so large. These figures cannot be far from the experimental error but that they are real is shown by the infra-red curves shown opposite (Figure IV). They are possibly too high, leading to a high value for the hydroxy acid content of the oil and this is indicated in the figures obtained by Methods A and B for S. hispidus and S. verrugosus (see Table VI, p.140).

3. Method C:

The basis of the infra-red determination of hydroxy ester content is that dilute solutions of compounds having alcoholic hydroxyl groups all show an absorption band at about 2.8μ (3600cm.$^{-1}$) which is attributed to the O-H stretching vibration[29].

Quantitative measurement is hampered (a) by association of hydroxyl groups in solution (very dilute solutions must be used) and (b) by interfering bands close to the OH band.

Measurements were made in two ways, two very different sets of values resulting (for details see p.):-

(i). Attempts to construct the pattern for the interfering bands and estimate the residual absorption due to hydroxy ester by comparison with calibration lines (prepared from mixtures of know concentration) gave low results in all cases but one (compare Tables IV and XIV). This method is considered to be inaccurate due to the uncertainty of the interfering band pattern and low values are considered likely.

(ii). The other mode of estimation was to compare the persistence (i.e. the height above a base line) of the OH-band with that of pure methyl 9-hydroxyoctadec-12-enoate, disregarding the interfering bands. This is expected to give rather high results and in general this is so although agreement with the chemically derived values is generally fairly good (see Table IV).

That the method is as yet unreliable is shown by the measurements on S. congoensis and S. intermedius. The hydroxy ester contents of the mixed esters and of the esters of the methanol and petroleum extracts were determined and from the last two values the hydroxy acid content of the mixed acids of the two oils was calculated and found to be 2.2units % low and 0.6units % high in comparison with the values measured.

4. Method D:

The method used by Gunstone[11] has not been used in the present work but discussion of the results obtained by it

and a comparison with the other methods is relevant here.

The main disadvantage is in the reliance placed on the stability of the acetylated hydroxy ester during distillation. Any degradation of this compound during the distillation will result in a low value for the hydroxy acid content of the oil. Evidence for this can be seen from the figures for S. hispidus (Table VI).

Approximations used in the calculation lead to a slight uncertainty in the method, though the values involved are small and unlikely to affect the final result very much.

5. Conclusions:

Method A is the most convenient method and is of satisfactory accuracy and this is preferred for the analysis of Strophanthus oils and other oils of low monohydroxy acid content. The method is not one of total analysis since saturated acids are obtained as a composite fraction but, if a sufficient quantity of oil were available, these components could be determined by fractional distillation after low temperature crystallisation or lead salt separation thus giving a total analysis.

Method C is not yet sufficiently developed to provide an accurate alternative to A or B.

Table VI.

Comparison of Analytical Methods.

	Method A				Method B				Method C				Method D			
	Satd	C18'	C18"	OHC18'	Satd	C18'	C18"	OHC18'	Satd	C18'	C18"	OHC18'	Satd	C18'	C18"	OHC18'
S.h.	55.0		30.0	15.0	21.5	34.0	28.5	16.0					21.0	35.5	30.0	13.5
S.Occur.	21.0	36.0	33.0	10.0[a]									21.0	38.5	30.5	10.0
S.V.	23.5	44.5	26.0	6.0	22.0	44.5	26.0	7.5	23.0	44.0	26.0	7.0				
S.T.	25.5	36.0	28.5	10.0					25.5	39.0	28.5	7.0				
S.cong.					23.5	39.0	27.5	10.0	24.0	39.5	27.5	9.0 11.5[b]				

[a] OHC18' value taken from previous work (Method D)[11].

[b] OHC18' in mixed acids, determined directly by infra-red measurement.

All analyses of S. hispidus are previous work[11,12].

Details of Experimental.

1. Preparation of fractions:

The general methods of preparing the mixed acids, free from unsaponifiable material, partition of these acids and preparation of the ester and acetylated ester fractions will be described for S. verrucosus. The resulting fractions were those used in the Method B analysis of this oil.

Some of the oils were shown to contain traces of solvent and all oils were therefore heated on the steam bath under vaccuum for 1 hour to remove any volatile material.

(a). Preparation of mixed acids (ex non-sap.):

S. verrucosus seed oil (101.8g.) in ethanol (500ml.) solution was refluxed for 0.5 hr. with potassium hydroxide (45g.) in water (45ml.). About half the ethanol was then distilled off and the remaining solution cooled and diluted with water.

This soap solution was extracted three times with ether (portions of 500ml.) and the ether extract washed three times with water, alternately with 3N.potassium hydroxide and water (3 times each) and finally with water till neutral. The ether extract was dried and distilled and the mixed acids were recovered by ether extraction after acidification of the soap solution and all washings:-

Non-saponifiable material ..... 2.13g.

Mixed acids (ex N.S.) ..... 93.30g.

(b). Partition of mixed acids:

Petroleum (B.pt. 40-60°) and 80% methanol were first equilibrated in a large separatory funnel.

To 900ml. of the petroleum in each of two separatory funnels (1 & 2) was added about 46g. of the mixed acids and 500ml. of the same solvent was placed in funnels 3 & 4. 80% Methanol (900ml.) was then added to the first funnel and, after equilibration, passed through each of the other three funnels in turn. This was followed by three other portions of 80% methanol (900ml. each) and the acids recovered from the two extracts:-

Petroleum soluble acids ..... 86.68g.

Methanol soluble acids ..... 5.51g.

(c). Preparation of esters:

The methanol soluble acids (5.51g.) were allowed to stand overnight at room temperature in solution in methanol (25ml.) containing dry hydrogen chloride (0.3g., ca.1%). The mixture was then diluted with water and extracted with ether, the extract being washed with 3%.potassium hydroxide solution, with water till neutral and finally dried and distilled to give the esters (5.71g.)

The petroleum soluble acids (83.68g.) were similarly esterified to give 86.82g.

(d). Preparation of acetylated esters:

The esters of the hydroxy acid concentrate (5.71g.) were refluxed for 3hr. with acetic anhydride (17ml., 3 vol.)

and then water (12ml., ca.2 vol.) added and reflux continued for a further hour. The mixture was subsequently diluted with water and extracted with ether, the extract being washed with water, 3N.potassium hydroxide and again with water till neutral and finally dried and distilled to give acetylated esters (6.23g.).

The non-hydroxy esters (54.20g.) similarly provided 53.54g. of acetylated esters.

x x x

For analytical methods A and C the partition step was omitted and the mixed acids esterified and acetylated as above.

2. Analytical procedures.

(a). General:

Saponification equivalents were determined in the usual way[35] and iodine values by the Wijs procedure[37]. Ultra-violet absorption ($E^{1\%}_{1cm}$) at 234mμ was determined before and after isomerisation[37], isomerisation being carried out with 7.5% potassium hydroxide in ethylene glycol at 180° for 60 minutes.

(b). Saturated esters:

The saturated ester content of the mixed esterswas determined by oxidation by Kartha's method[30b] and separation of acidic scission products on an alumina column[30a]:-

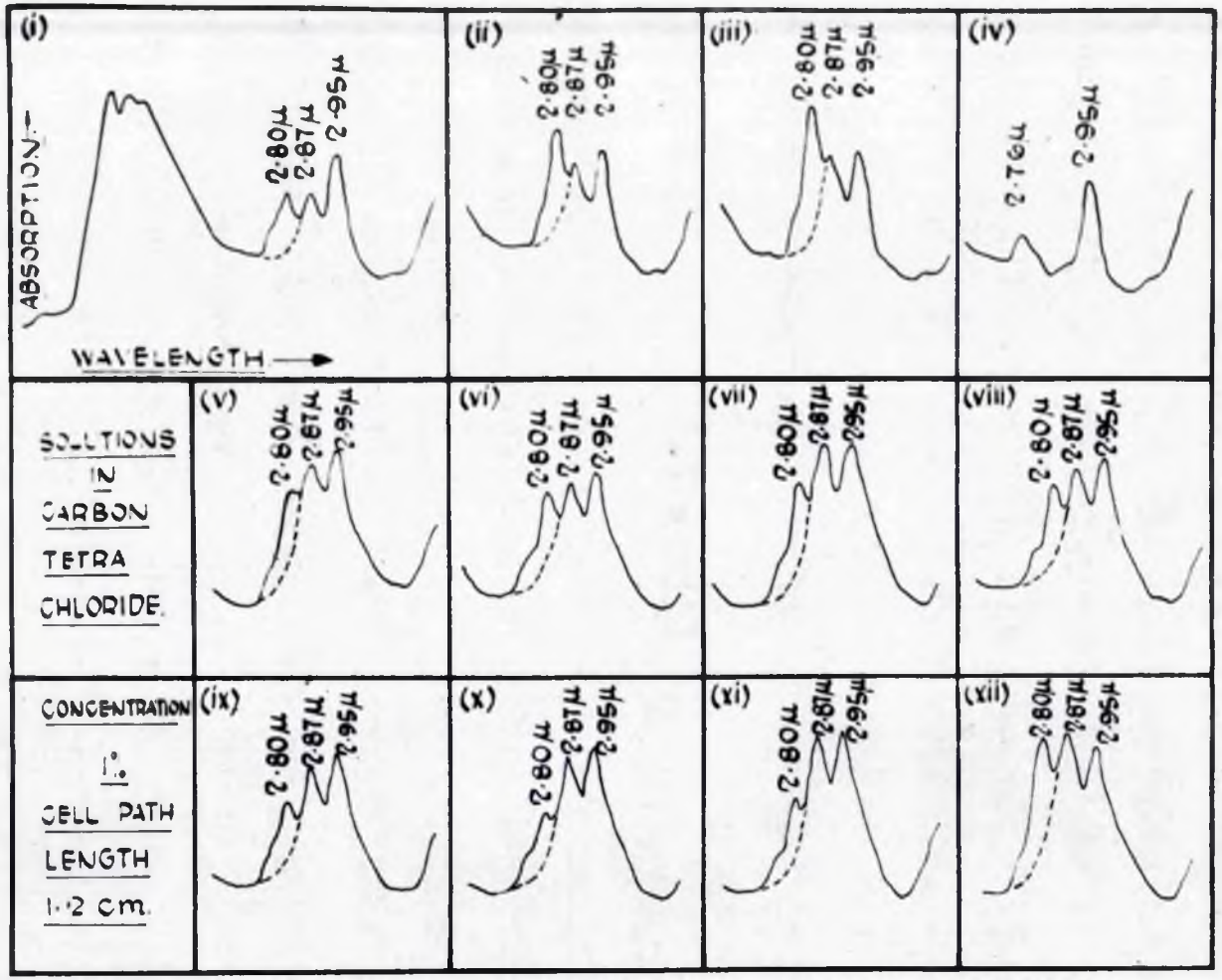
The mixed esters (ca.0.5g.) were dissolved in hot

acetone (20ml.) and acetic acid (1.2ml.) and potassium permanganate (1.5g.) was added, and the mixture refluxed for about 15 min. More acetic acid (0.6ml.) and permanganate (1.5g.) were then added and reflux continued for a further 30 min. This procedure was repeated twice more (total addition of $KMnO_4$ is 6.0g. and acetic acid is 3.0ml.) and reflux continued to a total time of 3hr. The solvent was removed under reduced pressure, water added to the residue and sulphur dioxide gas bubbled into the acidified solution till decolourisation was complete. The resulting solution was carefully extracted with four portions of chloroform (20ml.) and the chloroform extract washed once with water (50ml.) and dried (Na_2SO_4).

The dry solution was run through a tinted alumina column (25g., ca. 10 x 2cm.) and the saturated esters eluted with chloroform (ca.250ml.). The acidic components remain in the top half of the column as can be seen from the change of colour of the methyl orange incorporated in the alumina. The solvent was distilled from the eluate and the remaining esters weighed, their iodine value determined and a correction made for any positive value assuming it to be due to oleic acid.

In an experiment designed to check this procedure a mixture of esters and azelaic and nonanoic acids was percolated through the column. The esters were recovered in high yield (98%) after 200ml. of eluate had been

Figure V.



INFRA RED SPECTRA OF MIXTURES OF HYDROXY AND NON-HYDROXY ESTERS:
(i),(ii)and(iii) respectively 5.07,11.10 and 14.36% Methyl 9-hydroxyoctadec-12-enoate in Methyl oleate, (iv) Methyl oleate, (v) S.Welwitschi, (vi) S.gratus, (vii) S.Schuchardtii, (viii) S.amboensis, (ix) S.verrucosus, (x) S.Thollonii, (xi) S.Nicholsonii, (xii) S.Eminii.

collected showing that the separation was efficient.

A test oxidation on methyl palmitate (I.V.= 0) gave 98% recovery (I.V.= 0) and a test determination on the mixed esters of S. Courmontii gave a value of 20.9% compared to Gunstone's value for the same oil of 20.8%.

The first two of these tests involved very much more saturated esters than are present in the Strophanthus samples and recovery of saturated esters from these latter is likely to be better than 98% using the same volume of eluent.

Determinations were done in duplicate and agreement was always good.

(c). Infra-red determinations:

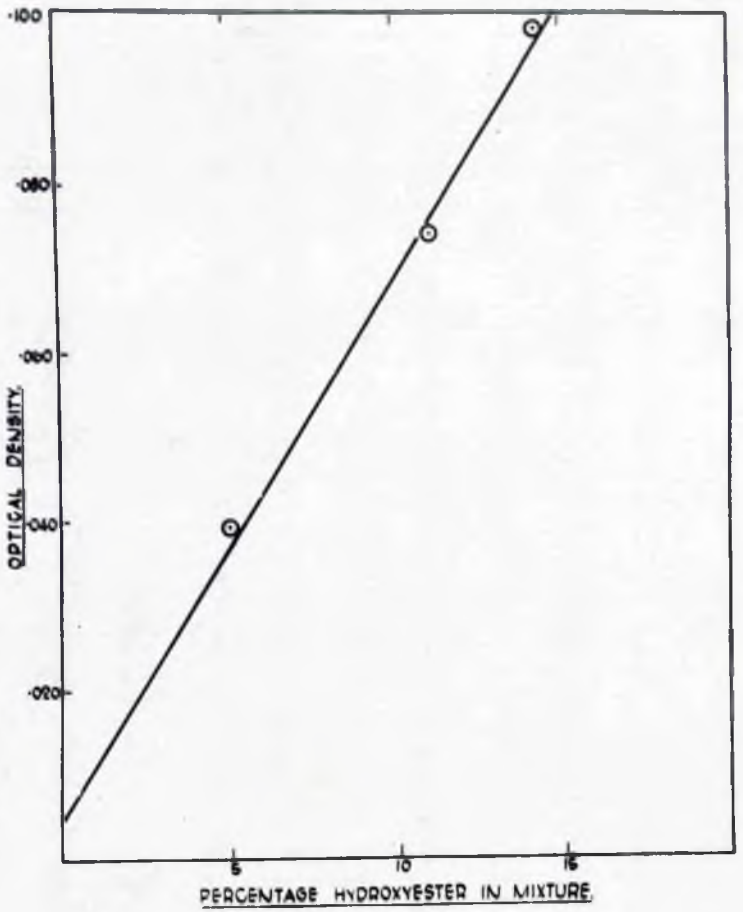
The infra-red measurements of hydroxy ester content were carried out by Dr. D. Chapman and Dr. J.F. Macey in the Research Department of Unilever Limited, Port Sunlight.

A Grubb-Parsons Recording Double Beam Spectrometer was used with a slit width of 1.2mm. Two identical sodium chloride cells of path length 1.2cm. each were filled with carbon tetrachloride and balanced. The solvent in the sample cell was then replaced by the ester solution (1%, i.e. hydroxy ester is ca. 0.1%) and the spectrum recorded. This procedure was carried out for pure methyl-9-hydroxyoctadec-12-enoate and on mixed esters, hydroxy esters and non-hydroxy esters of S. congolensis and S. intermedius. These curves are shown in Figure IV, p. 136.

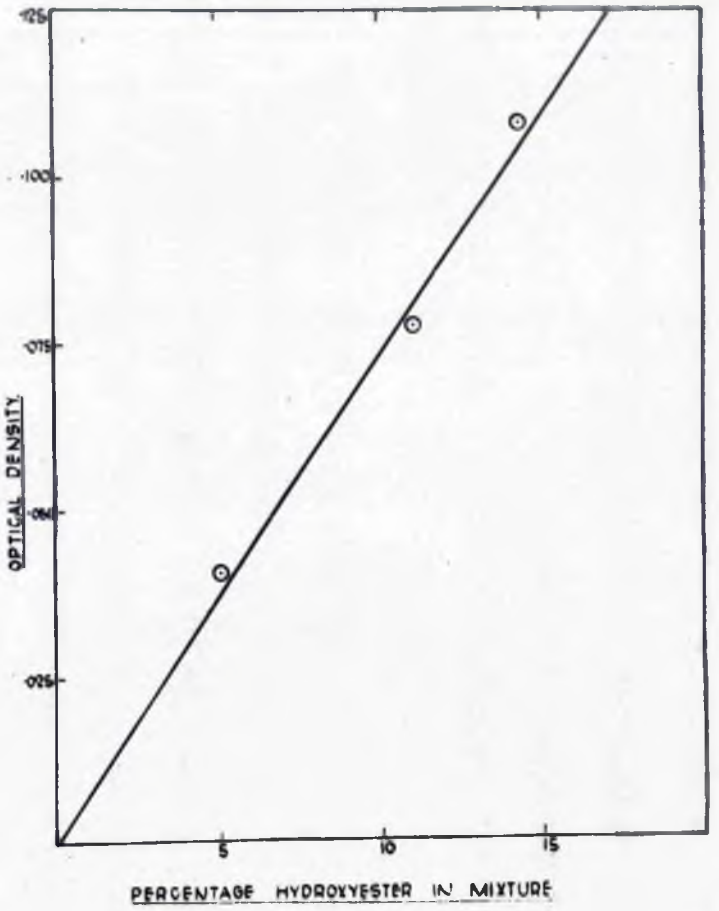
This procedure was also used to give curves (Figure V, opp.)

Figure VI.

GRAPH (i) OF OPTICAL DENSITY OF HYDROXYL BAND V. PERCENTAGE HYDROXYESTER



GRAPH (ii) OF OPTICAL DENSITY OF HYDROXYL BAND V. PERCENTAGE HYDROXYESTER



for eight Strophanthus mixed esters, three reference solutions containing 5.07, 11.10 and 14.36% respectively of methyl 9-hydroxyoctadec-12-enoate in methyl oleate, and for pure methyl oleate.

The spectra, in the 2.7 - 3.0μ region, recorded for mixtures of hydroxy-and non-hydroxy esters exhibited four absorption bands. Two of these (near 2.76μ and 2.95μ) were present in the spectrum of methyl oleate solution, the band near 2.80μ was the unassociated hydroxyl band and the band near 2.87μ could not be assigned.

Estimation of hydroxyl ester content was made in two ways:-

(i). by measurement of the persistence of the hydroxyl band at 2.8μ compared with that of the pure hydroxy ester or those of the mixtures of known concentration and making no allowance for the interfering band at 2.87μ. This method is considered to give slightly high results. The values are given in Tables XIV and IV.

(ii) by construction of the pattern of the bands at 2.87μ and 2.95μ and subtraction of the absorption at 2.8μ due to the tail of these bands from the total absorption at that wavelength. The resulting measures of optical density for the three known mixtures were found to lie near a straight line when plotted against the percentage hydroxy ester, (see Figure VI opposite). This straight line provided the calibration from which the concentrations of hydroxy ester

in the various unknown samples could be estimated. Two completely separate estimations were carried outby this method and the divergence between the various pairs of values was between about -5% and +5%.

The values are given in Tables XII and XIII.

3. Results in detail.

The following tables give the data from which the compositions of the oils, shown in Table IV, were calculated. The characteristics of the oils have already been given in Table III.

Table VII - Saponification Equivalents - Method A.

Oil	Saponification Equivalents of Esters				OH 18' (wt.%)
	Before acetylation		After acetylation		in mixed acids
S.g.	294.5 294.9 295.3 294.8	294.9	272.6 272.7 272.6 272.6	272.6	10.3
S.E.	288.2 288.4 288.7 285.6	287.7	267.7 267.8 267.7	267.7	9.7
S.E.	295.3 295.6 295.2 295,6	295.4	265.3 265.1 265.2 265.3	265.2	14.4
S.N.	294.4 294.4 294.5 294.5	294.5	275.4 274.9 274.6 274.8	274.9	9.0
S.a.	294.5 294.5 294.5 294.5	294.5	274.8 274.9 275.3 274.6	274.9	9.0
S.Sch.	296.2 296.1 295.8 295.6	295.9	271.0 270.7 270.7 269.6	270.4	11.8
S.T.	294.8 294.5 294.9 294.3	294.6[a]	272.9 272.8 272.9 272.6	272.8	10.1
S.Y.	294.3 293.6 294.6 293.9	294.1	280.3 280.5 280.2 280.3	280.3	6.2

[a] These values are calculated from the saponification equivalents of the mixed acids.

Table VIII - Saturated Ester Values and Ultra-violet After Alkali Isomerisation - Method A.

Oil	Satd. Esters (wt.%)	$E^{1\%}_{1cm}$ (234mμ), mixed acids	C18"
S.G.	21.2, 21.7 } 22.0	291.1, 288.7, 282.3, 283.4 } 286.4	31.6
S.F.	24.2, 24.4 } 24.3	244.5, 240.6, 240.2, 238.1 } 240.9	26.6
S.Cour.	20.8, 21.0 } 20.9	296.2, 297.0, 298.3, 297.5 } 297.3	32.8
S.E.	26.4, 26.6 } 26.5	287.7, 290.7, 286.4, 290.5 } 288.7	31.9
S.H.	24.8, 25.0 } 24.9	330.1, 334.7, 331.8 } 332.2	36.7
S.a.	24.1, 24.1 } 24.1	220.3, 214.7, 219.3 } 218.1	24.1
S.Sch.	23.7, 23.5 } 23.6	191.3, 192.5, 196.2 } 193.3	21.3
S.P.	25.3, 25.5 } 25.4	254.3, 259.9, 257.6 } 257.3	28.4
S.V.	23.1, 23.3 } 23.2	236.5, 237.7, 234.2, 235.2 } 235.9	26.0

Table IX. Iodine Values - Method A.

Oil	I.V. of Acetylated Esters	Mean Mol. Wt. (calc.)	I.V. of Acids (calc.)
S.g.	89.8, 90.0 } 89.9	299.0	95.7
S.W.	81.7, 81.5 } 81.6	291.4	86.9
S.Cour.	89.6, 90.1 } 89.9	292.7*	95.8
S.E.	88.4, 88.0, 88.6 } 88.3	301.1	94.5
S.M.	97.1, 96.8 } 97.0	298.0	103.1
S.a.	84.9, 84.7 } 84.8	298.0	90.1
S.Sch.	82.1, 82.1 } 82.1	300.6	87.5
S.T.	90.1, 90.1 } 90.1	298.6	95.9

* This value is calculated from :-

$$\text{Mean Mol.Wt.} = \frac{42G(E - 14)}{100M}, \text{ since}$$

no value of D was available (see p. 161).

Table X. Saponification Equivalents - Method B.

Oil (Fraction)	Saponification Equivalent of Esters				OHC_{18}, (wt.%) in fraction
	Before acetylation		After acetylation		
S.v. (OH acids)	308.6 308.6	308.6*	184.1 184.3 184.5	184.3	88.5
S.v. (non-OH)	288.9 288.5 288.5	288.6	283.2 283.1 283.0	283.1	2.5
S.cong. (OH acids)	299.5 299.7 299.3 299.4	299.5*	187.7 188.3 188.2 188.2	188.1	79.7
S.cong. (non-OH)	289.6 288.8 288.7 287.8	288.7	283.2 282.8 281.3 281.9	282.3	2.9
S.i. (OH-acids)	307.6 307.5 307.4 307.5	307.5*	197.4 196.9 196.1 196.4	196.7	73.8
S.i. (non-OH)	292.9 292.8 293.3 292.9	293.0	289.8 290.0 289.2 289.4	289.6	1.5

* These values were calculated from the saponification equivalents of the acids.

Table XI.

Iodine Values and U.V. Absorption after Isomerisation-Method B.

Oil (Fraction)	Iodine Value	$E^{1\%}_{1cm}$ (234μ)	C_{18}-(wt%) in fraction
S.v. (mixed acids)		235.2 235.8 236.7 237.5 } 236.3	26.1
S.v. (non-OH acids)	93.6 93.6 } 93.6	251.8 245.3 245.3 246.1 } 247.1	27.3
S.cong. (mixed acids)		252.9 254.9 254.0 251.4 } 253.3	27.9
S.cong. (non-OH acids)	93.7 93.6 } 93.7	266.2 270.0 266.1 268.7 } 267.8	29.6
S.i. (mixed acids)		261.3 262.3 } 261.8	28.8
S.i. (non-OH acids)	93.7 94.2 } 94.0	278.3 278.7 } 278.5	30.7

Table XII.

Preparation of Calibration Lines for Infra-red Measurements.

OHC_{18}, ester content of mixture (wt.%)	(i)		(ii)	
	Optical density	Spread from line (%)	Optical density	Spread (%)
5.07	.0388	-0.33	.0403	-0.46
11.10	.0740	+0.24	.0775	+0.42
14.36	.0982	-0.30	.1081	-0.50

The spread of each point from the calibration line is given as percentage of hydroxy ester.

Table XII. % Hydroxy esters by I.R. estimation - Method C.

Esters	OHC_{18} ester by persistence	(i) Optical density	(i) OHC_{18} ester	(ii) Optical density	(ii) OHC_{18} ester	Av. of i + ii
S.g. (mixed)	9.70	.0522	7.43	.0566	7.79	7.61
S.W. (mixed)	10.90	.0444	6.20	.0480	6.60	6.40
S.E. (mixed)	15.32	.0908	13.48	.0939	12.95	13.22
S.M. (mixed)	9.10	.0508	7.20	.0522	7.15	7.18
S.g. (mixed)	9.75	.0541	7.70	.0554	7.61	7.65
S.Sch. (mixed)	11.40	.0670	9.75	.0737	10.15	9.95
S.T. (mixed)	7.20	.0375	5.15	.o395	5.42	5.29
S.v. (mixed)	7.00	.0454	6.35	.0478	6.56	6.46
S.cong. (mixed)	11.4					
S.cong. (OH)	74.2					
S.cong. (non-OH)	2.7					
S.i. (mixed)	9.4					
SVi. (OH)	69.6					
S.i. (non-OH)	2.2					

Table XIV.

Component acids of oils using average $OHC_{18'}$ acid content obtained by optical density measurement in the infra-red.

Oil	Satd.	$C_{18'}$	$C_{18''}$	$OHC_{18'}$
S.g.	22.0 (25.0)	(39.0) 36.0	31.5	7.5
S.W.	24.5 (30.0)	(42.5) 37.0	26.5	6.5
S.E.	26.5 (26.0)	(28.0) 28.5	32.0	13.5
S.M.	25.0 (22.0)	(31.0) 34.0	37.0	7.0
S.a.	24.0 (23.5)	(44.0) 44.5	24.0	8.0
S.T.	25.5 (21.5)	(40.5) 44.5	28.5	5.5
S.V.	23.0 (23.0)	(44.5) 44.5	26.0	6.5
S.Sch.	23.5 (23.5)	(45.0) 45.0	21.5	10.0

Compare with Table IV, p. 129.

4. The dihydroxy acid present in S. verrucosus and S. sarmentosus seed oils.

The dihydroxy acid was isolated from the methanol soluble fractions of S. verrucosus and S. sarmentosus by crystallisation from acetone and ethyl acetate, respectively, at 0° and it was shown to have a glycol value of 98.2%.

The recrystallised acid (18mg., M.pt. 139-141°) was oxidised (see p. 94) in aqueous solution (50ml.) containing permanganate/periodate oxidant solution (10ml.) and enough potassium carbonate to give a pH of 8 - 9. The solution was allowed to stand for 15hr. before oxidation was stopped and the acids recovered as a partly solid residue (17.6mg.) which separated into a petroleum soluble fraction (7.4mg.) which gave the p-bromophenacyl ester of nonanoic acid (M.pt. 59-65°, mixed M.pt. with authentic sample 60-65°), and an insoluble residue (8.0mg., M.pt. 80-95°) which was recrystallised to give azelaic acid (M.pt. and mixed M.pt. 103-6°)

The structure is thus 9:10-dihydroxystearic acid and that it is the same enantiomorph as the acid present in castor oil is shown by the fact that there is no depression of the mixed melting point. Mixing of opposite enantiomorphs would have resulted in the racemate (M.pt. 132°).

Appendix to Part III.

1. Derivation of Equation for Determination of Hydroxy acid Content.

The following equation relates the content of mono-hydroxy acid (% wt.) in admixture with non-hydroxy acids to the saponification equivalents of the mixed esters before and after acetylation:-

Sap.equiv. of mixed esters ..... E

Sap.equiv. of acetylated mixed esters ..... D

Sap.equiv. of non-hydroxy esters ..... N

Molecular wt. of hydroxy acid ..... M

Wt. % of hydroxy acid in mixed acids ..... G

Composition of mixed acids:-

	OH acids		non-OH acids		Total
Weight %	G	+	100 - G	=	100
Moles.	$\frac{G}{M}$	+	$\frac{100 - G}{N}$	=	$\frac{100}{E - 14}$
wt. after acetylation and esterification	$\frac{G(M+56)}{M}$	+	$\frac{(100-G)(N+14)}{N}$		
This will react with	$\frac{2G}{M}$	+	$\frac{100-G}{N}$	moles of KOH.	

$$\therefore \text{Sap. equiv. of acetylated esters} = \frac{\frac{G(M+56)}{M} + \frac{(100-G)(N+14)}{N}}{\frac{2G}{M} + \frac{100-G}{N}} = D \quad \ldots\ldots(i)$$

Similarly the sap. equiv. of the esters before acetylation is:-

$$\frac{\frac{G(M+14)}{M} + \frac{(100-G)(N+14)}{N}}{\frac{G}{M} + \frac{100-G}{N}} = E \quad \ldots\ldots(ii)$$

There are now two unknowns (G and N) in two equations and these can be solved:-

From (i):- $$N = \frac{M(D-14)(100-G)}{(56G+100M-2DG)} \quad \ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

From (ii):- $$N = \frac{M(E-14)(100-G)}{(14G+100M-EG)} \quad \ldots\ldots\ldots\ldots\ldots \text{(iv)}$$

Equating these:-

$$\frac{M(D-14)(100-G)}{(56G+100M-2DG)} = \frac{M(E-14)(100-G)}{(14G+100M-EG)}$$

whence:- $$G = \frac{100M(D-E)}{(D-42)(E-14)}$$

2. Derivation of Equation for Determination of Mean Molecular Weight of Acetylated Esters.

Mixed acids contain G% of a single monohydroxy acid of molecular weight ..... M

Sap. equiv. of mixed esters ..... E

Sap. equiv. of acetylated mixed esters ..... D

Sap. equiv. of non-hydroxy esters ..... N

Composition of mixed acids:-

	OH acids		non-OH acids		Total
Weight %	G	+	100 - G	=	100
Moles.	$\frac{G}{M}$	+	$\frac{100-G}{N}$	=	$\frac{100}{E-14}$ ..(v)

Weight of acetylated esters $= \frac{G(M+56)}{M} + \frac{(100-G)(N+14)}{N}$

$$\therefore \text{Mean Mol. Wt.} = \frac{\frac{G(M+56)}{M} + \frac{(100-G)(N+14)}{N}}{\frac{100}{E-14}} \quad \ldots\ldots\ldots \text{(vi)}$$

From (v):- $$N = \frac{M(E-14)(100-G)}{100M-G(E-14)}$$

Substituting this value of N in (vi):-

$$\text{Mean Mol. Wt.} = E + \frac{42G(E-14)}{100N}$$

or, since $$G = \frac{100M(E-D)}{(D-42)(E-14)}$$ (p. 161)

$$\text{Mean Mol. Wt.} = E + \frac{42(E-D)}{(D-42)} \quad \text{or} \quad \frac{D(E-42)}{(D-42)}$$

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Part IV.

Configurational Studies.

Introduction.

I. Optically active hydroxy acids ($C_{x,n}$).

1. General:

Oxygenated fatty acids, other than ω-hydroxy acids, have one or more asymmetric centres at the positions of the oxy-functions and are thus capable of existing in enantiomorphic forms. Where these acids are produced naturally only one of these forms is, in general, formed and optical activity results. Synthetic acids are racemic unless an optically active group is already present in the molecule.

The number of possible enantiomorphic forms of an acid is 2^n, where n is the number of asymmetric centres in the molecule. Thus a dihydroxy acid can show four optical isomers and a tetrahydroxy acid sixteen.

2. Monohydroxy acids (Table XV, p. 171):

(a). Ricinoleic acid is the most common optically active monohydroxy acid (see p. 58). Hawke[1] has rigorously purified this acid and reports a specific rotation of +7.79° (no solvent). Other values reported are +7.86° (no solvent)[2], +7.15° (acetone)[3], and +9.3° (acetic acid)[4], the variation being probably due to solvent effects.

(b). The other monohydroxy acid of proved optical activity is 9-hydroxyoctadec-cis-12-enoic acid present in Strophanthus oils and in the present work this acid has been purified and a specific rotation of +3.5° (acetic acid) measured.

The rotations for the trans-isomers of these acids and other related acids have also been measured.

3. Dihydroxy acids(Table XVI, p. 172):

(a). erythro-9:10-dihydroxystearic acid occurs naturally in one of its forms in castor oil[5] and in Strophanthus oils (present work) and is considered to be laevo-rotatory, though the value is very small[5].

Racemic threo- and erythro-9:10-dihydroxystearic acids have been resolved into their components[6], the threo-isomers showing high rotations (± 24°) and the erythro-forms negligible rotations. Whether a true separation of the erythro-forms has been effected is open to doubt since the resulting acid has a melting point of 133° (cf. 141° for the naturally occurring acid and 132° for the racemate). The specific rotations of compounds derived from these were also given[6].

Other claims of resolution of these acids and related compounds[7] are unconvincing in view of the rotations obtained and some of these resolutions could not be repeated[6].

(b). 12:13-dihydroxy acids showing optical activity have been obtained from 12:13-epoxyoleic acid[8] which occurs naturally with a dextro-rotation.

(c). 15:16-dihydroxylinolenic acid ($[\alpha]_D$ +8.2° calc.) has been shown to be present in Camelina sativa seed oil (present work) and the saturated acid (+3.5°) has been prepared.

4. Trihydroxy acids (Table XVII, p.173):

(a). 9:10:12-trihydroxystearic acids were prepared from natural ricinoleic acid by Kass and Radlove[9], all four isomers being obtained as optically active forms.

(b). 9:12:13-trihydroxystearic acids have been prepared in the present work and their rotations measured.

5. Tetrahydroxy acids (Table XVIII, p. 174):

Eight 9:10:12:13-tetrahydroxystearic acids having optical activity were prepared by Bharucha and Gunstone[6] from naturally occurring 12:13-epoxyoleic acid.

II. Absolute optical configurations.

Ricinoleic acid is the only long chain hydroxy acid whose absolute configuration is known. This acid, on degradation, produces β-hydroxynonanoic acid with specific rotation $+2^{\circ}26'$ (ethanol)[10]. Synthetic L-β-hydroxynonanoic acid of proven configuration and specific rotation -3° (ethanol) has been prepared by Serck-Hanssen and Stenhagen[11] which, when mixed with the dextro-rotatory acid from natural sources, gave the racemic compound. These authors therefore consider ricinoleic acid to have the D-configuration and thus be 12-D-hydroxy-cis-9-octadecenoic acid.

Table XV - Monohydroxy compounds

Compound	M.pt.	$[\alpha]_D$	Solvent	Observed Rotation	Ref.
12-OH-oleic acid	-	+ 9·3°	AcOH		4
12-OH-elaidic acid	52-3°	+ 6·7	?		*
12-OH-stearolic acid	52-3·5°	+ 10·4	AcOH	+ 2·07°	
12-OH-stearic acid	78-9°	- 0·7	AcOH	- 0·14°	
		- 0·4	Pyridine		2
Methyl-12-OH-stearate		- 0·3	AcOH	- 0·11	
9-OH-iso-oleic acid	30-2	+ 3·5	AcOH	+ 0·28	
9-OH-iso-elaidic acid	56·5-9·5	+ 1·3	AcOH	+ 0·07	
9-OH-stearic acid	81-2	+ 0·4	AcOH	+ 0·03	

* Hilditch, "Chemical Constitution of Natural Fats", 1956, 3rd. Ed. p.517 (London: Chapman and Hall)

Table XVI - Dihydroxy compounds

Acid	M.pt.	$[\alpha]_D$	Solvent	Observed Rotation	Ref.
erythro-9:10-diOH-stearic	141°	- 0·15°	MeOH		5
	133	+ 0·25	MeOH	+ 0·01°	6
threo-9:10-diOH-stearic	99·5	± 24	MeOH	± 2·4	6
erythro-12:13-diOH-stearic	125·5-6·5	(+ 3)	AcOH	(+ 0·02)	8
		- 1·7	EtOH	- 0·03	8
erythro-12:13-diOH-oleic	87-8	+ 1·2	AcOH	+ 0·12	8
		- 3·6		- 0·29	8
threo-12:13-diOH-stearic	95-6	(+ 1)	AcOH	+ 0·01	8
		- 1·3	EtOH	- 0·05	8
threo-12:13-diOH-oleic	53-4	- 6·8	AcOH	- 0·69	8
		- 5·7	EtOH	- 0·51	8
threo-15:16-diOH-stearic	89·5-90	+ 3·3	EtOH	+ 0·26	
threo-15:15-diOH-linoleic	-	+ 8·2 (calc)	EtOH	+ 0·16	

Figures in brackets are within the experimental error.

Table XVII - Trihydroxy compounds

-Stearic acid	M.pt.	$[\alpha]_D$	Solvent	Observed Rotation	Ref.
erythro-9:10:12-triOH	138°	- 116°	AcOH		9
		- 3·9	EtOH		9
"	112	- 6·6	AcOH		9
		- 2·9	EtOH		9
threo-9:10:12-triOH	110	- 38·7	AcOH		9
		- 26·6	EtOH		9
"	87	+ 21·8	AcOH		9
		+ 19·1	EtOH		9
erythro-9:12:13-triOH	148·5-50	+ 5·5	AcOH	+ ·11°	
"	102·5	(+ 0·1)	AcOH	(+ ·01)	
threo-9:12:13-triOH	89·5-90	+28·5	AcOH	+2·28	
"	?	laevo	AcOH	laevo	

Figures in brackets are within the experimental error

Table XVIII - Tetrahydroxy compounds. (Ref.8)

-Stearic acid	M.pt.	$[\alpha]_D$	Solvent	Observed Rotation
erthro-9:10-erythro-12:13-tetraOH-	177°	- 10°	AcOH	- 0·04°
		(+ 6)	EtOH	(+ 0·01)
"	156	(+ 14)	AcOH	(+ 0·02)
erythro-9:10-threo-12:13-tetraOH-	165	(- 6)	AcOH	(- 0·01)
"	117	-	-	-
threo-9:10-threo-12:13-tetraOH-	156	- 44	AcOH	- 0·35
		- 7	EtOH	- 0·03
"	130	+ 14	AcOH	+ 0·10
		+ 5	EtOH	+ 0·03
threo-9:10-threo-12:13-tetraOH-	148	-	-	-
"	122	- 9	AcOH	- 0·04

Figures in brackets are within the experimental error.

III. Methods available for the establishment of configuration.

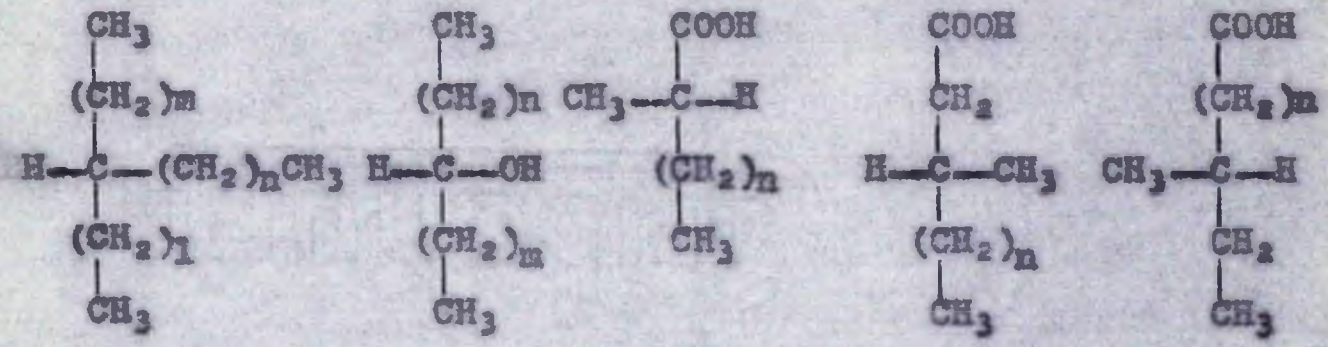
Mills and Klyne[12], in an excellent review of this subject, say:- "The configurations of two compounds may be correlated by purely chemical methods, such as degradation or synthesis that does not involve an asymmetric centre, ..., or a displacement reaction that involves an asymmetric centre but is known to be stereospecific. In other cases, a kinetic study of a displacement reaction at an asymmetric centre, or the achievement of a partial asymmetric synthesis, may provide the necessary information. Sometimes, purely physical methods may be used, as in the use of molecular rotations, the detection of 'quasi-racemic compounds' from melting point diagrams, and the use of X-ray or electron-diffraction procedures. Enzymatic methods may be valuable for many biologically important compounds." These methods are well described in the review cited.

Two of these approaches were studied in attempts to discover the configurations of these long chain hydroxy acids, and these have been termed (a) Empirical approach and (b) Synthetic approach.

(a). Empirical approach:

The statement that "A single measurement of rotation may suffice to fix the configuration of a compound beyond reasonable doubt"[12] is true for some homologous series of acyclic compounds for which several members of the series have already been investigated. For example all known

members of the series of D-branched hydrocarbons (I, where $l > m > n$), D-secondary alcohols (II, $m > n$), L-α-methyl acids (III), D-β-methyl acids (IV, $n > 1$) and L-anteisoacids have dextro-rotation:-



I II III IV V

and it may safely be predicted that other members will be dextro-rotatory.

While this method of correlation may be applied to members of other series containing one asymmetric centre, provided the configurations of representative members of the series have been determined by other methods (e.g. synthetic or degradative), it cannot in general be applied to series of compounds containing two or more asymmetric centres. The Rule of Optical Superposition[13] hardly ever holds in its original quantitative form, the contribution from any single centre being influenced in a complex way through the vicinal action of other asymmetric centres and structural features such as unsaturation. This vicinal action falls away rapidly the further the groups are separated by saturated carbon atoms.

Other methods of correlation must be used for these

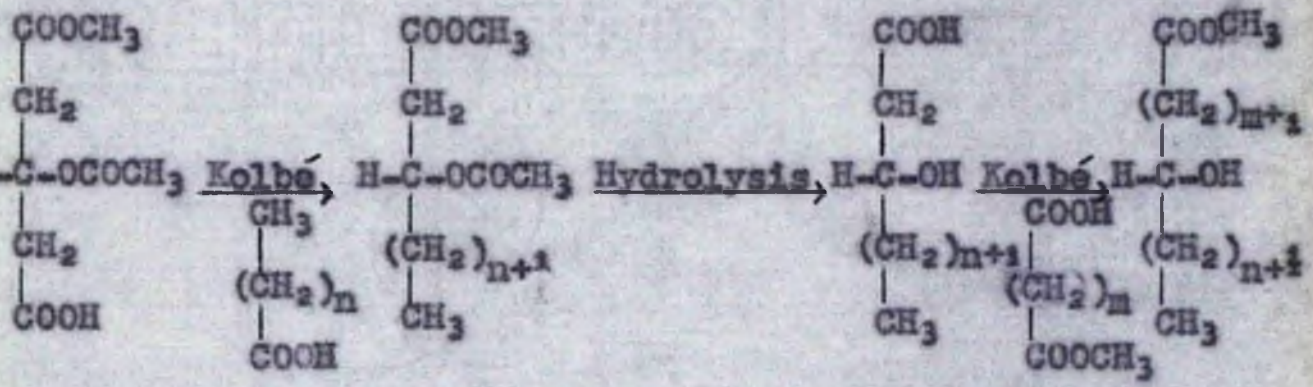
compounds but, since these were not applied in the present work, they will not be discussed here.

)b). Synthetic approach:

The configurations of long chain compounds containing one or more asymmetric groups can be determined by their synthesis from smaller molecules containing these groups in known configuration. Many branched chain fatty acids have been synthesised in this way and their configurations thus determined[14].

Although ricinoleic or ricinstearic acids have not been similarly synthesised, the antipode of its oxidation scission product (3-D-hydroxynonanoic acid) has been synthesised[11] from methyl hydrogen β-acetoxyglutarate of known configuration[15].

It is clearly possible to synthesise ricinstearic acid, 9-hydroxystearic acid and other monohydroxystearic acids from one or other of the enantiomers of methyl hydrogen β-acetoxy glutarate and obtain products of known configuration:-



It is also possible to visualise syntheses of long chain acids containing vicinal dihydroxy groups, starting from an

enantiomer of tartaric acid or some similar short-chain dihydroxy compound of known configuration.

x x x

These two approaches, synthetic and empirical, may be used together to correlate the configurations of a series of positional isomers; for example, if a representative series of monohydroxystearic acids of known configuration is synthesised and their rotations measured, the measurement of the rotation of any other member of the series should be enough to fix its configuration.

Discussion and Experimental.

(a). Empirical approach.

Discussion.

I. Isolation and preparation of hydroxy acids.

1. 9-Hydroxyoctadec-12-enoic acid (M.pt. 30-32°) was obtained pure from the methanol soluble fraction from the partition (see p. 142) of S. verrucosus mixed acids by fractional crystallisation from acetone, at low temperature, and from petroleum.

2. The four 9:12:13-trihydroxystearic acids were obtained by oxidation of the hydroxy acid concentrate from seven Strophanthus oils as follows:-

(i). erythro-9:12:13-trihydroxystearic acids:

Permanganate oxidation by the Lapworth and Mottram procedure gave a mixture of the erythro-trihydroxy acids which were separated by their different solubilities in chloroform (M.pts. 148.5-50°, and 102-5°; Lit. and 108-10° [17] respectively). These acids had specific rotations of +5.5° and 0.0° respectively.

(ii). threo-9:12:13-trihydroxystearic acids:

Performic acid oxidation of the hydroxy acid concentrate gave a liquid crude product from which one isomer was separated (M.pt. 89.5-90°), having a specific rotation of +28.5°.

Repeated attempts at fractional crystallisation and chromatographic separation did not yield the other isomer

pure. By analogy with the trihydroxy acids obtained from ricinoleic acid[9], of which three are laevo-rotatory and the low melting threo-acid is dextro-rotatory, it was expected that, since the three isomers isolated are dextro- (or zero) rotatory, the fourth would have a specific rotation of the order of -15°. This expectation would appear to be justified since fractions were obtained with low dextro and laevo-rotations but -4.6° was the most pronounced laevo-rotation obtained for any fraction and the low melting isomer could not be isolated in a pure state.

The results seem to indicate the formation of either a simple eutectoid or a 'quasi-racemic compound' (ref.[12] p.202) although the large range in the melting point of each fraction cannot be explained.

II. Measurement of optical rotations.

The optical rotation measurements were carried out with sodium-D light on a Franz Schmidt & Haensch (Berlin) polarimeter with a potential accuracy of 0.01°. Stainless steel polarimeter tubes of 3mm. diameter were used, the length (1 or 2dm.) chosen being dependent on the colour of the solution. All readings quoted are the mean of eight or more determinations, approaching the balance point alternately from each side. Although the potential accuracy of the instrument is 0.01° only observed readings of 0.03° or more have been taken as real.

Of the compounds whose rotations were measured

9-hydroxyoctadec-cis-12-enoic acid, threo-15:16-dihydroxystearic acid, the concentrate of threo-15:16-dihydroxylinoleic acid and the four 9:12:13-trihydroxystearic acids were prepared in this work. Samples of 12-hydroxystearic acid, 9-hydroxyoctadec-trans-12-enoic acid, 9-hydroxystearic acid and ricinstearolic acid were available and were purified and/or esterified as required.

III. Conclusions:

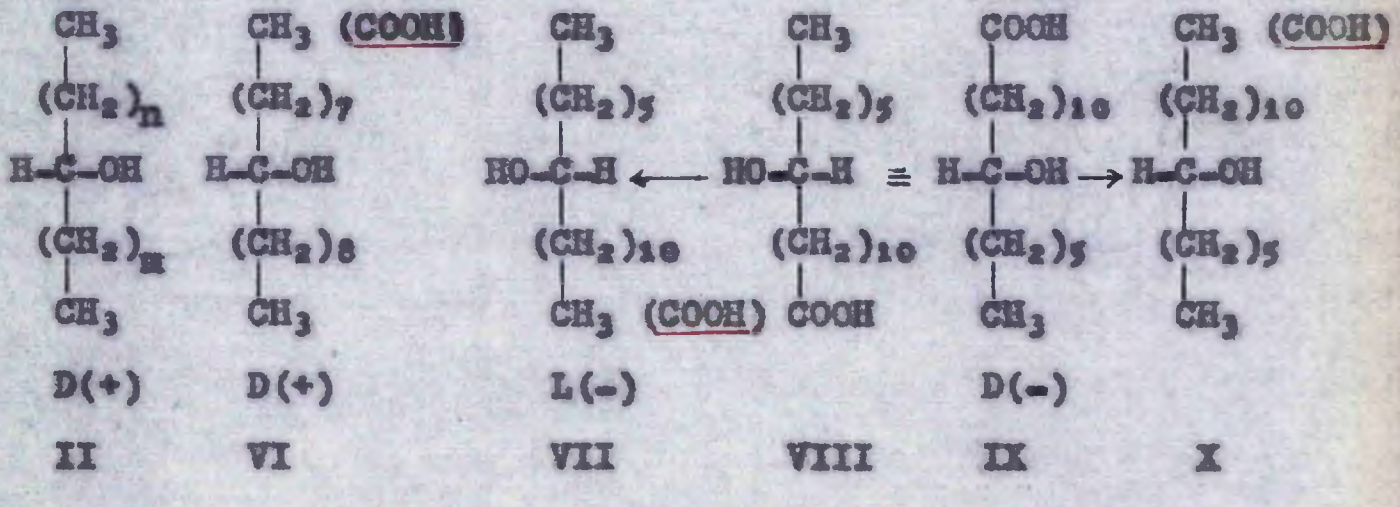
1. Ricinoleic acid has been shown to have the D-configuration, when written with the carboxyl group at the top (IX)[11], and presumably 12-hydroxyelaidic and 12-hydroxystearic acids will have the same configuration, since they are derived from ricinoleic acid by reactions which should not affect the double bond.

2.(a). The isomeric 9-hydroxy acids probably have the D-configuration also, since natural asymmetric compounds of the same type are generally produced in the same configuration (e.g. amino acids and anteiso acids, both of which belong to the L-series).

(b). Confirmatory evidence is available using the generalisation that in secondary alcohols of the type II ($m > n$) all members of the D-series are dextro-rotatory. McGhie et al.[6] have shown with 9:10-dihydroxystearic acids, modification of the end group (-COOH, $-COOCH_3$, $-CH_2OH$) has little or no effect on the observed rotation and it may be assumed that this would hold if the -COOH group were converted to $-CH_3$. Thus, considering 9-hydroxystearic acid as equivalent to

9-hydroxyoctadecane, it follows that, since it has dextro-rotation, it has the configuration VI (cf. II, $m > n \equiv 8 > 7$) and therefore belongs to the D-series.

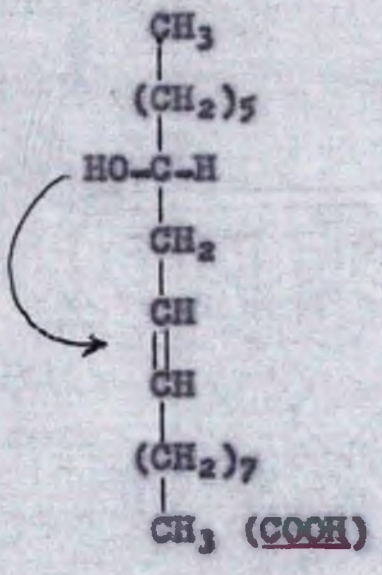
As a corollary, D-ricinstearic acid (IX) when considered as the hydroxy hydrocarbon (X) has $n > m$ (cf.II). Thus to conform with this convention, it should be rotated through 180° and be written as VII (cf.VIII) and is thus equivalent to an L-sec.-alcohol which accords with its laevo-rotation.



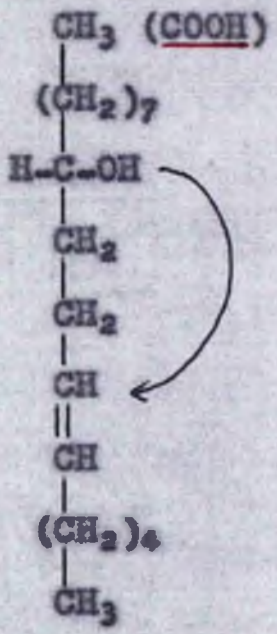
(c). The fact that the unsaturated acids have larger rotational values than the corresponding saturated acids must be due to vicinal action by the centres of unsaturation. It is noticeable that the increase in value for 9-hydroxy-octadecenoic acid, where the two centres are separated by two methylene groups, is much less than the increase for ricinoleic acid where there is a separation of only one methylene group.

The reversal of sign of rotation on going from ricinstearic to ricinoleic acid, while the sign of the 9-hydroxy acids

remains the same, must also be due to vicinal action of the double bond and a tentative explanation is given as follows:-



XI



XII

If the two acids (or the derived hydroxy hydrocarbons) are written as described in (b), (VII and VI), and the double bonds inserted, then projections XI and XII result. Now, if it is formally imagined that the increase in rotation, from saturated to unsaturated acids, is due to a directional influence from the hydroxy group to the double bond, at the OH side of the molecule (shown by arrows) then the influences are in opposite directions and if the 9-hydroxy acid gains a dextro-rotatory increase, then the increase in the 12-hydroxy acid will be laevo. These are the observed facts and this tentative suggestion, if true, lends confirmatory evidence to the suggestion that the two acids belong to the same series (D).

3. No correlation on the basis of optical superposition can be carried out for the poly-hydroxy acids and much work remains to be done before these can be satisfactorily correlated and their configurations assigned.

Experimental.

I. 9-Hydroxyoctadec-cis-12-enoic acid:

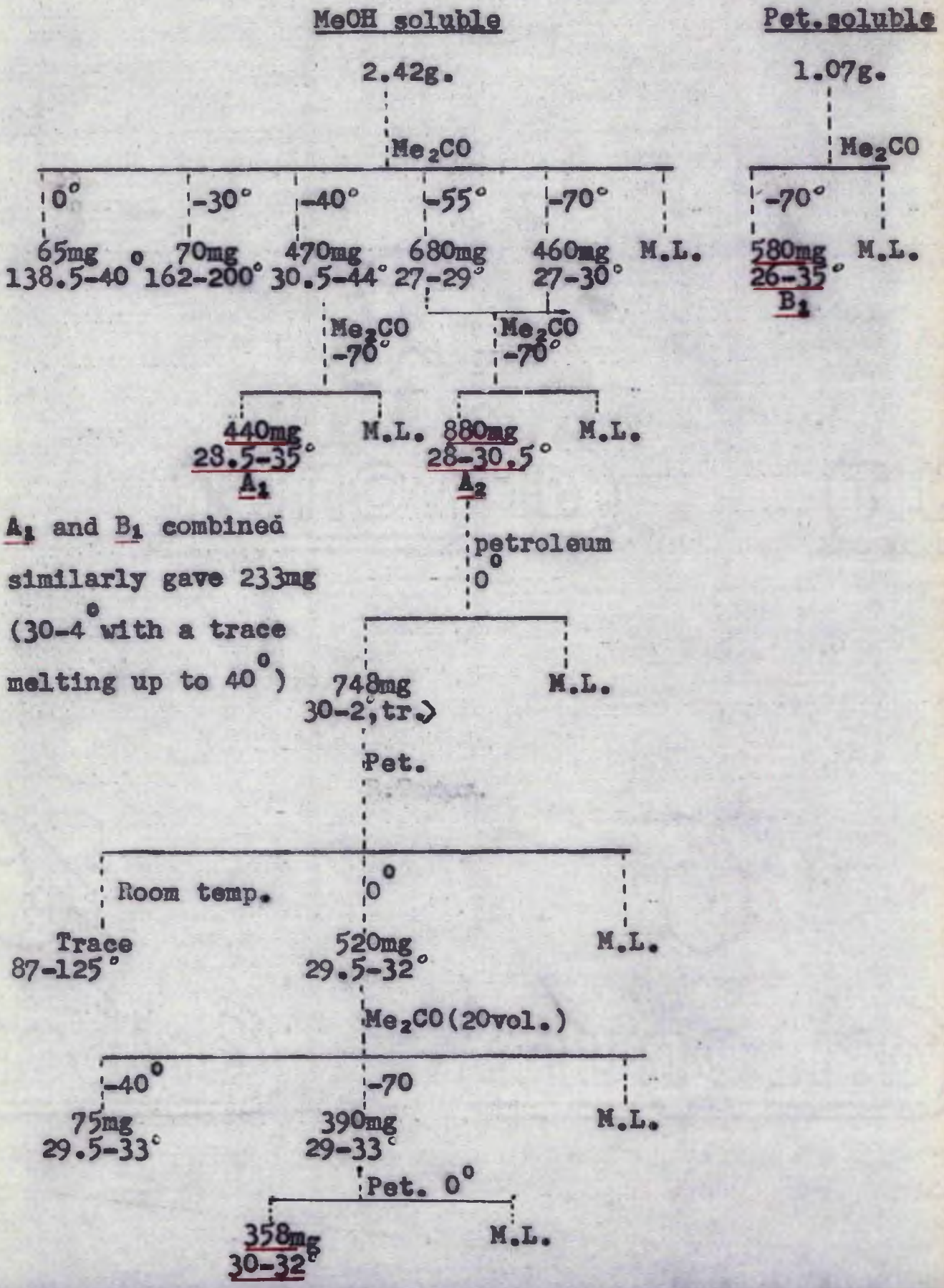
Some S. verrucosus hydroxy acid concentrate (ca. 4g.) was dissolved in 1% aqueous potassium hydroxide and the solution extracted with ether to remove any unsaponifiable matter. The recovered acids (3.59g.) were dissolved in 80% methanol and partitioned with petroleum (see p. 142), six portions (50ml. each) of petroleum being taken through the system and the methanol and petroleum fractions worked up separately to give 2.42g. and 1.07g. of acids respectively.

The methanol soluble fraction (2.42g.) was dissolved in dry acetone (50ml.) and cooled to 0° to give 65mg. of an acid (M.pt. 138.5-140°) which was shown to be (-)-erythro-9:10-dihydroxystearic acid (see p. 158).

The remaining acids were fractionally crystallised from acetone and from petroleum (B.pt. 40-60°) as shown in Figure VII, to give pure 9-hydroxyoctadec-cis-12-enoic acid (358mg., M.pt. 30-2°). A small amount of higher melting impurity which crystallised with the required acid in the earlier stages is possibly a trace of hydroxystearic acids and was separated by crystallisation from petroleum at room temperature.

Figure VII.

Purification of 9-hydroxyoctadec-cis-12-enoic acid.



II. Preparation of four 9:12:13-trihydroxystearic acids:

The mixed acids recovered from the analyses of seven Strophanthus oils (S. gratus, S. Welwitschii, S. Emini, S. Nicholsonii, S. amboensis, S. Schuchardtii and S. Thollonii) were combined (156.6g.) and partitioned between petroleum and 80% methanol (see p. 142) to give a methanol soluble hydroxy acid concentrate (13.47g.).

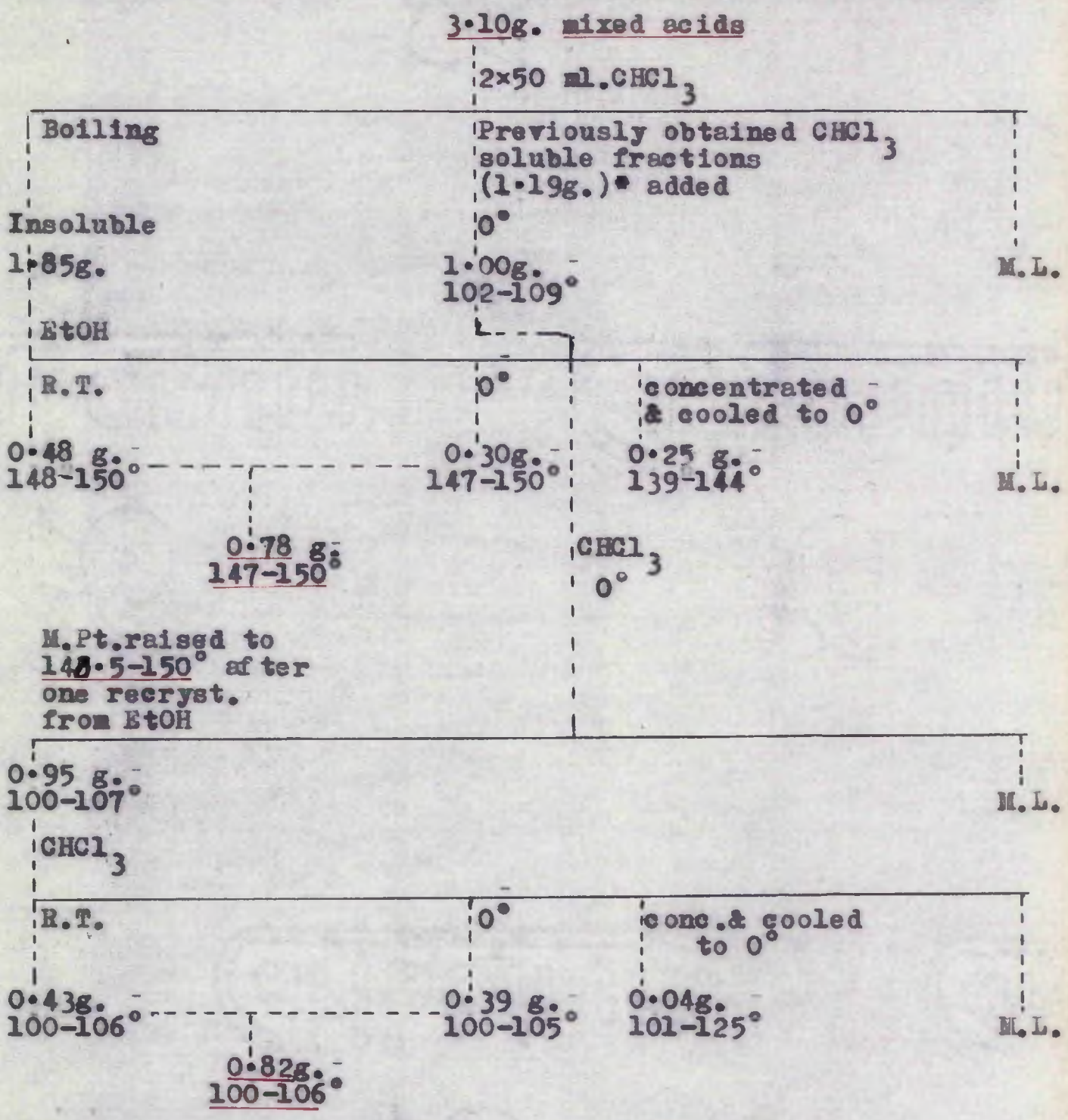
(a). Preparation of two erythro-9:12:13-trihydroxystearic acids:

A portion of the above concentrate (4.0g.) in 1% sodium hydroxide solution (400ml.) was diluted with ice water (3200ml.) and stirred during the quick addition of 1% potassium permanganate solution (320ml.). After five minutes the solution was decolourised with sulphur dioxide gas, acidified with concentrated hydrochloric acid and allowed to stand overnight before the crude product was filtered.

This product was extracted twice with boiling petroleum (B.pt. 40-60°) to remove any non-hydroxy acids and unoxidised starting material and the two isomers separated and purified as shown in Figure VIII.

Figure VIII

Separation of mixed erythro-9:12:13-trihydroxystearic acids



*Obtained during oxidation of S.verrucosus, S.congoensis and S.intermedius hydroxy acid concentrates for identification purposes.

(b). Preparation of two three-9:12:13-trihydroxystearic acids:

A portion of the hydroxy acid concentrate (9.65g.) was acetylated (see p. 142), to prevent internal dehydration between the hydroxyl and the two hydroxyl groups to be introduced, and the acetylated acids (10.57g.) dissolved in formic acid (26ml.). Hydrogen peroxide (4.0ml. of a solution containing 270.9g.H_2O_2/litre) was added and the mixture kept at 40° for 2hr. by means of cold and hot water baths. After removal of the formic acid under reduced pressure, the residue was boiled with 3N. aqueous sodium hydroxide (150ml.) for 1hr. and the soap solution poured into excess of ice cold 3N hydrochloric acid with stirring and left overnight.* The crude product was extracted with chloroform, the extract washed with portions of water till neutral and the oxidised acids recovered (9.41g. of viscous liquid).

The liquid impurities were separated and one of the isomers isolated as shown in Figure IX. Fractions A_1 and A_2 were combined and several recrystallisations from ethyl acetate and acetone gave the pure higher melting threo-acid (M.pt. 89.5-90°).

* This long contact with strong acid probably caused extensive dehydration to pyrans and furans to occur, giving rise to the large amount of liquid impurity which was found.

Fractions B_1, B_2 and B_3 were combined and subjected to extensive fractional crystallisation from ethanol containing increasing amounts of water. A further fraction of the higher melting isomer (0.25g., M.pt. 86-8°) was separated but, from the melting points of the other six fractions obtained, no separation of the lower melting isomer appeared to have occurred and these fractions were recombined.

Nitromethane, acetonitrile, ethyl acetate, chloroform, carbon tetrachloride and benzene were all tried as solvents for fractional crystallisation and in no case did any real separation appear to have been effected and all fractions were recombined (1.25g., Fraction C).

Fractional crystallisation from acetone was then attempted and the rotations, rather than the melting points, used as a guide to separation. Some separation has obviously occurred (see Figure X) and Fraction C_1 was set aside and the others recombined and, in chloroform solution, added to a column of activated silica gel (20 x 2cm.) and eluted with chloroform and then with methanol.

Six arbitrary fractions were collected and again some separation is evidenced but by no means a clear cut separation. Lack of time prevented a fuller chromatographic study.

Figure IX

Separation of the mixed threo-9:12:13-trihydroxystearic acids

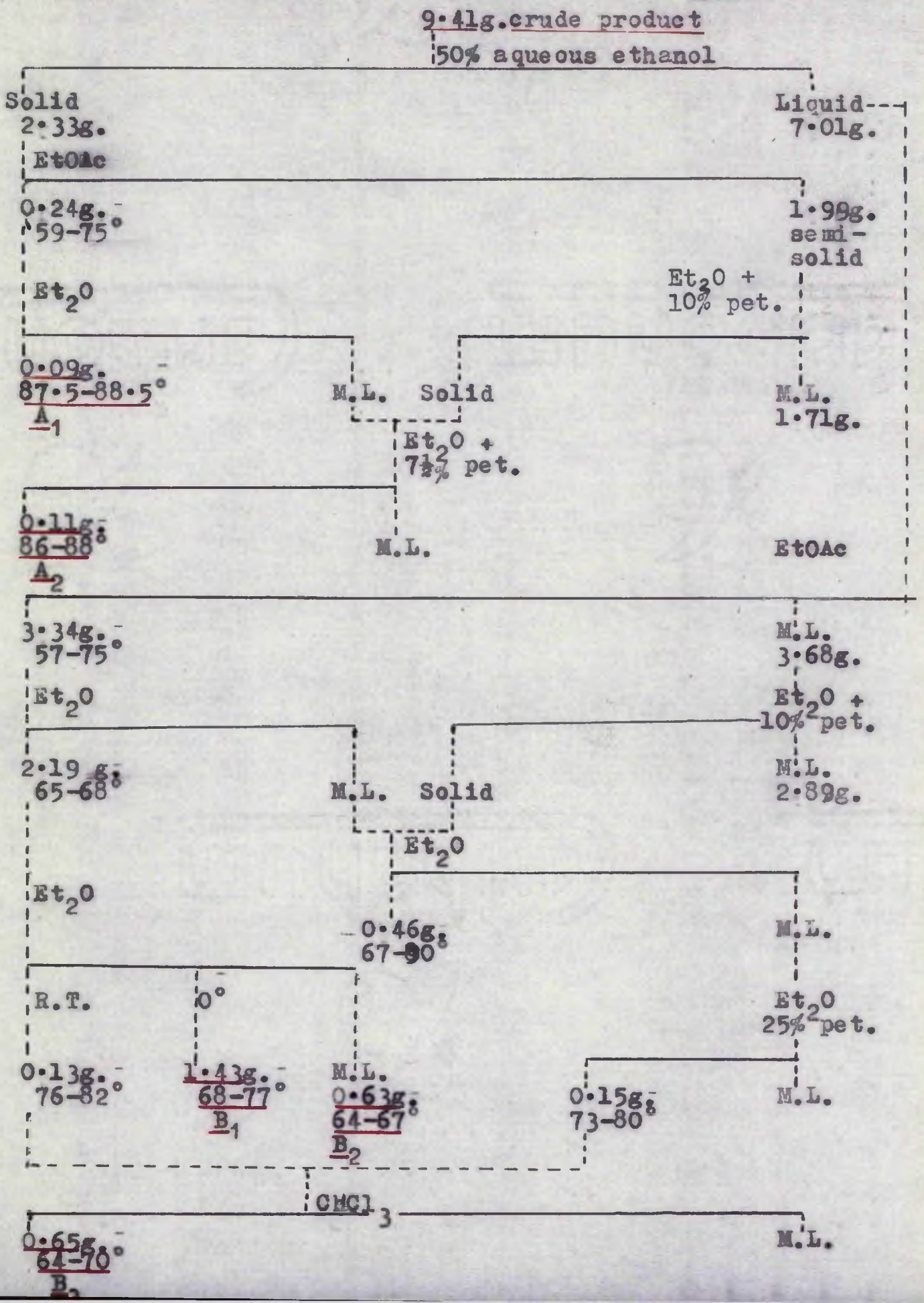
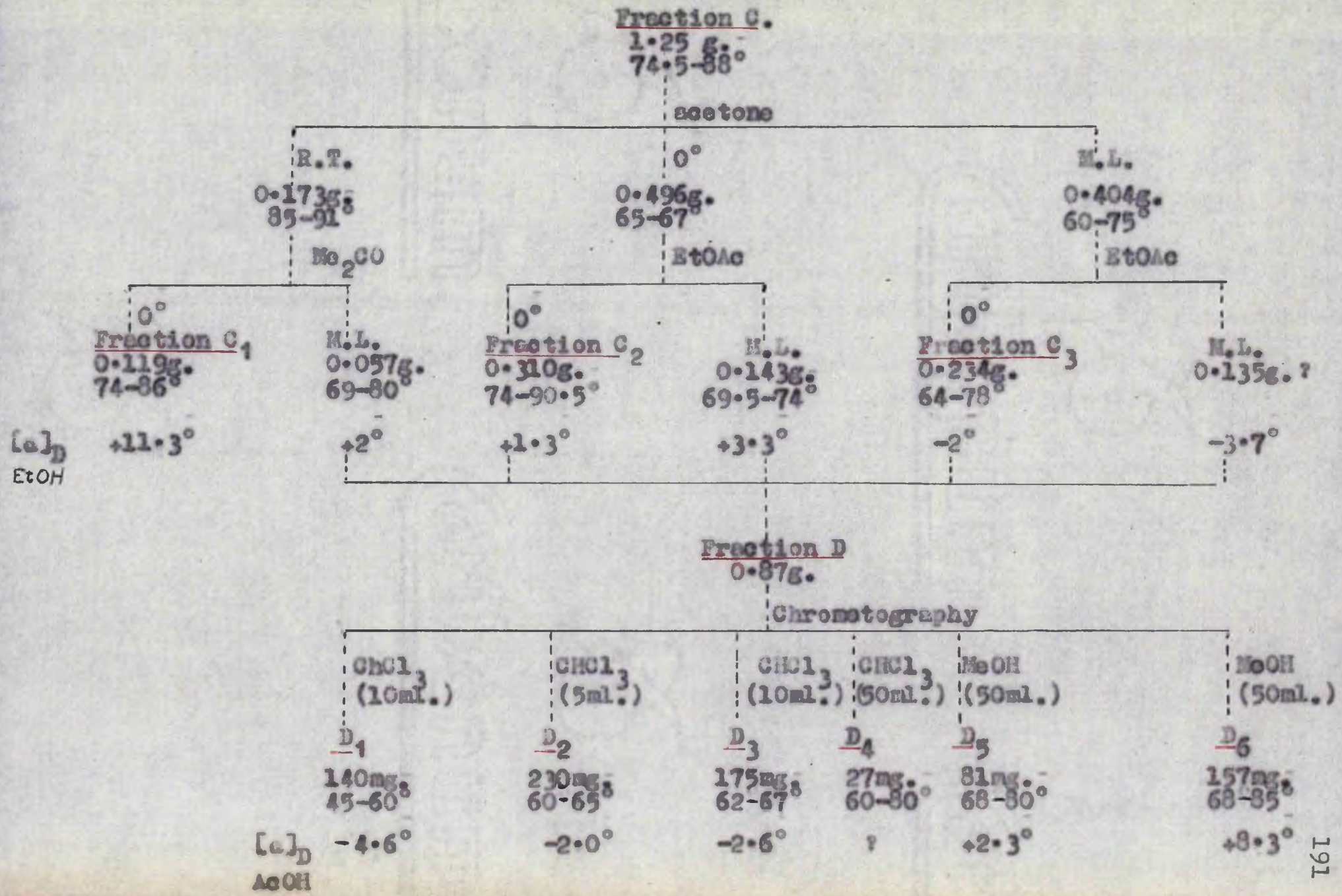


Figure X.

Further seperation of mixed threo-9:12:13-trihydroxystearic acids



(b). Synthetic approach.

Discussion.

Although the series of racemic dihydroxystearic acids, from 6:7 through to 12:13, has been prepared[18], by oxidation of olefinic compounds and reduction of diketo compounds, no synthesis of an optically active dihydroxy long-chain acid of known configuration has been reported.

Resolution of a racemic compound into its enantiomers does not give any indication of the configuration of either of these and a direct approach, by synthesis, leading to active products of known configuration was attempted.

It was hoped that it might be possible to extend the chain of (+) or (-)-tartaric acid, without affecting the asymmetric centres, to give a series of threo-dihydroxystearic acids of known configuration. The 9:10- and 12:13-dihydroxy acids so synthesised could then be compared with the 9:10-enantiomers obtained by McGhie et al.[6] by resolution and with the 12:13-enantiomer obtained by Bharucha and Gunstone[8] from cis-12:13-epoxyoleic acid.

The common methods of chain extension cannot be applied to tartaric acid for the following reasons:-

(i). The hydroxy groups must be protected by methylation and these ether groups occupy positions β- to the carboxyl group. It is known[19] that β-alkoxyhalides are very inactive towards metathetical reagents and this rules out the possibility of extension to a 3:4-substituted adipic acid

by reaction of the di-iodide with a metal cyanide, followed by hydrolysis and of extension using a substituted sodio-malonic ester or a sodio-acetylene.

(ii). Possible modes of chain extension via the acid chloride will always result in an α-keto-methoxy derivative, except in the case of the Arndt-Eistert homologation reaction which is discussed below, and reduction of the carbonyl group causes cleavage of the methoxyl group with olefine formation[20].

(iii). Although the dialdehyde or aldehydo-ester of dimethoxy-succinic acid could probably be prepared by the method of Weygand et al.[21], involving lithium aluminium hydride reduction of the N-monomethylanilides, (but by no other method[22]) the chances of racemisation by enolisation were considered too great for the possibility of their extension by condensation in a Doebner or Knoevenagel reaction[23] to be investigated.

(iv). Anodic coupling of carboxylic acids (Kolbé reaction) is negligible when one of the components has α-hydroxy or α-alkoxy substitution[24] and extension of the chain by at least one unit at each end is therefore imperative for this reaction to be used successfully.

x x x

Although Tewari and Tewari have stated[25] that the usual methods of chain extension fail when applied to the preparation of substituted adipic acids from the corresponding derivatives of succinic acid, the Arndt-Eistert

synthesis has been investigated. It can be applied to the homologation of α-substituted acids and it has been shown that if the α-carbon is asymmetrical, the starting configuration is retained in the product[26].

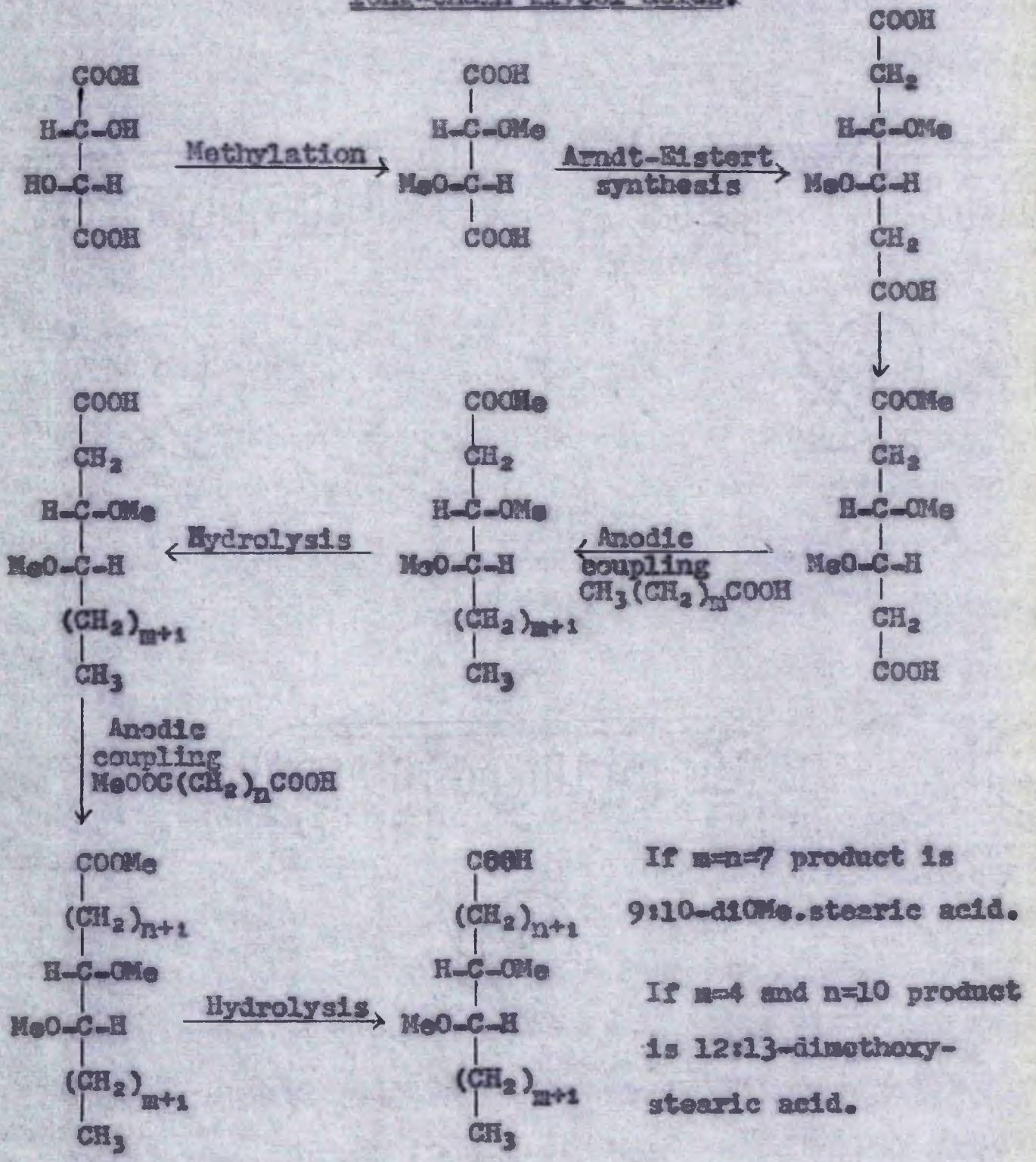
The proposed reaction sequence is shown in Figure XI and consists of methylation of the hydroxyl groups, bishomologation by Arndt-Eistert synthesis to give the di-β-substituted adipic acid, anodic coupling of the half ester with a monobasic acid followed, after separation and hydrolysis of the desired product, by a second anodic coupling with the monoester of a dibasic acid. If m + n = 14, hydrolysis of the separated product would give a threo-dimethoxystearic acid of the same configuration as the starting material.

The 9:10- or 12:13-dihydroxy products could be compared with the known acids either by methylation of the latter or by demethylation of the former to dihydroxy acids. Demethylation can be accomplished by heating with hydriodic acid or by reaction with acetic anhydride and p-toluene-sulphonic acid and hydrolysis of the diacetate so produced[27].

This proposed scheme is capable of variation by extending only one end of the dimethoxysuccinic acid at a time. Arndt-Eistert extension of methyl hydrogen dimethoxysuccinate and anodic coupling of the substituted glutaric half ester so produced followed by hydrolysis of the ester group and a second homologation and anodic coupling reaction would lead to the same products.

Figure XI.

Proposed sequence of extension of (+)-tartaric acid to long-chain glycol acids.



It should be noted that no possibility exists for a similar synthesis of optically active erythro-dihydroxy-stearic acids since the corresponding tartaric acid is the meso or internally compensated form. Synthesis of these acids will require the preparation, resolution and characterisation of the antipodes of unsymmetrical dihydroxy dibasic acids.

Attempted conversion of tartaric acid to 3:4-dimethoxy-succinic acid.

1. Arndt-Eistert reaction with adipic acid:

A preliminary investigation of the bishomologation of adipic acid to suberic acid by the Arndt-Eistert reaction was undertaken. This procedure involves the reaction of the acid chloride with excess of diazomethane to form a diazoketone which is then made to undergo a Wolff rearrangement to a derivative of the next higher acid[28].

The diazoketone, bis-diazoacetylbutane was readily obtained[29] by treatment of adipoyl chloride with excess diazomethane. The rearrangement of this diazoketone by a number of reagents was studied and the results summarised below.

The results indicate that methods 3 and 6 are the most suitable and the latter was preferred because of the homogeneity of the reaction medium.

No.	Reagent	Ref.	Product	Yield	Crude acid		Pure acid	
					%	M.pt	%	M.pt
1	Ag_2O - EtOH	30	diEt.ester	72%	42	-	12	132-9
2	Ag_2O - MeOH	31	diMe.ester	75	47	126-39	9	132-9
3	$AgNO_3$ - NH_4OH	29	diamide	94 pure				
4	-collidine - benzyl alcohol	32	dibenzyl ester	69	35	129-35	20	135-9
5	-collidine - octanol-2	33	dioctyl ester	54	49	133-39	30	137-40
6	Ag-benzoate - triethylamine	34	diethyl-amide	100 ex N_2				

2. Preliminary experiments with racemic tartaric acid:

A preliminary study of the reaction sequence described in Figure XI was carried out as far as the preparation of dimethoxysuccinyl chloride.

Methylations of methyl dl-tartrate[35] by the classical Purdie-Irvine method[36], using silver oxide and methyl iodide, and of the silver salt of dl-tartaric acid by the modified method of Patterson and Patterson[37] gave far lower yields (41% and 24%) of dimethoxysuccinic acid than those reported.

The acid chloride was prepared (M.pt. 68-9°) by the action of phosphorus pentachloride in benzene[38] in 46% yield but other methods normally leading to acid chlorides gave

the hitherto unknown anhydride (M.pt. 129-31°) and thence the monoanilide (M.pt. 129-31°) which confirmed its constitution.

3. Experiments on d-tartaric acid:

Since more work has been done on the optically active tartaric acid derivatives and since the purity of the products can be more readily assessed from optical rotation measurements the investigation of the racemic compounds was discontinued and attempts to carry out the synthesis from d-tartaric acid were made.

The methylation procedures described by Purdie and Irvine and by Patterson and Patterson again gave unsatisfactory yields. The reaction was followed by measurement of optical rotation and was uncompleted even after four consecutive methylations of methyl d-tartrate. A modified procedure was developed which resulted in higher yields of purer product.

The dimethoxysuccinate was hydrolysed to the acid from which was prepared d-dimethoxysuccinyl chloride. Attempts to rearrange the bis-diazoketone, derived from this chloride, by Newmens method were unsuccessful and the study was discontinued.

x x x

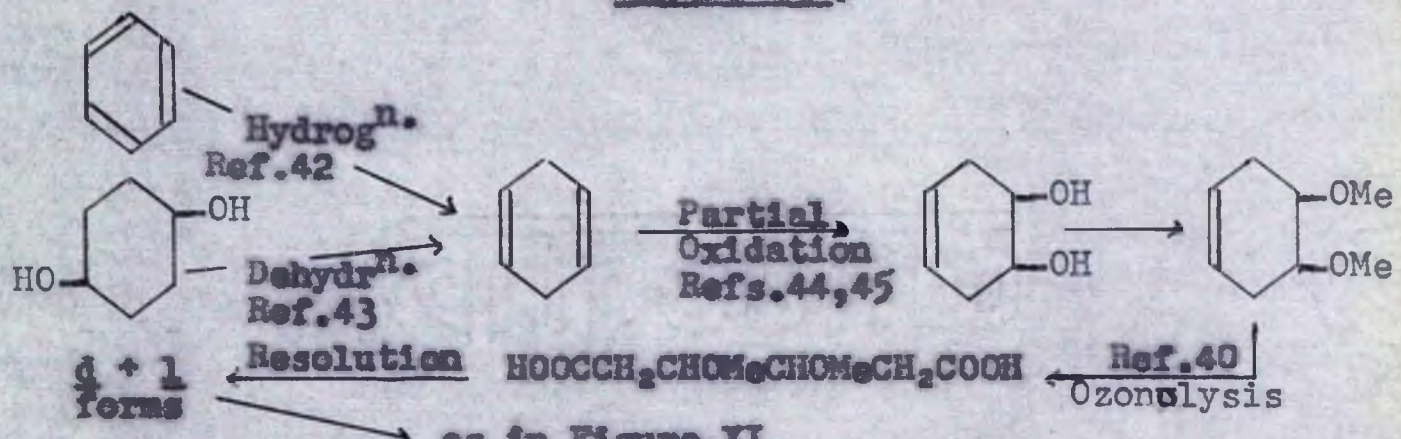
A recent paper by Posternak and Sucz[39] describes the preparation and resolution of the antipodes of 3:4-threo-dihydroxyadipic acid and these authors have determined the optical configurations of the two enantiomers.

This makes a new synthetic approach possible, if the threo-3:4-dimethoxyadipic acid is prepared from symmetric compounds, and is resolved and related to the compounds of Posternak and Sucz. This dimethoxy acid is one of the intermediates in the reaction sequence proposed (Figure XI) for the extension of tartaric acid and it is now possible to circumvent the first few steps of that procedure which have been found to be very difficult to carry out.

threo-3:4-Dihydroxyadipic acid can be prepared directly by ozonolysis of trans-cyclohexene-4:5-diol[40] or as its ester by cis-hydroxylation of methyl trans-1:4-dihydromuconate[39;41]. The dihydroxy acid very readily forms the dilactone and the method of opening the cyclohexene ring has the advantage, over the other, that the hydroxyl groups can be protected by methylation before cleavage of the double bond.

The following reaction scheme is therefore considered preferable to that previously described for the preparation of threo-dimethoxystearic acids of known configuration; but lack of time prevented its investigation.

Figure XII.



Experimental.

1. Preparation of reagents:

(a). Thionyl chloride was purified by fractional distillation from quinoline (1ml. quinoline/5ml. thionyl chloride) followed by fractional distillation from raw linseed oil (1ml. linseed oil/3ml. thionyl chloride). About 50% recovery of pure thionyl chloride (B.pt. 76-8°) was obtained.

(b). Methanol and ethanol were purified by refluxing for 3hr. with magnesium and distilling the anhydrous alcohol.

(c). Dioxan was purified by standing over potassium hydroxide pellets and then distilled from fresh potassium hydroxide (B.pt. 101°).

(d). Methyl iodide was purified by shaking with dilute sodium thiosulphate solution to bleach the colour, washing with water and after drying over calcium chloride, distilling (B.pt. 42.5°). This reagent was kept in a brown bottle with a drop of clean mercury to prevent colouring.

(e). Acetonitrile was purified by standing over phosphorus pentoxide for a few hours and then distilling from fresh P_2O_5. (B.pt. 80-2°).

(f). Nitrosomethylurea was prepared from methylamine hydrochloride, sodium cyanate and sodium nitrite[46] a yield of 81% being obtained.

(g). Diazomethane was prepared by adding nitrosomethylurea (65g., ca.0.6 mole.) to a mixture of 50% aqueous potassium hydroxide (180ml.) and ether (600ml.) cooled to 5°, and then the diazomethane, along with ether distilled through a triple surface

condenser carrying an adaptor dipping below the surface of ether (120ml.) in the collecting flask, which was cooled in an ice-salt mixture. The distillate was collected until it was colourless and the ether solution, containing 16 - 18g. of diazomethane (63 - 70%) dried by standing for a short time over KOH pellets and then for ten minutes over sodium wire.

(h). Silver oxide was prepared by adding a solution of potassium hydroxide (100g.) in water (1000ml.) to a solution of silver nitrate (400g.) in water (2000ml.), the product being filtered, washed well with water, ethanol and ether and finally dried in a vaccuum desiccator.

(i). Silver benzoate was prepared by adding a solution of silver nitrate (28.3g.) to an aqueous solution of benzoic acid (20g.), neutralised with potassium hydroxide (9.3g.). The precipitated salt was washed well by decantation with water, ethanol and ether and dried.

2. Bishomologation of adipic acid:

(a). Preparation of adipoyl chloride:

Adipic acid (20g.) was refluxed with pure thionyl chloride (40ml.,100% excess of 2 equivalents) for 3hr. and the excess thionyl chloride distilled off under reduced pressure to give the liquid adipoyl chloride (24.91g., 100%). This material was used without any purification.

(b). Preparation of 1:4-bis-diazoacetylbutane:

Adipoyl chloride (12.5g., 0.068mole.) in anhydrous ether (100ml.) solution was dropped into a solution of diazomethane

(ca.0.6 mole.) in dry ether (ca.500ml.) over a period of 5min. A brisk effervescence took place and a crystalline separation occurred within a few minutes. The mixture was left overnight at room temperature, excess diazomethane distilled off with ether and destroyed, and the concentrated mixture cooled and filtered to give the diazoketone (10.5g., 79.4%) M.pt. 68-71°; Lit. 69-71° [29]). Evaporation of the mother liquors gave 2.3g. of a red oil which was discarded.

(c). Rearrangement of 1:4-bis-diazoacetylbutane:

Five methods of rearrangement were tried and later a sixth one described by Newman[34].

(i). Silver oxide - ethanol:

To a hot solution of the diazoketone (1g.) in anhydrous ethanol (20ml.) a slurry of silver oxide (0.5g.) in anhydrous ethanol was added in stages, a fresh portion added as soon as nitrogen evolution from the previous addition had stopped. The mixture was refluxed (ca.6hr.) until a test sample did not effervesce on addition of concentrated hydrochloric acid, and then filtered hot. The filtrate was diluted with water, extracted with ether and the extract washed with concentrated ammonia to remove silver salts and then with water till neutral. After drying, the ether was distilled off to give a liquid ester product (0.86g.) which was hydrolysed to give crude suberic acid (0.38g., 42%) which was recrystallised from ether (0.11g., 12%, M.pt. 131-8°, Lit. 141°)

(ii). Silver oxide - methanol:

The diazoketone (1g.) was added in portions to a boiling mixture of silver oxide (0.5g.) in anhydrous methanol (20ml.) and the reaction continued as in (i) to give 0.78g. of crude dimethyl suberate which was hydrolysed to suberic acid (0.42g., 47%, M.pt. 126-39°) which was purified from ether to give 0.08g. (9%, M.pt. 132-9°).

(iii). Silver nitrate - ammonia:

When the diazoketone (1g.) in warm dioxan (10ml.) was treated with a mixture of 20% aqueous ammonia and 10% aqueous silver nitrate (1ml.) little of the expected evolution of nitrogen occurred. Commercial silver oxide was added a little at a time and this promoted vigourous reaction which was allowed to die down before the mixture was heated on the water bath for 1hr. The mixture was filtered hot and the filtrate cooled to precipitate the diamide of suberic acid (0.84g., 94%, M.pt. 213-7°, Lit. 217°).

(iv). γ-collidine and benzyl alcohol:

The diazoketone (1g.) in a mixture of benzyl alcohol and γ-collidine (15ml. of each) was heated rapidly, in an oil bath, to 180° and held at that temperature during vigourous nitrogen evolution (5min.) and then for a further 5min. before cooling. The solvents were distilled off under vaccuum from the water bath and the dibenzyl ester distilled from an oil bath (1.25g., B.pt. 150-68° at 0.5mm.),. Hydrolysis and ether extraction of the acidified soap solution gave

crude suberic acid (0.31g., 35%, M.pt. 129-35°) which was purified by crystallisation from ether (0.18g., 20%, M.pt. 135-9°)

(v). γ-collidine and octanol-2:

Reaction as in (iv) gave the dioctyl ester (1.12g., B.pt. ca.128° at 0.8mm.) which was hydrolysed to crude suberic acid (0.44g., 49%, M.pt. 133-9°) and purified from ether (0.27g., 30%, M.pt. 137-40°)

(vi). Silver benzoate and triethylamine:

The diazoketone (1g.) was dissolved in anhydrous ethanol and a few drops of a 10% solution of silver benzoate in triethylamine was added. Nitrogen evolution began at once and accelerated during the reaction which was complete in less than 10min. Nitrogen evolution was 233ml. at S.T.P., corresponding to 100% reaction. The product was not isolated.

x x x

The low recovery of acid from the hydrolysate in experiments (i), (ii), (iv) and (v) may be due to inefficient extraction of the acid with ether and continuous extraction or the use of another solvent might have improved the yields from these rearrangements.

3. Preparation of dl-dimethoxysuccinyl chloride.

(a). Preparation of dl-2:3-dimethoxysuccinic acid:

(i). via Dimethyl tartrate:

Racemic acid (75g., 0.5 mole.) was dissolved in anhydrous methanol (650ml.) and the cold solution saturated with

hydrogen chloride gas and allowed to stand overnight. The excess reagents were distilled off from the water bath, effective removal of water being ensured by azeotropic distillation with benzene, followed by heating under vaccuum. The residue was dissolved in methanol (500ml.), resaturated with hydrogen chloride, left overnight and the solvents again removed to give dl-dimethyl tartrate (89g., 100%) which was not further purified.

To a solution of dimethyl tartrate (89g., 0.5 mole.) in methyl iodide (426g., 3 mole) was added silver oxide (348g., 1.5 mole.) and a vigourous reaction ensued which was controlled by cooling. The mixture was subsequently heated under reflux for 1hr., the excess methyl iodide distilled off and the residue extracted with ether in a Soxhlet extractor for 3hr. The ether extract afforded crude dimethyl dimethoxysuccinate (102.2g., 96%).

Fractional distillation, under reduced pressure, through an electrically heated and packed column (Towers) gave a fraction of 64.8g. (61%, B.pt. 138° at 16.5mm.).

Hydrolysis of the dimethoxy ester was effected by boiling with aqueous alcoholic potassium hydroxide (48g.) for 1hr. The hydrolysate was titrated to neutrality with concentrated hydrochloric acid and continuously extracted with ether for 60hr. Distillation of the ether and crystallisation of the product from water gave pure dl-dimethoxysuccinic acid (41.2g., 38.5%, M.pt. 169-71°, Lit. 168-71° [47]), a less pure

fraction (2.72g., 2.5%, M.pt. 159-68°) and a syrupy residue which was discarded.

Before weights and melting points were determined the crystallised fractions, which separate as a hydrate, were dried in an oven at 110°.

(ii). via Silver tartrate:

Racemic acid (19.5g.) in water was neutralised with aqueous sodium hydroxide (10.4g.) and silver tartrate precipitated by the addition of silver nitrate (44.2g.) in water. The silver salt was washed several times with water by decantation, filtered, washed with ethanol and ether and dried in air, giving silver dl-tartrate (37.9g., 80%).

Silver tartrate (50g., 0.137mole.) was added in portions over 0.5hr. to a mixture of silver oxide (91.8g., 0.396mole.) and methyl iodide (400g.), heated under reflux. The excess methyl iodide was distilled off, the residue extracted with ether in a Soxhlet extractor for 3hr. and the ether removed to give the methylated ester (19.6g., 67%), which was hydrolised and worked up as in (i) to give 65% yield of crude dimethoxy acid (16.0g.) which, after unsuccessful attempts at crystallisation from ethanol, acetone and mixtures of these with ether, was finally crystallised from water to give dl-dimethoxysuccinic acid (5.97g., 24%, M.pt. 164-8°).

(b). Preparation of dl-dimethoxysuccinyl chloride:

dl-Dimethoxysuccinic acid (5g.) was partially dissolved in benzene (50ml.), phosphorus pentachloride added (13g.) and

the mixture stood overnight at room temperature. Reaction was completed by refluxing for 1hr., the benzene distilled off and the residue crystallised from dry ether after thorough washing with light petroleum, to give the chloride (2.75g., 46%, M.pt. 67-71°), the melting point being sharpened to 68-9° after two recrystallisations from ether.

This gave a strong precipitate with silver nitrate in aqueous solution and reacted with cold methanol to give crude dimethyl dimethoxysuccinate (M.pt. 33-5°)

(c). Attempts to prepare 2:3-dimethoxysuccinyl chloride resulting in the anhydride:

(i) With thionyl chloride:

dl-Dimethoxysuccinic acid (2g.) was refluxed with thionyl chloride (6g.) for 3hr. and the volatile components distilled under reduced pressure to give a solid residue (1.9g.) which was crystallised from anhydrous ether at room temperature (0.95g., M.pt. 130-5°) and the mother liquors on cooling to 0° gave a further 0.30g. (M.pt. 128-33°). This was recrystallised several times from ether to a melting point of 129-31°). Found: C, 45.86; H, 5.05%; $C_6H_8O_5$ requires: C, 45.00; H, 5.04%.

When dissolved in warm water and treated with silver nitrate solution, no precipitate was formed, indicating complete absence of reactive chlorine.

The monoanilide was prepared by heating with aniline in benzenesolution and the crude product was purified by several

recrystallisations from acetone solution to give the mono-anilide of dl-dimethoxysuccinic acid (M.pt. 129-31°). Found: C, 57.35; H, 6.14; N, 5.62. $C_{12}H_{15}NO_5$ requires: C, 56.91; H, 5.97; N, 5.53.

(ii). With thionyl chloride and zinc chloride:

The dimethoxy acid (2g.) was refluxed with thionyl chloride (6g.) and zinc chloride (0.1g.) for 3hr. and the crude product crystallised from ether to give the anhydride (1.42g., 79%, M.pt. 130-3°).

(iii). With phosphorus trichloride:

The acid(2g.), after boiling for 1hr. with phosphorus trichloride (15ml.) and crystallising the crude product from ether gave the anhydride (1.04g.,58%, M.pt. 129-32°) and unchanged dimethoxy acid (0.29g., M.pt. and mixed M.pt. 166-8°).

(d). Preparation of anhydride:

The dimethoxy acid was heated at 100° with acetic anhydride (2 vol.) for 1hr., the product allowed to crystallise on cooling, filtered and washed with dry ether several times to yield the anhydride (80%, M.pt. 131-3°). This gave no depression of melting point when mixed with the products of e(i), (ii) and (iii) above.

The monoanilide (M.pt. 124-5°) was prepared and the melting point raised (126-8°) when mixed with that prepared in e(i).

3. Preparation of d-dimethoxysuccinyl chloride.

"Literature" melting points and specific rotations, unless indicated by reference, are taken from Heilbron and Bunbury's "Dictionary of Organic Compounds", 1943, (London:Eyre and Spottiswoode). Specific rotation values quoted are those given for the same solvents and similar concentrations and temperatures to those used.

(a). Preparation of silver d-tartrate:

Pure d-tartaric acid (150g.) of melting point 167-70° and specific rotation +11.98°, for 20% solution in water (lit. M.pt. 170°, $[\alpha]_D^{20}$ = +11.98°, c = 20 in H_2O), was dissolved in water, neutralised with sodium hydroxide solution and the silver salt precipitated by the addition of silver nitrate (340g.) in water. The salt was washed by decantation with water, filtered, washed with ethanol and then ether and dried (354g., 97.3%).

(b). Preparation of d-dimethoxysuccinate:

(i). Silver d-tartrate (177g,) was added over 1.5hr. to a mixture of silver oxide (369g.) and methyl iodide (1512g.) and the product isolated by ether extraction (24hr.) in a Soxhlet extractor, after removal of excess methyl iodide. A low yield (66.6g., 66%) of impure dimethyl dimethoxysuccinate was obtained (n_D^{19} 1.4330, $[\alpha]_D^{25}$ = +60.3°, c = 2 in acetone; Lit. n_D^{20} 1.4340[47], $[\alpha]_D^{20}$ = +79.6°). Hydrolysis of a portion gave impure d-dimethoxysuccinic acid (M.pt. 143-51°, Lit.153°) with specific rotation +72.6°, c = 2 in acetone (Lit. +95.8°).

Three remethylations of a portion of the ester by the same method raised the refractive index to 1.4340 and the specific rotation to +74°, which is still not pure.

Remethylation of the remainder of the ester (44.5g.) by the method described in b(ii) gave 39.6g. of improved dimethoxy ester (n_D^{17} 1.4359, $[\alpha]_D^{25}$ = +77.6°).

(ii). Silver d-tartrate (177g.) and methyl iodide (710g.) were divided equally between four 500ml. flasks which were shaken for 12hr. to convert the salt to d-dihydroxysuccinic ester (dimethyl tartrate). Silver oxide (360g.) was added, three portions of ca.30g. to each flask at 12-hourly intervals. The spontaneous boiling which accompanied each addition was contained by placing reflux condensers on the flasks until boiling had ceased, when the flasks were transferred to the agitator for the remainder of the 12 hour period. Acetonitrile (50ml.) was added to each flask after each addition of silver oxide to keep the mass liquid enough for effective agitation.

After the final addition the flasks were shaken for a final 12hr. (36hr. reaction in all), the solvents removed, the residues combined and extracted in a Soxhlet with ether for 3hr. to give dimethyl dimethoxysuccinate (78g., 78%; n_D^{19} 1.4344. $[\alpha]_D^{20}$ = +75.8°).

A portion of this ester was hydrolysed to give d-dimethoxy-succinic acid (M.pt. 156-7°, $[\alpha]_D^{26}$ +91.7°).

(iii). Dimethyl tartrate (163g.) was prepared by reaction with cold saturated methanolic hydrogen chloride (see p. 204-5). The product was a glassy solid (100%) which was not purified.

The ester (178g.) was methylated in two lots by the method b(ii) above, with silver oxide (348g.) and methyl iodide (426g.) to give 175.3g. (87%) yield of dimethoxy ester (n_D^{18} 1.4369, $[\alpha]_D^{26} = +70.5^\circ$)

(c). Purification of dimethyl dimethoxysuccinate:

The products from the three methylations (b(i), (ii) and (iii)) were combined (285g.) and distilled through an electrically heated and packed column (Towers) at 18mm. pressure. The following fractions were obtained:-

Fraction	Wt.(g.)	B.pt.(18mm.)	M.pt.	$[\alpha]_D^{25}$
1	23.9	100-137°	-	+67.3°
2	76.8	137	48-54	+76.9
3	147.1	137	48-53	+76.4
4	13.0	137-140	48-53	+75.8
Residue	24.0	-	-	-

Fractions 2 and 3 are almost pure d-dimethyldimethoxy-succinate and were combined (Lit. B.pt. 130-2°/12mm., M.pt. 53-4°, $[\alpha]_D^{20} = +79.6^\circ$).

(d). Preparation of d-dimethoxysuccinic acid:

Dimethyl dimethoxysuccinate (221g.) was refluxed with 10% aqueous potassium hydroxide solution (1400ml.) for 2hr. and the cooled solution made just acid with concentrated hydrochloric acid and concentrated. The concentrated solution was continuously extracted with ether for 24hr., the ether distilled and water removed by continuous azeotropic

distillation with benzene. The crude acid (181g., 94.8%, M.pt. 140-2°) was purified by two crystallisations from acetone at 0° to give pure d-dimethoxysuccinic acid (113.9g., 60%, M.pt. 155-8°, $[\alpha]_D^{20}$ = +90.9°).

(e). Preparation of d-dimethoxysuccinyl chloride:

d-Dimethoxysuccinic acid (5g.) was converted to the acid chloride by the method described on p. 206 (Section 3(b)). Crystallisation of the crude product from anhydrous ether gave the chloride (3.72g., 62%, M.pt. 93-4°, $[\alpha]_D^{18}$ = +99.9°, c = 2, in benzene; Lit. M.pt. 90-3°, $[\alpha]_D^{20}$ = +104.1°, c = 2, in benzene[38]).

(f). Preparation of 1:2-bis-diazoacetylethane:

d-Dimethoxysuccinyl chloride (1g.) in anhydrous ether (30ml.) was added dropwise to a solution of diazomethane (ca.1.5g.) in ether, the reaction mixture allowed to stand overnight and the excess diazomethane and ether distilled off leaving a yellow oil (1.17g.) containing small lemon coloured crystals. No attempt was made to purify this.

(g). Rearrangement of the diazoketone:

The diazoketone (1.17g.), in anhydrous methanol solution, was rearranged by Newman's method[34] by the dropwise addition of a 10% solution of silver benzoate in triethylamine (10ml.). Nitrogen evolution was 74ml. at S.T.P. corresponding to ca.40% reaction. The product (2.83g.) was obtained by removal of solvents after boiling to reduce silver salts to silver. This crude product was contaminated with benzoic acid and its

ether solution was washed with 10% sodium carbonate and water and the material recovered from the ether was distilled to give a liquid product (0.40g., B.pt. 125-40° at 0.9mm.).

This rearrangement was repeated giving 43% reaction, from nitrogen evolution, and a similar product.

These products were combined (1.88g.) and hydrolysed, the soap solution continuously extracted with ether (8hr.) to remove neutral components (0.40g.) and the acidified residue continuously extracted with ether (12hr.) to give 1.55g. of acidic material (M.pt 95-115°).

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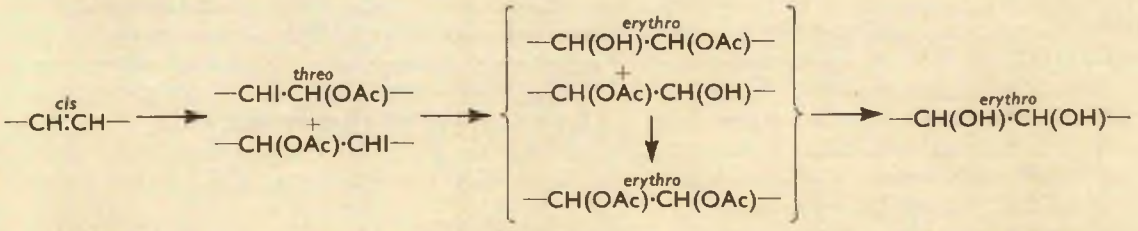
88. *Fatty Acids. Part V.* Applications of the Woodward* cis-*Hydroxylation Procedure to Long-chain Olefinic Compounds.*

By F. D. Gunstone and L. J. Morris.

Hydroxylation involving use of iodine and silver acetate in wet acetic acid is a satisfactory means of oxidising long-chain ethylenic compounds and has some advantages over other methods of *cis*-hydroxylation.

The oxidation of olefins to the corresponding glycol is an important reaction for which several reagents are available.[1] Unsymmetrically substituted olefins when so oxidised are converted into the *threo*- and *erythro*-glycols and if stereospecific reagents are used a single isomer may be isolated. *trans*-Hydroxylation is usually effected by organic peracids,[2] by reaction with Prévost's reagent,[3] or by reaction with halogens or hypohalous acids followed by hydrolysis of the halogenated products. *cis*-Hydroxylation results from oxidation with potassium permanganate, osmium tetroxide,[4] hydrogen peroxide in *tert.*-butyl alcohol with a suitable catalyst,[5, 6] sodium or potassium chlorate with traces of osmium tetroxide,[7] or potassium manganate.[8]

The oxidation of long-chain mono-olefinic acids to the corresponding dihydroxy-acids is an important method of identifying these compounds, and *cis*- and *trans*-hydroxylation are best effected by cold dilute alkaline potassium permanganate,[9] and by performic acid,[10] respectively; both are simple procedures and give the desired dihydroxy-acid in almost quantitative yield. The reaction with permanganate, however, has some disadvantages. Following Lapworth and Mottram's procedure very dilute solutions must be used (*ca.* 1 l. per g. of acid) so that the oxidation of large quantities becomes very tedious (attempts to reduce the volume have been reported by Traynard [11]). In addition, it is less satisfactory when applied to substances which are insoluble in aqueous alkali, though acetone may then be used as solvent. Of the other methods of effecting *cis*-hydroxylation only osmium tetroxide has been widely used and a method which overcomes these difficulties and avoids the use of the expensive and toxic osmium tetroxide is therefore desirable. We have found that reaction with iodine and silver acetate in wet acetic acid followed by alkaline hydrolysis of the mixed mono- and di-acetates is a highly satisfactory procedure for the *cis*-hydroxylation of long-chain olefinic acids. The method originates from Professor Woodward's laboratory and has been described for a few individual compounds all containing the ethylenic group in an alicyclic system.[12]



The reaction occurs in three stages.[13] In the first iodine and silver acetate interact to form a product which quickly reacts with the olefin giving an iodo-acetate by *trans*-addition.

* Part IV, *J.*, 1956, 1611.

These changes occur when the reagents in acetic acid are shaken at room temperature; the American workers add the iodine portion-wise but we have found this to be unnecessary. The second stage involves replacement of the halogen by a hydroxyl group which may become acetylated; this is effected by silver acetate in acetic acid. Winstein and Buckles [14] have shown that by using anhydrous solvent this reaction occurs with predominant retention of configuration but that the presence of water causes inversion to an increasing extent, almost complete inversion taking place when one equivalent of water is present; these authors have explained these observations in terms of neighbouring-group participation. (We had already used this highly stereospecific solvolysis for the conversion of a *cis*-epoxide into an *erythro*-glycol [15]). This reaction is carried out after addition of the required amount of water and with our olefins refluxing for 1 hr. is as satisfactory as 3 hr. on the water-bath as recommended in the American papers. Finally the mixture of mono- and di-acetates is isolated and hydrolysed.

By our modified procedure we have oxidised a number of olefinic compounds (see Table). With pure starting materials the products are obtained in high yield and readily purified. Though most of these oxidations were effected on 2—3 g. of olefin similar yields were obtained in one experiment on ten times this scale. The method is a little more tedious than Lapworth and Mottram's but it gives equally good yields of pure product, works just as well with neutral compounds, and may be conveniently effected on a larger scale than is usual with the permanganate method.

Experimental

Ethylenic Compounds.—Olive oil and castor oil were good-quality commercial materials; oleic acid, *cyclo*hexene, and acenaphthylene were those available from chemical suppliers, and methyl undecenoate was prepared from purchased undecenoic acid. Methyl hexadecenoate was derived from a concentrate of unsaturated C_{16} esters isolated from crocodile oil and consisting mainly of hexadec-9-enoate; methyl linoleate had also been prepared previously from 9 : 10 : 12 : 13-tetrabromostearic acid.

Pure methyl oleate was prepared from the mixed acids of olive oil: saturated acids were removed by crystallisation at $-20°$ and polyethenoid acids remained in solution at $-50°$; the monoethenoid concentrate was then esterified and distilled, and appropriate fractions combined. Some of the ester was hydrolysed and the acid, when isomerised [16] by heating it with selenium at 220—225°, afforded elaidic acid (m. p. 43—44°) a part of which was converted into the methyl ester. Reduction (lithium aluminium hydride) of oleic and elaidic acid gave the corresponding alcohols.[17]

Oxidation.—The following method applies to the oxidation of esters. The ethylenic compound (0·01 mole), silver acetate (0·022 mole), and iodine (0·01 mole) in glacial acetic acid (65 ml.) are shaken for 30 min. at room temperature. Wet acetic acid (10 ml. containing 0·2 ml., 0·011 mole, of water) is then added and the mixture is refluxed for 1 hr. After cooling the precipitated silver salts are filtered off and washed with a little acetic acid, and the solvent then removed from the combined filtrate and washings by distillation at 100° under reduced pressure. The residue is diluted with water and extracted with ether, and the extract washed with concentrated ammonia and then with water. The solvent is next removed and the product hydrolysed by boiling it with excess of 3N-aqueous alcoholic potassium hydroxide for 1 hr.; the mixture is then diluted and acidified (hydrochloric acid). The crude oxidation product is dried in a vacuum desiccator (containing concentrated sulphuric acid and potassium hydroxide) and crystallised. Methanol was used in these experiments except where otherwise stated.

In the oxidation of olefinic acids the (partially) acetylated glycol cannot be washed with ammonia and therefore after acetic acid has been removed and water and ether added, the mixture is treated with dilute hydrochloric acid, and the precipitated silver salts are removed and washed with ether. The ether solutions are combined and washed with water, the solvent removed, and the residue hydrolysed.

With neutral products there is no need to acidify the final hydrolysate: the product usually separates when the diluted solution is cooled.

The results of several oxidations are summarised in the Table and some additional information is added below.

The cis*-hydroxylation of some ethylenic compounds.*

Olefin	Crude product, yield (%)	Crude product, m. p.	Pure product, yield (%)	Pure product, m. p.	M. p. (lit.)
Pure olefins :					
Methyl oleate	99	126—128°	89	130—132°	132 [a]
Methyl elaidate	97	92—93	91	93·5—94·5	95 [a]
Elaidic acid	89	92—94	85	94—94·5	95 [a]
Oleyl alcohol	100	123—125	81	126	126 [b]
Elaidyl alcohol	94	82—84	79	82·5—83·5	82 [b]
*cyclo*Hexene	66	—	41	94—97	98 [c]
Acenaphthylene	89	180—204	28	203—208	213 [d]
Crude olefins :					
Olive oil	87	—	83 *	131—132	132 [a]
Olive oil †	94	112—125	97 *	125—132	132 [a]
Castor oil	95	—	30 *	108—111	112 [e]
			7 *	135—137	138 [f]
Methyl undecenoate	49	74—77	42	84—87·5	85—86 [f]
Methyl hexadecenoate	93	—	62	126—128	129 [g]
Methyl linoleate	95	—	14	173	174 [h]
			15	163—165	164 [f]
Oleic acid	95	—	56	123—127	132 [a]

* These percentages are based on the assumption that olive oil contains 75% of oleic acid and castor oil 90% of ricinoleic acid.

† All oxidations were effected on about 0·01 mole of olefinic compound except in this case where 0·1 mole was used.

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Comments on Some Oxidations.—(a) *Castor oil.* The crude oxidation product after crystallisation from ethyl acetate was separated into fractions soluble and insoluble in hot chloroform. After crystallisation from ethanol these gave the low-melting and the high-melting isomer, respectively. Similar yields (28% and 8%) resulted from oxidation of acetylated castor oil.

(b) *Methyl undecenoate.* The above procedure afforded only 8% of dihydroxy-acid but the yield was raised to 42% by shaking the initial reactants overnight and then refluxing them with wet acetic acid for 2·5 hr. (The double bond in this compound is known to be less reactive; performic acid for example requires a longer time of reaction than usual and gives a 44% yield [10] whilst oxidation with permanganate affords the diol in 31% yield.[18])

(c) *Methyl linoleate.* Because this ester contains two double bonds the quantity of iodine, silver acetate, and water was doubled. The crude oxidation product was first crystallised from 30% acetic acid and then boiled with acetone (200 ml. per g.). The insoluble fraction, after crystallisation from 50% ethanol, gave the higher-melting isomer; the soluble acids crystallised on concentration of the acetone solution and were recrystallised from 50% ethanol to give the lower-melting isomer.[19]

(d) cyclo*Hexene.* The standard procedure gave only 20% of the *cis*-diol but this yield was doubled when the reactants were shaken for 4·5 hr. and the product worked up : after hydrolysis the solution was neutralised (concentrated hydrochloric acid) and evaporated to dryness, and the residue extracted with chloroform; this extract gave the diol on crystallisation from ethyl acetate (Clarke and Owen [20] obtained yields of 46% with sodium chlorate and osmium tetroxide and 33% with potassium permanganate and magnesium sulphate).

(e) *Acenaphthylene.* The first stage of the reaction was prolonged to 2 hr. The crude product was decolorised with charcoal in acetic acid solution and then recrystallised from the same solvent (Found : C, 77·0; H, 5·5. Calc. for $C_{12}H_{10}O_2$: C, 77·4; H, 5·4%). Previously this diol has been prepared from acenaphthylene by bromination and subsequent hydrolysis,[21] by reduction of the diketo-compound,[21] or by osmium oxidation of the olefin; [4] the first two methods give mixtures of the *cis*- and the *trans*-diol.

We were unable to oxidise tetramethylethylene, phenanthrene, methyl ximenynate, methyl ricinstearolate, and stearolic acid; there was some evidence of reaction but only very small yields of unsatisfactory products were isolated.

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