SYNTHESIS AND PROPERTIES

OF

SOME C_{18} UNSATURATED ACIDS

being a thesis

presented by

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to the

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in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY

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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.

The research work was carried out in the Department of Chemistry, University of St. Andrews under the direction of Dr. F.D. Gunstone, D.Sc., F.R.I.C.

CERTIFICATE

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

Research Supervisor.

ACKNOWLEDGEMENTS

I wish to record my indebtedness to Dr. F.D. Gunstone for his invaluable guidance, help and encouragement throughout this work.

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It was indeed a pleasure in working and in discussions - academic or otherwise - with my colleagues, both in the new and the old laboratories.

Finally, my thanks are due to the University of St. Andrews for the award of a research grant which supported me financially during this research work.

Previous Publications.

1. Cashew Nut Germ Oil, J.A. Barve and J.G. Kane, Indian Oil and Soap Journal, 28,(1962),34.
2. Garcinia Cambogia Seed Fat, J.A. Barve and J.G. Kane, Indian Oilseeds Journal, 5,(1962),282.
3. Sesame Oil from West Bengal and Assam, J.A. Barve and J.G. Kane, Journal of Oil Technologist's Assoc. of India, Part II, Vol. 18, (1963).
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5. Zur Kenntnis Der Cashew-Öle I: Gewinnung und Analyse, H.P. Kaufmann and J. Barve, Fette. Seifen. Anstrichmittel, 69,(1967), 437.
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Appendix: Synthesis of Octadecadienoic acids

ABBREVIATIONS

ApL	Apiezon L grease
b.p.	Boiling point
c/s or Cps	Cycles per second
DEGS	Diethyleneglycolsuccinate polyester
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
ECL	Equivalent Chain Length or Carbon number
E.E.	Diethyl ether
FCL	Fractional Chain Length
GLC	Gas Liquid Chromatography
lit.	Literature
m.p.	Melting point
NMR	Nuclear Magnetic Resonance
P.E.	Petrol ether
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
Ag^+/TLC	Silver ion thin layer chromatography
12a; 6c; 4a etc.	a = acetylenic c = cis olefinic t = trans olefinic. The number indicates the carbon atom on which the unsaturation starts. When not otherwise mentioned these were always acids or esters with eighteeen carbons in the chain.
C(2) etc.	The number in the bracket indicates the no. of the carbon atom measured from carboxyl or carbomethoxy group.

Abbreviations (cont.)

J C(2) C(3) etc.	Coupling constant for the coupling between the protons on the 2nd and the 3rd carbon atom etc.

SUMMARY

The complete series of octadecynoic acids (Δ^2 - Δ^{17}) have been prepared and, from these, the corresponding trans octadecenoic acids. In the acetylenic series some acids are prepared for the first time. These acids have been obtained in sufficient quantity (5-10 g in most cases) and purity to permit a comparative study of their physical, chemical and biological properties. The properties already studied and reported in this thesis include melting point, silver ion thin layer chromatography, gas liquid chromatography, infra-red spectroscopy and NMR spectroscopy.

It was shown that the alternation of m.p. apparent in the trans acids with unsaturation in the Δ^5 to Δ^{13} region is not observed with the acetylenic acids. This difference in m.p. behaviour reflects a difference in molecular packing of these two series of acids in their crystalline forms.

Ag^+/TLC behaviour was as expected with the acetylenic, cis olefinic and trans olefinic esters showing increased Rf values in that order [solvent, petrol ether and ether (94:6)] except for the Δ^2 isomers (cis olefinic > acetylenic > trans olefinic).

Equivalent chain lengths for all acetylenic and trans olefinic esters were measured on polar (DEGS) and non-polar (ApL) columns. By a correlation of these results with those reported previously for diunsaturated C_{18}-esters, it has been possible to examine the idea of predicting the equivalent chain lengths of polyunsaturated

esters.

Infra-red studies indicate that although the position of the =C-H bending band ($\sim$ 963 cm^{-1}) in trans acids is independent of the position of unsaturation, this is not true of the intensity of the band.

NMR examination (100 MHz and 220 MHz) of the series of mono-unsaturated acids show that the order of deshielding effect of different unsaturated groups is acetylenic > cis olefinic > trans olefinic. The acetylenic group shows a deshielding effect on the protons of α, β and γ carbon atoms but double bonds only affect protons attached to the α carbon atom with a very weak effect on those of the β carbon atom. The acetylenic acids with central unsaturation (Δ^{6} to Δ^{13}) may be distinguished on the basis of the absorption bands between 8.55τ to 8.74τ, when a high frequency (220 MHz) instrument is used.

It is expected that the full value of these two series of acids will be realised when they are used in further chemical, physical and biological studies parallel to those already carried out on the cis olefinic esters.

INTRODUCTION

The systematic study of fats was started by Chevreul, more than one and a half century ago but it did not become a branch of organic chemistry until long after that. Organic chemists of that time found fats uninteresting because they were complex mixtures which were not easily separated into pure compounds. This difficulty remained, until T.P. Hilditch, with the help of his many students, developed methods for the analysis of oils and fats and gave fat chemistry a place as an important branch of chemistry. Since then, new developments in methodology, among which gas liquid chromatography and thin layer chromatography are the most important, have completely changed the tempo of lipid research.

Developing Interest in Trans Acids.

(i) Discovery of new trans acids:

Rising standards of living and population growth have increased the demand for edible oils and new oils must therefore be found for the industrial products traditionally made from edible fats. This has led to a search for new oils with interesting properties and with the new accurate and sensitive methods many new fatty acids have been found. For example, in 1960 only about 30 natural C_{18}-acids were known but between 1960 and 1965 more than 50 new C_{18}-acids were discovered.[1]

Among the newly discovered acids, several had isolated trans unsaturation which was novel because previously it had been thought that trans unsaturation occurred only in the conjugated acids from the vegetable oils. Among C_{18}-acids the 5t acid was detected in 1962[2] in Thalictrum polycarpum seed oil, the 9t,12t acid was obtained in 1963 [3] from Chilopsis linearis, the 5t,9c, 12c acid was discovered in Aguiligia vulgaris[4] in 1964 and the 3t,9c,12c,15c acid in Tecoma Stans[5] in 1965. These are only a few examples of the new trans acids where the structures were determined; Wolff and his colleagues[6] reported several other acids with trans unsaturation but did not establish their structures. It is now generally accepted that though in most cases the trans acids are only minor components, they are fairly widely distributed in vegetable fats.

(ii) Trans acids in animal kingdom:

Animal fats were known to contain larger number of trans acids and with the advanced methods of isolation and structure determinations many more are found as minor components, e.g. milk fat alone has been shown to contain the trans-9,10,11 and 16 octadecenoic acids together with trans acids of other chain lengths. These are thought to arise by bio-hydrogenation of dietary polyunsaturated acids.[7]

(iii) Trans acids produced by hydrogenation of fats:

Catalytic, partial hydrogenation of fats is extensively

used in the food industry and it is now well-known that isomerisation, both positional and geometric, takes place during such hydrogenation, e.g. several workers[8,9,10] have shown that hydrogenation of oleic acid gives cis- and trans-7,8,9,10 and 11 octadecenoic acids.

(iv) Need for metabolic studies of trans acids:

The wide occurrence of the trans acids in the plant and animal kingdom and the conversion of many cis acids to trans acids during processing of food fats make it necessary that the metabolic fate of the trans acids in the body should be investigated. Whilst chemists are searching for new fatty acids biochemists are employing the latest chromatographic procedures to study their biosynthesis and metabolism. Exciting discoveries have shown how one positional isomer of an unsaturated acid can stimulate growth while another retards it. It is becoming clear that enzymes are not only stereoselective in the position they attach but structurally specific in the acids they handle. For such researches pure fatty acids are required. Many of these do not occur naturally and even those that do occur are often difficult to isolate from the complex mixtures of fatty acids in which they occur. For the reasons, most pure fatty acids must be obtained synthetically.

(v) Fatty acids as chemicals:

Industry has realised the unique nature of a fatty acid

molecule in which a polar carboxylic group is attached to a long non-polar chain which can contain additional reactive unsaturated groups, and fatty acids have now become raw materials for diverse chemicals. This means that chemicals with specific properties are prepared from fatty acids and since a general knowledge of the properties of a group of fatty acids based on only one member of the group (such as oleic acid) may not be sufficient fuller knowledge of the chemical and physical properties of all the members of the group is advantageous.

Synthetic Studies.

For the above reasons it is important that for all the positional as well as geometric isomers of unsaturated acids a comparative study of their physical, chemical and biological properties be carried out. This can best be done when a complete series of positional and geometric isomers are available at the same time.

With this in view, synthetic studies were started in our laboratories. Dr. Ismail synthesised all the possible isomers of the cis-octadecenoates while Dr. Lie synthesised some cis-cis and trans-trans octadecadienoic acids of the general formula

$$CH_3(CH_2)_xCH=CH(CH_2)_yCH=CH(CH_2)_zCOOH.$$

The physical and some chemical properties of these acids and their derivatives have been further studied in these laboratories and

the biological studies are carried out elsewhere. The cis-octadecenoic acids were converted into cyclopropane derivatives by Dr. Christie (St. Andrews) and into corresponding alcohols, acetates, hydrocarbons etc. by Dr. Lie (St. Andrews). The epoxides of 18:1 cis acids were prepared and studied by G. Maerker (Philadelphia) while Dutton and Frankel (Peoria) have undertaken the hydrogenation studies of 18:2 series of (cis) acids.

Interesting papers have already been published on the effect of the 18:1(cis)isomers on the growth of Leptospira interrogans serotype patoc[11] and of monkey kidney cells (LLC-MK_2),[12] and on the enzyme-controlled behaviour of coenzyme A-esters[13] and the cholesteryl esters[14] of cis octadecenoates.

Similar studies are being undertaken on the cis-cis and trans-trans octadecadienoic acids of Dr. Lie.

Present Work.

In continuation of these studies we have now prepared the Δ^2- to Δ^{17}- Octadecynoic and octadec-trans-enoic acids. We have studied their GLC and TLC behaviour, determined their melting points and carried out infra-red and nuclear magnetic resonance spectroscopic studies.

Studies on the trans epoxides from our trans octadecenoic acids and the reaction of the trans olefinic esters and the corresponding alcohols with mercuric acetate are already underway in our laboratory. We expect both the acetylenic and the trans

series of acids to be used in metabolic studies such as those described above and interest has already been shown by other laboratories in this direction.

DISCUSSION

A. INTRODUCTION

1. General Methods of Synthesis of trans Acids.

Although fatty acids have long been known, attempts to develop general methods for their total synthesis did not begin until 1940, possibly because of their abundance in nature. Cis acids were synthesised first because of their greater interest and the most important general method for their preparation involves partial reduction of long chain acetylenic acids. Vaughn[15] et.al. showed that the metal acetylides can be alkylated and Henne and Greenlee[16] improved the methods and prepared several alk-1-ynes. Ahmad and Strong[17] developed this method as one of the most suitable methods for preparation of long chain fatty acids.

(i) Isomerisation of cis to trans acids:

Although it is reported that acetylenic acids can be reduced to either cis or trans olefinic acids by partial reduction, researchers have, in many cases, prepared the cis acids first and then converted them to their trans isomer by nitrous acid, selenium or some other reagent, the first two reagents being most popular. The nitrous acid gives a number of nitrogen-containing by-products (which however can be easily removed by chromatography[18]) while selenium requires a high temperature and there is a possiblity of double bond migration. In addition, these reagents

form equilibrium mixtures and though the trans isomer predominates (75-80%) it has still to be separated from the closely related cis isomer.

(ii) Anodic synthesis:

Brown and Walker[19] showed that acids can be prepared by anodic synthesis using the half esters of dibasic acids. Weedon et.al.[20] extended this method and prepared unsaturated acids by electrolysis of unsaturated monobasic acids and half ester of dibasic acids, thus

$$RCH{=}CH(CH_2)_nCOO^- + {}^-OOC(CH_2)_mCOOR' \rightarrow RCH{=}CH(CH_2)_{n+m}COOR'$$

(+ Products of symmetrical coupling)

This is a very elegant method for preparing the long chain acids without double bond migration or stereomutation but the chief drawback is that only a small quantity of the material can be prepared at a time and the yields are very low. Also both n and m must be greater than one i.e. $n+m > 2$, therefore Δ^2 and Δ^3 acids can not be prepared by this method and the short chain trans acids have to be prepared first by some other method.

(iii) Wittig synthesis:

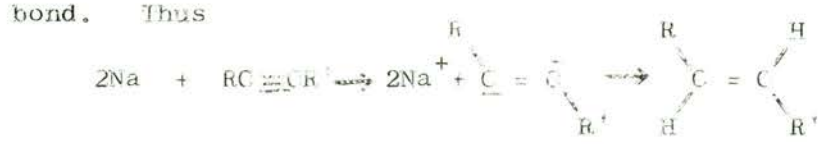
In the Wittig reaction the phosphorane derivative of an alkyl bromide reacts with an aldehyde (or ketone) to give an olefin thus:

$$Ph_3\overset{+}{P}-\overset{-}{C}HR + O{=}CHR' \rightarrow Ph_3\overset{+}{P}-CHR \quad Ph_3P\cdots CHR$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\quad O-CHR' \qquad\quad O\cdots CHR'$$
$$\rightarrow \quad Ph_3PO + RCH{=}CHR'$$

The olefin obtained is a mixture of the cis and trans isomer in which the trans form predominates. Bergelson and Shemyakin[21] claim that the reaction can be made stereospecific to give cis isomers by varying the solvent, temperature etc. and adding Lewis base. The reaction is done under fairly mild conditions and can be made to give very largely the one or the other isomer but when high purity is required the reaction becomes of little use as, especially in the preparation of fatty acids, the cis and trans isomers can not be separated easily (see synthesis of 2t acid).

(iv) Sodium and liquid ammonia reduction of acetylenic acids:

Campbell and Eby[22] first found that the dialkyl acetylenes can be reduced stereospecifically to give trans olefins by sodium in liquid ammonia. The mechanism of the reduction probably involves the stepwise addition of two electrons to the triple bond. Thus



If this mechanism is accepted then the stereospecificity of the reaction can easily be explained because obviously the most stable disposition of the intermediate di-anion is that involving maximal separation of the two negatively charged sp^2 orbitals i.e. the trans configuration. Though it is established that the addition of the electrons is stepwise[23], whether the second addition of electron precedes the addition of the first

proton is not definitely known.

Elsner and Paul[24] found that the reduction of the long chain alkynes with the double bond in the central region can not be carried out at the boiling point of liq. ammonia but occurs satisfactorily at the room temperature (in an autoclave).

This reaction normally goes to virtual completion (i.e. reduction to alkene stage), does not lead to formation of any cis isomer and does not give any migration of double bond. In this connection the reaction is superior to any other reaction of synthesising trans acids from the acetylenic acids. The reaction also does not give any saturated acids because isolated double bonds with only sp^2 orbitals can not be reduced by this method. The one limitation of the method is that it can not be used when the triple bond is conjugated with the carboxyl group (e.g. Δ^{2a} acids) because in this case 1:4 addition of electrons takes place and leads to a saturated acid. In addition, many operators have reported some difficulty in getting reproducible results. The reaction has been successful on some occasions and failed at other times, the reason for this has not always been clear. We find the reaction to be satisfactory when attention is paid to certain points of experimental details. These are taken up later.

2. Octadecynoic and Octadec-trans-enoic Acids:

A summary of previous work on the synthesis of octadecynoic

and octadec-trans-enoic acids is given in the following pages. The literature is surveyed upto June 1970. In addition to the above acids reference is made to cis isomers and to some acids of other chain lengths when this is relevant.

Δ^2 Acids:

An early attempt (1887)[25,26] to synthesise the 2t acid from oleic acid must have been based on the false assumption that oleic acid was a Δ^2 or Δ^3 octadecenoic acid.

The attempts of Ponzio[27] and of Le Sueur[28] to prepare this acid by boiling 2-iodo or 2-bromostearic acid with alcoholic potassium hydroxide must have given the Δ^{2t} isomer on mechanistic grounds, though it might have been contaminated with 3t and possibly other isomers because of the double bond migration under alkaline conditions.

More recently Meyer[29] synthesised the 2t acid by the above method and proved it to be a trans isomer by preparing the lower melting cis isomer from the trans isomer through its bromoacetoxy derivative. The 2-hydroxystearic acid obtained as a by-product in this reaction can be efficiently removed via its copper chelate.[30]

Grimmer and Hildebrandt[31] prepared the Δ^2 acetylenic ester, partially reduced this to the 2c ester and converted it to the 2t ester via methoxy mercuric acetate.

$$RBr \xrightarrow{1} RC\equiv CH \xrightarrow{2} RC\equiv CCOOH \xrightarrow{3} RC\equiv CCOOMe \xrightarrow{4} \overset{cis}{RCH=CHCOOMe}$$

$$\xrightarrow{5} RCH(OCH_3)CH(HgOAc)COOMe \xrightarrow{6} \overset{t}{RCH=CHCOOMe}$$

R = $C_{15}H_{31}$ (1) DMF/NaC$\equiv$CH (2) $NaNH_2/CO_2$ (3) HCl/MeOH

(4) Lindlars' catalyst/H_2 (5) $Hg(OAc)_2$/MeOH (6) Acid

However they report that this method gives a mixture of cis and trans isomers, so they also prepared the 2t acid from the octadec-cis-2-en-1-ol by oxidation with tert-butyl chromate. The oxidation is apparently accompanied by stereomutation.

$$RC\equiv CH \xrightarrow{1} RC\equiv CCH_2OH \xrightarrow{2} RCH^{cis}=CHCH_2OH \xrightarrow{3} RCH^{t}=CHCOOH$$

R = $C_{15}H_{31}$ (1) $NaNH_2$, HCHO (2) Lindlar's catalyst/H_2

(3) tert. butyl chromate.

Ismail[32] isomerised the 2c acid with selenium and purified the product containing the 2t isomer (84%) by silver nitrate TLC.

Δ^3 Acids:

The attempt by Eckert and Halla[33] to prepare the octadec-3-enoic acid by dehydrohalogenation of β-iodostearic acid should have yielded 3t-isomer but this was probably contaminated with the 2t acid.

More recently Ismail and Gunstone[42] reported that their attempt to oxidise octadec-3-yn-1-ol by chromic acid gave a mixture of Δ^2, Δ^3 and Δ^4 acetylenic acids. They therefore reduced the alcohol to octadec-cis-3-en-1-ol which could be successfully oxidised to 3c acid. The trans isomer was then obtained by stereomutation with selenium.

Because of the difficulty of preparation of Δ^3 acids, it is of interest to consider the preparation of acids of other chain

length having this unsaturation. These include 8:1 (3a)[34,35], 9:1 (3a)[36], 12:1 (3a)[37], 15:1 (3a)[38], and 16:1 (3t)[39]. In general two procedures are available: (a) oxidation of a Δ^3 unsaturated alcohol and (b) chain extension of a Δ^2 unsaturated alcohol.

(a) $RC\equiv C \xrightarrow{1} RC\equiv C(CH_2)_2OH \xrightarrow{2} RC\equiv CCH_2COOH$

(b) $RC\equiv CCH_2OH \xrightarrow{3+4} RC\equiv CCH_2CN \xrightarrow{5} RC\equiv CCH_2COOH$

(1) $CH_2\!-\!CH_2$ (O) /$NaNH_2$ (2) Chromic Acid/Acetone (3) PBr_3

(4) $Cu_2(CN)_2$/xylene (5) Hydrolysis

There is some conflict of opinion about the relative merits of these procedures.

Δ^4 Acids:

Eckert and Halla[33] were again the first to attempt the preparation of 4t acid from 4-iodostearic acid but their product was probably not a single isomer (cf. Δ^{3t}).

Boughton et.al.[40] synthesised the 4t acid by an elaborate sequence of reactions from n-tridecyl bromide. The reaction sequence is set out below.

$$RI \xrightarrow{1,2,3} RCH(OMe)COOH \xrightarrow{4,5} RCH(OMe)CO\underset{\displaystyle COOCH_2Ph}{\overset{\displaystyle COOCH_2Ph}{C}}CH_2COOCH_2Ph$$

$$\xrightarrow{6} RCH(OMe)CO(CH_2)_2COOH \xrightarrow{7} RCH(OMe)CH(CH_2)_2CO \xrightarrow{8}$$

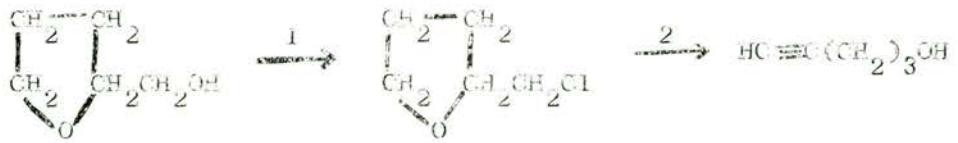
(O bridge between the CH(OMe) and CH(CH$_2$)$_2$CO groups)

$$\underset{\text{erythro}}{RCHBr\text{-}CHBr(CH_2)_2COOH} \xrightarrow{9} RCH{=}^{t}CH(CH_2)_2COOH$$

R = $C_{13}H_{27}$ (1) $NaC(OMe)(COOMe)_2$ (2) KOH (3) decarboxylation (4) Oxalyl chloride (5) $Na/PhCH_2OOCCH_2CH(COOCH_2Ph)_2$ (6) debenzylation, decarboxylation and hydrolysis (7) Raney nickel/H_2 (8) $HBr/AcOH/H_2SO_4$ (9) Zn

Ames and his colleagues[41] shortened the procedure considerably by condensing the dilithium derivative of propargyl alcohol with tridecyl bromide to get hexadec-2-yn-1-ol and then extending the chain by malonic ester synthesis to octadec-4-ynoic acid. However the trans-isomer was obtained via the cis isomer resulting from partial reduction. The 4c isomer was converted via the threo-diol to the erythro-dibromide and then debrominated with zinc and methanol.

Ismail and Gunstone[42] prepared the 4a acid by using the following sequence of reactions



(1) $SOCl_2$/Pyridine (2) $NaNH_2$/liq.NH_3 (3) chromic acid/Acetone (4) $SOCl_2$, $HNMe_2$ (5) $NaNH_2$/liq.NH_3, $C_{13}H_{27}Br$ (6) Hydrolysis.

They obtained the trans acid by isomerisation of the cis isomer with selenium.

Δ^5 Acids:

Posternak[43] prepared the Δ^{5a} acid in 1916, by preparing the di-iodide of the naturally occurring tariric acid (Δ^{6a}) and dehydrohalogenating it with alcoholic potassium hydroxide. He obtained a mixture of 5a, 6a and 7a acids from which he separated the 5a acid. He reacted hydrogen iodide with the 5a acid and reduced the product with zinc and acetic acid to obtain 5t acid. The isomeric purity of this preparation was not rigorously established but the melting point (47.5°) agrees well with that of the octadec-trans-5-enoic acid prepared by unequivocal synthesis.

Ames et.al[44] report that the condensation of N,N-dimethyl hex-5-ynamide with dodecyl bromide gives a better yield (35%) of acetylenic acid (amide) than the condensation of hex-5-yn-1-ol with dodecyl bromide followed by oxidation of the alcohol with chromic acid[38].

This method of using N,N-dimethylamides to protect the carboxylic group during the condensation in sodamide and liq. ammonia was used by Ismail and Gunstone[42] to prepare a series of acids including the octadec-5-ynoic acid. (see also the 4t acid). They then obtained the 5t acid by transmutation of the cis isomer with selenium.

Δ^6 Acids

Though tariric acid (octadec-6-ynoic acid) has been known since 1892, its first chemical synthesis was achieved by Lumb and

Smith[45] by using modified Ahmad-Strong synthesis as follows.

$$(RC\equiv C)_2Hg \xrightarrow{1} RC\equiv C(CH_2)_3Cl \xrightarrow{2} RC\equiv C(CH_2)_4COOH$$

$R = C_{11}H_{23}$ (1) Li/Dioxan, $I(CH_2)_3Cl$ (2) Malonic ester synthesis.

They overcame the difficulty noted by Strong et.al.[46] that short chain chloroiodides (less than 6c atoms) do not react with sodium derivatives of long chain alkynes in liq. ammonia, by using the lithium derivative of alkyne.

Unable to prepare the lithium derivative in liq. ammonia they reacted the mercuric salt of the alkyne with lithium in dioxan.

Khan[47] achieved the partial synthesis of this acid by brominating petroselenic acid and then dehydrobrominating it with sodamide in liquid ammonia.

Baker et.al.[48] prepared the acid by the 'anodic coupling' of the half ester of dodec-6-ynedioic acid with excess of octanoic acid. The products of symmetrical coupling can also be isolated.

Recently Ismail and Gunstone[42] prepared tariric acid by the following route

$$I(CH_2)_4Cl \xrightarrow{1} HC\equiv C(CH_2)_4Cl \xrightarrow{2} HC\equiv C(CH_2)_4COOH$$

$$\xrightarrow{3} HC\equiv C(CH_2)_4CONMe_2 \xrightarrow{4} RC\equiv C(CH_2)_4CONMe_2$$

$$\xrightarrow{5} RC\equiv C(CH_2)_4COOH$$

R = $C_{11}H_{23}$ (1) NaC≡CH/liq.NH_3 (2) NaI/NaCN, Hydrolysis

(3) $SOCl_2$, $HNMe_2$ (4) $NaNH_2$/liq.NH_3, $C_{11}H_{23}Br$ (5) Hydrolysis.

They obtained petroselaidic acid in small quantities by transmutation of cis isomer to trans acid and separation by Ag^+/TLC.

Δ^7 Acids:

As already indicated in connection with the Δ^5 acids Posternak[43] first prepared the 7a acid from tariric acid and converted this to the 7t isomer as given for 6t.

Huber[48] and Fusari et.al[49] synthesised the 7a acid in the same year using the general Ahmad-Strong synthesis set out below.

$$RC\equiv CH \xrightarrow{1} RC\equiv C(CH_2)_5Cl \xrightarrow{2} RC\equiv C(CH_2)_5COOH$$

R = $CH_3(CH_2)_9$ (1) $NaNH_2$/liq.NH_3, $I(CH_2)_5Cl$ (2) NaCN, Hydrolysis.

Huber[48] prepared the 7t acid by reducing the acetylenic acid to the cis olefinic acid and by stereomutating this with selenium. His melting point (44.5°) agrees well with that given by Posternak (45.5°).

Ismail and Gunstone[42] used the same general method, as given for 6a and 6t acids, to prepare 7a and 7t acids.

Δ^8 Acids:

Arnaud and Posternak[50] reported the synthesis of 8a acid by dehydrohalogenation of a mixture of 9,9-di-iodo and 10,10-di-iodo stearic acid. They prepared the octadec-8-enoic acid by treating 9-iodostearic acid with alcoholic potassium hydroxide as well as, surprisingly by dehydration of 10-hydroxystearic acid. It now seems unlikely that they obtained isomerically pure acids, but the 8-enoic acid obtained by them should be predominantly the trans isomer.

Huber[48] has prepared the whole series of acetylenic, cis and trans acids from Δ^7 through Δ^{12} with the reaction sequence as given in Δ^7 acids. He reports the melting point of the Δ^{8t} isomer to be 52.5°, which shows that the acids prepared by Vanin and Chernoyarova[51] and Pigulevskii and Simonova[52] were probably 8t acids though they believed them to be octadec-7-enoic acid.

Fusari et.al[49] independently synthesised the 8a and the 8t acids by the same method as Huber and in the same year.

Ismail and Gunstone[48] prepared the 8a acid as well as the cis and trans isomers by their general method of reacting ω-yne-dimethylamides with alkyl bromides (cf. Δ^6 acids).

Δ^9 Acids:

Oleic acid being the most common component of all the vegetable and animal fats, elaidic (Δ^{9t}) acid was prepared as early as

in 1819[53]. Stearolic acid (Δ^{9a}) was first prepared by Overbeck[54] in 1866. Many workers have carried out partial synthesis of oleic or elaidic acids and have generally obtained mixture of these two acids. The following discussion is restricted to previous work on the total synthesis of the 9a and 9t acids.

Because of the availability of the natural oleic acid and its rather easy transformation to elaidic acid and stearolic acid, it is not surprising that the total synthesis of elaidic acid was not carried out till 1951. Gensler et.al.[55] were the first to carry out chemical synthesis of pure elaidic acid as follows.

$$CH_2{=}CHCH_2(CH_2)_7COOCH_3 \xrightarrow{1} CH_2BrCH{=}CH(CH_2)_7COOCH_3$$

$$\xrightarrow{2,3} CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$$

(1) N-Bromosuccinimide (2) Heptylmagnesium bromide; Saponification

Ames and Bowman[56] attempted the total synthesis of oleic acid by their alkoxy-ketone route (cf. Δ^4 acid) in the same year but got a mixture of oleic and elaidic acid.

Huber[48] was the first to achieve a complete synthesis of stearolic acid by the Ahmad-Strong synthesis (cf. Δ^7 acid). He converted the acetylenic acid to oleic acid and this latter to elaidic acid by heating with selenium.

Baker et.al.[57] prepared stearolic acid by the anodic synthesis from pentadec-6-ynoic acid and methyl hydrogen glutarate. They

later improved the low yield (6%) in this experiment by using benzyl hydrogen glutarate.

Ismail and Gunstone[42] condensed octyl bromide with N,N-dimethyldec-9-ynamide and hydrolysed the product to obtain stearolic acid. They then converted the acetylenic acid to cis isomer by partial hydrogenation and heated the cis isomer with selenium to obtain trans acid.

Δ^{10} Acids:

All the previous work on the synthesis of octadec-10-enoic acid by the action of alcoholic alkali on 10-iodostearate (Saytzeff[25,26]), dehydration of 10-hydroxystearate (Saytzeff[25,26] and Vasely and Majtl[58]), treatment of oleic acid with zinc chloride (Bauer and Panagoulias[59]) or heating of 10-chlorostearic acid with nickel carbonate (Vanin and Chernoyarova[51]) must have led to the mixture of positional and possibly stereo isomers.

The first unequivocal synthesis of the acetylenic and cis and trans olefinic acids was achieved by Huber[48] with the alkyl-acetylene method.

Most recently Ismail and Gunstone[42] carried out the synthesis of all the three acids by the following sequence of reactions.

$$HOOC(CH_2)_8COOH \xrightarrow{1} HOOC(CH_2)_8COOMe \xrightarrow{2,3} Br(CH_2)_8COOMe$$

$$\xrightarrow{4} Br(CH_2)_8CONMe_2 \xrightarrow{5} I(CH_2)_8CONMe_2 \xrightarrow{6}$$

$$CH_3(CH_2)_6C\equiv C(CH_2)_8CONMe_2 \xrightarrow{7} CH_3(CH_2)_6C\equiv C(CH_2)_8COOMe$$

$$\xrightarrow{8} CH_3(CH_2)_6\overset{c}{CH}{=}\overset{c}{CH}(CH_2)_8COOMe \xrightarrow{9} CH_3(CH_2)_6\overset{t}{CH}{=}\overset{t}{CH}(CH_2)_8COOH$$

(1) Diethyl Sebacate/EtOH (2) $AgNO_3$/KOH, Br_2/CCl_4 (3) NaOH

(4) $SOCl_2$, $HNMe_2$ (5) NaI/Acetone (6) $NaNH_2$/liq. NH_3, Nonyne

(7) Hydrolysis, methylation (8) H_2/Lindlar's Catalyst

(9) Hydrolysis, Selenium.

Δ^{11} Acids:

Apart from many early preparations of octadec-11-enoic acid, which are of dubious authenticity, many unequivocal synthesis of the acetylenic, cis and trans acids have been reported. Strong et.al.[60] prepared the 11a acid by condensing sodio-octyne with 1-chloro-9-iodononane in liquid ammonia and converting the resulting chloride to the acid. The acetylenic acid was then converted by partial reduction to cis isomer which was in turn elaidinised to give vaccenic (11t) acid.

Huber[48], Fusari et.al[49], Hofmann and Sax[61] and Morton and Todd[62] independently used the same route as given by Strong et. al. to prepare all the three isomers of the acid, and obtained closely agreeing melting points.

Gensler and Thomas[63] used the Grignard reaction to obtain vaccenic acid thus:

$$H_2C{=}C(CH_2)_9Cl \xrightarrow{1} BrCH_2CH{=}CH(CH_2)_8Cl \xrightarrow{2} CH_3(CH_2)_5CH{=}$$

$$=CH(CH_2)_8Cl \xrightarrow{3} CH_3(CH_2)_5CH{=}CH(CH_2)_9COOH$$

(1) N-Bromosuccinimide (2) Pentylmagnesium bromide (3)Malonic

ester synthesis.

van Loon and van der Linden[64] claimed the partial synthesis of a 2:1 mixture of trans and cis isomers several years before the first total synthesis of the vaccenic acid. They started with palmitoleic acid which is a constituent of whale and other fish oil fatty acids and got the mixture after following steps.

$CH_3(CH_2)_5CH=CH(CH_2)_7COOMe \xrightarrow{1} CH_3(CH_2)_5CH=CH(CH_2)_8OH \xrightarrow{2}$

$CH_3(CH_2)_5CH=CH(CH_2)_8Br \xrightarrow{3} CH_3(CH_2)_5CH=CH(CH_2)_9COOH$

(1) Bouveault and Blanc reduction (2) PBr_3/Toluene (3) Malonic ester synthesis.

Linstead and his colleagues[65] achieved the same chain elongation of palmitoleic acid by the anodic condensation with methyl hydrogen succinate and obtained pure octadec-cis-11-enoic acid. They then converted the cis acid to pure vaccenic acid via threo-dihydroxy stearic acid.

Ismail and Gunstone[42] obtained the 11a acid by condensation of ω-bromoundec-N,N-dimethylamide with heptyne followed by hydrolysis. The acetylenic acid, after partial reduction to cis isomer, was heated with selenium to obtain a mixture of cis and trans acids from which pure vaccenic acid was separated by silver nitrate TLC.

Δ^{12} Acids:

On the basis of our present understanding of the alkaline dehydrobromination of the bromoacids and of the alkenoic acids

obtained by dehydration of monohydroxy acids, in addition to the properties reported by Fokin[66] for octadec-12-enoic acid, the isomeric purity of his preparations seems to be doubtful.

The first unequivocal synthesis of the 12a, 12c and 12t acids was carried out by Huber[48] by the alkyl acetylene route given in Δ^7 acids.

Bharucha and Gunstone[67] achieved a partial synthesis of the 12t acid by bromination and debromination of threo-12,13-dihydroxystearic acid obtained from naturally occurring 12,13-epoxy oleic acid (vernolic acid).

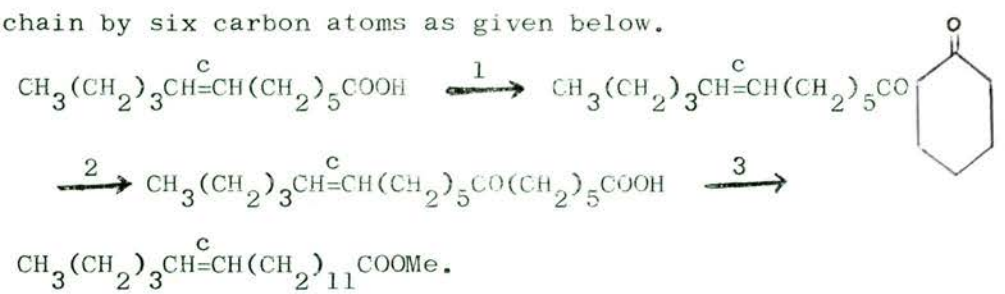
Baker and Gunstone[68] synthesised the acetylenic acid by anodic condensation of tetradec-8-ynoic acid with a half-ester of adipic acid.

Recently Ismail and Gunstone[42] obtained all the three isomers of Δ^{12} acid by the same method as used by them for Δ^{11} acids.

Δ^{13} Acids:

No synthesis of the acetylenic acid has been reported.

Ismail and Gunstone[42] prepared dodec-7-ynoic acid, converted it to the cis isomer by partial reduction and then extended the chain by six carbon atoms as given below.



(1) $SOCl_2$, 1-Morpholino-cyclohex-1-ene, HCl (2) KOH, HCl (3) N_2H_4/ethanolamine, KOH, HCl, methylation.

They comment that the C_{18} acetylenic acid was not prepared by this method because the migration of the triple bond occurred during the reduction of the keto group. However, they do not comment as to why the double bond, did not migrate under the same conditions and apparently gave very satisfactory results.

They obtained the trans acid by heating the cis isomer with selenium and separating the mixture on silver nitrate TLC.

Δ^{14} Acids:

No synthesis of octadec-14-ynoic acid has been reported.

Ismail and Gunstone[42] obtained the Δ^{14c} acid by the same method as given in the Δ^{13} acids and then elaidinised it with selenium. The pure trans acid was obtained from the mixture (80:20) by silver nitrate TLC.

Δ^{15} Acids:

An elaborate synthesis of octadec-15-enoic acid has been reported by Toyama and Yamamoto[69] starting with ethyl erucate. The main 4 steps are: (1) Preparation of ω-chlorotridecylaldehyde and thence 1-chloro-13-methoxy-12,13-dibromo-undecane; (2) Chain extension by 2 atoms on one side with Grignard reaction; (3) Introduction of unsaturation by an elimination reaction; (4) Chain extension by 3-C atoms in two steps on the other side of

the molecule. Thus:

$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOEt \xrightarrow{1,2,3,4} Cl(CH_2)_{12}CHO \xrightarrow{5}$

$Cl(CH_2)_{11}CHBrCH(OMe)Br \xrightarrow{6} Cl(CH_2)_{11}CHBrCH(OMe)CH_2CH_3$

$\xrightarrow{7} Cl(CH_2)_{11}CH=CHCH_2CH_3 \xrightarrow{8,9} CH_3CH_2CH=CH(CH_2)_{11}COOEt$

$\xrightarrow{10,11,12} CH_3CH_2CH=CH(CH_2)_{13}COOH$

(1) Na/BuOH (2) $SOCl_2$ (3) H_2O_2/AcOH (4) $Pb(OAc)_4$ (5) Br_2, HBr/MeOH (6) EtMgBr (7) Zn/BuOH (8) KCN/EtOH, Hydrolysis (9) esterification (10) NaBuOH (11) HBr (12) Malonic ester synthesis.

The introduction of the unsaturation being non-specific they obtained a mixture of cis and trans which was elaidinised by oxides of nitrogen to give a predominantly 14t acid.

Ismail and Gunstone[42] obtained the cis acid by the same method as given for Δ^{13} acids. Heating the acid with selenium gave a mixture of cis and trans, which they separated on silver nitrate TLC to obtain pure trans acid.

Δ^{16} Acids:

Kapp and Knoll[70] synthesised the octadec-16-enoic acid in 1943 starting from undec-9-enoic acid, as follows.

$CH_3CH=CH(CH_2)_7COCl \xrightarrow{1} CH_3CH=CH(CH_2)_7COC(COCH_3)(COOEt)(CH_2)_5COOEt$

$\xrightarrow{2} CH_3CH=CH(CH_2)_7CO(CH_2)_6COOH \xrightarrow{3} CH_3CH=CH(CH_2)_{14}COOH.$

(1) Na, $CH_3COCH(COOEt)(CH_2)_5COOEt$ (2) KOH, H_2SO_4, NaOH

(3) N_2H_4, KOH.

The configuration of the double bond was not determined, though the melting point, (62.8°-63.5°), when compared with that given by Ismail and Gunstone[42] for 16t acid ($65.5 - 66.5^{\circ}$), suggests it to be a trans isomer. The isomeric purity of the preparation will depend on the purity of the undec-9-enoic acid.

Ismail and Gunstone[42] obtained the cis acid by the same reaction sequence as given for Δ^{13} acids and elaidinised this with selenium. The trans acid was obtained by purifying the mixture of cis and trans isomers (26:74) by silver nitrate TLC.

Δ^{17} Acids:

Both Huber[48] and Kapp and Knoll[70] synthesised the octadec-17-enoic acid by the chain extension of undec-10-enoic acid. Kapp and Knoll achieved this by acetoacetic ester synthesis and Wolff-Kishner reduction as given for Δ^{16} acid.

Huber did the chain extension via di-undec-10-enyl cadmium thus:

$$CH_2=CH(CH_2)_8COOEt \xrightarrow{1} CH_2=CH(CH_2)_8CH_2MgBr \xrightarrow{2}$$
$$[CH_2=CH(CH_2)_9]_2Cd \xrightarrow{3} CH_2=CH(CH_2)_9CO(CH_2)_5COOEt.$$

(1) $LiAlH_4$, Bromination, Mg (2) $CdCl_2$ (3) $ClOC(CH_2)_5COOEt$.

Wolff-Kishner reduction gave the 17-octadecenoic acid.

Ismail and Gunstone[42] prepared dodec-11-enoic acid starting from nonane-1,9-diol and then extended the chain by 6 carbon atoms with the reaction sequence given in Δ^{13} acids.

During the reduction of the keto group they got considerable migration of the double bond (13.5%, Δ^{16} acid) and suggest that the chain extension should be carried out on the dodec-11-ynoic acid. This will make the purification of the final acid after reduction with Huang-Minlon reduction, easy, because the terminal acetylenic acid can be separated via its silver salt.

No synthesis of the acetylenic acid is reported.

B. SYNTHESIS

1. Synthesis of Octadecynoic Acids

This thesis reports the synthesis of two series of acids: octadec-2 to 17-ynoic acids and the octadec-trans-2 to 16-enoic acids. The purpose of this work was to prepare the 3, 13, 14, 15, 16 and 17- acetylenic acids which have not been synthesised before and so complete the series for the comparative study of their physical, chemical and biological properties and to synthesise all the acids in sufficient quantities (5-10 g) and as pure as possible so as to make such study possible.

It is desirable to use,as far as possible, a single flexible synthetic procedure capable of giving 5-10 g of pure product. The Ahmad-Strong synthesis was used successfully by Huber[48] to prepare the acids with the unsaturation in the middle of the chain but it has not been tried for the acids having the unsaturation near either end of the molecule. Ames and his colleagues[71] used ω-bromoacids to synthesise long chain unsaturated acids but long chain bromoacids ($> C_{12}$) required when unsaturation is near the methyl end, are not sufficiently soluble in liquid ammonia to give reasonable yields. The method also fails if short chain bromoacids ($> C_4$) are used (Ames et.al.[38]) so that unsaturation near the carboxyl end can not be introduced in this way.

Ames[44,72] used N,N-dimethylamides in place of the free acids to prevent precipitation of the carboxylic acids as their salts in the liquid ammonia syntheses and Ismail and Gunstone[42] prepared

several acetylenic acids in this way. The method still suffers from the disadvantage of insolubility when long chain compounds ($< C_{11}$) are used and in addition the amides have to be hydrolysed under rather severe alkaline conditions. Ismail and Gunstone[42] also used the enamine chain extension procedure devised by Hunig[73] to convert C_{12} acids to C_{18}-acids with unsaturation near the methyl end (Δ^{13} to Δ^{16}). This procedure did not give acetylenic acids and the conditions used for the reduction of the keto group (potassium hydroxide and ethanolamine at 176° for 30 mins.) gave some double bond migration (1%), some stereomutation (2 to 8%) and some saturation (2 - 2.5%) of the double bond. The method was particularly unsatisfactory for the Δ^{17} acid.

Anodic synthesis is a very elegant method of producing unsaturated acids without saturation, stereomutation or migration of the unsaturated centres. Nevertheless it suffers from two disadvantages: It can not be used with Δ^2 and Δ^3 acids and will not, therefore, furnish acids having unsaturation near either methyl or carboxyl end of the molecule and it is inconvenient for preparation on the 5-10 g scale.

On balance it was concluded that the condensation of α-ω-chloro-iodides with alk-1-ynes provided the best route to most of the acetylenic acids.

The purity of the acids was checked with silver nitrate TLC and by gas liquid chromatography on packed and also later on wall-coated open tube (capillary), polar (DEGS) and non-polar (ApL)

columns. von Rudloff oxidation and GLC examination of the dibasic and monobasic acids, is not a satisfactory procedure for checking the isomeric purity of acetylenic acids but some isomers were examined by this method after their reduction to cis-olefinic esters with Lindlar's catalyst. Impurities mentioned in the final product (cf. experimental) are generally not isomeric impurities but unidentified impurities which can be easily removed by column chromatography or TLC.

Octadec-2-ynoic Acid:

Pentadecyl bromide required for this synthesis was obtained by Ismail and Gunstone[42] from palmitic acid by the Hunsdiecker reaction. We prefer the recently introduced procedure of McKillop et.al.[74] involving the thallium salt. Thallium ethoxide is prepared by reacting ethanol vapour and air with thallium turnings in a specially prepared apparatus (see fig. 1). The heavy, oily thallium ethoxide settles at the bottom of the flask containing ethanol and is preserved like that. The ethoxide can then be pipetted from the flask when required. Thallium palmitate was prepared in high yield (97-99%) by adding the thallium ethoxide to a solution of the acid in petrol ether. This method is much simpler than the preparation of the silver salt, not least because it does not involve aq. solutions and the salt is therefore dried more easily. The bromide was then obtained

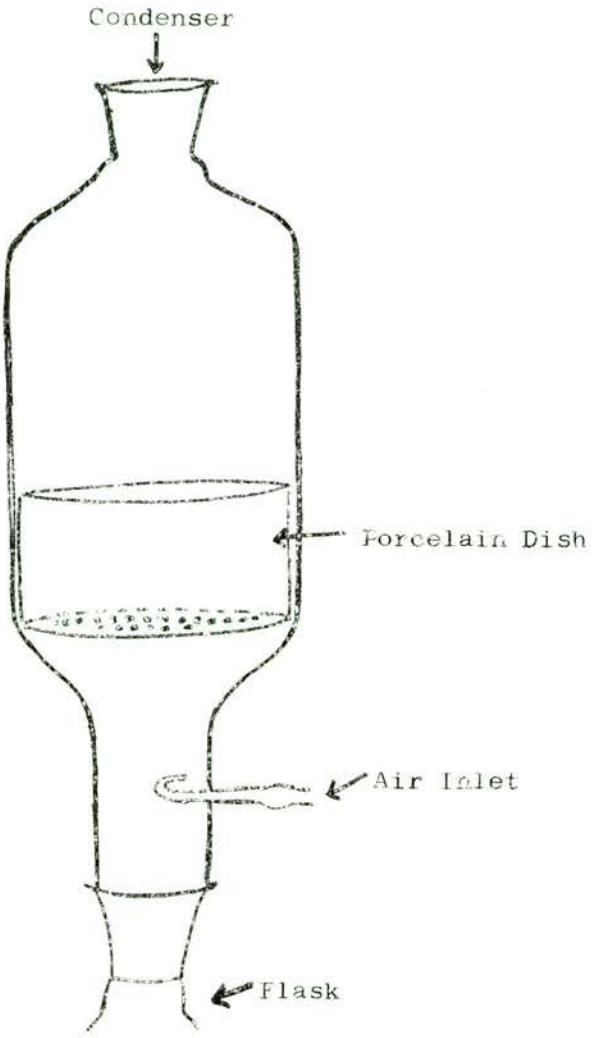


Figure 1.

Apparatus for preparation of Thallium Ethoxide

by adding bromine to the suspension of the thallium salt in carbon tetrachloride. Ismail and Gunstone[42] reacted the alkyl bromide with sodium acetylide in an autoclave because of the low solubility of pentadecyl bromide in liquid ammonia at -33° but DMF is a more suitable solvent as reported by Jenny and Meier[78] and we used this method to obtain the heptadecyne in good yield (77%). Hildebrandt and Grimmer[31] reacted heptadec-1-ynylmagnesium bromide and carbon dioxide at atmospheric pressure but like Ismail we carried out the reaction in an autoclave overnight.

The methylation of the Δ^{2a} acid proved difficult. When a small quantity was methylated with 14% boron trifluoride in methanol, GLC showed the presence of heptadec-1-yne (60%) while 3% boron trifluoride in methanol gave a methyl ester with less heptadec-1-yne(4%). Methylation by refluxing for 3 hrs. with methyl iodide and silver oxide produced a product which was almost entirely heptadec-1-yne (90%). The acetylenic acid was apparently decarboxylating during methylation and it was thus not possible to determine the purity of the acetylenic acid in this manner though the best methylation result (2% conc. H_2SO_4 in methanol, $\frac{1}{2}$hr. reflux) showed it to be at least 98% pure.

Indirect proof of the purity of the acid was obtained by reducing the acid with Lindlar's catalyst to octadec-cis-2-enoic acid and then methylating this with 3% boron trifluoride in methanol. The GLC then showed no heptadec-1-yne. The acid had

a melting point of 56-57° (without any further purification) which agrees well with that given by Grimmer and Hildebrandt[31] and Ismail and Gunstone[42] (56-57°). The nuclear magnetic resonance spectrum of the acid showed the complete absence of an ethynyl proton (-C≡CH).

Ismail and Gunstone[42] reported that their preparation gave some Δ^3 isomer (3.2%) and concluded that triple-bond migration occurred during the condensation of carbon dioxide and alkyl-magnesium bromide. Our product showed no migration either on GLC (which should be apparent as Δ^2 and Δ^3 acetylenic esters can be separated on GLC) or on von Rudloff's oxidation of the partially reduced acid.

Octadec-3-ynoic Acid:

Though the synthesis of octadec-3-ynoic acid has not been reported, two routes have been used for the preparation of Δ^3 acids of other chain lengths.

(1) $RC\equiv C-CH_2OH \longrightarrow RC\equiv C-CH_2Cl \longrightarrow RC\equiv C-CH_2CN$

$\longrightarrow RC\equiv CCH_2COOH$

(2) $RC\equiv CCH_2CH_2OH \longrightarrow RC\equiv CCH_2COOH$

While the preparation of the propargylic chloride is straight forward there are difficulties in the preparation of nitrile. Newmann and Wotiz[34] claimed to have prepared hept-2-ynonitrile with cuprous cyanide and xylene (90% yield) and Ames et.al.[38]

obtained tetradec-2-ynonitrile by the same procedure though with poor yields. On the other hand, Wotiz and Hudak[36] could not prepare oct-2-ynonitrile by this method and obtained a mixture of 1-cyano-oct-2-yne and 3-cyano-oct-1,2-diene when they used cymene (b.p. 176°) instead of xylene. More recently Kniparth and Stein[39] failed to carry out the reaction with 1-bromopentadec-2-ene. We got only a polymerised product on heating heptadec-2-ynyl chloride with sodium cyanide and DMSO.

The oxidation of the Δ^3 alcohol by chromic acid and acetone gives only poor or moderate (11-50%) yields of the Δ^3 acid.

Suga et.al.[75] reported that t-butyl chromate oxidised primary olefinic alcohols with β-unsaturation to the corresponding aldehyds with high yields. We tried this with heptadec-3-yn-1-ol but obtained the corresponding aldehyde only in low yields despite several modifications of the reaction conditions.

When they oxidised octadec-3-yn-1-ol with chromic acid and acetone, Ismail and Gunstone[42] got an impure product (34% 4a-acid, 50% 2a-acid) and they found it better to oxidise octadec-cis-3-en-1-ol to the 3c acid (0.5% 4c, 5% 2c) (50% yield). They report that von Rudloff oxidation of the 3a acid gave variable results. It is our experience that acetylenic acids are difficult to oxidise satisfactorily by this method and give a number of other products. It is therefore not clear whether the migration they observed, took place during chromic acid oxidation or the

von Rudloff oxidation.

We prepared hexadec-1-yne from tetradecyl bromide in DMF and reacted its Grignard derivative with ethylene oxide to obtain octadec-3-yn-1-ol. The alcohol was then oxidised with chromium trioxide and acetone to obtain the 3a acid (31%). The 2a, 3a and 4a methyl esters are easily separated on GLC (DEGS) but we obtained no peak corresponding to the ECL of either 4a or 2a. Our acid was only 95% pure but the impurities were two early running peaks. We could not purify the product further because it was found that these impurities could only be removed by silver nitrate TLC and that the 3a ester oxidised during purification. Repeated crystallisation may remove the unidentified impurities but the yield will be very poor.

Octadec-4-ynoic Acid:

Two procedures are available for the preparation of 4a acid. We preferred the chain extension of hexadec-2-yn-1-ol via malonic ester synthesis (Ames et.al.[41]) to the procedure used by Ismail and Gunstone[42] which requires rather severe alkaline conditions for the hydrolysis of a dimethyl amide and a low yielding oxidation of pent-1-yn-1-ol.

Hexadec-2-yn-1-ol was prepared from the dilithium derivative of propargyl alcohol and tridecyl bromide in liquid ammonia. The yield of the alcohol was moderate (36%) probably because of the low solubility of tridecyl bromide but the bromide can be easily

recovered and recycled. We tried to avoid this difficulty by using DMF as a solvent and carrying out the condensation reaction at room temperature. Surprisingly, we could not get any reaction at room temperature and at 70° only a polymerised product is obtained. Chain extension via malonic ester synthesis gave the acid which was over 99% pure according to GLC.

Octadec-5-ynoic Acid:

Tridec-1-yne, as its sodium derivative, reacts with ω-bromopentanoic acid (Ames et.al.[38]) but not with 1-chloro-3-iodopropane (Lumb and Smith[45]) in liquid ammonia.

We prepared 1-chloropent-4-yne from 1-chloro-3-bromopropane (10% yield) and condensed it with dodecyl bromide (yield 15%). The chain extension via nitrile gave octadec-5-ynoic acid. The yields, however, were very low and we did not repeat this procedure on a larger scale.

The attempt to condense chlorobromopropane with sodiotetradec-1-yne at 70°C in DMF was also unsuccessful.

Octadec-5-ynoic acid was finally prepared by condensation of N,N-dimethylhex-5-ynamide with dodecyl bromide as given by Ismail and Gunstone[42]. The acid was over 99% pure after one crystallisation from petrol ether. Some of it was partially reduced to the cis acid with Lindlar's catalyst and subjected to von Rudloff oxidation. No migration of unsaturated centre was observed.

Octadec-6 to 13-ynoic Acids:

Strong and Taylor[46] concluded that α-ω-chloroiodides (C_4- C_9) can be condensed in liquid ammonia with alk-1-ynes smaller than C_{13} so that octadec-7 to 11-ynoic acids can be prepared by this method. Huber[48], however, prepared the 7a to 12a acids by this method and with slight modifications we were able to prepare Δ^{5a} (see above) to 17a acids from α-ω-chloroiodides, though yields were sometimes low (Δ^5 and Δ^{16}).

The Δ^6 acid was prepared by the following sequence of reactions.

$$I(CH_2)_4Cl \xrightarrow{1} HC\equiv C(CH_2)_4Cl \xrightarrow{2} RC\equiv C(CH_2)_4Cl \xrightarrow{3}$$

$$RC\equiv C(CH_2)_4COOH$$

$R = C_{12}H_{25}$

(1) $NaC\equiv CH/Liq.NH_3$ (2) $NaNH_2/Liq.NH_3, C_{12}H_{25}Br$ (3) Chain extension via nitrile.

The yields were satisfactory and the product obtained was free of positional isomers.

Δ^7 to Δ^{13} acids were prepared by obtaining either C_{16} or C_{17} chlorides by the Ahmad-Strong synthesis followed by appropriate chain extension with malonic ester synthesis or via nitrile. These synthetic sequences presented no special problems.

Octadec-14-ynoic Acid:

Dodecane-1,12,-diol was converted to dichloride. On treatment with sodium iodide and acetone the dichloride gave a mixture of dichloride (60-66%), di-iodide and chloro-iodide. It was found advantageous to use this crude mixture without distillation which seemed to be accompanied by some decomposition. The dichloride gives a dibasic acid whilst di-iodide gives either dibasic acid or long chain hydrocarbons in further reaction. All of these are easily separated from the final monobasic acid either by crystallisation or column chromatography. Pent-1-yne was prepared from lithium acetylide and propyl bromide taking all the precautions mentioned by Ismail[32]. The final acid contained a considerable amount of dibasic acid (C_{14}) as expected. Most of it was removed by taking up the monobasic acid in petrol ether (in which the dibasic acid is not very soluble at room temperature) and recrystallising it. This removed all the other impurities but the product was contaminated by some dibasic acid. Final purification was effected by the column chromatography of the methylated product. The acid obtained was $\sim$ 100% pure according to GLC, and melted at 63.5-64°. The synthesis or the melting point of this acid has not been reported before.

Octadec-15-ynoic Acid:

Ethyl bromide was condensed with lithium acetylide in liquid ammonia, in an unsuccessful attempt to prepare but-1-yne. The

reaction was carried out under acetone cardice condenser and the ammonia was evaporated through an ice-water condenser and scrubbed by passing through drechsel bottles filled with petrol ether (b.p. 60-80°) but all the but-1-yne was carried off.

Subsequently lithium acetylide was prepared by the titration method (0.5 mole lithium) in liquid ammonia and treated with excess of ethyl bromide (0.75 mole) to avoid any unreacted lithium acetylide, which will lead in further reactions to ω-ynoic acids difficult to separate from the final product. Lithamide prepared in another flask was added to the mixture, stirred for 3 hrs. and reacted with crude 1-chloro-12-iodo-dodecane (containing 66% chloro-iodide). The whole reaction sequence was carried out under an acetone-cardice condenser followed by 8 hrs. reflux. The 1-chlorohexadec-13-yne was obtained in moderate yield (40%). The usual malonic ester synthesis gave the 15a acid, which was found to be pure (m.p. 65-65.5°) according to GLC after one crystallisation.

Octadec-16-ynoic Acid:

1-Chloro-14-iodotetradecane treated with prop-1-yne in the same way as for the Δ^{15} acid gave no C_{17}-product. This may have been due to an unsuccessful preparation of prop-1-yne or to the very low solubility of the chloro-iodide, but the matter was not pursued further.

Crude chloro-iodo-dodecane (60%) was condensed with sodium acetylide in DMF at room temperature and the product (24.5%) was purified by silica gel column chromatography. This reaction also gave a large amount of a by-product which was insoluble in petrol ether but was not further investigated. 1-Chlorotetradec-13-yne (as its sodium derivative) was treated with methyl bromide and the crude reaction product was successively converted to a C_{17}-acid by malonation, reduced to the C_{17}-alcohol, turned into C_{17}-chloride, treated with sodium cyanide and DMSO, and shaken with 25% hydrochloric acid in dry methanol. The portion of the product soluble in petrol ether was purified by chromatography on a column of florisil impregnated with silver nitrate. This gave a sample of methyl octadec-16-ynoate with two impurities [ECL 21.0 (10%) and 20.0 (5%) on DEGS and ECL 17.0 and 16.0 on ApL], which could not be removed even by silver nitrate TLC. From the ECL values the impurities seem to be heptadec-15-ynoic acid and hexadec-14-ynoic acid.

The impure product is suitable for examination of the ester by GLC and TLC but the melting point of the acid will have no significance. von Rudloff oxidation of the acid will not give information about the isomeric purity of the Δ^{16} acid because of the presence of lower homologues in the preparation. GLC, however, shows the absence of the Δ^{17} and Δ^{15} C_{18}-acids since the esters of these acids are well separated from the Δ^{16} ester. The

preparation has thus been achieved without migration of the unsaturated centre. An attempt to separate small amounts of pure Δ^{16} acetylenic acid by preparative GLC was unsuccessful.

This preparation is probably unsatisfactory because we did not purify the intermediates. The condensation of propyne and C_{12}-chloro-iodide gave such a poor yield that we chose not to lose material during purification at each subsequent stage, believing that purification of the final product was possible. Since all impurities can be removed by silver nitrate column chromatography except the lower homologues of the acid, it is important that the intermediates should be purified after each chain extension procedure.

This acid was prepared at the end of our studies and there was no time to repeat the preparation. For the future we would propose that the condensation of the chloroiodo-dodecane with sodium acetylide should be carried out in liquid ammonia rather than in DMF. The yield in liquid ammonia may be low but it should be possible to separate the unreacted chloroiodide by column chromatography and recycle it. The reaction with sodium cyanide in DMSO also gave an unsatisfactory product but whether this was due to the impure intermediate or to some unexpected property of 1-chloroheptadec-15-yne and its lower homologues, was not determined.

Octadec-17-ynoic Acid:

This acid, which is not prepared previously, was synthesised thus:

$$I(CH_2)_{12}Cl \xrightarrow{1} HC\equiv C(CH_2)_{12}Cl \xrightarrow{2} HC\equiv C(CH_2)_{13}COOH$$

$$\xrightarrow{3} HC\equiv C(CH_2)_{14}OH \xrightarrow{4} HC\equiv C(CH_2)_{14}Cl \xrightarrow{5}$$

$$HC\equiv C(CH_2)_{15}COOH.$$

(1) $NaC\equiv CH/liq.NH_3$ (2) Malonic ester synthesis (3) $LiAlH_4$

(5) $SOCl_2$/Pyridine (6) Malonic ester synthesis.

No purification of intermediates was attempted except that the dibasic acid obtained after first malonation was removed by crystallisation from petrol ether solution and the hexadec-15-ynyl chloride was passed through a short silica gel column which gave pure chloride. The second malonic ester synthesis gave a product which was isomerically pure Δ^{17a} according to GLC and melted at 66-67°.

Some of the acid was turned into methyl ester with 3% boron trifluoride in methanol and reduced with Lindlar's catalyst (5 mins.). The Δ^{17e} methyl ester obtained showed the presence of 2.5% of stearic acid (GLC) but no other impurity. (Both the 16c and 16t methyl ester differ from the 17e ester in their GLC behaviour.)

2. Synthesis of Octadec-trans-enoic Acids

The complete series of octadec-trans-enoic acids was prepared by Gunstone and Ismail[42] and some of their physical properties (GLC, m.p.) were examined. In common with many others they obtained the trans acids from the more readily available cis isomers by stereomutation. This procedure gives a mixture of cis and trans acids which can be separated only by careful silver nitrate chromatography. This restricts the amount of trans esters which can be separated and for chemical and biological study of these acids, it is desirable to have them in larger quantities free of cis or positional isomers.

No satisfactory method for the partial stereospecific reduction of acetylenes to trans olefin was available until 1941 when Campbell and Eby[22] suggested that dialkyl acetylenes can be reduced by sodium and liq. ammonia. Elsner and Paul[24] found that the reduction of octadecynes with sodium and liquid ammonia at atmospheric pressure as given by Campbell and Eby[22] was not possible but they succeeded by using an autoclave under pressure. This method of using sodium and liquid ammonia for the reduction of triple bond to trans-double bond is not previously used with octadecynoic acids but seemed very promising for the preparation of trans-acids on 5-10 g scale.

We also found that the octadecynoic acids (except Δ^2 and Δ^3)

can not be reduced at atmospheric pressure but can be reduced in an autoclave at room temperature in 10-12 hrs. A number of precautions however must be taken. Reaction between sodium or lithium and ammonia to form sodamide is a very very slow reaction but presence of transition metal ions makes it very fast and the alkali metal is quickly converted to amide. For this reason any contamination with iron must be scrupulously avoided and only distilled ammonia should be used. Stainless steel is normally not attacked by sodium and liquid ammonia and we had one or two good runs in the stainless steel autoclave but then the autoclave must have got slightly attacked and we could not carry out satisfactory reductions. We then used a glass sleeve inside the autoclave to carry out the reaction and replaced the metal stirrer by a magnet encased in glass (P.T.F.E. coated magnets are attacked by sodium and liquid ammonia). To ensure nearly complete reduction, the solution was stirred well with the magnetic stirrer and sodium dried THF (25% of liq. ammonia) was added as a co-solvent. Lithium was preferred to sodium because iron is a much less effective catalyst for the unwanted reactions between lithium and ammonia[76]. We also found that the reaction was nearly complete after a single reduction (Repeated reductions give some migration of double bond). If all the precautions are taken, all the acids from Δ^4 to Δ^{16} can be reduced almost completely (97-99%), overnight, without formation of any positional isomers.

Octadec-trans-2-enoic Acid.

Sodium and liquid ammonia reduction of octadec-2-ynoic acid gives stearic acid because the triple bond is conjugated with the C=O group and 1:4 addition of electrons takes place. So the trans acid had to be prepared by separate method.

Grimmer and Hildebrandt[31] obtained a mixture of cis and trans esters when they treated methyl octadec-cis-2-enoate with mercuric acetate. It was found in our laboratory[77] that when the Δ^2 cis methyl ester is reacted with mercuric acetate for 48 hrs. in dry methanol at room temperature, the olefin regenerated with dilute acid is predominantly the 2t ester with less than 2% cis isomer. So some of the acetylenic acid was reduced to cis-isomer with Lindlar's catalyst, turned into methyl ester and converted to trans ester by the above method. The ester contained 98% trans isomer. The von Rudloff oxidation showed no migration of double bond.

The Wittig reaction is a well known reaction for the synthesis of trans compounds and was tried first. Cetyl alcohol was converted via its tosylate to cetyl aldehyde and the aldehyde was condensed with carbethoxymethyl-triphenylphosphorane. The ethyl octadec-trans-2-enoate obtained (80%) contained 7.7% cis isomer according to GLC.

The Doebner synthesis is also known to yield trans isomers. Cetyl aldehyde was therefore condensed with malonic acid in

pyridine at 90°. The methylation of the acid presented some difficulty (see table 1) and it appeared that esterification was accompanied by double bond migration.

Table 1.

Esterification of Octadec-trans-2-enoic Acid

Esterification conditions	%3t isomer (by GLC)
Boron trifluoride-Methanol (14%); 2 Min. reflux	22
Boron trifluoride-Methanol (3.5%); 30 Min. reflux	11
Silver oxide-Methyl iodide; 2 hrs. reflux	14
Diazomethane	31

Since the free acid and its ester (with 11% of 3t isomer) both gave palmitic acid and pentadecanoic acid (11%) when subjected to von Rudloff oxidation, it seemed that the original acid already contained some 3t isomer.

Octadec-trans-3-enoic Acid:

When the double bond is near the carboxylic group it is reduced by sodium and liquid ammonia much faster than when it is in the middle of the chain. The Δ^3 acetylenic acid could be reduced at atmospheric pressure to 91% trans content in 8hrs. Crystallisation of the product did not increase the trans content.

Von Rudloff oxidation as well as GLC showed no migration of the double bond.

Octadec-trans-4 to 16-enoic Acids.

The acetylenic acids were reduced with lithium and liquid ammonia in an autoclave. None of them (except Δ^8 and Δ^9) showed any migration of the unsaturated centre and all of them were reduced to 96% to 99% trans acids. No further purification was attempted except that the acids were crystallised once from petrol ether. This generally improved the trans content by 1 to 2%. The Δ^8 and Δ^9 acids were prepared at the beginning of our work when the reduction procedure was not standardised. The Δ^8 acid had to be reduced 4 times and Δ^9 acid twice before reduction was complete. This repeated reaction gave some migration of double bond (4% on each side for Δ^8 and 2 to 2.5% on each side for Δ^9) in these two cases.

C. PROPERTIES

I Melting Point:

The melting points of the octadecynoic and octadec-*trans*-enoic acids are given in Table 2 and illustrated graphically in Figure 2. No special purification was carried out before determining the melting points and the melting points are uncorrected. Ismail and Gunstone[42] have determined the melting points of the complete series of *trans* isomers and of some of the acetylenic acids. Our melting points agree well with theirs. The following points are noted:

(1) The melting points of five of the octadecynoic acids (3a, 13a, 14a, 15a and 17a) are reported for the first time.

(2) When unsaturation is in the central region of the C_{18}-chain (Δ^5 to Δ^{13}), the acetylenic acids, unlike the *cis* and *trans* olefinic acids, do not show an alternation in their melting points. Instead they lie in a shallow saucer-shaped curve, with maximum and minimum values of 52° and 46°.

(3) With these values, the melting points straddle those of the *trans* acids. The acetylenic acid has a higher m.p. when unsaturation starts on an odd C atom (Δ^5 - Δ^{13}) and a lower m.p. when unsaturation starts on an even C atom (Δ^6 - Δ^{12}). The difference in two series is smallest with the Δ^9 isomer (2°).

(4) When the unsaturation is not in the central region the melting

Table 2.

MELTING POINTS

Positon of the unsaturation	Octadecynoic Acid °C	Octadec-trans-enoic Acid °C
Δ^2	56.5 - 57.0	57.5 - 58.5*
Δ^3	73.0 - 74.0	64.0 - 65.0
Δ^4	74.0 - 74.5	59.0 - 59.5
Δ^5	51.5 - 52.0	45.0 - 46.0
Δ^6	49.5 - 50.0	52.5 - 53.0
Δ^7	47.0 - 48.0	43.5 - 44.5
Δ^8	46.5 - 47.0	51.0 - 51.5
Δ^9	46.0 - 46.5	43.5 - 44.5
Δ^{10}	45.5. - 46.5	52.0 - 52.5
Δ^{11}	46.0 - 46.5	43.0 - 43.5
Δ^{12}	46.0 - 47.0	51.0 - 52.0
Δ^{13}	48.5 - 49.0	43.5 - 44.5
Δ^{14}	63.5 - 64.0	53.0 - 53.5
Δ^{15}	65.0 - 65.5	58.0 - 58.5
Δ^{16}	-	65.5 - 66.5*
Δ^{17}	66.0 - 67.0	55.5 - 56.5

* Values taken from Gunstone and Ismail[42]

MELTING POINT CURVES

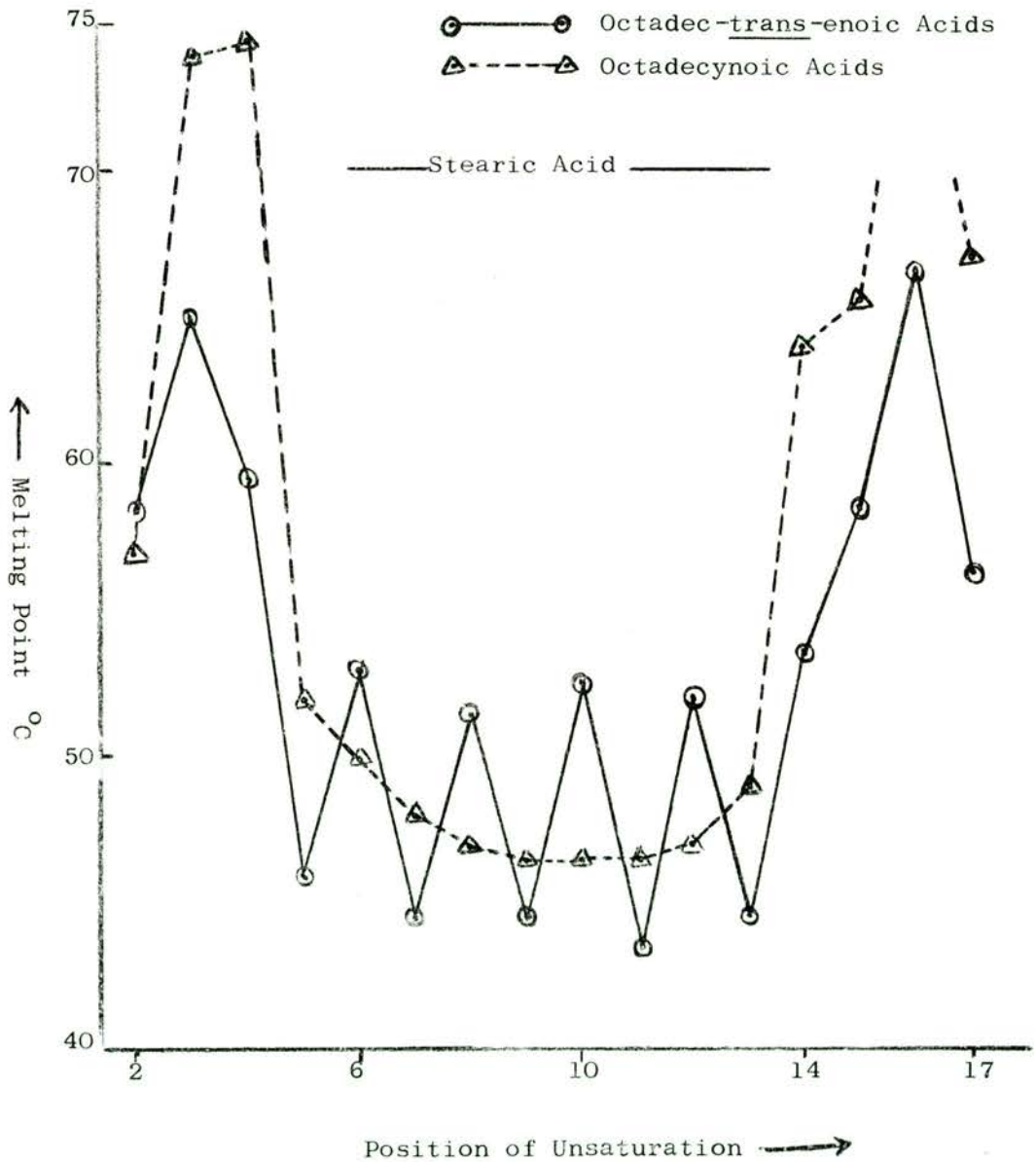


Figure 2.

points are higher, especially for the Δ^{3a} and Δ^{4a} (and probably Δ^{16a}) acids, which melt higher than does stearic acid.

(5) The increase in m.p., as the triple bond moves from the central region of the chain to either end, is greater than the increase shown by the trans olefinic acids.

These changes in m.p. among the isomeric octadecynoic acids and the lack of alternation when the triple bond is in the central region (compare the marked alternation in trans acids) is indicative of changes of packing in the crystals of these isomeric molecules, which can be fully explained only after a detailed study of the crystal structure. Nevertheless it is useful to discuss these results in relation to a recent and interesting paper by Howton[78] on the m.p. of long chain acetylenic acids.

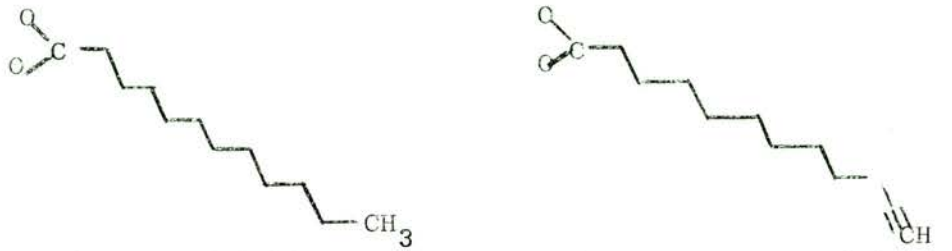
Howton has drawn attention to the fact that the m.p. of the octadecynoic acids do not alternate when the unsaturation is in the central region (Δ^5 to Δ^{12}) of the carbon chain. Our results confirm this observation and extend it to the Δ^{13} isomer. The melting point is much higher as the double bond approaches either end because of the so called end effects. As reported for some other Δ^3 and Δ^4 acetylenic acids, our Δ^3 and Δ^4 acids also melt higher than the corresponding saturated acid. This may be due to the long linear segments (C_4) near the end of the molecules which will allow better packing. At the other end, we could not

determine the melting point of Δ^{16a}, because a pure sample was not available, but our Δ^{17a} melts slightly lower (66^{o}-67^{o}) than the saturated acid. In commenting on the end group effects, Howton considers that the m.p. of ynoic acids exceed that of the corresponding saturated acid for the Δ^{3}, Δ^{4}, ω and ω-1 acids and concludes that the only single example of an ω-acid (ω-C_{10}) which melts below that of the corresponding saturated acid, may have an erroneously low m.p. We have no information on this acid but our ω-C_{18}-acid also melts slightly lower than the stearic acid. In this ω- series, the difference in m.p. between the ynoic acid and the parent saturated acid lies in this increasing order.

$$C_{10},\ C_{18},\ C_{14},\ C_{8};\quad C_{9},\ C_{23},\ C_{17},\ C_{11} \text{ and } C_{13}.$$

It may be pointed out that in this series all the even acids fall below all the odd acids. The difference between the m.p. of the ω-unsaturated acids and their saturated analogues is -5^{o} to $+5^{o}$ for the even acids and +7 to $+15^{o}$ for the odd acids.

Howton suggests that when the end alkyl plane (CH_2-CH_3) is inclined to the plane of first 2C atoms ($\overset{O}{\underset{O}{>}}C$-$CH_2$) the melting point is lowered. In the ω-acetylenic acids we have a 3C linear segment (CH_2—C≡CH, see fig. 3) and in a transoidal arrangement of the molecule, the even, ω-unsaturated acid will have inclined end alkyl plane while the saturated acid will have the end alkyl plane parallel to the plane of the first 2C atoms.



C_{12}-Saturated Acid C_{12}-ω-unsaturated Acid

Figure 3.

So the effect of better packing due to a longer linear end segment may be offset by the inclination of the end alkyl group.

II Thin Layer Chromatography on Silver Nitrate Impregnated Silica Gel Plates

It is well known that silver ions form complexes with olefins by the overlap of the vacant 5s and the filled 4d orbitals of the silver ion with the π 2p and the antibonding π orbitals of the olefin. Nichols[79] reported that the argentation constants [i.e. The ratio of the concentration of olefin-silver complex (BAg) to the concentration of uncomplexed material (B) multiplied by concentration of silver ions (Ag^+) or $(BAg)/(B)(Ag^+)$] for cis and trans olefins differ greately and that methyl oleate and elaidate can thus be separated by counter current distribution between aqueous methanolic silver nitrate and iso-octane.

Since then many researchers have used silver nitrate chromatography on columns or on thin layers to separate (a) long chain fatty esters with different degrees of unsaturation and (b) cis and trans isomers of long chain alkenoates. Bergelson and co-workers[80] found that positional isomers of monoenoic esters also give different Rf values and having separated the 7c, 9c, and 11c octadecenoates,claimed that all the positional isomers of the monoenoic acids can be separated by silver nitrate TLC. Morris et.al.[81] also separated several cis and trans isomers of octadecenoates by this method at -25^{o} and predicted the Rf values of others. Gunstone and Ismail[82] having carried out the Ag^+/TLC of

all the cis and trans octadecenoates showed that cis alkenoates can always be separated from their trans isomers but that all positional isomers (cis or trans) of the octadecenoates can not be separated from each other.

We are not aware of any study of the behaviour of all the octadecynoates by Ag^+/TLC, though Morris and Marshall[83] have reported that methyl stearolate has a higher Rf value (0.6) than methyl oleate (0.45), when developed in toluene at -25^{o}.

Silver nitrate TLC of the Δ^2 to Δ^{16} acetylenic esters is shown in fig. 4, and the separation of the a, c and t isomers is shown in fig. 5. The following points are apparent:

(i) As with the cis and trans octadecenoates the positional isomers of octadecynoate have different Rf values from each other, which lie on a curve. The 2a ester has the highest Rf value; this value falls sharply for the 3a ester and then gradually to a minimum value at the 6a/7a ester and rises steadily again to a maximum at the 16a ester. The 17a ester forms a silver salt and is held on the base line.

(ii) It follows that many isomeric octadecynoates can be separated from each other but not all of them.

(iii) In all the cases it is possible to separate acetylenic esters from the corresponding cis and trans olefinic isomers. The order of Rf values is t > c > a with our solvent system (94 Petrol ether: 6 ethyl ether). The only exception is the Δ^2 ester

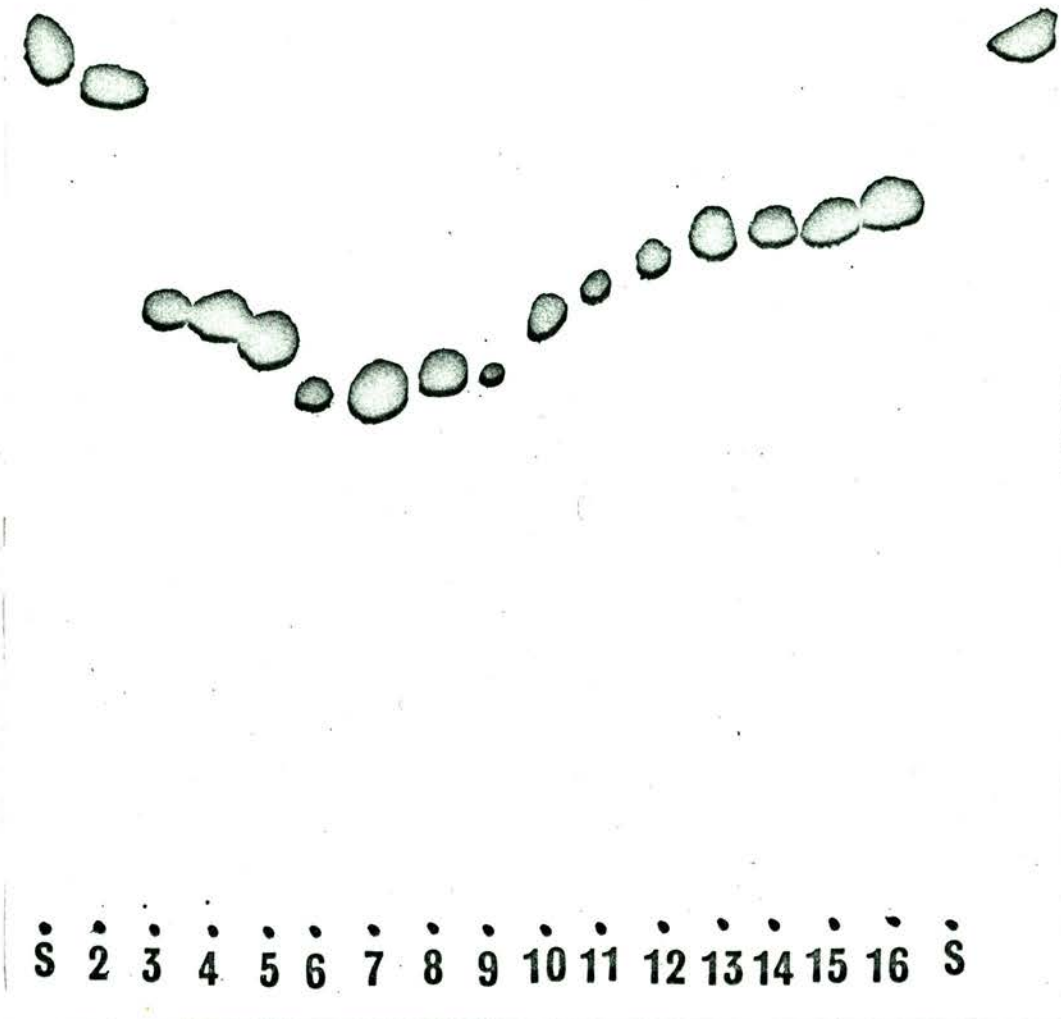


Figure 4.

Ag^{+}/TLC of Δ^{2} to Δ^{16} Methyl Esters of Acetylenic Acids

10% $AgNO_3$ Solvent: PE/EE (94:6)

(The nos. on plate denote the position of unsaturation

S = Me Stearate)

in which the order of elution is c > a > t.

The high value for the Δ^2 ester is not unexpected since the conjugation of the unsaturated group with carbomethoxy group will lead to reduced availability of electrons for reaction with silver ion. The differential behaviour of the remaining esters is more difficult to explain and the suggestion previously made by Ismail[32] is probably not satisfactory.

Our results with the 9a, 9c and 9t esters differ from those reported by Morris and Marshall[83]. We have confirmed their results using toluene at -25°C as developing solvent and found that in this solvent, but not in petrol ether/ether mixture used by us, there is a change of order of elution with the change in temperature (see Table 3).

Table 3.

The Order of Elution of Δ^9 a, c, t Esters on Ag^+/TLC

Solvent	temperature	Order of increasing Rf value
PE:EE:: 94:6	20°	t > c > a
PE:EE:: 94:6	-25°	t > c > a
Toluene	20°	t > c > a
Toluene	-25°	t > a > c

This reversal of the Rf values of the acetylenic and the cis olefinic ester must reflect the change in the argentation

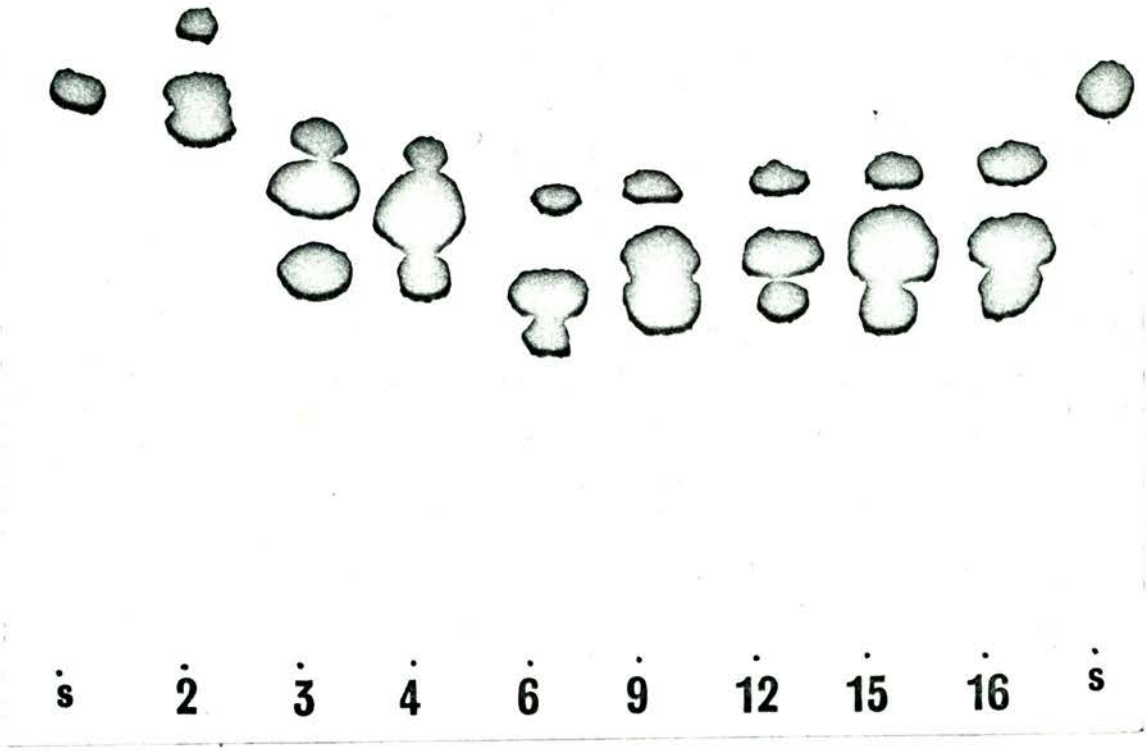


Figure 5.

Separation of a, c, and t isomers on Ag^{+}/TLC.

(The nos. on plate denote the position of unsaturation

S = Me. Stearate)

20% $AgNO_3$ Solvent: PE/EE (94:6)

Two developments at ca. 20°C.

constant of these esters with temperature but the reason for this change is not clear.

Muhs and Weiss[84] found the argentation constants of alkynes to lie between cis and trans alkenes by GLC. They also claim that alkynes form only 1:1 complex with silver ion so that the extra pair of filled orbitals in alkynes has little or no effect on the bonding with silver. Lucas and co-workers[85] on the other hand report that when the solubility (at $+25^{o}$) of any alkyne in aq. silver nitrate solution is plotted against the silver ion concentration, the plot has an upward curvature. This indicates a greater solubility than would be given by 1:1 complex and is believed to be the result of 1:2 complex (olefin: Ag^{+}). If the finding of Lucas et.al.[85] is correct, it is natural that the alkynoic esters which will form 1:2 complex (at least on plates heavily impregnated with silver nitrate) should have lower Rf value than the cis monoenes which form only 1:1 complex.

III GAS LIQUID CHROMATOGRAPHY

James and Martin[86] published in 1952 a description of separations of model mixtures of fatty acids by gas liquid chromatography and put into the hands of Lipid chemists a powerful analytical technique. Its usefulness to biochemists and Lipid chemists can be seen from its very rapid development and from the fact that practically every recent paper published on the analysis of fats and lipids, contains GLC data.

GLC allows a very rapid analysis, both quantitative and qualitative, of the mixture of fatty esters which are otherwise very difficult to separate, and requires only small amounts of the substance, usually a few µg. The development of open tube wall coated columns, has made it possible to separate not only fatty acid esters with different chain lengths and degree of unsaturation but also positional isomers of unsaturated acids with the same chain length.

Though the definitive identification of a fatty ester can not be based on GLC analysis alone, the tentative identification of an ester in a mixture of very similar esters is possible by comparison with known substances especially if carried out on both polar and non-polar columns. In fact the retention time of an unknown ester relative to the retention time of the standard saturated esters can lead to a tentative identification. This

relative retention can be conveniently described as the 'carbon number'[87] or equivalent chain length (ECL)[88]. Holman et.al.[89] and Miwa and his colleagues[88] have compiled the ECL data for a large number of diverse fatty acid esters.

Gunstone et.al.[90,91] have recently published ECL values for a complete series of octadec-cis and trans-enoates and for some dienoic, diynoic and monoynoic esters. Christie[92] has published the data on the complete series of methyl interrupted octadecadienoates.

(a) ECL values of octadecynoates and octadec-trans-enoates

We have now studied the GLC behaviour of the complete series of octadecynoates and re-examined the octadec-trans-enoates on both polar (Diethylene glycol succinate - DEGS) and non-polar (Apiezon L - ApL) columns. In his study of the trans esters on ApL, Ismail[32] used mixtures of cis and trans esters which did not always separate from each other. ECL values on a DEGS column were not studied on this series.

The ECL data for the octadecynoate series is given in Table 4 and for the octadec-trans-enoate is given in Table 5. The values given in the tables are the mean of two values which did not differ from each other by more than 0.02 to 0.03. The columns used were 50 m. open tube wall coated columns purchased from Perkin-Elmer Ltd.

Table 4.

ECL Values for Methyl Octadecynoates

Isomer Δ	DEGS	ApL
2	21.13	18.57
3	20.64	18.05
4	19.97	17.88
5	19.90	17.86
6	19.98	17.81
7	20.03	17.80
8	20.02	17.80
9	20.02	17.78
10	20.09	17.82
11	20.17	17.86
12	20.25	17.93
13	20.33	17.99
14	20.42	18.01
15	20.64	18.12
16	21.46	18.50
17	21.16	17.98

Table 5.

ECL Values for Methyl Octadec-trans-enoates

Isomer Δ	DEGS	ApL
2	19.57	18.61
3	18.67	17.86
4	18.32	17.75
5	18.34	17.76
6	18.35	17.72
7	18.33	17.71
8	18.36	17.71
9	18.35	17.72
10	18.38	17.74
11	18.40	17.75
12	18.45	17.78
13	18.50	17.81
14	18.52	17.81
15	18.60	17.86
16	18.89	18.00
17	18.81	17.90

We found ECL values for both octadec-trans-enoate and octadecynoates to be reproducible on the ApL column. The maximum deviation from the values reported by Ismail[32] 5 years ago was 0.05 and most of them agreed within 0.02 - 0.03 which is the margin of reproducibility reported by both of us. The only exception is the value for Δ^{2t} for which Ismail reported 18.80. There is also satisfactory agreement (max. deviation 0.07) between our DEGS values and those published by Scholfield and Dutton[93]. Holman et.al.[89] have reported values for the 6a, 7a, 8a, 9a and 11a esters on a packed DEGS column which are much higher (0.32 to 0.42) than our values. It is our experience that packed columns give higher values for acetylenic esters than the wall coated open tubular columns, that they have used helium (we nitrogen) as eluant may also have some effect. Again there is a fair agreement between our values for acetylenic esters (max. deviation 0.09) and those given by Scholfield and Dutton[83] for some esters.

(b) Separation of positional isomers

Although the ECL values of the esters do not depend on the number of theoretical plates of the column the separation of the two substances is directly dependent on the number of theoretical plates. Christie[92] reported ca. 40,000 theoretical plates for his non-polar (ApL) column and ca. 15,000 for his polar column

(NPGS) and claimed recognisable separations with as little as 0.02 ECL difference. The resolution power of our wall coated open tubular columns was only ca. 5,800 theoretical plates for non-polar and ca. 7,300 theoretical plates for the polar column. With this efficiency we could achieve recognisable separations (two peaks separated at the apex) above 0.15 (for polar column) and 0.11 (for non-polar) ECL differences. For the base line separation the peaks had to be at least 0.2 ECL apart. The tables 6, 7 and 8 show representative separations on the polar and non-polar columns between cis and trans isomers and between positional (trans and acetylenic) isomers.

Table 6.

Separation of cis and trans isomers.

Isomer Δ	Separation		Difference in ECL	
	DEGS	ApL	DEGS	ApL
2	base line	base line	1.35	0.63
16	base line	separated	0.28	0.11
13	shoulder on the peak	No separation	0.12	0.02
9	No separation	Shoulder on the peak	0.05	0.09

Table 7.

Separation of Acetylenic Isomers.

Isomers △	Separation		Difference in ECL	
	DEGS	ApL	DEGS	ApL
2 + 3 + 4	base line	base line	0.51,0.33	0.51,0.17
9 + 12 + 15	base line	separated	0.23,0.39	0.14,0.21
11 + 13	separated	-	0.16	-
14 + 15	-	shoulder on the peak	-	0.08
11 + 12 + 13	No separation	No separation	0.08,0.08	0.07,0.06

Table 8.

Separation of trans-Isomers

Isomers △	Separation		Difference in ECL	
	DEGS	ApL	DEGS	ApL
2 + 3 + 4	base line	separated	0.9,0.35	0.74,0.11
12 + 15	separated	Shoulder on the peak	0.15	0.09
13 + 15	Shoulder on the peak	No separation	0.10	0.05
9 + 12	No separation	No separation	0.10	0.05

(c) Prediction of ECL values:

Apart from the value of the above GLC data for tentatively assigning double bond or triple bond position in an unknown octadec-trans-enoic or octadecynoic esters, it is interesting to see whether these values can be used to predict the ECL value of polyenoic acids. Ackman[94] used the concept of contribution of functional groups to the basic ECL value [the fractional chain length (FCL)] to predict the ECL's of branched chain ester. Gunstone and Lie[91] applied this concept to the polyunsaturated (cis)esters and compared obtained values for the dienoates with those calculated from the corresponding monoenoates. For example, on DEGS column,

$$\text{FCL } \Delta^{5c}: 18.41 - 18.00 = 0.41$$

$$\text{FCL } \Delta^{12c}: 18.56 - 18.00 = 0.56$$

$\therefore$ calculated ECL for 5c, 12c ester = 18.00 + 0.41 + 0.56
= 18.97

similar calculations are made for ECL on ApL column,

$$\text{FCL } \Delta^{5c}: 17.66 - 18.00 = -0.34$$

$$\text{FCL } \Delta^{12c}: 17.73 - 18.00 = -0.27$$

$\therefore$ calculated ECL for 5c, 12c ester = 18.00 + (-0.34) + (-0.27)
= 17.39

The agreement was satisfactory when the two unsaturated centres were well separated but there was a considerable difference between observed and calculated values when the two double bonds were

closer together. The necessary correction was greatest with conjugated dienoates, significant with methylene interrupted dienes and apparent when the two unsaturated centres were separated by two methylene groups. Christie[92] who had earlier determined the ECL's of all the methylene interrupted octadecadienes also calculated the ECL's by this method and found that the necessary correction was fairly constant except when the double bonds were very near to either end of the chain (2,5; 13,16; 14,17).

We have now calculated the values of the diynes and trans-trans dienes and compared them with the values given by M. Lie. (Tables 9-12).

Table 9.

Calculated and Observed ECLs of Octadec-tt-dienes on ApL

Isomers Δ	Observed	Calculated	Methylene Groups between D.Bs. n =	δ
5,12	17.53*	17.53	5	0.00
6,12	17.51*	17.49	4	0.02
7,12	17.58*	17.48	3	0.10
6,11	17.57	17.47		0.10
8,12	17.48*	17.48	2	0.00
6,10	17.44*	17.46		-0.02
9,12	17.61	17.49	1	0.12
6,9	17.56*	17.44		0.12
10,12	18.62	17.51	0	1.11
6,8	18.60	17.43		1.17

* These values have been determined by F. Jacobsberg, immediately after our work and under the same conditions and are therefore used in preference to those given by M. Lie.

Table 10.

Calculated and Observed ECLs of Octadec-tt-dienes on DEGS.

Isomers Δ	Observed	Calculated	Methylene Groups between D.Bs. n =	δ
5,12	18.83	18.79	5	0.04
6,12	18.91	18.80	4	0.11
7,12	18.96	18.78	3	0.18
6,11	18.91	18.75		0.16
8,12	18.85	18.81	2	0.04
6,10	18.78	18.73		0.05
9,12	19.04	18.80	1	0.24
6,9	18.98	18.70		0.28
10,12	20.61	18.83	0	1.78
6,8	20.59	18.71		1.88

Table 11.

Calculated and Observed ECLs for Octadecadiynes on ApL

Isomers Δ	Observed	Calculated	Methylene Groups between T.Bs. n =	δ
7,15	17.94	17.93	6	0.01
8,15	17.95	17.93	5	0.02
5,12	17.85	17.80		0.05
9,15	17.95	17.89	4	0.06
6,12	17.83	17.76		0.07
7,12	17.89	17.75	3	0.14
6,11	17.88	17.69		0.19
8,12	17.90	17.75	2	0.15
6,10	17.85	17.63		0.22
9,12	18.23	17.71	1	0.52
6,9	18.18	17.59		0.59
10,12	19.60	17.75	0	1.85
6,8	19.61	17.61		2.00

Table 12

Calculated and Observed ECLs for Octadecadiynes on DEGS.

Isomers Δ	Observed	Calculated	Methylene Groups between T.Bs. n =	δ
7,15	22.72	22.67	6	0.05
8,15	22.82	22.65	5	0.17
5,12	22.29	22.14		0.15
9,15	22.77	22.65	4	0.12
6,12	22.35	22.22		0.13
7,12	22.40	22.28	3	0.12
6,11	22.32	22.14		0.18
8,12	22.43	22.26	2	0.17
6,10	22.27	22.06		0.21
9,12	23.39	22.26	1	1.13
6,9	23.21	21.98		1.23
10,12	24.94	22.34	0	2.60
6,8	25.02	21.98		3.04

(d) Conclusions:

Calculation of ECL for ApL columns: The difference in the calculated and observed ECL values for dienes and diynes is summarised in Table 13. The values are grouped according to the number (n) of methylene group intervening between the two unsaturated centres. The calculation of this difference depends on the determination of three ECL values. Since the accuracy of each determination is $\pm$ 0.02 differences up to $\pm$ 0.06 are probably not significant.

Gunstone and Lie[91] have already concluded, for the cis-cis dienoates, that the difference in calculated and observed ECL's is insignificant for dienes with $n > 2$ but increases when $n < 2$. It is small when $n = 2$ (0.07 to 0.08), significant when $n = 1$ (0.12, 0.14) and greatest when $n = 0$ (0.29, 0.26). For the series of methylene interrupted (n=1) dienes further information is available from the results of Christie[92]. Apart from the dienes which have unsaturation very close to either end of the molecule (2,5; 3,6; 13,16 and 14,17) the discrepancy between the calculated and observed values averaged 0.14 which agrees very well with the two values given by Gunstone and Lie. It is considered that these differences between observed and calculated values reflect the interaction of unsaturated centres which are either conjugated or methylene interrupted.

It is of interest to extend this approach to results obtained by Lie for the trans-trans-dienoates and the diynoates, which is

now possible with the results for the trans octadecenoates and octadecynoates reported here.

Table 13.

Difference in ECL (Observed-Calculated) on ApL.

Isomer	(n)	cis-cis	trans-trans	diynes
7,15	(6)	0.01	-	0.01
8,15	(5)	0.02	-	0.02
5,12		0.03	0.00	0.05
9,15	(4)	0.06	-	0.06
6,12		0.03	0.02	0.07
7,12	(3)	0.00	0.10	0.14
6,11		0.01	0.10	0.19
8,12	(2)	0.07	0.00	0.15
6,10		0.05	0.02	0.21
9,12	(1)	0.12	0.12	0.52
6,9		0.14	0.12	0.59
10,12	(0)	0.29	1.11	1.85
6,8		0.26	1.17	2.00

Comparing the difference between observed and calculated ECL's on an ApL column the following observations are made.

(i) The largest deviation always appears with the conjugated isomers and differs somewhat for cis-cis dienes (ca. 0.28), trans-trans dienes (ca. 1.14) and diynes (ca. 1.93).

(ii) Methylene interrupted diunsaturated esters show the next highest difference which is small, but significant, for both series of dienes (ca. 0.12) and somewhat larger for the diynes (ca. 0.56).

(iii) Apart from these two series of diunsaturated esters (n = 0 and 1), the difference tends toward an insignificant value except for the cis-cis dienoates (perhaps) with n = 2 when the difference is ca. 0.08, interestingly for the trans-trans dienoates with n = 3 (ca. 0.10) but not when n = 2 and for diynoates when n = 2 (ca. 0.18) and 3 (ca. 0.17)

(iv) The fact that in some cases the difference between observed and calculated values is beyond the limits of experimental error and reproducible indicates that the unsaturated centres are not acting in a simple additive fashion. This discrepancy (or correction factor) always has a positive value whilst the FCL's are in almost all cases negative, so that the additional double bond has less diminishing effect on ECL than its FCL. It follows, therefore, that in polyenoic acids a case may arise in which a double bond when added in a particular position may not diminish

the ECL at all. This in fact is true and can be seen from the following example. Δ^{15} cis double bond has FCL -0.10 but when it is added to a 9,12 unsaturated diene or 6,9,12 unsaturated triene the ECL does not change.

	ECL		ECL
9c,12c	: 17.50*	6c,9c,12c	: 17.27*
9c,12c,15c	: 17.49*	6c,9c,12c,15c	: 17.26*

(v) Whilst it may be possible with the help of these correction factors to calculate ECL's which may be of some value in helping to identify unknown ester, the reason why these correction factors must be applied is uncertain. They must be linked in some way with a particular process occurring in GLC which is dependent on the structure of isomeric unsaturated esters.

Calculations of ECL's on DEGS Columns:

Calculations of ECL for a diunsaturated ester again involved the determination of three ECL's. Although these values are fairly reproducible (± 0.03) when obtained under constant operating conditions, the nature of a polar column varies throughout its life and these changes may occur fairly quickly on a capillary column. It is therefore more difficult to make comparison between results obtained at different times or different places (i.e. under conditions when the properties of the column may be different).

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* Values observed by Gunstone and Mrs. P. Winlow

Table 14.

Difference in ECL (Observed-Calculated) on DEGS.

Isomer (n)	cis-cis	trans-trans	diynes
7,15 (6)	- 0.05	-	0.05
8,15 (5)	- 0.01	-	0.17
5,12	- 0.05	0.04	0.15
9,15 (4)	0.03	-	0.12
6,12	- 0.01	0.11	0.13
7,12 (3)	0.05	0.18	0.12
6,11	- 0.04	0.16	0.18
8,12 (2)	0.07	0.04	0.17
6,10	0.05	0.05	0.21
9,12 (1)	0.15	0.24	1.13
6,9	0.13	0.28	1.23
10,12 (0)	-	1.78	2.60
6,8	1.41	1.88	3.04

For these reasons, therefore, rather less reliance can be put on our present results where calculated ECL's based on FCL measured in 1970 (for trans olefinic and for acetylenic ester) are compared with observed ECL's measured by Lie in 1968.

Nevertheless the results in table 14 show the same general pattern as was observed with the results on ApL column. There is a large difference between observed and calculated values for the conjugated esters in all three series and a smaller but significant difference for the methylene interrupted esters. In both cases the deviations are largest for diynoates followed by trans-trans dienoates and smallest for cis-cis dienoates. The trans-trans dienoates again show a significant discrepancy when n = 3 but not when n = 2 or 4. The diynoates (n = 2 to 5) show a deviation of 0.12 - 0.21, which may be significant but which may be due to a constant error between measurements made at a 2 year interval.

IV Infrared Spectroscopy

Jones, McKay and Sinclair[95] observed a uniform progression of absorption bands between 1180 and 1380 cm^{-1} in the infra-red spectra of saturated fatty acids in the solid state. Meiklejohn et.al.[96] found that the number of bands in this region is equal to half the no. of carbon atoms in the chain when the saturated acid contains more than 12 carbon atoms. Working with some trans C_{18}-acids Susi[97] came to the conclusion that in trans unsaturated acids the number of bands in the 1180 to 1380 cm^{-1} region is related to the number of the methylene groups between the carboxyl group and the double bond and that the number and arrangement of these bands is unique for each of the positional isomers. Dr. Ismail[32] extended Susi's work and after studying trans Δ^2 to Δ^{16} octadecenoic acids reported this as an excellent procedure for the unambiguous determination of the position of unsaturation in trans octadecenoic acids. No similar work has been reported on the acetylenic acids.

Grimmer and Hildebrandt[31] studied several α-β unsaturated acids in solution and reported that the trans isomers have their CH bending absorption at 970-972 cm^{-1} instead of at the usual 962-965 cm^{-1} and C=C stretching is at 1660-1667 cm^{-1}, slightly higher wave length than for the corresponding cis acids.

We have studied the Δ^2 to Δ^{16} trans octadecenoates as 0.5% solutions in carbon disulphide by infra-red spectroscopy. All

of them show the expected bands for the C-H bending, C=O (ester group) stretching and CH stretching. The position of the characteristic trans absorption at about 965 cm^{-1} does not depend on the position of unsaturation and gives absorption at 961 to 963 cm^{-1} for all the isomers except the Δ^2. In the Δ^2 ester where unsaturation is conjugated with the carbonyl group, the band appears at a slightly higher wave number i.e. at 976 cm^{-1} (Grimmer and Hildebrandt[31] give 970-972 cm^{-1}). The Δ^{2t} ester also shows a C=C stretching absorption at 1660 cm^{-1} (lit.[31] 1660-1667 cm^{-1}) which is not observed in other acids. There are also strong bands at 1265 cm^{-1} and 1040 cm^{-1}, not observed in other isomers.

The infra-red spectra of the 2a, 5a, 15a and 17a acids (as 0.5% solutions in carbon tetrachloride) were also studied and apart from the Δ^2 and Δ^{17} isomers, all had similar spectra devoid of any unusual characteristic. No peak, characteristic of $RC\equiv CR'$ group, was obtained in the spectra of these isomers.

The 2a ester gives a strong, sharp band at 2241 cm^{-1} ($RC\equiv C-R'$ stretching) and a sharp band at 1075 cm^{-1} and a broad band at 1250-1255 cm^{-1} associated with the conjugated ester group. The 17a ester gives the three bands characteristic of the $HC\equiv C-R$ group viz. a sharp strong band at 3320 cm^{-1} (C-H stretching), a weak band at 2120 cm^{-1} ($C\equiv C$ stretching) and a broad, strong band at 630 cm^{-1} (C-H bending).

Quantitative measurement of trans unsaturation:

Infra-red chromatography is used both to detect and to determine the trans unsaturation. Shreve et.al.[98] first showed that absorption around 965 cm^{-1} is a measure of the amount of isolated trans unsaturation in a sample and this has provided the basis of a standard method[99] for the quantitative estimation of isolated trans unsaturation. In this method a standard curve, of absorption (at ~965 cm^{-1}) of methyl elaidate against its concentration in the solution, is obtained and absorptions of the samples (at ~ 965 cm^{-1}) are related to this curve. Recently Allen[100] showed that trans unsaturation can be measured quantitatively by measuring the ratio of the absorption at ~ 965 cm^{-1} to that of a second band, at ~1165 cm^{-1} in esters or at 870 cm^{-1} in acids, thus avoiding the necessity of external standard or the cumbersome procedure of preparing solutions of accurately known concentrations.

Most workers have used elaidic acid as a standard for measuring trans unsaturation. It is interesting to see, therefore, whether the intensity of the ~965 cm^{-1} band is the same for all positional isomers of trans octadecenoic acids. It is to be remembered that the estimation of trans unsaturation is often done on partially hydrogenated fats which can contain a number of positional isomers.

Using Allen's method[100], we measured the ratio of absorbance at ~963 cm^{-1} to that at ~ 1165 cm^{-1} for several isomers. The results are reported in Table 15 and 16.

Table 15.

Absorbance Ratio A963/A1165 for Different Positional Isomers *.

Absorbance Ratio	5t	6t	7t	8t	9t	10t	11t
A963/A1165	0.920	1.035	0.500	0.732	0.905	0.907	1.115

* Measurements carried out on 0.5% solution of methyl ester in carbon disulphide and are mean of at least 2 values.

Table 16.

Absorbance Ratio (A963/A1165) at Different Concentrations.*

Concentration %	Δ^{6t}	Δ^{8t}	Δ^{10t}
0.8%	1.055	0.862	0.920
0.5%	1.045	0.858	0.915
0.3%	1.045	0.860	0.927

* Measurements on carbon disulphide solutions of the methyl esters.

The table 16 shows that values obtained over a range of different concentrations agree satisfactorily as claimed by Allen[100]. Our value (0.905) for methyl elaidate also agrees very well with that expected (0.896) from the equation given by him viz.

$$\%\ \underline{\text{trans}}\ \text{unsaturation (methyl elaidate)} = 121.86(A10.3/A8.55) - 9.18.$$

The values for different isomers, however, do not agree and the variation is 0.5 to 1.1 (table 15). We found that the values vary significantly when repeated over a period of days, e.g. value for 8t ester in table 15 is 0.732 while that in table 16 is 0.858 for the same concentration, the values for 6t and 10t esters in both tables agree fairly well.

We did not discover the conditions under which we could get constant values and though the values recorded in table 15 may not have absolute value they indicate a variation in the relative intensities of the peak at $\sim 965\ cm^{-1}$ and at $\sim 1165\ cm^{-1}$, for different positional isomers. It follows that the quantitative measurement for <u>trans</u> unsaturation in a sample containing <u>trans</u> isomers other than elaidic acid may not be reliable.

V Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) was discovered in the 1920's and first used to study fatty acids and related compounds in 1958. The phenomenon of NMR is based on the fact that nuclei of some atoms have a spinning motion and are affected by an applied magnetic field. If in addition to the magnetic field, an oscillating radio frequency is applied the nucleus will resonate between different energy levels at a definite frequency and absorb a little energy in doing so. This absorption in energy can be amplified and recorded. That protons have got this property is very useful to the organic chemist not only because they are found in practically all the organic compounds but also because no other method is available to determine the structural environment of hydrogen.

Although it is sometimes possible to calculate chemical shifts and coupling constants for individual protons, this is difficult for most compounds and the identification has to be based on comparison with other similar compounds of known structure. For this purpose it is important to produce data for as many known, pure compounds as possible. Since Hopkins and Bernstein[101] studied the long chain fatty acids and related compounds, a number of papers have been published on the NMR of saturated[101,102,103], monoenoic[104,105], dienoic[104, 106, 107] and polyenoic acids[108,109].

Purcell and Susi[110] studied several octadecynoic acids and Gunstone and Ismail[111] published NMR data for the $\Delta^{2,3,4,5}$ and Δ^{10} octadecynoic acids but the Δ^{16} and Δ^{17} acids were not studied. In our laboratory a systematic NMR study of the positional isomers of C_{18} unsaturated acids and esters is being made. Gunstone and Ismail[111] studied the complete series of cis-monoenoic acids while Gunstone et.al.[112] published data on several C_{18} dienoic acids (both cis-cis and trans-trans isomers) and diynoic acids. In continuation of this work we have now studied the complete series of trans-octadecenoic and of octadecynoic acids.

(i) Experimental Details:

Initially the NMR-spectra were recorded on a Varian HA-100 instrument with 100 MHz at room temperature. All spectra were recorded from the internal standard, tetramethylsilane (τ = 10), downfield using approximately 10% solutions (0.5 ml) of the acids or esters in analar carbon tetrachloride. Near the end of our work, S.R.C. offered the services of a Varian HA-220 instrument which recorded the NMR-spectra at 220 MHz. Several acids have been examined on this instrument and we plan to examine all the octadecenoic (cis and trans isomers) and all the octadecynoic acids. The spectra are recorded at room temperatures with carbon tetrachloride solutions and with tetramethylsilane as internal standard.

(ii) 60 MHz, 100 MHz and 220 MHz NMR:

Most of the NMR data on long chain fatty compounds reported before 1967 was obtained at 60 MHz. With high frequency instruments three important changes occur: (a) Higher frequency spectra are more sensitive so that smaller amounts can be used. (b) The chemical shifts (c/s) change but the coupling constants do not, so there is a better separation between the groups of peaks. (c) Because the chemical shifts change but not the coupling constant, the multiplets due to higher order change to a first order pattern and the spectra are simplified and thus more easily interpreted. The net result of these three properties is that higher frequency spectra have sharper peaks which are resolved from other peaks and distorted triplets and broad peaks are changed to simple triplets, quadruplets etc. It is then easier to measure coupling constants.

In the earlier work on NMR in our laboratories it was found that 60 MHz spectra showed a large broadening of the base of the isolated chain methylene peak ($\sim$ 8.74 τ). When we recorded 100 MHz spectra on the same substances the spectra were considerably improved and a separation was obtained between the peak for the methylene adjacent to the carboxyl group and the peak for allylic or propargylic protons. However, there were still shoulders and broad bands near the base of the chain methylene peak. To get more information in this region we recorded the

220 MHz spectra.

The 220 MHz spectra simplified this region considerably and the signal for the end methyl group which generally appeared as a distorted triplet with the 100 MHz instrument, now appeared as a multiplet or, in some cases, as a sharp triplet. With this high frequency instrument we also obtained a new peak slightly downfield from the main chain methylene peak, which we consider to be due to protons on the C(3) atom, β to the carboxyl or carbomethoxy group. With some isomers additional peaks were obtained in this region which could not always be explained (see later).

(iii) General Comments on the Peaks Appearing in the NMR of a Long Chain Fatty Acid or Ester:

Detailed comments on each unsaturated acid/ester are given later but some general comments about the signals due to protons in different environments are introduced at this point.

In an unsaturated monoenoic acid or ester there are 7 groups of protons which have a different environment from the others and show up as a distinct signal on the NMR spectrum (see table 17). In our present study, whenever we studied acids we neglected the carboxylic proton which absorbs below 0 (on the τ scale) and gives variable peaks according to the concentration of the solution, temperature etc. It is also not much affected by the changes in

the long chain. When methyl esters are studied the protons of the ester methyl group absorb at 6.36 to 6.44 τ. The position of this peak, (like the carboxylic proton peak) is unaffected by the changes in the long chain.

End Methyl Group

These three protons give a signal which appears as a triplet at 9.10-9.12τ. With 60 MHz or 100 MHz instruments it generally appears as a distorted triplet and sometimes only two peaks can be distinguished; the 220 MHz instrument resolves this distortion and gives a sharp triplet or multiplet. No change in its position occurs till the unsaturated centre approaches the methyl end of the molecule and obvious changes in both its shape and position occur from Δ^{14} to Δ^{16}. The Δ^{17} isomers, of course, have no end methyl group.

Chain Methylene Groups.

On 100 MHz, the large peak due to the protons of the chain methylene groups appears around 8.74τ, unaffected by the position of the unsaturation, in monoenoic acids. In the 9a acid, however, this signal is split into two peaks at 8.70τ and 8.62τ and this is even clearer in the 220 MHz spectrum. A study of the 220 MHz spectra of all these acids reveals that the cis and trans monoenoic acids do not show this effect to a marked extent but in the acetyelnic acids, a side band appears in the Δ^{7} isomer, increases

in size through the Δ^8 and Δ^9 isomers and in the Δ^{10} acid this peak appears virtually as a doublet. This splitting of the chain methylene peak disappears again as the triple bond moves near the methyl end (i.e. Δ^{14} onwards).

β Methylene Group [C(3)protons]

The protons on the carbon atom β to the carboxyl group are slightly more deshielded than the chain methylene protons and have a slightly downfield chemical shift. Though not seen in the 60 MHz or 100 MHz spectrum, the 220 MHz spectrum shows this signal in all the acids (except a few from which it is absent for understandable reasons) at 8.30 to 8.42τ, as a broad unresolved multiplet. The stearic acid also shows this peak proving that it is not confined to unsaturated compounds. When decoupling was carried out on the Δ^6 acid by irradiating at 8.35τ the triplet due to the protons on the C(2) collapsed to a singlet showing that the 8.35τ peak is due to the protons on the carbon atom adjacent to C(2).

Propargylic or Allylic protons:

Propargylic or allylic protons are deshielded more than C(3) protons and produce a multiplet at 8.00 to 8.07τ (allylic) or at 7.90 to 7.94τ (propargylic). The positon of this peak is affected (in part) as the unsaturated centre approaches the carboxylic group (from Δ^5).

Table 17

Main Peaks in Octadecynoic and trans-Octadecenoic Acids

m n q s s q n p r
$CH_3(CH_2)_xCH_2CH{=}CHCH_2(CH_2)_yCH_2CH_2COOH$

Isomer	m		n		p		q		r		s	
	a	t	a	t	a	t	a	t	a	t	a	t
Δ^2	9.12	9.11	8.76	8.76	8.40*	8.55*	7.67*	7.84*	-	-	-	4.29*, 3.14*
Δ^3	9.12	9.12	8.75	8.77	-	-	7.86	7.99	6.80*	7.10*		4.55
Δ^4	9.12	9.13	8.76	8.76	-	-	7.93	8.07	7.53	7.68	-	4.60
Δ^5	9.12	9.13	8.74	8.75	8.24	8.34	7.93, 7.82	8.00	7.58	7.73	-	4.68
Δ^6 to Δ^{13}	9.14-9.09	9.13-9.11	8.75-8.68	8.75-8.68	8.38-8.30	8.42-8.34	7.94-7.90	8.08-8.00	7.70-7.67	7.73-7.70	-	4.74-4.64
Δ^{14}	9.04†	9.12†	8.72†	8.73†	-	-	7.93†	8.04†	7.71†	7.72†	-	4.69†
Δ^{15}	8.92	9.06	8.76	8.78	8.39	8.39	7.97	8.09	7.73	7.73	-	4.71
Δ^{16}	8.28*	8.38*	8.76	8.76	8.43	8.38	7.95	8.06	7.80	7.78	-	4.67
Δ^{17}	8.25*	5.11*	8.76	8.77	8.47	8.45	7.89	7.98	7.80	7.81	-	4.31

* The group is in a different environment than usual.

† Values taken from 100 MHz spectrum.

α-Methylene Group [C(2) protons]

The carboxyl group has a stronger deshielding effect than the triple bond or a double bond and hence the protons on the C(2) carbon atom adjacent to a carboxyl group produce a triplet signal (J=7) downfield (7.67-7.73τ) from that due to propargylic or allylic protons. In esters, rather than acids, the C(2) protons absorb slightly upfield at 7.74-7.81τ (J=7). As the unsaturated centre approaches the C(2) protons, they are deshielded more and give a peak still further downfield.

Olefinic Protons

Olefinic protons, in the central region of the chain, absorb at 4.64-4.77τ. trans-Protons generally absorb slightly downfield of cis-protons but the difference is insignificant. Both protons are equivalent in all isomers except the Δ^{17} and Δ^{2} acids. In the Δ^{2c} ester the protons absorb at 4.30τ [C(2)] and at 3.85τ [C(3)]. In the 17e ester there are three olefinic protons, the two protons on C(18) absorb around 5.11τ while the single proton on C(17) absorbs at 4.31τ.

(iv) General Comments on the Deshielding Influences:

Gunstone et.al.[112] while studying some diynoic and dienoic acids came to the conclusion that the effectiveness of the deshielding effects of different groups can be given by the following

sequence

$$-COOH > -COOMe > -CH \equiv C-C \equiv C- > \overset{c}{-CH=CH}-\overset{c}{CH=CH}- > -C \equiv C- >$$

$$\overset{t}{-CH=CH}-\overset{t}{CH=CH}- > \overset{c}{-CH=CH}- > \overset{t}{-CH=CH}-$$

It is of interest to study the deshielding effect of the triple and the double bonds on their own as well as in conjunction with other groups such as the carboxylic acid or ester.

(a) Deshielding Effect of -COOH and -COOMe

The carboxylic acid group has a stronger deshielding influence than the ester group thus the C(2) prtons adjacent to the carboxyl group in the 8t acid give a peak at 7.70τ but those in the 8t-methyl ester give a peak at 7.78τ.

(b) Unsaturated groups (a,c,t):

1. Methylene groups next to the unsaturated centre

An isolated unsaturated centre has a deshielding effect on the adjacent (α) carbon atom and, possibly, on the next carbon atom (β). The propargylic and allylic protons are considerably more deshielded than those on the chain carbon atoms. The following are average values for propargylic and allylic protons under the deshielding influence of no other group except the unsaturated centre.

$-CH_2-\overset{c}{C \equiv C}-CH_2-$	7.92τ
$-CH_2-\overset{t}{CH=CH}-CH_2-$	8.01τ
$-CH_2-CH=CH-CH_2$	8.05τ

Thus on a 220 MHz spectrum the protons next to a triple bond have a chemical shift $\sim$ 20 c/s greater than those next to a cis double bond and cis-allylic protons are shifted $\sim$9 c/s more than trans-allylic protons. The spectra contains no signal which can be attributed to the protons on the carbon atom β to the unsaturated centre. This means neither a double bond nor a triple bond exerts sufficient deshielding on these (β) protons to pull them out of the broad methylene peak at $\sim$8.74τ. However, the unsaturated groups still can show some long range effect as given below.

2. Effect of the Unsaturated Centre on the End Methyl Protons

The following table shows the τ values for the end methyl group in Δ^{14}, Δ^{15} and Δ^{16} acids/esters.

	a	c	t
CH_3 X	8.28	8.43	8.38
CH_3CH_2 X	8.92	9.03	9.06
$CH_3CH_2CH_2$ X	9.04*	9.11*	9.12*
$CH_3(CH_2)_n$ X	9.11-9.12	9.11-9.12	9.11-9.12

X denoates unsaturated centre

* values from 100 MHz spectrum

The above values show that double bonds have a deshielding effect on the protons of a methyl group in the α and in the β position but not in the γ positon. The deshielding effect of a triple bond however stretches to a methyl group in the γ position.

3. Effect of the Unsaturated Centre in Conjunction with the Carboxyl Group

Table 18 shows the combined deshielding effect of the unsaturated centre and the acid/ester group on methylene groups lying between them.

Table 18

Combined Deshielding Effect of Acid/Ester Group and Unsaturated Centre

Deshielding Groups	Δ^3	Δ^4		Δ^5			Other Isomers	
	C(2)	C(2)	C(3)	C(2)	C(3)	C(4)	C(2)	C(3)
C≡C/COOH	6.80	7.52	7.57	7.58	8.24	7.82	7.70	8.40
^{c}CH=CH/COOMe	6.96	7.72	7.72	7.76	8.40	7.98	7.78	8.40
^{t}CH=CH/COOH	7.10	7.68	7.68	7.73	8.34	8.00	7.70	8.37

From the above table it is again apparent that the triple bond has the strongest deshielding effect followed by the cis double bond and then the trans double bond. (The apparently anomolous values of some cis isomers are because they are taken from the spectrum of methyl esters. As already shown above the ester group has a weaker deshielding effect than the carboxylic acid group.) The cis double bond exerts a slight β effect [C(2) protons in the Δ^4 ester show a signal at 7.72τ instead of at 7.78τ] but no γ effect. The trans double bonds exert no apparent deshielding influence on protons attachedto β or γ carbon atoms [C(2) proton signals in Δ^4 and Δ^5 acids at 7.68τ and 7.73 respectively as against the

normal value of 7.70τ].

(c) Conjugated Systems (unsaturated acyl compounds)

In the Δ^2 acids/esters the unsaturated centre is conjugated with the acyl group and quite a different NMR absorption pattern is produced. The τ values for the signals produced by protons on C(4) and C(5) are as follows

	C(4)	C(5)
2a acid	7.67	8.40
2c ester	7.38	8.40
2t ester	7.84	8.55

Rather unexpectedly, the values show that the cis double bond when conjugated with an acyl group has a stronger deshielding effect than the triple bond conjugated with an acyl group and the trans double bond conjugated with acyl group has the weakest deshielding effect of the three. The trans double bond which has no β effect of its own, now in conjugation with acyl group, shows a slight deshielding effect on the protons of the β carbon atom [C(5)]. The apparently anomolous behaviour of the cis double bond conjugated with the carboxyl group is discussed in the following section.

(v) NMR Spectra of Individual Acids/Esters

Δ^2 Acids and Esters:

The 2c and 2t acids* have been studied by Purcell et.al.[107],

* In the following discussion of individual acids and esters, the term acid is used for both acid and ester unless it is significant to mention whether it was acid or ester.

the 2t acid by Hopkins[113], and the 2a and 2c acids by Gunstone and Ismail[111]. The fragment of the Δ^2 acid which is most interesting from NMR point of view is shown below.

$$-CH_2CH_2CH=CHCOOH$$

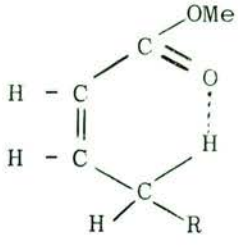
a) No mention has previously been made of signals arising from the protons on C(5) but with a high frequency instrument these signals can be observed. The following are their τ values.

acetylenic : 8.40τ cis-olefinic: 8.40τ and trans-olefinic : 8.55τ

b) The C(4) protons, which are on a carbon atom adjacent to a conjugated system, are deshielded more than the usual allylic or propargylic protons and produce signals further downfield.

	acetylenic acid	cis-ester	trans-ester
Δ^2	7.67τ	7.38τ	7.84τ
Δ^6 to Δ^{13}	7.92τ	7.97τ	8.00τ

Less expected is the particularly large deshielding influence of the cis double bond. This may be due to the fact that because of the cis-configuration of the double bond the carboxyl group is brought nearer to the C(4) protons as shown



thus enhancing the deshielding influence of the conjugated unsaturated ester function. A similar effect does not occur with the trans olefinic or acetylenic esters. [In 2a acid the C(4) protons show a triplet with J C(4)C(5)= 5-7 c/s and in 2c and 2t esters these show an overlapping double triplet with J C(3) C(4)=~7 c/s and J C(4) C(5) = ~ 7 c/s

c) In most of the positional isomers of octadecenoic acids the olefinic protons are equivalent but because of the proximity of the ester group they are non-equivalent in the Δ^2 isomers and have completely different chemical shifts. The C(2) proton produces a doublet at 4.31τ in 2c and a doublet at 4.29τ in 2t, because of the spin coupling with C(3) proton. The coupling constants are Jcis = ca. 11 Cps (lit.[107], 11.31 Cps) and Jtrans = ca. 16 Cps (lit.[107], 15.51 Cps).

The C(3) protons couple with both the C(4) protons and the C(2) proton and give a double triplet signal centred at 3.14τ for trans-ester and 3.85τ for cis-ester. The coupling constants are J C(2) C(3) trans = ~ 15 c/s; J C(3) C(4) trans = ~ 7 c/s (lit.[107], 15.51 and 6.67 c/s resp.) and J C(2) C(3) cis = ~11 c/s; J C(3) C(4) cis = ~ 7 c/s. (lit.[107], 11.31 and 5.78 c/s resp.).

Δ^3 Acids and Esters:

NMR spectra (60 MHz) of the 3a and 3c acids have been studied by Gunstone and Ismail[111] and those of the 3t acid by Kleiman et. al.[114]. The interesting fragment of the molecule is $-CH_2CH=CHCH_2COOH$.

a) The C(2) protons, deshielded by both the unsaturated centre and the carboxyl group produce a doublet (J C(2) C(3) = 6.6 c/s) downfield from the usual signal for C(2) protons (a triplet at 7.70τ), in the olefinic acids/esters and a singlet in the acetylenic acid. The following are the chemical shifts:

acetylenic acid: 6.80τ; cis-acid: 6.96τ; trans-ester: 7.10τ.

b) The propargylic protons on C(5) are deshielded slightly more than the normal propargylic protons through a long range effect of the carboxylic group and give a signal at 7.86τ (instead of the usual signal at 7.90-7.94τ). Allylic protons in this position are not affected and have a signal in the normal range (~ 8.00τ).

c) The ethylenic protons are equivalent, and the signal for the cis-protons appear at 4.50τ i.e. slightly downfield from their normal position at 4.64-4.74τ. The signal due to the cis-olefinic protons is shifted slightly downfield (about 11c/s) of that from the trans-olefinic protons, which is the reverse of the normal position.

d) There are two sharp singlets at 8.45τ and 8.15τ, each corresponding on the integration curve to a single proton. We are not able to explain these peaks.

Δ^4 Acids and Esters:

The 4a and 4c acids have been studied by Gunstone and Ismail[111] and the 4t acid by Hopkins[113]

$$-CH_2CH=CHCH_2CH_2COOH$$

The following points are of interest:

a) In the 4a acid the triple bond exhibits a small deshielding effect on the C(2) protons and shifts the signal by ~ 37.5 c/s downfield from the usual position, the cis-double bond has a smaller but still significant effect and shifts the signal by ~13 c/s downfield but the trans double bond has little or no effect and the C(2) protons give a signal at 7.68τ (normal position 7.70τ).

b) The allylic/propargylic protons on C(3) are under the deshielding effect of the unsaturated centre and also the β deshielding effect of the carboxyl group and hence are deshielded more than the other propargylic/allylic protons on C/6). The τ values for the two pairs of propargylic/allylic protons in these compounds are listed below:

	allylic/propargylic proton signals	
	C(3)	C(6)
acetylenic acid	7.57 [a]	7.93
cis-olefinic ester	7.72 [b]	7.98
trans-olefinic acid	7.68 [b]	8.07

[a] triplet (J C(2)C(3) = ~6 c/s)

[b] unseparated from signal due to C(2) protons

c) Hopkins[113] reports that the 4t olefinic protons give a signal at 4.54τ instead of at the normal value of 4.65τ. We find these olefinic protons to be shifted only slightly downfield and our value of 4.60τ is little different from that given by Gunstone and Ismail[111] for the 4c ester (4.63τ).

Δ^5 Acids and Esters:

Gunstone and Ismail[111] studied the 5c and 5a compounds but the NMR spectrum of the 5t acid has not been reported.

$$-CH_2CH{=}CHCH_2CH_2CH_2COOH$$

a) In olefinic acid or ester, all protons generally give signals more or less at the usual positions. Gunstone and Ismail[111] detected a peak at 8.30τ in their 60 MHz spectrum of 5c ester and alloted it to the C(3) protons. On a high frequency instrument

the signal for the C(3) protons is clearly distinguished in most of the acids independently of the position of the unsaturated centre and the signal which we obtained at 8.38τ was in the usual range (8.3 to 8.4τ).

b) In the acetylenic acid however the C(2) proton signal is shifted slightly downfield (~ 26 c/s) from its normal position (7.70τ) because of the γ effect of the acetylenic bond. The C(3) proton signal is also shifted slightly downfield (~26 c/s) froms it usual position at 8.36τ and the two propargylic proton groups on C(4) and C(7) give completely separated but distorted triplets at 7.82τ [C(4) protons] and 7.93τ [C(7) protons; usual propargylic proton signal].

Δ^{6} to Δ^{13} Acids:

These acids give the peaks expected of a long chain unsaturated ester and are reported as undistinguishable from one another[111]. In these isomers no proton is present in a distinct environment which differs in any way from one isomer to another, there are therefore no distinctive signals. However on a high frequency spectrometer, the large peak around 8.7τ shows distinct patterns which, though difficult to explain, can be used empirically for the tentative identification of Δ^{6a} to Δ^{13a} acids. Purcell and Susi[110] have already observed this phenomenon in the 60 MHz spectra of acetylenic acids.

Acetylenic Acids:

The acids outside the Δ^6 to Δ^{13} range show a single broad peak at 8.72 to 8.76τ but most of the Δ^6 to Δ^{13} acids show one or more additional peaks. In the 6a acid the major signal (8.75τ) shows several small bands downfield, the 7a, 8a, 9a, 10a and 12a acids each show two peaks, the 11a acid has only one signal (8.68τ) a little lower than usual and the 13a acid has its signal at 8.70τ with some side bands (see table 18). The Δ^{14} acid shows only the normal single peak in this region.

Table 18.

The Chain Methylene Peak in 6a to 14a acids (τ values)

Normal position	6a	7a	8a	9a	10a	11a	12a	13a	14a
8.72 to 8.76 single broad peak	8.75 with several side bands	8.75 and 8.55	8.74 and 8.60	8.71 and 8.64	8.69 and 8.73	8.68	8.70 and 8.64	8.70 with several side bands	8.72

It appears from the above table that all but 9a and 12a acids can be identified from each other and even the pattern of these two acids is sufficiently different to distinguish one from the other.

trans and cis Acids:

Both the cis and trans acids also show these side bands in some of the acids but they are sharp, very close to the main

methylene peak and the variation is not as systematic as in the acetylenic acids. It is therefore not feasible to distinguish the positional isomers on this basis. It is however remarkable that the 9t acid shows two distinct bands at 8.68τ and 8.72τ while the 9c acid shows only one broad band at 8.75τ and hence these two acids can be distinguished from each other.

Δ^{14} Acids:

The 14c acid was studied by Gunstone and Ismail[111] who found it to differ from the 2c to 13c acids in that it has a clearly defined triplet at 9.08τ due to the end methyl group (J = 6.0 c/s). We found that all the three acids (a, c and t) have clearly defined triplets due to the end methyl group but the triplets for both the olefinic compounds were centred at their usual position (9.12τ) and had a J= 7.0 c/s while the acetylenic acid had the triplet centred at 9.04 (J = 7.0 c/s) because of the γ effect of the triple bond on the end methyl group. The signal due to the propargylic protons was also simplified considerably and appeared as a well defined but a broad triplet (J = 6 c/s) instead of a broad band as in the Δ^{2a} to Δ^{13a} acids.

Δ^{15} Acids:

The only distinctive feature of the Δ^{15} spectra is the position of the triplet due to the end methyl group. In the

15a acid it is centred at 8.92τ, in the 15c at 9.03τ and in the 15t at 9.06τ, showing the difference in deshielding of the end methyl group by the a, c and t unsaturated centres.

Δ^{16} Acids:

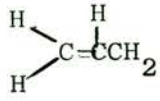
Gunstone and Ismail[111] noted that in the 16c acid the end methyl group gives a doublet at 8.38τ with J = 5.2 c/s.

The 220 MHz spectrum of the 16t acid shows a multiplet for the end methyl group at 8.38τ. The methyl protons may be coupling not only with the C(17) proton but also through the unsaturated centre with protons further away. Δ^{16c} gives a split peak at about 8.44τ which is not a doublet but two overlapping peaks due to protons on C(3) [8.45τ] and the end methyl group (8.43τ). The signal due to the end methyl group appears more like a broad singlet rather than a doublet as mentioned by Ismail[32]. In the 16a acid the end methyl protons are further deshielded and give a signal at 8.28τ. This is a triplet because of the coupling, through the unsaturated centre, with the C(15) protons but the coupling constant is very small (ca. 2-3 c/s).

Δ^{17} Acids:

The NMR spectrum of the octadec-17-ynoic acid is not reported in literature though Hopkins[115] recently reported one for a C_{19}-acid with end acetylenic group. Gunstone and Ismail[111] have studied the 17e acid. The end acetylenic proton gives a signal

at 8.2τ[115] while the signal due to the protons on the end methylene group appear at 5.05τ[111].

a) The ethylenic acid 

(1) We obtained an uneven triplet, due to the two protons on C(18), centred at 5.11τ. The two protons are not equivalent and hence this signal may be two overlapping doublets [signal due to each proton split by the proton on C(17)] with two different coupling constants J = 15 c/s and 11 c/s.

(2) The C(17) proton gave a complex signal (which could be a double triplet) centred at 4.31τ (lit.[111] 4.40τ).

(3) The allylic protons on C(16) produced a signal at 8.01τ (lit.[111] 7.85τ).

b) The acetylenic acid $HC\equiv CCH_2CH_2$

(1) The end acetylenic proton gives a triplet centred at 8.25τ (lit.[115] 8.2τ). The triplet arises because of the coupling with C(16) protons through the unsaturated centre. The coupling constant is very small (J = ca. 2.5 c/s).

(2) The protons on C(16) produce a well resolved double triplet centred at 7.89τ. Obviously the C(16) proton signal is split into a triplet by the spin coupling with C(15) protons (J = ca. 7 c/s) and the triplet is further split due to the coupling with C(18) proton through the unsaturated centre.

(3) There is a small multiplet centred at 6.65τ which we can not identify.

(4) In this particular spectrum the signal due to the C(3) protons at 8.47τ is clearly resolved into 7 peaks. The multiplet is of $A_2M_2X_2$ type and the coupling constants are J_{AM} = 6.25 c/s and J_{AX} = 14 c/s.

EXPERIMENTAL

Solvents.

Petrol Ether and Ethyl Ether: Petrol ether (b.p. 40-60° unless otherwise mentioned) and ethyl ether were used as were obtained commercially for all general work and where not otherwise mentioned. Dry ether and tetrahydrofuran were prepared by keeping the solvent over calcium chloride for a couple of days, distilling it and then allowing it to stand over fine sodium wire for several days. Before drying it with calcium chloride, the ether was freed of any peroxide by washing it with ferrous sulphate solution followed by water.

Dry Ethanol and Methanol: Magnesium turnings (5 g) are first converted to magnesium ethoxide or methoxide in 99% pure alcohol (100 ml) and then more alcohol (900 ml) is added. The mixture is refluxed for several hours and then distilled and kept in a ground glass stoppered flask. For the malonic ester synthesis only freshly prepared dry ethanol was used.

Other Solvents: Carbon tetrachloride used for spectroscopy was purchased from B.D.H.-special for spectroscopy.

Carbon disulphide was first refluxed over calcium hydride for 1 hour and then distilled. The distilled carbon disulphide was then kept over calcium chloride.

Benzene was normally dried over calcium chloride and distilled. Thiophene-free benzene was prepared by shaking it with concentrated

sulphuric acid as given by Vogel[116], and dried over sodium wire. Diglyme and dimethyl formamide (DMF) were dried over calcium hydride overnight and then distilled. Dimethyl sulphoxide (DMSO) was distilled before use.

Drying.

Extracts were dried over powdered anhydrous sodium sulphate.

Distillation.

Distillation of intermediates under vacuum was carried out through long (6") or short (3") columns packed with glass helices depending on their volatility and stability to high temperature.

The Fenske column was 0,6 m high and was always used with a Perkin triangle system as made by Towers.

For purification of some of the intermediates, 'Abeggs' spinning band column (supplied by Büchi) was used. The spinning band was of stainless steel and the distillation time to reflux time ratio was generally 2 secs : 30 secs.

Crystallisation.

Crystallisation of the final C_{18} monoenoic and C_{18} monoynoic acids was always carried out in 10% solution in petrol ether. The solution was generally kept overnight at 4-5°.

Melting Points.

The melting points of the acids were determined by a micro-melting point unit and are uncorrected.

Silver-nitrate Column Chromatography.

Florisil (for chromatographic analysis) obtained from B.D.H. was washed with acid as directed by K.K. Carroll[117] and then impregnated by 20% silver nitrate as reported by D. Wilner[118]. The column was as fast flowing as normal silica column. Column chromatography was generally used only to separate substances which showed large separations on TLC plates.

Thin Layer Chromatography.

For qualitative work, silica plates coated with 0.25 mm layer of MN-Silica Gel G and activated by heating for 90 mins. at 120°C were used and sprayed with 10% solution of phosphomolybdic acid in ethanol. For qualitative Silver nitrate TLC, the plates were impregnated with 10% silver nitrate. For preparative silver nitrate TLC, the plates were coated with 0.75 mm layer of MN-Silica Gel G impregnated with 20% silver nitrate. The plates were sprayed with 2,7-dichlorofluorescein. The fluorescein extracted along with the product by ether was removed by washing the ether extract by dilute sodium bicarbonate solution.

Gas Liquid Chromatography.

A 'Pye' series 104 chromatograph with flame ionisation detector was used throughout the work for routine testing as well as for the carbon number determination by capillary columns. The eluting gas was oxygen free nitrogen. The columns used for routine testing were 5' packed columns. The stationary phases used were diethylene glucol succinate (10 or 20% coating on HMDS Chromosorb W 80-100 mesh; supplied by Perkin-Elmer) and Apiezon L (3% coating on the support material same as above). Capillary columns (50 m) coated with the above mentioned stationary phases were obtained from 'Perkin-Elmer' and used for determining the accurate carbon numbers of the final products.

Infra Red Spectroscopy.

A 'Perkin-Elmer 137' infra-red spectrophotometer was used for the routine identification work. For quantitative infra-red determinations, a 'grating infra-red spectrophotometer 621' by Perkin-Elmer was used. Infra-red determinations were normally done by dissolving the substance (50 mg) in carbon tetrachloride (1 ml) and using one mm thick sodium chloride cells.

Nuclear Magnetic Resonance (NMR) Spectroscopy.

Most of the analytical determinations of the intermediates were done at 60 MHz with a 'Perkin-Elmer R-10'. 10% solutions

were made in carbon tetrachloride and tetramethylsilane was used as an internal standard.

For determinations of the coupling constants and the accurate chemical shifts in the pure acetylenic and trans fatty acids and esters, a 'Varian HA-100 NMR' working at 100 MHz was used. The concentrations and internal standard were same as above.

NMR spectra of some of the acids were also measured on a 220 MHz 'Varian HR-220 spectrometer'. The service was provided by the S.R.C. on an instrument at I.C.I. P and P Laboratory, Runcorn, Cheshire.

Methylation.

Methylation of small quantities of acids, for GLC testing, was carried out by dissolving the acid (a few mg) in 14% boron trifluoride in methanol(ca. 2 ml) and boiling it on a steam bath for ca. 60 sec. The product was then poured into excess demineralised water, extracted with ether, washed once with saturated solution of sodium chloride, dried and evaporated to suitable concentration.

Methylation carried out on gram quantities was effected by dissolving the acid in dry methanol (ca. 20 volumes) containing concentrated sulphuric acid (0.4 to 0.6 volumes) and refluxing it for an hour. The recovery of the methyl ester was as above.

von Rudloff Oxidation (as modified by Tulloch and Craig[119]).

The procedure was used to determine the purity of the acids. Commercial tertiary butanol (700 ml) was first oxidised with a 6% aqueous potassium permanganate solution (ca. 50 ml) by heating it at 60°C and rotating it at the same time for several hours. Then it is distilled and used.

Stock solution was prepared by dissolving sodium metaperiodate (5.21 g) and potassium permanganate (0.099 g) in distilled water (250 ml).

The acid (30-35 mg) was dissolved in tert. butanol (25 ml) and to it was added 0.5% aqueous potassium carbonate (5 ml), distilled water (5 ml) and the stock oxidant solution (10 ml). The mixture was shaken overnight at ambient temperature and the excess oxidant was reduced by gaseous sulphur dioxide. The mixture was made alkaline by addition of little pottasium hydroxide and the t-butanol was evaporated completely by heating on a steam bath in a stream of nitrogen. The water was then acidified by addition of dilute hydrochloric acid and extracted three times with ether (25 ml). The ether extract was washed twice with sodium chloride solution and dried over sodium sulphate. The monobasic and dibasic acids obtained after the evaporation of the ether are then methylated with 14% boron trifluoride in methanol as given above and tested with GLC.

Sodamide or lithamide preparation[120].

Liquid ammonia was run into a conical flask from an ammonia cylinder lying on the ground with its outlet tube pointing upwards. The liquid ammonia was then transferred to a three necked flask fitted with a mechanical stirrer through a mercury seal, a drying tube filled with potassium hydroxide pellets (changed afterwards to either a gas inlet tube or dropping funnel as required) and a cardice condenser. Powdered ferric nitrate (ca. 0.5 to 1.0 g) was added and the stirring started. Sodium or lithium was cut into suitably small pieces (ca. 0.5 g sodium or ca. 0.25 g lithium) and successive pieces were added whenever the blue colour first produced changed to grey. After all the sodium or lithium had been added the mixture was further stirred for about 30 min.

Sodium or lithium acetylide.

Sodium acetylide was prepared in two ways. Either by passing the acetylene gas into the sodamide suspension in liquid ammonia[121] or by what is called as the titration method[122].

An acetylene cylinder was joined to the inlet tube of the reaction flask through a mercury safety trap, a Dreschel bottle cooled with solid carbon dioxide to condense out acetone, another filled with concentrated sulphuric acid and one empty as a safety trap. The gas was passed at a fairly brisk rate through the sodamide, prepared as above, for about 90 min. while stirring.

In the titration method, liquid ammonia was placed in a three necked flask, fitted with a gas inlet tube, a stirrer with a mercury seal and a cardice condenser. The acetylene purified as above was passed through the stirred liquid ammonia at a rate of 4-5 bubbles a second and sodium or lithium cut in suitably small pieces was added to it. Each piece of sodium was added after the blue colour produced by the preceding piece had disappeared. Acetylene was stopped immediately after the blue colour produced by the last piece had disappeared completely.

The Conversion of Diols to Dichlorides[123].

A diol (1 mole) was placed in a 1 litre three-necked flask, fixed with a mechanical stirrer, water condenser and a dropping funnel. The flask was kept in an ice-water-bath and pyridine (12 ml) was added. Thionyl chloride (4 moles) was added dropwise to the mixture taking care not to allow the temperature to rise above 5-10^{o}C. After all the thionyl chloride had been added the mixture was stirred for 3 hrs. at room temperature and refluxed for a further hour. The mixture was then allowed to cool and ice water was added very carefully, while stirring the mixture vigorously, to destroy the excess of thionyl chloride. More water was added and the dichloride which was heavier than the water was separated. The water was extracted once with ether and the extract was mixed with the dichloride. It was then

washed with sodium bicarbonate solution followed by water. The ether solution was dried over powdered sodium sulphate, the ether evaporated, and the dichloride distilled under a water pump vacuum using a short column packed with helices.

Conversion of Dichlorides to Iodochlorides[123].

Sodium iodide (0.5 mole) was dissolved at room temperature in acetone (700 ml), which had been dried over anhydrous sodium sulphate. The dichloride (0.5 mole) and acetone (250 ml) were placed in a 1 litre three necked flask fitted with a mechanical stirrer through a mercury seal, a water condenser and a dropping funnel. The sodium iodide solution was then added to the refluxing dichloride solution at such a rate that all the solution was added in about 90 min. The mixture was further refluxed for ca. 3 hrs. and then most of the acetone was distilled off. The mixture was diluted with about a litre of water and extracted by ether (2 x 250 ml). The ether extract was washed once with water, dried over sodium sulphate and the ether evaporated. The product was finally fractionated under oil pump vacuum using either a Fenske column or a spinning band distillation apparatus.

Chain extension by one carbon atom[124].

Sodium cyanide (0.12 mole) was dissolved in DMSO (100 ml) in a two necked round bottom flask (500 ml), fitted with a water

condenser and a dropping funnel. The solution of alkyl chloride (0.1 mole) in DMSO (10 to 20 ml) was added while stirring the cyanide solution with a magnetic stirrer and the mixture was heated at 120-130° for 3½ hours. After cooling, it was diluted with a 5% solution of sodium chloride (500 ml) and extracted with ether (75 x 4). The extract was washed with 10% sodium chloride solution (3 times) and dried over sodium sulphate. A 25% solution of hydrochloric acid (gas) in methanol (100 ml) was added to the nitrile, obtained after evaporation of solvent and the mixture was kept overnight at room temperature. The methyl ester was then recovered by diluting the mixture with water and extracting it with ether.

Chain extension by two carbon atoms.

Malonic ester synthesis[125]: Super dry ethanol (175 ml) was stirred in a two necked round bottom flask (250 ml), fitted with a reflux condenser and a dropping funnel, during the addition of sodium metal (0.1 mole) cut into small pieces. Freshly distilled diethyl malonate (0.11 mole) was then added through dropping funnel and the mixture was refluxed for an hour before being cooled to room temperature. Sodium iodide (0.11 mole) was added and the alkyl chloride dissolved in super dry ethanol (0.1 mole, 25 ml) was added dropwise while stirring. The mixture was then refluxed for 24 hrs. and the malonic ester derivative recovered, by diluting the mixture with water (500 ml), acidifying with dilute

hydrochloric acid and extracting with ether.

The substituted malonic ester was saponified by refluxing with alcoholic potassium hydroxide (2N, 200 ml) for 2 hours. The mixture was diluted with water and the unsaponified matter was removed by extraction with ether. The water solution was then acidified with dilute hydrochloric acid and the organic acid was extracted with ether (75 ml x 3). The ether extract was dried with sodium sulphate after washing with 10% sodium chloride and the malonic acid derivative obtained after evaporation of the solvent. This acid was refluxed overnight with a mixture of DMSO (200 ml) and dilute sulphuric acid (0.25N, 200 ml) and the monobasic acid was recovered, by extracting with ether, washing the ether extract by 10% sodium chloride solution, drying it over sodium sulphate and evaporating the solvent.

Distillation of Ammonia[126].

For reduction of -C≡C- to trans CH=CH, reduction with lithium or sodium in liquid ammonia was used. The liquid ammonia obtained directly from the cylinder is often contaminated with iron which acts as a catalyst to change lithium to lithamide thus making it useless for reduction. Hence for reduction, distilled ammonia was always used. A three-necked 1 litre flask, fitted with a gas inlet tube and a cardice condenser was kept in a dry-ice bath. The outlet tube of the condenser was placed near the exhaust fan

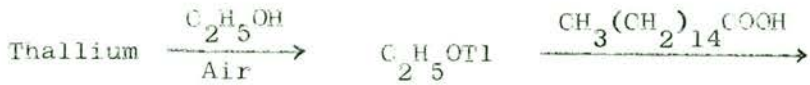
of the fume cupboard and the condenser was kept filled with a mixture of cardice and acetone. The ammonia cylinder,'in upright position', was joined to the inlet tube and ammonia was passed at such a rate that the acetone-cardice mixture did not boil over.

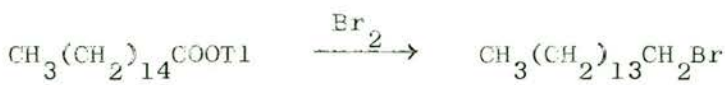
Lithium-Ammonia Reduction in an Autoclave.

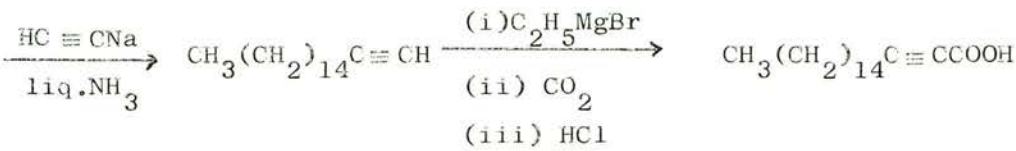
As the monoynes were not reduced at atmospheric pressure, they were reduced in an autoclave[127]. Since a stainless steel autoclave is attacked by lithium and ammonia a glass sleeve which fitted inside the autoclave was used and instead of a metal stirrer a magnet encased in glass was used as a stirrer. The acid (5 g) was dissolved in sodium dried tetrahydrofuran (ca. 100 ml) and kept in the autoclave. First a few ml. of distilled ammonia were added carefully to the stirring solution, avoiding excessive frothing from the reaction of acid with ammonia and evaporation of ammonia. Finally all the distilled ammonia (ca. 300 ml) was added, followed by lithium (2.5-3.0 g), cut into small pieces (ca. 0.2 g each). The first piece was added and when the initial reaction and frothing had subsided the subsequent pieces were added as rapidly as possible. The autoclave was closed and the mixture stirred overnight at ambient temperature. The pressure obtained was between 10 and 12 atmospheres. Next morning the pressure was relased, the autoclave opened and the

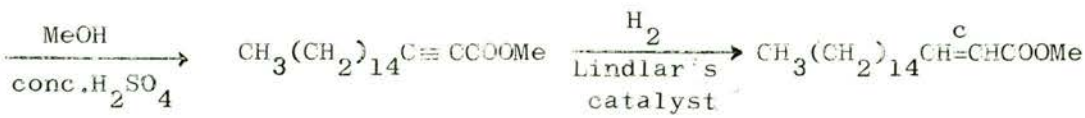
excess lithium was destroyed by careful addition of solid ammonium chloride. The ammonia was allowed to evaporate, water added, and the mixture was made acidic with dilute hydrochloric acid and then extracted with ether (4 x 50 ml.). The ether extract was washed free of acid with saturated sodium chloride solution and dried over sodium sulphate. The ether was evaporated and the product was crystallised from petrol ether.

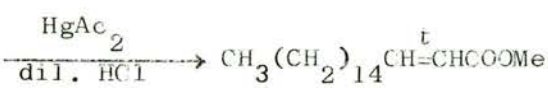
Methyl Octadec-trans-2-enoate











Thallium Ethoxide[128].

Thallium metal (51 g; 0.25 atom) was turned into fil ings with a pencil sharpner. These were kept in a porcelain sieve enclosed in a specially prepared glass tube, (see fig. 1). The glass tube was attached to a 250 ml round bottom flask and a water condenser with a soda-lime drying tube at the top. Super dry ethanol (125 ml) was placed in the flask and refluxed through the filings. A vigorous stream of air, dried with conc. sulphuric acid and passed through a tower of sodalime was let in by a side tube, so that the filings came in contact with ethanol vapour and oxygen at the same time. After about 18 hrs. reflux, practically all the thallium was dissolved and thallium ethoxide had settled as a heavy oil at the bottom of the flask.

Thallium Palmitate.

Palmitic acid (51.2 g, 98% pure; 0.2 mole) was dissolved in petrol ether (500 ml) and thallium ethoxide (14 ml; d = 3.5; 0.197 mole) was pipetted in, while stirring with a magnetic stirrer. It was stirred for a further 15 mins. and filtered through a Buchner funnel. The precipitate was then dissolved in ethanol (ca. 1 litre) by heating and recrystallised at room temperature m.p. 118-119°C (lit.[74] 116-117°C). Yield 97% (87 g).

Pentadecyl Bromide[74].

Thallium palmitate (86.5 g; 0.192 mole) was slurried in carbon tetrachloride (ca. 1200 ml) and a solution of bromine (45.5 g; 0.285 mole) in carbon tetrachloride (ca. 50 ml) was added dropwise, while stirring with a magnetic stirrer. The mixture was refluxed for 4 hrs, cooled, washed twice with water. The solution was then dried over anhydrous sodium sulphate, the carbon tetrachloride evaporated and the product purified by column chromatography (adsorbent:aluminium oxide;eluent:P.E.). The bromide (33.6 g; 51.5%) was tested with GLC (10% DEGS, 190°C) and was found to be pure.

Heptadec-1-yne[78,(a)].

Sodium acetylide was prepared by the titration method from sodium (5.22 g; 0.227 atom), ammonia (500 ml) and acetylene. Dimethylformamide (DMF) was added and the ammonia was evaporated completely. Pentadecyl bromide (33g ; 0.1135 mole) dissolved in DMF (ca. 50 ml) was added and the mixture was heated at 70°C for 3 hrs. The mixture was cooled, extracted with ether (3 x 100 ml), washed with dilute HCl and with 10% sodium chloride solution, dried over sodium sulphate and the solvent evaporated. The product was chromatographed on silica gel column (eluent: petrol ether) and was found to be pure (24 g, 90%) when tested with GLC (DEGS, 170°C).

Octadec-2-ynoic Acid[31].

Bromoethane (9.7 g; 0.075 mole) was added to magnesium filings (1.94 g; 0.08 mole), sodium dried ether (ca. 100 ml) and a trace of iodine contained in a three necked flask, fitted with a mechanical stirrer, water condenser fitted with a drying tube and a dropping funnel. After the addition was complete, the mixture was refluxed for a further ½ hr. It was then cooled and heptadec-1-yne (11.8 g; 0.050 mole) dissolved in sodium dried ether (ca. 25 ml) was added to it dropwise over 10 mins. After refluxing again for 1½ hrs., it was cooled and transferred to a cardice-cooled autoclave. Cardice (ca. 200 g) was added and the autoclave was sealed. The mixture was stirred overnight in the autoclave when the pressure reached 25 psi. The carbon dioxide was allowed to escape, the mixture was diluted with water, acifified with sulphuric acid (2N, ca. 100 ml) and extracted with ether (2 x 50 ml). The ether layer was washed free of acid with 10% sodium chloride solution and extracted with aqueous ammonia (2N, 3 x 75 ml) to remove the acid from the unreacted neutral material. The neutral material (3.5 g) was recovered from the ether as usual. The alkaline soap solution was acidified with dilute hydrochloric acid and the acid (7.75 g; 78.8%) recovered. The crude acid was purified by recrystallisation from petrol ether. m.p. 56-57°C. (lit.[42] 56-57°C). von Rudloff oxidation of the partially reduced acid (Δ^{2c}) gave only palmitic acid.

Octadec-trans-2-enoic Acid.

(i) Octadec-2-ynoic acid (100 mg) was dissolved in THF (25 ml) and placed in a conical flask (100 ml). Distilled liquid ammonia (50 ml) was added, followed by a small piece of lithium and the mixture was stirred by a magnetic stirrer. Ammonia was replenished from time to time and the mixture was kept blue for 1½ hrs, by further addition of small pieces of lithium whenever the blue colour started to fade. The acid was then recovered, methylated and examined by GLC. It was found to be methyl stearate (m.p. 37-37.5° lit.[129] 38.8°, ECL DEGS, 18.0; ApL 18.0).

(ii) Octadec-2-ynoic acid was first methylated by refluxing it with methanol, containing 2% concentrated sulphuric acid, for ½ hr., and the methyl ester was then partially reduced with hydrogen in presence of Lindlar's catalyst. The cis-methyl ester (1 g) was dissolved in dry methanol (40 ml), mercuric acetate (1.5 g) was added to it and the solution was kept at ambient temperature for 48 hours. The solution was then cooled in an ice-water bath and 10% concentrated hydrochloric acid in methanol (30 ml) added while stirring with a magnetic stirrer. The temperature of the mixture was kept below +5°. The solution was stirred for a further 30 min. at room temperature, diluted with water and extracted with ether. The extract was dried over sodium sulphate and

the solvent evaporated. Examined by GLC (DEGS, 190), the methyl ester (1 g) showed the presence of only ca. 2% methyl octadec-cis-2-enoate (ECL, 18.28) along with the trans isomer (ECL, 19.58). Pure ester was obtained by preparative silver nitrate thin layer chromatography. On von Rudloff oxidation, it gave only palmitic acid.

Other Attempts to Prepare Octadec-trans-2-enoic Acid.

(i) Doebner Synthesis

Cetyl Tosylate[130].

Cetyl alcohol (50.82 g, 0.21 mole) was dissolved in pyridine (70 ml) and p-toluene sulphonyl chloride (47.7 g, 0.25 mole) was added to it, in small lots, while stirring. The mixture was stirred for 3 hrs. at room temperature, water (2ml) was then added and the mixture was stirred for a further period of 10 hrs., at room temperature. After addition of sulphuric acid (2N, 500 ml) the mixture was extracted with ether (150 ml x 3). The ether extract was washed with 10% sodium chloride solution till neutral, dried over sodium sulphate and the solvent was evaporated. The product (66.1 g, 78.5%) was used without any further purification.

Conversion of Cetyl Tosylate to Palmitaldehyde[130].

A three necked, round bottom flask (500 ml), fitted with a gas inlet tube and a mechanical stirrer, was preheated to 165°

in a hot oil-bath. The stirrer was started and the solution of cetyl tosylate (60 g) in DMSO (300 ml) was added followed immediately by sodium bicarbonate (30 g). The temperature dropped from 165° to 155° and the mixture was stirred at this temperature under nitrogen. After 6 minutes the flask was removed and cooled under water, the contents poured into ice and the mixture was extracted with petrol ether (3 x 150 ml). The extract was then washed twice with water, dried over sodium sulphate and the solvent was removed. The crude product was purified by passing through a short silica gel column with petrol ether containing 10% ethyl ether (35 g, 96.5%). The aldehyde was shown to be pure by GLC (DEGS, 190°).

Preparation of Octadec-trans-2-enoic Acid[130].

Malonic acid (16 g, 0.15 mole) was added in small portions to dry pyridine (35 ml). When it was all dissolved, a solution of palmitaldehyde (16 g, 0.067 mole) in pyridine (25 ml) was added, followed by piperidine (1 ml). The mixture was allowed to react at 55°C for 1 hr. and then at 90°C for 5 hrs under nitrogen. Concentrated hydrochloric acid (35 ml) diluted with ice-water (125 ml) was added to the mixture and it was then extracted with ether (75 ml x 3). The ether extract was washed with aq. ammonia (2N, 40 ml x 4) and the washings were acidified with 2N sulphuric acid. The acidified water was then extracted with

ether (75 ml x 3), the ether extract was washed with sodium chloride solution (10%) and dried over sodium sulphate. The product obtained on evaporation of solvent was purified by column chromatography (100g. silica gel; eluent, 96:5:: petrol ether: ethyl ether) (13.3g, 70%). Subjected to von Rudloff oxidation the acid gave 89% palmitic and 11% pentadecanoic acid.

(ii) Wittig Synthesis

Preparation of Carbethoxy methyl-triphenyl-phosphonium-bromide[131].

Triphenyl phosphine (181 g, 0.5 mole) was dissolved in dry benzene (600 ml) by stirring with a magnetic stirrer at room temperature and ethyl bromoacetate (78.5 g, 0.47 mole) was added over 30 mins. The mixture was stirred overnight at ambient temperature and filtered through a Buchner funnel. The crystals were washed with benzene and petrol ether and dried under water pump vacuum at ~50°C for several hours. (165 g, 82.5%).

Carbethoxymethylene-triphenyl-phosphorane[131].

Carbethoxymethyl-triphenyl-phosphonium-bromide (42.9 g, 0.1 mole) was dissolved in water (1 litre) and dilute aqueous sodium hydroxide was added to it dropwise, while stirring, till the supernatant water showed pink colour with phenolphthalein. The precipitate was filtered through a Buchner funnel, washed with water and dried in a porous plate. It was then dissolved in hot acetic acid and recrystallised by addition of petrol ether.

(3.40 g, 97.7%, m.p. 118°, lit.[131] 116-117°)

Ethyl Octadec-trans-2-enoate.

Palmitaldehyde (10g, 0.0417 mole) and carbethoxymethylene-triphenyl-phosphorane (14.55 g, 0.0417 mole) were dissolved in dry benzene (200 ml) and the solution was refluxed under nitrogen overnight. The solvent was then evaporated under vacuum and ether was added to dissolve the octadec-trans-2-enoic acid; the triphenyl-phosphonium oxide was insoluble. It was filtered off and the ether was evaporated. The remaining solid was again dissolved in petrol ether, the small amount of triphenylphosphonium oxide which separated, was filtered off and the solvent was evaporated. The ethyl ester so obtained (10.35 g, 80%), when tested with GLC (ApL, 210°), was shown to contain 7.7% of the cis-isomer (on the basis of ECL values) and 0.4% of some other impurity (not palmitaldehyde or triphenylphosphonium oxide).

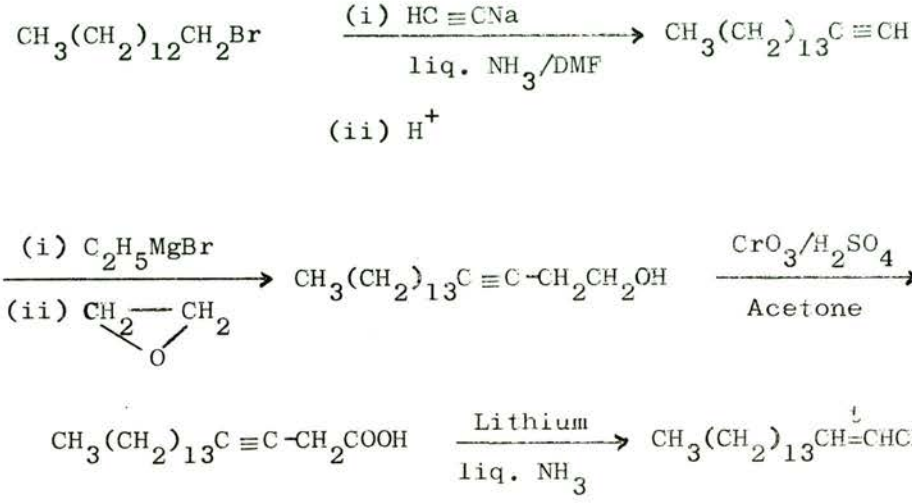
Transesterification of Ethyl Octadec-trans-2-enoate.

Ethyl octadec-trans-2-enoate (100 mg) was refluxed for 2½ hrs in 0.5% sodium methoxide in methanol (15 ml). When examined by GLC (DEGS, 190°) it showed that the transesterification was incomplete and that there may be some conversion to octadec-cis-2-enoate and octadec-trans-3-enoate.

An attempt to saponify the ethyl ester with 2N methanolic

potassium hydroxide at room temperature (stirred for $2\frac{1}{2}$ hrs) and to reesterify it (refluxed for 1hr with 2% concentrated sulphuric acid in dry methanol) gave a mixture of methyl octadec-trans-2-enoate (75%) with the cis-isomer (25%).

Octadec-trans-3-enoic Acid.



Hexadec-1-yne[78a].

Sodamide was prepared from sodium (13.8 g) and liquid ammonia (500 ml) and acetylene was passed into it for 1½ hrs. DMF (250 ml) was then added and the ammonia was evaporated. Tetradecyl bromide (obtained from Koch-Light Laboratories, GLC pure, 138.5 g), dissolved in DMF (200 ml), was added to the sodium acetylide and the mixture was stirred overnight at room temperature. It was then diluted with water and extracted with ether. The ether extract was washed with dilute hydrochloric acid followed by 10% sodium chloride solution, dried over sodium sulphate and freed from solvent. The product was distilled under oil-pump vacuum through a short column packed with glass helices. The hexadec-1-yne obtained [60 g, 54%, b.p. 86° /0.1 mm (lit.[32] 100°/0.5 mm)] was shown to be pure by GLC (DEGS, 150°).

Octadec-3-yn-1-ol[35].

Magnesium filings (3.28 g, 0.135 mole) together with dry ether (75 ml) and a speck of iodine, were placed in a three necked, round bottom flask (500 ml) fitted with a stirrer, a water condenser and a dropping funnel. Bromoethane (13.7 g, 0.125 mole) dissolved in dry ether (25 ml) was then added carefully. The mixture was refluxed for ½ hr. under nitrogen, sodium dried thiophene-free benzene (100 ml) was added to it and the solvent distilled off till the distilling solvent showed a temperature of 78°C.

The flask was then cooled down to room temperature, the condenser was changed to reflux position and a solution of hexadec-1-yne (19.6 g, 0.0885 mole) in dry thiophene-free benzene (25 ml) was added to it over 30 mins. The mixture became a thick white slurry, so more benzene (100 ml) was added and the mixture was refluxed for 4½ hrs. on a hot oil-bath.

Ethylene oxide (20 g) was placed in a round bottom flask (100 ml) and cooled with ice-water. The outlet tube from the flask was connected with a plastic tube to the inlet tube of the reaction vessel. The inlet tube was adjusted so that it was just above the surface of the stirred reaction mixture. The reaction vessel containing the slurry of hexadec -1-ynylmagnesium bromide in benzene was cooled in an ice-water bath and the reflux water condenser was changed to one containing ice and salt. The flask containing the ethylene oxide was then carefully heated to 13-15^{o}C. The slurry dissolved to a clear solution and soon set to a jelly. The ethylene oxide addition was stopped and the product was allowed to stand overnight at room temperature. The flask was then heated till the solvent started refluxing and the jelly dissolved. After refluxing for ½ hr. the solvent was distilled off, the product was cooled, water (100 ml) and hydrochloric acid (2N, 100 ml) were added and the mixture was extracted with ether (75 ml x 4). The ether extract was washed successively

with 10% sodium chloride solution, saturated sodium bicarbonate solution and again with 10% sodium chloride solution and dried over sodium sulphate. The solvent was evaporated and the product was purified by column chromatography (absorbent: silica gel; eluent: petrol ether with increasing proportion of ethyl ether 0%, 5% .....100%). (12.67 g, 54%).

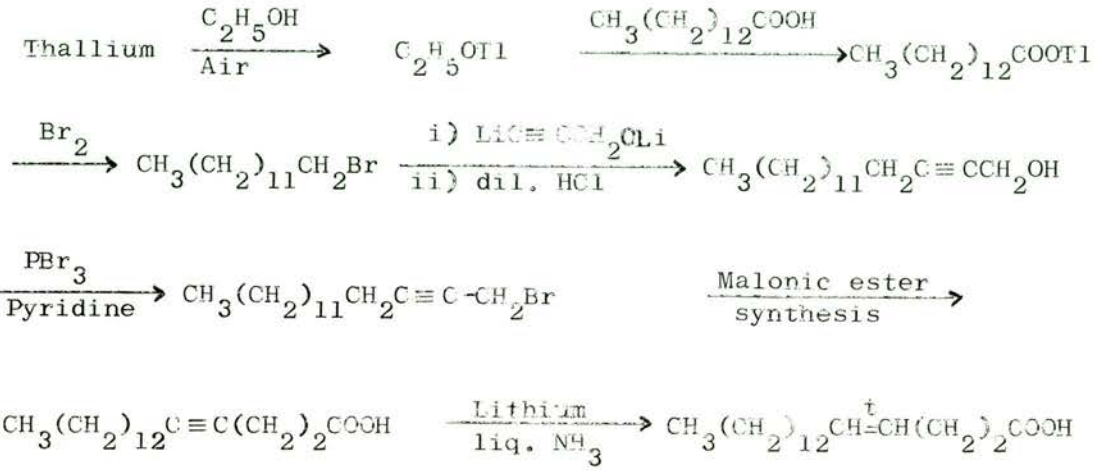
Octadec-3-ynoic Acid[32].

Octadec-3-yn-1-ol (12.39 g) was dissolved in acetone (192 ml) and the solution was stirred whilst being surrounded by ice-water. Concentrated sulphuric acid (1 ml) followed by a solution of chromium trioxide (6.3 g) in water (3.75 ml), was added dropwise taking care that the temperature of the solution did not rise above 10°C. The mixture was stirred for a further 4 hrs. at 15-20°C and then poured into water (400 ml). The mixture was extracted with ether (75 ml x 4) and the extract was washed free of inorganic salts with water. The acid was then extracted from the ether by washing with aqueous ammonia (2N, 100 ml x 5) to which 3% sodium chloride had been added. The combined washings were extracted with ether (50 ml x 3), acidified with sulphuric acid (4N) and the liberated acid was extracted with ether (75 ml x 3). The ether extract was washed with 10% sodium chloride solution, dried and the solvent was evaporated to give crude acid (3.86 g, 29.7%). After being crystallised from petrol ether, the acid (m.p. 73-74°) was 95% pure, the other 5% impurities were non-polar material.

Octadec-trans-3-enoic Acid.

Octadec-3-ynoic acid (1 g) was dissolved in THF (25 ml) and distilled liquid ammonia (50 ml) was added to it. It was then reduced at atmospheric pressure by addition of small pieces of lithium. The mixture was kept stirring and blue for 8 hrs. Reduction was 91% complete and there was no peak on the GLC trace corresponding to either the 4t or 2t ester. von Rudloff oxidation of the purified product (purified by crystallisation from petrol ether) gave pentadecanoic acid as the only monobasic acid.

Octadec-trans-4-enoic Acid



Thallium Ethoxide[128].

Thallium (51 g, 0.25 atom) was reacted with ethanol vapour in a stream of carbon dioxide-free air, as given before, to give thallium ethoxide (49 g, 79%).

Thallium Myristate

Thallium myristate was prepared from myristic acid (99% pure, 45.6 g, 0.2 mole) and thallium ethoxide (49 g, 0.198 mole) in the same way as thallium palmitate (cf. Δ^2 methyl ester). Crystallisation of the product from ethanol gave pure thallium myristate (m.p. 120°C, lit.[74], 120-121°C; 81.6g, 95.5%).

Tridecyl bromide[74].

Tridecyl bromide was prepared by making a slurry of thallium myristate (73.2g, 0.17 mole) in carbon tetrachloride (500 ml) and adding bromine (41 g, 0.255 mole). The product was passed through a short chromatographic column (absorbent: aluminium oxide; eluting solvent: petrol ether) to obtain pure tridecyl bromide (36.1 g, 81%; GLC: DEGS /170°).

Hexadec-2-yn-1-ol.

Lithamide was prepared in a three necked round bottom flask (2 lit.) from lithium (2.54 g, 0.363 atom) and liquid ammonia

(1200 ml) and a solution of propargyl alcohol (9.25 g, 0.165 mole) in dry THF (50 ml) was added dropwise. After stirring the mixture for 1½ hrs., tridecyl bromide (29 g, 0.11 mole) solution in dry THF (100 ml) was added and the mixture was refluxed under a cardice condenser for a further 3 hrs. The ammonia was then allowed to evaporate overnight and the crude hexadec-2-yn-1-ol was recovered, after dilution with water, by extracting with ether. The crude product was purified (17.08 g, 64.5%) by column chromatography (adsorbent: silica gel; eluent: petrol ether followed by petrol ether containing increasing proportions of ethyl ether).

1-Chlorohexadec-2-yne.

Hexadec-2-yn-1-ol (10.55 g, 0.0445 mole) and pyridine (2 ml) were stirred in a round bottom flask and cooled in an ice-water bath during addition of thionyl chloride (0.175 mole). The mixture was then stirred at room temperature for 2 hrs. and refluxed for an hour. The crude 1-chlorohexadec-2-yne was recovered after careful dilution with water and extraction with ether. Chromatography on a short silica gel column (eluent: petrol ether) provided pure 1-chlorohexadec-2-yne (9.5 g, 83.5%).

Octadec-4-ynoic Acid by Malonic Ester Synthesis.

Small pieces of sodium metal (0.86 g, 0.036 mole) were dissolved

in super dry ethanol (100 ml) and diethyl malonate (6.4 g, 0.04 mole) was reacted with the sodium ethoxide by refluxing for an hour. 1-Chlorohexadec-2-yne (9.25g, 0.036 mole) was added and the mixture was refluxed overnight. The malonic ester derivative was recovered and the crude product was saponified with potassium hydroxide (8 g) in 80% aq. ethanol (150 ml). Unsaponifiable matter was removed (2.86 g), the malonic acid derivative was recovered (5.25 g, 45%) and refluxed with DMSO (50 ml) and dilute sulphuric acid (0.25N, 50 ml) overnight. Octadec-4-ynoic acid was obtained by extraction with ether (4.52 g, 99%) and was purified by recrystallisation from petrol ether (m.p. 74-74.5°C, lit.[42] 74-75°C).

Octadec-trans-4-enoic Acid.

Octadec-4-ynoic acid when reduced at atmospheric pressure with liquid ammonia and lithium gave only 54% reduction after 1 hr. When the acid (3 g) was reduced in an autoclave at room temperature with lithium (1.5 g) and liquid ammonia, 92% reduction was effected after 6 hours. The product, crystallised from petrol ether at 5°C, still contained ca. 6.5% octadec-4-ynoic acid (GLC, DEGS/190°). A small amount of the acid was purified by silver nitrate thin layer chromatography (m.p. 59-59.5°, lit.[42], 58.5-59.5°).

von Rudloff oxidation of the octadec-trans-4-enoic acid gave myristic and succinic acids only.

Octadec-trans-5-enoic Acid

$$Br(CH_2)_3Cl \xrightarrow[liq.NH_3]{HC\equiv CNa} HC\equiv C(CH_2)_3Cl \xrightarrow[NaI]{NaCN,\ C_2H_5OH} HC\equiv C(CH_2)_3CN$$

$$\xrightarrow[MeOH]{i)\ HCl(gas)} HC\equiv C(CH_2)_3COOH \xrightarrow{SOCl_2} HC\equiv C(CH_2)_3COCl \xrightarrow{(CH_3)_2NH}$$

ii) KOH

$$HC\equiv C(CH_2)_3CON(CH_3)_2 \xrightarrow[liq.NH_3]{i)\ NaNH_2} CH_3(CH_2)_{11}C\equiv C(CH_2)_3CON(CH_3)_2$$

ii) $CH_3(CH_2)_{11}Br$

$$\xrightarrow[EtOH]{NaOH} CH_3(CH_2)_{11}C\equiv C(CH_2)_3COOH \xrightarrow[liq.\ NH_3]{Lithium} CH_3(CH_2)_{11}\overset{t}{CH}=CH(CH_2)_3COOH$$

1-Chloropent-4-yne[133].

Sodium acetylide was prepared by passing acetylene gas for 1½ hrs. into a sodamide suspension in liquid ammonia prepared from sodium (32 g, 1.37 mole). Bromochloropropane (197g, 1.25 mole) solution in dry ether (75 ml) was added over 30 mins. The mixture was refluxed for 3 hrs. using a cardice condenser and the ammonia was then allowed to evaporate overnight. The crude ω-chloropentyne was purified by distillation through a short packed column (75 g, 58.5% b.p. 108-109°, lit.[133] 88°/350 mm) and shown to be pure by GLC (DEGS, 175°C).

1-Cyanopent-4-yne.

1-Chloropent-4-yne when reacted with DMSO and sodium cyanide gave only a polymarised product. 1-Cyanopent-4-yne was hence prepared by refluxing 1-chloropent-4-yne (64 g, 0.6275 mole) with a solution of sodium iodide (113 g, 0.643 mole) and sodium cyanide (113 g, 2.26 mole) in aq. ethanol (80%, 1250 ml) for 48 hrs. About half of the ethanol was distilled off and the nitrile was recovered by extraction with ether after diluting the mixture by water (1 litre) (58 g, 99%).

Hex-5-ynoic Acid.

The crude 1-cyanopent-4-yne was turned into methyl hex-5-ynoate by keeping it overnight with 25% hydrochloric acid (gas) in dry

methanol (200 ml). The recovered ester was then saponified with 10% potassium hydroxide solution in aq. ethanol (90%, 400 ml) by refluxing for 30 mins., unsaponifiable matter was removed and the acid was recovered by extraction with ether. The crude acid was purified by distillation through a short packed column (45.2 g, 61%) (b.p. 112-115°C; lit.[134] b.p. 106°/9 mm).

Hex-5-ynoyl Chloride.

Thionyl chloride (95 g, 0.8 mole) was added to stirred hex-5-ynoic acid (40.3 g, 0.36 mole) over 1 hr. and the mixture was then stirred at room temperature for an hour and refluxed for a further half hour. The excess thionyl chloride was removed under water pump vacuum and the product was distilled to obtain pure hex-5-ynoyl chloride (35 g, 75%, b.p. 60-65°/15 mm).

N,N-Dimethylhex-5-ynamide[32].

An ether solution of the acid chloride (34.1 g, 0.261 mole in 200 ml) was added dropwise to an ice cold solution of dimethylamine (45g, 1 mole) in ether (250 ml) and the mixture was stirred for a further half hour in the ice bath. The mixture was then diluted with saturated salt solution and ether layer was separated. The water was extracted twice more with ether and the product was recovered in the usual manner. N,N-dimethylhex-5-ynamide was purified by column chromatography (adsorbent: silica gel; eluent: 75 ethyl ether:25 petrol ether) (22g, 61%).

N,N-Dimethyloctadec-5-ynamide[44,72].

N,N-dimethylhex-5-ynamide (21.6 g, 0.155 mole) solution in dry ether (50 ml) was added to a sodamide suspension (4.3 g, sodium, 0.186 mole) and stirred for 1½ hrs. Dodecyl bromide (obtained from Koch-Light Laboratories, GLC pure, 42 g, 0.170 mole) in dry ether (100 ml) was then added and after refluxing for 2 hrs. using a cardice condenser, the ammonia was allowed to evaporate overnight. The product was recovered in the usual manner and purified (24.2 g, 51%) by column chromatography (adsorbent: silica gel; eluent: ethyl ether:petrol ether:: 3:1).

Octadec-5-ynoic Acid.

Octadec-5-ynoic acid was obtained by refluxing the N,N-dimethyloctadec-5-ynamide (21.2 g) with an aq. solution of sodium hydroxide (5N. 300 ml) and ethanol (300 ml) for 8 hrs., and purified by crystallising twice from petrol ether (GLC pure, 15 g, 77.5%; m.p. 51.5-52°; lit.[42] 51-52°).

Octadec-trans-5-enoic Acid.

Octadec-5-ynoic acid (6 g) dissolved in THF (100 ml) was reduced with lithium (3 g) and liquid ammonia (400 ml) in an autoclave. The acid, after one crystallisation from petrol ether, contained less than 1% of octadec-5-ynoic acid (GLC, DEGS

/190°) (m.p. 45-46°; lit.[42] 46.5-47.5°). von Rudloff oxidation of the acid gave only tridecanoic acid and glutaric acid.

Octadec-trans-6-enoic Acid

$$Cl(CH_2)_4Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_4Cl \xrightarrow[liq.\ NH_3]{NaC\equiv CH} HC\equiv C(CH_2)_4Cl$$

$$\xrightarrow[liq.\ NH_3]{\text{i) } NaNH_2 \\ \text{ii) } CH_3(CH_2)_{10}Br} CH_3(CH_2)_{10}C\equiv C(CH_2)_4Cl \xrightarrow[DMSO]{NaCN} CH_3(CH_2)_{10}C\equiv C(CH_2)_4CN$$

$$\xrightarrow[MeOH]{HCl\ (gas)} CH_3(CH_2)_{10}C\equiv C(CH_2)_4COOMe \xrightarrow[EtOH]{KOH}$$

$$CH_3(CH_2)_{10}C\equiv C(CH_2)_4COOH \xrightarrow[liq.\ NH_3]{Lithium} CH_3(CH_2)_{10}\overset{t}{CH}{=}CH(CH_2)_4COOH$$

1-Chloro-4-iodobutane.

Sodium iodide (150 g, 1.0 mole) was dissolved in dry acetone (1 litre) and 1,4-dichlorobutane (obtained from B.D.H., 127 g, 1 mole) was added while heating. The product was recovered in the usual manner after refluxing the mixture for 1½ hrs. and purified by distillation through a Fenske column (79 g, 36%; b.p. 81°/15 mm, lit.[135] 93-94.5°/17 mm). GLC (DEGS, 160°) showed the 1,4-chloroiodobutane to contain only 5% of dichlorobutane.

1-Chlorohex-5-yne.

An ether (100 ml) solution of chloroiodobutane (79 g, 0.36 mole) was added to a sodium acetylide suspension (sodium 8.3 g, 0.36 mole) and after refluxing the mixture for 4 hrs. using a cardice condenser, the ammonia was allowed to evaporate. The product was recovered in the usual way and purified by distillation through a short packed column (26 g, 62.5%, b.p. 131°, lit.[46] 47-48°/17 mm).

1-Chloroheptadec-5-yne.

1-Chlorhex-5-yne (26g, 0.223 mole) dissolved in dry ether (50 ml) was added to a stirred suspension of sodamide (sodium 5.64 g, 0.245 mole) in liquid ammonia and allowed to react for 1½ hrs. An ether solution of undecyl bromide (63 g, 0.268 mole in 100 ml) was then added and after refluxing the mixture for 3 hrs.,

and allowing the ammonia to evaporate 1-chloroheptadec-5-yne was extracted with ether. The product was distilled under oil-pump vacuum (16 g, 26.6%) and redistilled for further purification (b.p. 124°/0.15 mm).

Octadec-6-ynoic Acid.

1-Cyanoheptadec-5-yne was obatined by refluxing 1-chloro-heptadec-5-yne (10.87 g, 0.04 mole) with sodium cyanide (2.45 g, 0.05 mole) and DMSO (200 ml) for 3½ hrs.

The crude cyanide (10.8 g) was allowed to stand overnight with a 20% hydrochloric acid (gas) solution in dry methanol (150 ml) and the resulting ester (11.1 g) was recovered in the usual manner.

The crude methyl octadec-6-ynoate was saponified with ethanolic potassium hydroxide (6%, 100 ml) by refluxing for ½ hr. The unsaponifiable matter was removed and the crude acid was recovered (10.5 g overall yield 94%). The acid was purified by crystallisation from petrol ether at +5°C (m.p. 49.5-50°, lit.[42] 50-51°). GLC showed the acid to be >99% pure.

Octadec-trans-6-enoic Acid.

Octadec-6-ynoic acid (6.2 g) dissolved in THF (100 ml) was reduced by lithium (3 g) and liquid ammonia (400 ml) in the autoclave overnight. Octadec-trans-6-enoic acid, after

crystallisation from petrol ether, was shown to contain 1.6% of acetylenic acid but was otherwise pure according to GLC (5.88 g, m.p. 52.5-53°, lit.[42] 53-54°C). The von Rudloff oxdiation of the acid gave adipic acid, lauric acid and only a trace (< 0.75%) of undecanoic acid.

Octadec-trans-7-enoic Acid.

$$Cl(CH_2)_5Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_5Cl \xrightarrow[liq.\ NH_3]{NaC\equiv CH} HC\equiv C(CH_2)_5Cl$$

$$\xrightarrow[liq.\ NH_3]{\text{i) } NaNH_2 \\ \text{ii) } CH_3(CH_2)_9Br} CH_3(CH_2)_9C\equiv C(CH_2)_5Cl \xrightarrow[DMSO]{NaCN}$$

$$CH_3(CH_2)_9C\equiv C(CH_2)_5CN \xrightarrow[MeOH]{HCl(gas)} CH_3(CH_2)_9C\equiv C(CH_2)_5COOMe$$

$$\xrightarrow[EtOH]{KOH} CH_3(CH_2)_9C\equiv C(CH_2)_5COOH \xrightarrow[liq.NH_3]{Lithium} CH_3(CH_2)_9\overset{t}{CH}=CH(CH_2)_5COOH$$

1-Chloro-5-iodopentane

1,5-Dichloropentane (245 g, 1.74 mole) was added to a stirred solution of sodium iodide (261 g, 1.74 mole) in dry acetone (1200 ml) and the mixture was refluxed for 1½ hr. After distilling off most of the acetone the crude chloroiodopentane was recovered and distilled under water-pump vacuum through a Fenske column (119 g, 34%; b.p. 98-102°/12 mm, lit.[48] 125-127°/36 mm). GLC showed the product to be 95% pure (DEGS, 160°).

1-Chlorohept-6-yne[46].

Sodium acetylide was prepared from sodium (13.7 g, 0.55 mole) in liquid ammonia and chloroiodopentane (116.5 g, 0.5 mole) in dry ether (100 ml) was added. The product was recovered after the usual reaction and distilled through spinning band column under water pump vacuum (50 g, 38.5%, b.p. 80°/31 mm, lit.[32] 78-79°/28 mm).

1-Chloroheptadec-6-yne.

1-Chlorohept-6-yne(25 g, 0.191 mole) was reacted with sodamide (4.83 g sodium, 0.210 mole) for 1½ hrs. and then n-decylbromide (46.5 g, 0.210 mole) in ether (100 ml) was added. The 1-chloro-heptadec-6-yne was purified by distillation under oil-pump vacuum (21.5 g, 41.8%; b.p. 119°/0.3 mm).

1-Cyanoheptadec-6-yne.

1-Chloroheptadec-6-yne (21.5 g, 0.08 mole) after refluxing with sodium cyanide (4.8 g, 0.096 mole) and DMSO (250 ml) for 3½ hrs. gave 1-cyanoheptadec-6-yne. The product (19.7 g, 95%) was shown to be pure, except for a minor volatile impurity, by GLC (ApL/210°C).

Octadec-7-ynoic Acid.

1-Cyanoheptadec-6-yne (19.7 g, 0.076 mole) was allowed to stand overnight with methanolic hydrochloric acid (30 g, HCl gas in 120 ml) and the methyl octadec-7-ynoate was recovered in the usual manner.

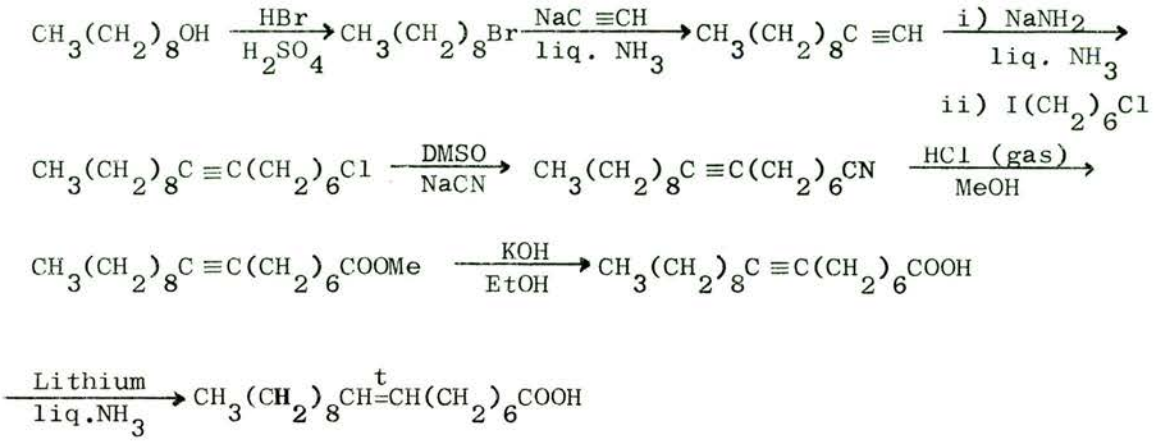
The ester was saponified with ethanolic potassium hydroxide (8 g in 200 ml 80% ethanol) by refluxing for half an hour and the acid was recovered by extraction with ether after the removal of unsaponifiable matter. The acid, purified by crystallisation from petrol ether (16.5 g, 77.5%), was found to be ca. 98% pure by GLC (DEGS, 190°) (m.p. 45.5-46.5°, lit.[42] 45.5-46.5°).

Octadec-trans-7-enoic Acid.

Octadec-7-enoic acid (5 g) gave, on overnight reduction with THF (100 ml), lithium (2.5 g) and liq. ammonia (400 ml) in an

autoclave, octadec-trans-7-enoic acid. GLC showed the acid (once crystallised from petrol ether) to be ca. 99% pure, (m.p. 43.5-44.5°C, lit.[42] 44.5-45.5°C). von Rudloff oxidation of the acid gave only pimelic acid and undecanoic acid.

Octadec-*trans*-8-enoic Acid.



Nonyl Bromide.

Aqueous hydrobromic acid (48%, 190 ml) was placed in a three-necked round bottom flask (1 litre) fitted with a mechanical stirrer and a water condenser and conc. sulphuric acid (35 ml) was added carefully while stirring. Nonan-1-ol (130 g, 0.9 mole) was then added, followed by more conc. sulphuric acid (25 ml) and the mixture was refluxed for 4 hrs. The mixture was diluted with water and the bromide was separated. It was washed successively with water, conc. hydrochloric acid, water, sodium bicarbonate solution and water and dried over sodium sulphate. The product was distilled under water-pump vacuum through a long packed column (b.p. 111-113^{o}/22 mm; lit.[136] 219.5^{o}/745 mm ; 149 g, 80%). The GLC analysis (ApL/130^{o}) showed the bromide to be pure.

Undec-1-yne.

Nonyl bromide (103.5 g, 0.5 mole) dissolved in dry THF (200 ml) was added to a stirred solution of sodium acetylide (13.5 g sodium, 0.6 mole) in liquid ammonia (1200 ml) and the mixture was stirred overnight. The product was recovered in the usual manner and purified by distillation through a spinning band column, under water-pump vacuum. (45 g, 59.5%; b.p. 84-86^{o}/15 mm; lit.[137] 81.5^{o}/ 10.5 mm).

1-Chloroheptadec-7-yne.

Undec-1-yne (38 g, 0.25 mole) dissolved in dry ether (100 ml) was reacted with a suspension of sodium acetylide (6.0 g sodium, 0.272 mole) in liquid ammonia (700 ml) for 1½ hrs. and chloroiodohexane (74 g, 0.3 mole) dissolved in dry ether (75 ml) was added. The mixture was refluxed under cardice condenser for 3 hrs., liquid ammonia was evaporated overnight and the product was recovered as usual by extraction with ether. The distillation of the product under oil-pump vacuum gave 1-chloroheptadec-7-yne (36.5 g, 54%; b.p. 151°/2 mm, lit.[48] 118-120°/0.5 mm) which was ca. 94% pure according to GLC (ApL/210°).

1-Cyanoheptadec-7-yne.

1-Chloroheptadec-7-yne (27 g, 0.1 mole) was added to a sodium cyanide (5.88 g, 0.12 mole) solution in DMSO (200 ml) and the mixture was refluxed for 3½ hrs. The nitrile was extracted with ether in the usual manner, dried over sodium sulphate and the solvent was evaporated.

Octadec-8-ynoic Acid.

All the crude nitrile obtained was allowed to stand overnight with a solution of hydrogen chloride (27.5 g) in dry methanol (135 ml). The methyl ester was recovered and saponified with ethanolic potassium hydroxide (12 g in 200 ml) under reflux for

1 hour. After removal of unsaponifiable material, the acid was recovered and crystallised from petrol ether [24.6 g, 88%; m.p. 46.5-47.0°, lit.[42] 47.0-47.5°, gave only a single peak on GLC (ApL/210°) after methylation].

Octadec-trans-8-enoic Acid.

Two attempts to reduce the acetylenic acid dissolved in THF, with lithium and liquid ammonia in an autoclave, gave only partial reduction. Finally, the acid (5.4 g) was dissolved in THF (75 ml) and reduced with lithium (3 g) and distilled liquid ammonia (450 ml) in a glass lined autoclave while stirring with a glass encased magnet. GLC showed the acid (once crystallised from petrol ether) to be completely octadec-8-enoic acid (DEGS, 190°; ECL: 18.54) (5.0 g, 92.5%, m.p. 51-51.5°, lit.[42] 51.5-52.5°).

von Rudloff oxidation of the acid gave C_8 (92%), C_9 (4%), C_7 (4%) dibasic acids along with C_{10} monobasic acid accompanied by small amounts of C_9 and C_{11} monobasic acids.

Octadec-*trans*-9-enoic Acid.

$$CH_3(CH_2)_7Br \xrightarrow[\text{liq. } NH_3]{NaC\equiv CH} CH_3(CH_2)_7C\equiv CH \xrightarrow[\text{liq. } NH_3]{\text{i) } NaNH_2 \text{ ii) } I(CH_2)_6Cl}$$

$$CH_3(CH_2)_7C\equiv C(CH_2)_6Cl \xrightarrow{\text{Malonic ester synthesis}} CH_3(CH_2)_7C\equiv C(CH_2)_7COOH$$

$$\xrightarrow[\text{liq. } NH_3]{\text{Lithium}} CH_3(CH_2)_7\overset{t}{CH}=CH(CH_2)_7COOH$$

Dec-1-yne.

1-Bromo-octane (115.8 g, 0.6 mole) in dry ether (100 ml) was added to a suspension of sodium acetylide made from sodium (15.18 g, 0.66 mole) in liquid ammonia (1200 ml) and the mixture was refluxed using a cardice condenser for 4 hrs. The ammonia was allowed to evaporate and the product was recovered in the usual manner. Distillation of the product, through a short packed column under water-pump vacuum gave pure dec-1-yne (49.8 g, 60%, b.p. 172-173°, lit.[138] 174°).

1-Chlorohexadec-7-yne.

Dec-1-yne (43 g, 0.312 mole) was reacted with sodamide (7.2 g sodium, 0.312 mole) in liquid ammonia (1200 ml) for 1½ hrs. and a solution of chloroiodohexane (61.6 g, 0.25 mole) in ether (100 ml) was added. The mixture was refluxed using a cardice condenser for 3 hrs., ammonia was evaporated overnight and the product was recovered. Pure 1-chlorohexadec-7-yne (43.2 g, 67.5%) was obtained by distillation under water-pump vacuum (b.p. 205-206°/32 mm).

Octadec-9-ynoic Acid by Malonic Ester Synthesis.

Sodium (3.47 g, 0.15 mole) was dissolved in superdry ethanol (200 ml), diethyl malonate (26.6 g, 0.166 mole) was added and the mixture was refluxed for an hour. Sodium iodide (25 g,

0.166 mole) was added to the cooled mixture followed by 1-chloro-hexadec-7-yne (38 g, 0.15 mole) and the reaction carried out overnight under reflux. The product was recovered as usual and saponified with ethanolic potassium hydroxide (36 g in 500 ml). The unsaponifiable matter was removed by extraction with ether and the acid was recovered. The malonic acid derivative was then decarboxylated with DMSO (625 ml) and sulphuric acid (0.25N, 625 ml) by refluxing overnight. The monobasic acid was crystallised from petrol ether (35.7 g, 85%; m.p. 46-46.5°, lit.[42] 45.5-46.5°) and the GLC analysis of its methyl ester showed it to be more than 99% pure.

Octadec-trans-9-enoic Acid.

The acid (7 g) was reduced in the metal autoclave with lithium and liquid ammonia but was only 30% reduced. The same acid (6.2 g) was dissolved in dry THF (100 ml) and again reduced with lithium (3.2 g) and distilled liquid ammonia (500 ml) in a glass lined autoclave. The acid crystallised once from petrol ether (m.p. 43.5-44.5° lit.[42] 44.5-45.5°) contained 3% acetylenic acid (GLC, DEGS, 190°). No further purification was attempted. The C_9 dibasic acid (95.3%) was accompanied by the C_8 (2.5%) and C_{10} (2.2%) dibasic acids, when the olefinic acid was submitted to von Rudloff oxidation.

Octadec-*trans*-10-enoic Acid.

$$CH_3(CH_2)_6OH \xrightarrow[H_2SO_4]{HBr} CH_3(CH_2)_6Br \quad \ldots\ldots\ldots\ldots\ldots \text{(I)}$$

$$CH_2{=}CH(CH_2)_8COOH \xrightarrow{Br_2} BrCH_2CHBr(CH_2)_8COOH \xrightarrow[liq.\ NH_3]{NaNH_2}$$

$$HC{\equiv}C(CH_2)_8COOH \xrightarrow[liq.\ NH_3]{\text{i) } NaNH_2 \quad \text{ii) (I)}} CH_3(CH_2)_6C{\equiv}C(CH_2)_8COOH \xrightarrow[liq.\ NH_3]{\text{Lithium}}$$

$$CH_3(CH_2)_6\overset{t}{CH}{=}CH(CH_2)_8COOH$$

10,11-Dibromoundecanoic Acid.

Undec-10-enoic acid (275 g, 1.5 mole) was dissolved in dry ether (1200 ml) and placed in a three necked round bottom flask (2 litre) fitted with a stirrer, water condenser and a dropping funnel. Bromine was added drop by drop till the solution gave a distinct orange colour, while cooling the mixture in an ice-water bath. The water bath was then removed, the solution was stirred for a further 2 hrs. and the solvent and excess bromine were evaporated on the rotary evaporator under vacuum. The acid was crystallised once from petrol ether for purification. (472 g, 92%; m.p. 38-39°, lit.[139] 38.5°).

Undec-10-ynoic Acid.

The solution of the dibromo acid (115 g, 0.33 mole) in dry THF (150 ml) was added to the sodamide suspension (30 g sodium) in liquid ammonia (1500 ml) over ½ an hour. The mixture was refluxed for 2 hrs. using a cardice condenser, ammonia was allowed to evaporate overnight and the acid was recovered after acidifying and diluting with water. The product was dissolved in petrol ether to make 10% solution and crystallised at +5°C. The methyl ester of the undec-10-ynoic acid (51 g, 82%; m.p. 41-42°, lit.[140] 42°) was found to be pure on GLC (ApL/150°).

Heptylbromide.

n-Heptanol (58 g, 0.5 mole) was added to a mixture of hydrobromic acid (48%, 100 ml) and conc. sulphuric acid (15 ml) while stirring and the mixture was refluxed for $3\frac{1}{2}$ hrs. after addition of more conc. sulphuric acid (15 ml). The product was recovered in the usual manner and distilled under water-pump vacuum for purification (80 g, 90%; b.p. 81^{o}/30 mm, lit.[141] 94-94.5^{o}/50 mm). The product was shown to be pure by GLC (ApL/125^{o}).

Octadec-10-ynoic Acid.

A solution of undec-10-ynoic acid (61 g, 0.33 mole) in dry THF (250 ml) was added to lithamide suspension (5 g lithium, 0.715 mole) in liquid ammonia (1200 ml) and allowed to react for 2 hrs. n-Heptyl bromide (72 g, 0.348 mole) dissolved in THF (250 ml) was then added and after refluxing the mixture for 3 hrs. under cardice condenser the ammonia was allowed to evaporate overnight. The acid was recovered as usual, methylated by refluxing with methanol (500 ml) and conc. sulphuric acid (12.5 ml) and distilled under oil-pump vacuum to remove all the more volatile impurities. The residue was shown to be pure methyl octadec-10-ynoate (41.85 g, 45%) by GLC (ApL/200^{o}). The hydrolysis of the ester with methanolic potassium hydroxide (17 g in 250 ml) gave the pure acid (m.p. 45.5-46.5^{o}, lit.[42] 45.5-46.5^{o}).

Octadec-trans-10-enoic Acid.

Octadec-10-ynoic acid (7 g) was dissolved in THF (50 ml) and was reduced with lithium (3.2 g) and liquid ammonia (400 ml) in an autoclave (without a glass sleeve) overnight. The conversion of the acetylenic acid was shown to be >98% complete by GLC (DEGS/190^{o}) but there was a second impurity (12.7%) with an ECL of 21.58. The acid was therefore converted to methyl ester by borontrifluoride and methanol and purified by silica gel column chromatography (370 g, adsorbent; eluent: 700 ml petrol ether followed by 200 ml 99:1, 500 ml 98:2 and finally 95:5::petrol ether:ethyl ether). The methyl octadec-trans-10-enoate (6 g) showed only one peak with ECL 18.5 on the GLC trace (DEGS/190^{o}). Some of the methyl ester was saponified to acid and crystallised from petrol ether (52-52.5^{o}C, lit.[42] 52.5-53.5^{o}C). Both the methyl ester and the acid gave on von Rudloff oxidation only C_{10}-dibasic and C_{8}-monobasic acid.

Octadec-trans-11-enoic Acid.

$$HO(CH_2)_9OH \xrightarrow[\text{Pyridine}]{SOCl_2} Cl(CH_2)_9Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_9Cl \ldots . (I)$$

$$CH_3(CH_2)_5OH \xrightarrow[H_2SO_4]{HBr} CH_3(CH_2)_5Br \xrightarrow[\text{liq. } NH_3]{NaC\equiv CH} CH_3(CH_2)_5C\equiv CH$$

$$\xrightarrow[\text{liq.}NH_3]{\text{i) } NaNH_2 \\ \text{ii) (I)}} CH_3(CH_2)_5C\equiv C(CH_2)_9Cl \xrightarrow[DMSO]{NaCN} CH_3(CH_2)_5C\equiv C(CH_2)_9CN$$

$$\xrightarrow[MeOH]{HCl\ (gas)} CH_3(CH_2)_5C\equiv C(CH_2)_9COOMe \xrightarrow[MeOH]{KOH} CH_3(CH_2)_5C\equiv C(CH_2)_9COOH$$

$$\xrightarrow[\text{liq. } NH_3]{Lithium} CH_3(CH_2)_5\overset{t}{CH}=CH(CH_2)_9COOH$$

1,9-Dichlorononane.

Thionyl chloride (250 ml) was added to a mixture of nonane-1,9-diol (90 g, 0.56 mole) and pyridine (6 ml) and stirred for 3 hrs. at room temperature. After refluxing it for a further 1 hr., the dichloride was recovered and purified by column chromatography (Adsorbent: Aluminium oxide; eluent: petrol ether) (110 g, 99%).

1-Chloro-9-iodononane.

Sodium iodide (84.5 g, 0.563 mole) solution in dry acetone (700 ml) was added to the 1,9-dichlorononane (110 g, 0.563 mole) also dissolved in dry acetone (100 ml). 1-Chloro-9-iodononane was recovered, after refluxing the mixture for 3 hrs., in the usual manner and fractionated on a spinning band column. (54 g, 33.3%, b.p. 142-144^{o}/6 mm, lit.[48] 123-126^{o}/4 mm). GLC (DEGS/ 160^{o}) showed the presence of 6.7% dichloride but no di-iodide.

Hexylbromide.

n-Hexanol (102 g, 1 mole) was added to a mixture of hydrobromic acid (48%, 190 ml) and conc. sulphuric acid (60 ml) and the mixture was refluxed for 3 hrs. The mixture was then diluted with water (750 ml) and extracted with ether. The ether extract was washed successively with water, conc. hydrochloric

acid, water, sodium bicarbonate solution and water. The solvent was evaporated after drying the extract over sodium sulphate and the n-hexylbromide obtained was purified by distillation under water-pump vacuum through a short packed column (140 g, 85%, b.p. 51°/18 mm, lit.[136] 153°/751 mm).

Oct-1-yne.

n-Hexyl bromide (112 g, 0.68 mole) dissolved in dry ether (100 ml) was added to sodamide (19.8 g sodium, 0.86 mole) in liquid ammonia (700 ml) and refluxed, using a cardice condenser, for 2 hrs. The recovered product was distilled through a short packed column (58.5 g, 78%, b.p. 125-127° lit.[16] 126.3°). GLC (DEGS/50°) showed the oct-1-yne to be pure.

1-Chloroheptadec-10-yne.

Oct-1-yne (16.5 g, 0.15 mole) was reacted with sodamide (3.6 g sodium, 0.157 mole) in liquid ammonia (1200 ml) for 2 hrs. and 1-chloro-9-iodononane (54 g, 0.187 mole) dissolved in dry ether (50 ml) was added. After refluxing the mixture, using a cardice condenser, for 3 hrs., the ammonia was evaporated and the product recovered in the usual manner. Purification was carried out by distilling the more volatile impurities under oil-pump vacuum, and by passing the residue through a short florisil column to remove coloured impurities. The 1-chloroheptadec-10-yne (17.8 g,

53%) was found to be nearly pure by GLC (DEGS/190°).

1-Cyanoheptadec-10-yne.

1-Chloroheptadec-10-yne (17 g, 0.063 mole) was added to a solution of sodium cyanide (7.2 g, 0.152 mole) in DMSO (250 ml) and the mixture was heated at 120-130° for 3½ hrs. The product was recovered in the usual manner, (14.8 g, 90%).

Octadec-11-ynoic Acid.

The nitrile (14.8 g, 0.0565 mole) was allowed to stand overnight with a solution of hydrochloric acid (gas) in dry methanol (20% in 100 ml) and the methyl ester was recovered. The ester was then saponified with methanolic potassium hydroxide (100 ml of 6% solution), unsaponifiable material was removed and the acid recovered by extraction with ether (15 g, 95%). The octadec-11-ynoic acid was purified (m.p. 46-46.5°, lit.[42] 46.5-47.5°) by crystallisation (x 2) from petrol ether and GLC of its methyl ester showed it to be 98.5% pure.

Octadec-trans-11-enoic Acid.

Octadec-11-ynoic acid (6 g) was dissolved in THF (50 ml) and reduced overnight with lithium (3.2 g) and liquid ammonia (250 ml) in a glass lined autoclave. The recovered acid was purified by

crystallisation from petrol ether and was found to contain 1% octadec-11-ynoic acid according to GLC (m.p. 43-43.5°, lit.[42] 43.5-44.5°). It gave only C_{11} dibasic and C_7 monobasic acids on von Rudloff oxidation.

Octadec-trans-12-enoic Acid.

$$HO(CH_2)_{10}OH \xrightarrow[Pyridine]{SOCl_2} Cl(CH_2)_{10}Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_{10}Cl \quad .....(I)$$

$$CH_3(CH_2)_4OH \xrightarrow[H_2SO_4]{HBr} CH_3(CH_2)_4Br \xrightarrow[liq.\ NH_3]{NaC\equiv CH} CH_3(CH_2)_4C\equiv CH$$

$$\xrightarrow[liq.NH_3]{\text{i) } NaNH_2} CH_3(CH_2)_4C\equiv C(CH_2)_{10}Cl \xrightarrow[NaCN]{DMSO} CH_3(CH_2)_4C\equiv C(CH_2)_{10}CN$$

ii) I

$$\xrightarrow[MeOH]{HCl} CH_3(CH_2)_4C\equiv C(CH_2)_{10}COOMe \xrightarrow[EtOH]{KOH} CH_3(CH_2)_4C\equiv C(CH_2)_{10}COOH$$

$$\xrightarrow[liq.\ NH_3]{Lithium} CH_3(CH_2)_4\overset{t}{CH}=CH(CH_2)_{10}COOH$$

1,10-Dichlorodecane.

Thionyl chloride (476 g, 4 moles) was added to the mixture of 1,10-decanediol (174 g, 1 mole) and pyridine (12 ml) and stirred overnight. The dichloride was recovered after refluxing the mixture for 3 hrs. in the usual manner and purified by distillation under oil-pump vacuum (185 g, 89%; b.p. 93°/0.4 mm, lit.[48] 115-120°/4 mm).

1-Chloro-10-iododecane.

Sodium iodide (75 g, 0.5 mole) dissolved in dry acetone (700 ml) was added to a solution of 1,10-dichlorodecane (105 g, 0.5 mole) in acetone (250 ml) and the mixture was refluxed for 4 hrs. After recovering the product in the usual way, the product was fractionated through a short vigreux column under oil-pump vacuum. Some pure 1,10-dichlorodecane was obtained (51 g) together with 1-chloro-10-iododecane (57.5 g, 74%, b.p. 111-115°/0.4 mm, lit.[48] 140°/3 mm) which contained 1.2% dichloride and 4% di-iodide (DEGS/190°).

Pentylbromide.

Pentan-1-ol (44 g, 0.5 mole) was added to a mixture of hydrobromic acid (48%, 100 ml) and conc. sulphuric acid (30 ml) and the mixture was refluxed for 3 hrs. The usual recovery procedure

gave pentyl bromide which was then distilled for purification (64.5 g, 85%; b.p. 128-129°, lit.[136] 128.5°/762 mm).

Hept-1-yne.

Pentyl bromide (50 g, 0.33 mole) solution in dry ether (50 ml) was added dropwise to a sodium acetylide suspension (11 g sodium, 0.47 mole) in liquid ammonia (750 ml) and the mixture was refluxed, using a cardice condenser, for 3 hrs. After allowing the ammonia to evaporate overnight the product was recovered and purified by distillation (27 g, 84.5%, b.p. 99-100°, lit.[16] 99.8°).

1-Chloroheptadec-11-yne.

Hept-1-yne (19.5 g, 0.195 mole) was reacted with a lithamide suspension (1.4 g lithium, 0.195 mole) in liquid ammonia (750 ml) for 2 hrs. and 1-chloro-10-iododecane (49.5 g, 0.163 mole) solution in dry ether (100 ml) was added. The product was recovered after refluxing the mixture, using a cardice condenser, for 4 hrs. and evaporating the ammonia overnight. The crude product was purified by fractional distillation with a spinning band column under oil-pump vacuum (23 g, 51%, b.p. 173°/4-5 mm, lit.[48] 159-162°/3.5 mm).

1-Cyanoheptadec-11-yne.

Sodium cyanide (4.8 g, 0.096 mole) was dissolved in DMSO

(250 ml) and 1-chloroheptadec-11-yne (19.7 g, 0.08 mole) was added dropwise. The nitrile was recovered, after heating the mixture at 120-130°C for 3½ hrs., in the usual manner.

Octadec-12-ynoic Acid.

The crude 1-cyanoheptadec-11-yne was allowed to stand with a 25% solution of hydrochloric acid (gas) in methanol (100 ml) overnight. The methyl ester was removed after diluting the mixture with water and extracting with ether and was saponified with a methanolic potassium hydroxide (5% solution, 200 ml). After removing the unsaponifiable matter (2.5 g) octadec-12-enoic acid was recovered and purified by crystallisation (x 2) from petrol ether (11.5 g, 65%; m.p. 46-47°, lit.[42] 46-47°). The GLC of the methyl ester showed it to be 98.5% pure.

Octadec-trans-12-enoic Acid.

Octadec-12-ynoic acid (6 g) dissolved in THF (100 ml) was reduced with lithium (3 g) and liquid ammonia (400 ml) in a glass lined autoclave, overnight. The product was crystallised once from petrol ether (m.p. 51-52°, lit.[42] 52-53°) and its methyl ester showed the presence of only 1.5% of the acetylenic isomer on GLC (DEGS/190°). von Rudloff oxidation of the acid gave only C_{12}-dibasic and C_6-monobasic acids.

Octadec-trans-13-enoic Acid.

$$HO(CH_2)_{10}OH \xrightarrow[Pyridine]{SOCl_2} Cl(CH_2)_{10}Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_{10}Cl \quad .....(I)$$

$$CH_3(CH_2)_3Br \xrightarrow[liq.NH_3]{NaC\equiv CH} CH_3(CH_2)_3C\equiv CH \xrightarrow[liq.NH_3]{\substack{i)\ NaNH_2 \\ ii)\ (I)}} CH_3(CH_2)_3C\equiv C(CH_2)_{10}Cl$$

$$\xrightarrow[synthesis]{Malonic\ ester} CH_3(CH_2)_3C\equiv C(CH_2)_{11}COOH \xrightarrow[liq.\ NH_3]{Lithium}$$

$$CH_3(CH_2)_3\overset{t}{C}H=CH(CH_2)_{11}COOH$$

Hex-1-yne.

Butyl bromide (109 g, 0.8 mole; obtained from B.D.H. Laboratories), dissolved in dry ether (50 ml), was added to a sodium acetylide suspension (23.5 g sodium, 1 mole) in liquid ammonia (700 ml) and the mixture was stirred under a cardice condenser for 2 hrs. followed by a further two hrs. under water condenser. 2N-ammonium hydroxide (200 ml) was then added dropwise to the mixture, followed by water (200 ml) and the aqueous mixture was extracted with ether (4 x 100 ml). The ether extract was washed successively with 2N-hydrochloric acid, water, sodium bicarbonate solution and water and then dried over sodium sulphate. The ether solution of hexyne was then carefully distilled through a short vigreux column to remove ether. The product was purified by distillation through a 3" packed column (35 g, 53.5%; b.p. 70-71°, lit.[16] 71.4°). The hex-1-yne was found to be pure by GLC (DEGS/50°).

1,10-Dichlorodecane.

1,10-Dichlorodecane was prepared from 1,10-decanediol (1 mole) and thionyl chloride (4 moles) as given before (see Octadec-trans-12-enoic acid).

1-Chloro-10-iododecane.

1,10-Dichlorodecane (105 g, 0.5 mole) was reacted with sodium iodide (75 g, 0.5 mole) in acetone (700 ml) to obtain 1-chloro-10-iododecane as given before (see octadec-trans-12-enoic acid).

1-Chlorohexadec-11-yne.

A dry ether (25 ml) solution of hex-1-yne (20 g, 0.236 mole) was added to a stirred suspension of sodamide (6 g sodium, 0.276 mole) in liquid ammonia (500 ml) and allowed to react for 1 hr. 1-Chloro-10-iododecane (59.5 g, 0.197 mole) in ether (50 ml) was added and the mixture was refluxed using a cardice condenser for 3 hrs. The cardice condenser was then replaced by a water condenser and the ammonia was allowed to evaporate overnight. After recovery by the usual procedure, the 1-chlorohexadec-11-yne was distilled under oil-pump vacuum through a short vigreux column. (38 g, 76%; b.p. 127-128^{o}/0.6 mm). The GLC analysis showed it to be 98% pure.

Octadec-13-ynoic Acid.

Sodium (2.52 g, 0.11 mole) was dissolved in superdry ethanol (150 ml) and diethyl malonate (19.75 g, 0.121 mole) was added to it. After refluxing the mixture for 1 hr., sodium iodide (18.5 g, 0.121 mole) and the 1-chlorohexadec-11-yne (28.2 g, 0.11 mole) were added and the mixture was refluxed for a further 12 hours.

The malonic ester derivative was recovered and saponified by ethanolic potassium hydroxide (27 g in 275 ml) by refluxing for 2 hrs. After removing the unsaponifiable material (9.8 g) the acidic material was recovered (19.9 g) and decarboxylated by refluxing overnight with DMSO (250 ml) and sulphuric acid (0.25N, 250 ml). The recovered octadec-13-ynoic acid was purified by crystallisation from petrol ether (17.5 g, 76%; m.p. 48.5-49^{o}). Its methyl ester gave a single peak on both polar and non-polar GLC columns (DEGS/190^{o}, ApL/210^{o}).

Octadec-*trans*-13-enoic Acid.

Octadec-13-ynoic acid (6.0 g) was dissolved in THF (100 ml) and reduced with lithium (3 g) and liquid ammonia (400 ml) in a glass-lined autoclave. The acid was recovered in the usual manner and crystallised once from petrol ether (m.p. 43.5-44.5^{o}, lit.[42] 43.5-44.5^{o}). GLC of its methyl ester showed the presence of only 1.5% acetylenic isomer.

von Rudloff oxidation gave only the C_{13}-dibasic acid.

Octadec-*trans*-14-enoic Acid.

$$HO(CH_2)_{12}OH \xrightarrow[Pyridine]{SOCl_2} Cl(CH_2)_{12}Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_{12}Cl \quad .....(I)$$

$$CH_3(CH_2)_2Br \xrightarrow[NaNH_2]{NaC\equiv CH} CH_3(CH_2)_2C\equiv CH \xrightarrow[liq.\ NH_3]{\substack{i)\ NaNH_2 \\ ii)\ (I)}}$$

$$CH_3(CH_2)_2C\equiv C(CH_2)_{12}Cl \xrightarrow[DMSO]{NaCN} CH_3(CH_2)_2C\equiv C(CH_2)_{12}CN \xrightarrow[MeOH]{HCl(gas)}$$

$$CH_3(CH_2)_2C\equiv C(CH_2)_{12}COOCH_3 \xrightarrow[MeOH]{KOH} CH_3(CH_2)_2C\equiv C(CH_2)_{12}COOH$$

$$\xrightarrow[liq.\ NH_3]{Lithium} CH_3(CH_2)_2\overset{t}{CH}{=}CH(CH_2)_{12}COOH$$

Pent-1-yne.

Lithamide was prepared by the titration method from lithium (7 g, 1 mole) in liquid ammonia (750 ml) and propyl bromide (123 g, 1 mole) was added to it over $\frac{1}{2}$ hr., while stirring and refluxing under cardice condenser. The mixture was refluxed for a further 3 hrs. and petrol ether (b.p. 80-100°, 50 ml), followed by ice-cold 2N-ammonium hydroxide (300 ml), was added. The escaping ammonia was passed through two Drechsel bottles containing petrol ether (b.p. 80-100°) to trap the pent-1-yne. The aqueous mixture was then extracted by petrol ether (b.p. 80-100°, 2 x 50 ml), all the petrol ether extracts were mixed, washed with 2N-hydrochloric acid followed by saturated sodium chloride solution and dried over sodium sulphate. Pure pent-1-yne was obtained by distillation through a 9" packed column (45 g, 66.5%; b.p. 40°C, lit.[142] 39-40°).

1,12-Dichlorodecane.

Thionyl chloride (250 ml) was added dropwise to a stirring mixture of dodecane-1,12,-diol (102 g, 0.5 mole) and pyridine (10 ml) and the mixture was stirred at room temperature for 6 hrs. followed by reflux for 3 hrs. The dichloride was recovered by the usual procedure and purified either by distillation under oil-pump vacuum or sometimes by passing through a short silica gel column (eluent: Petrol ether) (106 g, 89%; b.p. 108-112°/0.3 mm, lit.[143] 170-172°/10 mm). GLC (ApL/210°) showed the product to be pure.

1-Chloro-12-iodo-dodecane.

Sodium iodide (67 g, 0.446 mole) dissolved in dry acetone (650 ml) was added to the solution of 1,12-dichlorododecane (106 g, 0.447 mole) in dry acetone (250 ml) and the mixture was refluxed for 3 hrs. The product was recovered in the usual manner and distilled under oil-pump vacuum. The 1-chloro-12-iodo-dodecane obtained (41.75 g, 67% allowing for recovered dichloride; b.p. 105-110°/0.03 mm) was found to contain 6.3% di-iodo- and 3% di-chlorododecane (ApL/210°). [Later this purification step was found unnecessary and the product was used without further purification.]

1-Chloroheptadec-13-yne.

Pent-1-yne (10.2 g, 0.15 mole)was reacted with a lithamide suspension (1.1 g lithium, 0.15 mole) in liquid ammonia (750 ml) for 1½ hrs. and 1-chloro-12-iodo-dodecane (41.25 g, 0.125 mole) solution in dry THF (100 ml) was added to it. Ammonia was allowed to evaporate overnight after refluxing the mixture for 4 hrs. using a cardice condenser and the product was recovered by the usual procedure. The crude product (43 g) which was found to contain ca. 80% 1-chloroheptadec-13-yne by GLC (DEGS/190°) was not purified further.

1-Cyanoheptadec-13-yne.

The crude 1-chloroheptadec-13-yne (27 g, 0.1 mole) was added dropwise to a stirred solution of sodium cyanide (5.88 g, 0.12 mole) in DMSO (125 ml) and the mixture was heated at 120° for 3 hrs. The product was recovered by extraction with ether in the usual manner.

Octadec-14-ynoic Acid.

The nitrile, obtained above, was dissolved in methanolic hydrochloric acid (25 g in 120 ml) and put aside overnight. The usual recovery procedure gave the methyl octadec-14-ynoate (16 g). The ester was hydrolysed by methanolic potassium hydroxide (10 g in 200 ml) by refluxing for 1 hr. and the acid was recovered after removing the unsaponifiable matter. The acid was dissolved in hot petrol ether (100 ml) and the solution allowed to cool down to room temperature, a precipitate was obtained which was removed by filtration (the precipitate on recrystallisation from alcohol melted at 124-125°; m.p. of C_{14}-dibasic acid, 125.8° [146]) and the monobasic acid was crystallised at +5°C. The methyl ester of the acid was found to be impure by GLC (DEGS/190°), and was purified by column chromatography (adsorbent: silica gel; eluent: 95:5::petrolether:ethyl ether) (9.4 g, 32% overall). The ester was hydrolysed back to acid (m.p. 63.5-64°) by saponification with methanolic potassium hydroxide (5 g

in 100 ml) by refluxing for 1 hr.

Octadec-trans-14-enoic Acid.

Octadec-14-ynoic acid (4.5 g) was dissolved in dry THF (75 ml) and reduced overnight with lithium (2.5 g) and liquid ammonia (300 ml) in a glass-lined autoclave. The recovered acid was purified by crystallisation from petrol ether (m.p. 53-53.5°, lit[42] 53-53.5°) and was found to contain ca. 1.5% acetylenic isomer by GLC (DEGS/190°). von Rudloff oxidation gave only C_{14}-dibasic acid.

Octadec-trans-15-enoic Acid.

$$HO(CH_2)_{12}OH \xrightarrow[Pyridine]{SOCl_2} Cl(CH_2)_{12}Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_{12}Cl \text{.....(I)}$$

$$CH_3CH_2Br \xrightarrow[liq.NH_3]{NaC\equiv CH} [CH_3CH_2C\equiv CH] \xrightarrow[liq.NH_3]{\substack{i)\ NaNH_2 \\ ii)\ (I)}} CH_3CH_2C\equiv C(CH_2)_{12}Cl$$

$$\xrightarrow[synthesis]{Malonic\ ester} CH_3CH_2C\equiv C(CH_2)_{13}COOH \xrightarrow[liq.\ NH_3]{Lithium}$$

$$CH_3CH_2\overset{t}{C}H=CH(CH_2)_{13}COOH$$

1-Chlorohexadec-13-yne.

Lithium acetylide was prepared by titration method from lithium (3.5 g, 0.5 mole) and liquid ammonia (400 ml) and ethyl bromide (82 g, 0.75 mole) solution in dry ether (50 ml) was added to it. The mixture was allowed to react under acetone-cardice condenser for three hours and a lithamide suspension prepared separately from lithium (3.85 g, 0.55 mole) and liquid ammonia (200 ml) was then added to it. After refluxing this mixture for 1½ hrs., 1-chloro-12-iodo-dodecane [for preparation see octadec-trans-14-enoic acid. The product contained 66% chloro-iodide, 19% dichloride, and 15% di-iodide.] solution in dry ether (33 g in 100 ml) was added over ½ hr. and the mixture was refluxed for a further 8 hrs. using an acetone-cardice condenser. The ammonia was then allowed to evaporate, the product was recovered following the usual procedure and purified by column chromatography (adsorbent: silica gel; eluent: petrol ether; 6.8 g, 40%).

Octadec-15-ynoic Acid.

Sodium (0.67 g, 0.029 mole) was dissolved in super-dry ethanol (100 ml) and diethyl malonate (4.65 g, 0.029 mole) was added to it. The mixture was refluxed for 1 hr., cooled down and sodium iodide (4.35 g, 0.029 mole) and 1-chlorohexadec-13-yne (6.7 g, 0.026 mole) added. The product was recovered after refluxing the mixture

overnight and saponified with ethanolic potassium hydroxide (5 g in 100 ml). The malonic acid derivative obtained (6.58 g) after removal of unsaponifiable matter, was decarboxylated with DMSO (100 ml) and 0.25N-sulphuric acid (100 ml). The recovered octadec-15-ynoic acid, crystallised from petrol ether (5.3 g, 73%), was found to be pure by GLC (DEGS/190^{o}; m.p. 65-65.5^{o}).

Octadec-*trans*-15-enoic Acid.

Octadec-15-ynoic acid (2 g) solution in dry THF (50 ml) was reduced by lithium (2 g) and liquid ammonia (200 ml) in a glass-lined autoclave overnight. The octadec-*trans*-15-enoic acid was recovered and crystallised from petrol ether (1.8 g, 90%; m.p. 58-58.5^{o}, lit.[42] 58-59^{o}). Its methyl ester showed the presence of ca. 2% acetylenic isomer on GLC (DEGS/190^{o}). von Rudloff oxidation gave only C_{15}-dibasic acid.

Octadec-trans-16-enoic Acid.

$$HO(CH_2)_{12}OH \xrightarrow[Pyridine]{SOCl_2} Cl(CH_2)_{12}Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_{12}Cl \xrightarrow[liq.NH_3/DMF]{NaC\equiv CH}$$

$$HC\equiv C(CH_2)_{12}Cl \xrightarrow[liq.NH_3]{i)\ NaNH_2} CH_3C\equiv C(CH_2)_{12}Cl \xrightarrow[synthesis]{Malonic\ ester}$$

ii) CH_3Br

$$CH_3C\equiv C(CH_2)_{13}COOH \xrightarrow{LiAlH_4} CH_3C\equiv C(CH_2)_{14}OH \xrightarrow[Pyridine]{SOCl_2}$$

$$CH_3C\equiv C(CH_2)_{14}Cl \xrightarrow[DMSO]{NaCN} CH_3C\equiv C(CH_2)_{14}CN \xrightarrow[MeOH]{HCl(gas)}$$

$$CH_3C\equiv C(CH_2)_{14}COOCH_3 \xrightarrow[EtOH]{KOH} CH_3C\equiv C(CH_2)_{14}COOH$$

$$\xrightarrow[liq.\ NH_3]{Lithium} {}^{t}CH_3CH{=}CH(CH_2)_{14}COOH$$

1-Chlorotetradec-13-yne.

Sodium acetylide was prepared in the usual manner from sodium (6.7 g, 0.29 mole) and liquid ammonia (250 ml), DMF (250 ml) was added to it and the ammonia evaporated. 1-Chloro-12-iodo-dodecane [for preparation see octadec-trans-14-enoic acid. The product used, contained 66% chloro-iodide, 26% di-iodide, and 8% dichloride] (84.5 g, equivalent to 0.216 mole) dissolved in DMF (100 ml) was then added to it and the mixture was stirred for 3 hrs. at room temperature. The mixture was diluted with water (500 ml), extracted with ether (3 x 100 ml) and the ether extract was washed successively with water, hydrochloric acid (2N), water, dilute sodium bicarbonate solution and again with water. The ether extract was dried over sodium sulphate and the product recovered by evaporating the solvent. The product was only partially soluble in petrol ether so it was purified by column chromatography (adsorbent: silica gel; eluent: petrol ether) (12 g, 24.5%).

1-Chloropentadec-13-yne.

1-Chlorotetradec-13-yne (9.9 g, 0.035 mole) solution in dry ether (200 ml) was added to a sodamide suspension (0.9 g sodium, 0.039 mole) in liquid ammonia (250 ml) and stirred for 3 hrs. Methyl bromide (33.6 g, 0.35 mole) was then condensed into the reaction flask and the mixture was refluxed for 7 hrs. using an acetone-cardice condenser. After evaporating the ammonia overnight,

the product was recovered in the usual manner (9.96 g) and was found to contain 55.2% of 1-chloropentadec-13-yne according to GLC. It was used without further purification.

Heptadec-15-ynoic Acid.

Sodium (1.61 g, 0.07 mole) was dissolved in super-dry ethanol (80 ml), diethyl malonate (10.72 g, 0.067 mole) was added and the mixture was refluxed for an hr. After cooling the mixture, sodium iodie (10.02 g, 0.067 mole) followed by crude 1-chloropentadec-13-yne (9.96 g, equivalent to 0.061 mole chloride) was added and the mixture was refluxed overnight. The malonic ester derivative was recovered in the usual manner and saponified with ethanolic potassium hydroxide (16 g in 175 ml). The dibasic acid (10.1 g) was recovered after removing the unsaponifiable matter and decarboxylated with DMSO (100 ml) and sulphuric acid (0.3N, 100 ml). The heptadec-15-ynoic acid obtained (8.64 g) was used without any further purification.

Heptadec-15-yn-1-ol[144].

Lithium aluminium hydride (3 g, 0.079 mole) was stirred for 10 mins. with dry ether (150 ml) in a three necked round bottom flask (500 ml) fitted with a stirrer, dropping funnel and water condenser fitted with a drying tube at the top. The heptadec-15-ynoic acid (8.5 g) solution in dry ether (125 ml) was then added

dropwise while stirring and the mixture was stirred for a further 20 mins. at room temperature. Water was added carefully to destroy excess lithium aluminium hydride, followed by sulphuric acid (4N) till the mixture was clear. The ether layer was separated, washed with sodium chloride, dried over sodium sulphate and the solvent evaporated (8.03 g).

1-Chloroheptadec-15-yne.

Thionyl chloride (16.7 g, 0.14 mole) was added to the mixture of heptadec-15-yn-1-ol (8 g) and pyridine (0.5 ml) and after stirring for 3 hrs. at room temperature, the mixture was refluxed for for a further 3 hours. After the usual recovery procedure the product was passed through a short silica-gel column (eluent: petrol ether) to remove any unreacted alcohol (8.7 g).

1-Cyanoheptadec-15-yne.

1-Chloroheptadec-15-yne (8.7 g) was heated at 120-130^{o} for 3½ hrs. with a solution of sodium cyanide (3.26 g, 0.0666 mole) in DMSO (200 ml). The mixture was diluted with a sodium chloride solution and extracted with ether repeatedly. A dark viscous liquid (6 g) was recovered.

Octadec-16-ynoic Acid.

The dark liquid (6 g) was allowed to stand overnight with

methanolic hydrochloric acid (gas) (100 ml of 25% solution). The usual recovery procedure gave a dark product (6 g) which was soluble in ethyl ether but only partially soluble in petrol ether. The petrol ether insoluble part was filtered off and the soluble portion was purified by silver nitrate - column chromatography, (adsorbent: 200 g florisil coated with 20% silver nitrate; eluent: petrol ether —> 90:10::Petrol ether:ethyl ether). The methyl octadec-16-ynoate (960 mg) obtained was still impure according to GLC (DEGS/190^{O}), so the ester was purified with preparative silver nitrate thin layer chromatography. The purified product (800 mg) was found to be methyl octadec-16-ynoate (85%) together with two impurities with ECL 21.0 (10%) and 20.0 (5%) (DEGS/190^{O}). The ester (700 mg) was saponified with 1% methanolic potassium hydroxide (30 ml), unsaponifiable material was removed and the acid was recovered. The methyl ester of the recovered acid still showed both the impurities when injected on GLC.

Octadec-trans-16-enoic Acid.

Octadec-16-ynoic acid (500 g) dissolved in dry THF (50 ml) was reduced overnight with lithium (1 g) and liquid ammonia (250 ml) in a glass-lined autoclave. The recovered acid was recrystallised from petrol ether (400 mg), its methyl ester did not show any acetylenic isomer on GLC but two impurities with ECL 18.0 (10%) and 17.0 (3%) (DEGS/190^{O}).

Methyl Octadec-17 enoate

$$HO(CH_2)_{12}OH \xrightarrow[Pyridine]{SOCl_2} Cl(CH_2)_{12}Cl \xrightarrow[COMe_2]{NaI} I(CH_2)_{12}Cl$$

$$\xrightarrow[liq.\ NH_3]{NaC\equiv CH} HC\equiv C(CH_2)_{12}Cl \xrightarrow[synthesis]{Malonic\ ester} HC\equiv C(CH_2)_{13}COOH \xrightarrow{LiAlH_4}$$

$$HC\equiv C(CH_2)_{14}OH \xrightarrow[Pyridine]{SOCl_2} HC\equiv C(CH_2)_{14}Cl \xrightarrow[synthesis]{Malonic\ ester}$$

$$HC\equiv C(CH_2)_{15}COOH \xrightarrow[MeOH]{BF_3} HC\equiv C(CH_2)_{15}COOMe \xrightarrow[H_2]{Lindlar's\ catalyst}$$

$$H_2C{=}CH(CH_2)_{15}COOMe$$

1-Chloro-tetradec-13-yne.

The 1-chloro-12-iodo-dodecane [for preparation see octadec-trans-14-enoic acid. The product had the composition of 60.5% chloro-iodide, 22.4% dichloride and 17% di-iodide] (54.5 g) dissolved in dry ether (100 ml) was added to the sodium acetylide suspension (2.53 g sodium, 0.11 mole) in liquid ammonia (500 ml) and after refluxing the mixture for 3 hrs. under cardice condenser, ammonia was allowed to evaporate overnight. The product, recovered in the usual manner, was passed through a short silica-gel column (eluent: petrol ether) but not purified otherwise (42 g).

Hexadec-15-ynoic Acid.

Sodium (4.6 g, 0.2 mole) cut in small pieces was dissolved in super-dry ethanol (150 ml), diethyl malonate (35.2 g, 0.22 mole) was added and the mixture was refluxed for one hour. After cooling the mixture, sodium iodide (33 g, 022 mole) and 1-chlorotetradec-13-yne (22 g) were added and the mixture refluxed again overnight. After recovering the product with the usual procedure, it was saponified by ethanolic potassium hydroxide (50 g in 500 ml) and the acidic material was recovered after removing the unsaponifiable matter. The acidic material was then refluxed for 1 hr. with petrol ether, cooled down to room temperature and the insoluble matter filtered off. After evaporating the petrol ether, the procedure was repeated and then the petrol ether soluble material (9.53 g) was

esterified with boron trifluoride in methanol (3.5% solution, ½ hr. reflux). The GLC (ApL/210°) of the ester showed only two peaks with ECL 16.0 (hexadec-15-ynoic acid) and 20.6 (24.6%).

Hexadec-15-yn-1-ol[145].

The methyl hexadec-15-ynoate (9.5 g) solution in dry ether (50 ml) was added to a stirring suspension of lithium aluminium hydride (3 g) in dry ether (150 ml) and the mixture was stirred for a further 15 mins. at room temperature. Water was added carefully to destroy excess lithium aluminium hydride, followed by sulphuric acid (4N) and the product was recovered by extraction with ether. It was put through a short silica gel column (eluent, petrol ether:ether::75:25) and was found to be pure (8 g) by TLC, (developing solvent: 25% ether in petrol ether).

1-Chlorohexadec-15-yne.

Thionyl chloride (50 ml) was added to the mixture of hexadec-15-yn-1-ol (8 g, 0.0336 mole) and the mixture was refluxed for 2½ hrs., after stirring at room temperature for 1 hr. The usual recovery procedure gave the crude product which was purified by passing through a short alumina-column (eluent: petrol ether) (6 g, 70%).

Octadec-17-ynoic Acid.

Sodium (0.644 g, 0.028 mole) was dissolved in super-dry ethanol (100 ml) and diethyl malonate (4.48 g, 0.028 mole) was added. The mixture was refluxed for one hour, cooled down and sodium iodide (4.2 g, 0.028 mole), followed by 1-chlorohexadec-15-yne (3.25g, 0.013 mole), was added. The mixture was refluxed overnight and the product recovered. The malonic ester derivative was then saponified with ethanolic potassium hydroxide (6.2 g in 100 ml), unsaponifiable matter was removed and the acidic material recovered as usual. This was decarboxylated by refluxing it with DMSO (50 ml) and sulphuric acid (0.3N, 50 ml) overnight. The octadec-17-ynoic acid obtained was crystallised once from petrol ether (3.1 g, 87.5%; m.p. 66-67^{o}). Its methyl ester, when analysed with GLC (DEGS/190^{o}) was found to be pure.

Methyl Octadec-17-enoate.

Octadec-17-ynoic acid was methylated with boron trifluoride and methanol and the solution of methyl ester (500 mg) in ethyl acetate (15 ml) together with quinoline (10 mg) was shaken vigorously with Lindlar's catalyst (30 mg) and hydrogen for five minutes. The catalyst was filtered off, the solution was washed with dilute hydrochloric acid followed by saturated salt solution and dried over sodium sulphate. The GLC analysis of the ester, after evaporating the ethyl acetate, showed the presence of 2.5% of methyl stearate but no Δ^{16} ester.

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APPENDIX

SYNTHESIS OF OCTADECADIENOIC ACIDS

Introduction

As mentioned in the introduction to this thesis, with the advent of very sensitive and accurate chromatographic methods and improved methods for isolation and structure determination, many acids of novel structure have been and are being discovered. These discoveries have modified the concept that *cis* unsaturation is 'the rule' in vegetable fats while *trans* unsaturation is 'an exception'. Also it is now well accepted that isolated *trans* double bonds can be found in non-conjugated polyenoic acids and are not necessarily confined to conjugated polyenoic acids. It is also known that natural *cis* polyenoic acids may change to acids containing *trans* unsaturation during the processing of fats.

With the availability of the new methods and the increasing evidence provided by chemists that polyenoic acids containing *trans* unsaturation are present in the processed food products, medical and biological researchers are increasingly interested in these acids and require pure synthetic acids for studying their metabolism in human and animal body.

Also, it is of academic interest to study molecules which contain two different types of unsaturation. The acetylenic, *trans* and *cis* bond each orient the molecule in a different way

and each gives distinctly different chemical and physical properties to the molecule. The interaction of two (or more) of these groups present in the same molecule and their total effect on the chemical and physical properties should therefore be investigated.

Comparatively little work has been done on this type of C_{18} or other long chain acids. It is not intended to review all the previous work, in this appendix. Instead, we refer only to C_{18} acids of this type. de Gaudemarais and Arnaud[1] prepared, 18:9a, 12t; 18:9c,12t; 18:9t,12a; 18:9t,12c; 18:9a,12c and 18:9c,12a acids. More recently, Gunstone and Jacobsberg[2] have achieved a partial synthesis of all these acids by different methods, starting from naturally occurring C_{18}-acids such as Vernolic or Crepenynic acid.

In the beginning of our research work we tried to prepare the 18:2 acids, of the general formula

$$CH_3(CH_2)_x\ (U)\ (CH_2)_y\ (W)\ (CH_2)_zCOOH$$

where U or W is either cis or trans or acetylenic group. Our attempt was unsuccessful for the reasons given later but we feel that these attempts should be reported.

Discussion

(i) Previous work.

The general method used by de Gaudemarais and Arnaud[1] for the preparation of cis-trans unsaturated acids was as follows.

$$Cl(CH_2)_6I \xrightarrow{1} Cl(CH_2)_6C\equiv CH \xrightarrow{2} HC\equiv C(CH_2)_7COOH \xrightarrow{3}$$

$$BrMgC\equiv C(CH_2)_7COOMgBr \xrightarrow{4} CH_3(CH_2)_7\overset{t}{CH}=CHCH_2C\equiv C(CH_2)_7COOH \xrightarrow{5}$$

$$CH_3(CH_2)_7\overset{t}{CH}=CHCH_2\overset{c}{CH}=CH(CH_2)_7COOH$$

1. $NaC\equiv CH$/Liq. NH_3 2. Malonic ester synthesis 3. EtMgBr
4. CuCl; $CH_3(CH_2)_7\overset{t}{CH}=CHCH_2Br$ 5. Lindlar's Catalyst/H_2

We tried to repeat this 'Grignard condensation', which is attractive for the preparation of methylene interrupted acids such as crepenynic (18:2 9c12a) acid even though it cannot be used as a general method for preparation of diunsaturated acids with more than one methylene group between the unsaturated groups. In our hands, however, this method did not work and there are reports that other workers have also failed.

(ii) Synthesis.

The more general route we tried is outlined below.

$$I(CH_2)_yI \xrightarrow{1} HC\equiv C(CH_2)_yC\ \ CH \xrightarrow{2} CH_3(CH_2)_xC\equiv C(CH_2)_yC\equiv CH$$

$$\xrightarrow{3} CH_3(CH_2)_x\overset{t}{CH}=CH(CH_2)_yC\equiv CH \xrightarrow{4} CH_3(CH_2)_x\overset{t}{CH}=CH(CH_2)_yC\equiv C(CH_2)_zCl$$

$$\xrightarrow{5} CH_3(CH_2)_xCH=CH(CH_2)_yC\equiv C(CH_2)_zCOOH \xrightarrow{6}$$

$$CH_3(CH_2)_x\overset{t}{CH}=CH(CH_2)_y\overset{c}{CH}=CH(CH_2)_zCOOH$$

1. $NaC\equiv CH$/liq. NH_3 2. $NaNH_2$/liq. NH_3;$CH_3(CH_2)_xBr$ 3. Na/liq. NH_3 4. $NaNH_2$/liq.NH_3;$I(CH_2)_zCl$ 5. Chain extension by 1 carbon atom via nitrile [or by 2 carbon atoms via malonic ester] 6. Lindlar's catalyst/H_2.

This procedure is based on the method of Dobson and Raphael[3], in which a diyne containing an ethynyl group can be reduced by sodium and liquid ammonia to the trans-enyne if the ethynyl group is first protected by formation of the sodium salt. They were able to convert undeca-1,7-diyne to undeca-trans-7-en-1-yne (75%) in one reduction. We were only able to reduce tridec-1,7-diyne and dodec-1,6-diyne by sodium and liquid ammonia to a mixture of diyne (45%) and enyne (55%). When the reduction was repeated 4 times the yield of enyne was increased to ~90% but not any further. The separation of pure enyne by spinning band distillation was fairly easy. Chain extension by the above reaction sequence gave the 18:6a,12t, and 18:7a,12t acids and the reduction of these acids with Lindlar's catalyst and hydrogen gave the 18:6c, 12t and 18:7c,12t acids. However, the von Rudloff oxidation of the acids showed that the trans double bond had migrated about 7% on both sides and hence the acids were unacceptable for our purposes.

(iii) Comments.

(1) The method, we used, is quite satisfactory from the point of

view of yields and does not include any particularly difficult reactions.

(2) The method becomes unacceptable because there is considerable trans double bond migration. This can take place during the repeated reductions or during the following condensation reaction involving sodamide. On submitting the enyne to von Rudloff oxidation we found that the migration had indeed taken place during the reductions (refer also to 8t and 9t acids), though the possibility of a very slight migration during the condensation reaction in presence of sodamide cannot be disproved.

(3) From our later experience in reduction by sodium and liquid ammonia, we feel that this method may still be feasible if the following points are observed

a) The migration of the double bond takes place mainly or wholly when the reduction procedure has to be repeated several times, so reduction must be carried out once only and conditions must be found to improve the effectiveness of this process.

b) The reduction of diyne, if carried out overnight, in a glass-lined autoclave with a good stirrer, should go to near completion (i.e.to near 100% enyne) without double bond migration.

c) No sodamide should be allowed to form during the reaction (by avoiding any contamination with iron or other transition metals) and no sodamide need be added in the beginning to form sodium salt of the ethynyl group as the ethynyl group reacts directly with the

solution of sodium in liquid ammonia to form a salt.

d) Migration may take place in the subsequent reactions in presence of sodamide when there is a methylene interrupted system as such compounds are easily isomerised under these conditions[4] and some other method may then have to be used. This reduction may also fail for conjugated acids because of 1:4 addition of electrons.

Experimental.

Experimental details for the preparation of 18:6a,12t only are given. Attempts were also made to prepare the 18:7a,12t acid by similar procedure.

1,4-Di-iodobutane.

Dichlorobutane (127 g, 1 mole) was reacted with sodium iodide (330 g, 1.1 mole) solution in dry acetone (1500 ml) in the usual way. The di-iodide obtained was distilled through a ½ m. Fenske's column for purification (75%, b.p. 85-90°/0.8 mm, lit.[5] 147-152°/26 mm).

Trideca-1,7-diyne.

Sodium acetylide prepared by the titration method (18.5 g, 0.8 mole sodium) was reacted with 1,4-di-iodobutane (109 g, 0.4 mole) in liquid ammonia (1200 ml). 1,7-Octadiyne was recovered by the usual procedure and further reacted with sodamide in liquid ammonia followed by bromopentane. The recovered product was fractionated

by distillation under water-pump vacuum (~ 30%, b.p. 110-112°/ 16 mm).

Tridec-trans-7-en-1-yne.

Trideca-1,7-diyne (27.35 g) solution in dry THF (100 ml) was added to a sodamide suspension (5.6 g sodium) in liquid ammonia (500 ml) and stirred for 2 hrs. Small pieces of sodium (4 g) were then added and the solution kept blue for 1½ hrs. Excess sodium was destroyed by addition of solid ammonium chloride and the product recovered. According to GLC analysis this contained 57% tridec-trans-7-en-1-yne. This was increased to 82%, 89%, and 92% after successive reductions. The product was distilled through a spinning band distillation column under water-pump vacuum (11.8 g, b.p. 102°/10 mm).

1-Chloroheptadec-trans-11-en-5-yne.

The sodium derivative of the tridec-trans-7-en-1-yne (11.8 g) was condensed with 1,4-chloroiodobutane (18 g) in the usual manner in liquid ammonia. Distillation of the product under oil-pump vacuum gave the C_{17}-chloride (10.3 g, b.p. 157-162°/1 mm).

Methyl Octadec-trans-12-en-6-ynoate.

The C_{17}-chloride (10.3 g) was reacted with sodium cyanide (2.5 g) in DMSO (150 ml) and the nitrile obtained was allowed to stand overnight with methanolic hydrochloric acid (25%, 150 ml). The crude methyl ester recovered (9 g), contained ca. 10% of a more polar impurity which was easily removed by silver nitrate column chromatography.

von Rudloff Oxidation.

The methyl ester of octadec-trans-12-en-6-ynoic acid was reduced by hydrogen in the presence of Lindlar's catalyst to methyl octadeca-cis-6-trans-12-dienoate and oxidised by the von Rudloff oxidation method. The expected C_6-dibasic acid was accompanied by ca. 7% each of the C_7- and C_5-dibasic acids along with traces of the C_8- and C_4-dibasic acids.

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