Some Physical, Chemical, and Biological Properties of Natural and Synthetic Unsaturated C_{18} Acids[1]

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ABSTRACT

Series of octadecenoic (*cis* and *trans*), octadecynoic, octadecadienoic (*cis,cis* and *trans,trans*), and octadecadiynoic acids have been synthesized and employed in a comparative study of some physical (melting point, Raman spectra, NMR spectra, Ag^+ thin layer chromatography, and gas liquid chromatography) and biological properties. The results show that the position of unsaturation has a considerable influence on the physical and biological properties of isomeric unsaturated acids. Several chemical reactions of linoleic acid and of the related oxygenated acids ricinoleic or vernolic (12,13-epoxyoleic) furnish cyclopropane compounds and 1,4- (or 1,5-) epoxides. The formation of these is rationalized in terms of neighboring group participation. The oxymercuration-demercuration reaction is the basis of a new method of examining acids with Δ3, Δ4, or Δ5 unsaturation.

INTRODUCTION

Although the study of natural products has always been a prominent part of organic chemistry, fatty acids have not been highly considered in comparison with the more favored carbohydrates, isoprenoids, and alkaloids, to mention only a few. Historically, organic chemists preferred those compounds which could be readily isolated in a pure state, and this generally implied that they were crystalline solids or volatile liquids. If they were colored, that was an added advantage. The fatty acids (and, to a lesser extent, the amino acids) did not meet these criteria; and, in addition, most fatty compounds were considered to be mixtures of a small range of acids of simple structure.

This is no longer true. New chromatographic and spectroscopic procedures make these mixtures easy to handle and study. Over 300 natural fatty acids are now known, and biochemists continually ask questions that present interesting challenges to the organic chemists working in this field. It is 30 years ago this autumn since I became a research student in Liverpool, working with T.P. Hilditch; fatty acid chemistry is a very different activity from what it was at that time.

Nevertheless, and despite the many meetings organized by the AOCS and all that has been written in the 50 volumes of the Society's journal, there is still a lot that we do not know about the chemistry of fatty acids. There has been a concentration of study on a few acids, such as oleic acid and linoleic acid, and it has been generally assumed that what was true of oleic acid held for other monoenoic acids and that what was true of linoleic acid held for other polyenoic acids. This may be so when we consider the gross chemical behavior of these compounds. It is less likely to hold for those physical and biological properties which are more specifically dependent on the shape of the whole molecule and on the conformation it assumes at the moment of investigation.

During the past decade we have been engaged in studies designed to learn more about fatty acid chemistry. Some of my students have prepared series of closely related acids and have then made—sometimes in collaboration with other research groups—a comparative study of their properties. Other students have examined some old and some new reactions of the more common acids (such as oleic and linoleic acid) and of acids with an additional oxygenated function (such as ricinoleic and vernolic acid). In these studies the underlying theme of neighboring group participation has become apparent.

In this lecture I hope to review some of our recent investigations. Inevitably our work has impinged on the studies of other research groups; and I will refer to some of

[1]F.D. Gunstone received the 1973 Award in Lipid Chemistry at the AOCS 47th Annual Fall Meeting, Chicago, September 1973. This Award Address was presented at the Plenary Session.

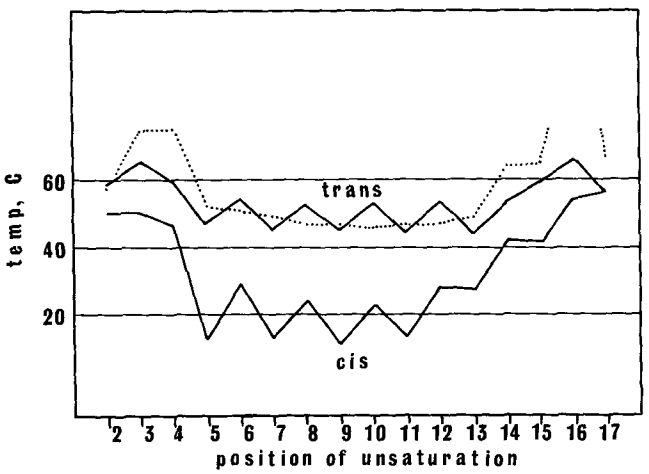


FIG. 1. Melting points of octadecenoic (*cis* and *trans*) (———) and octadecynoic acids (· · · ·).

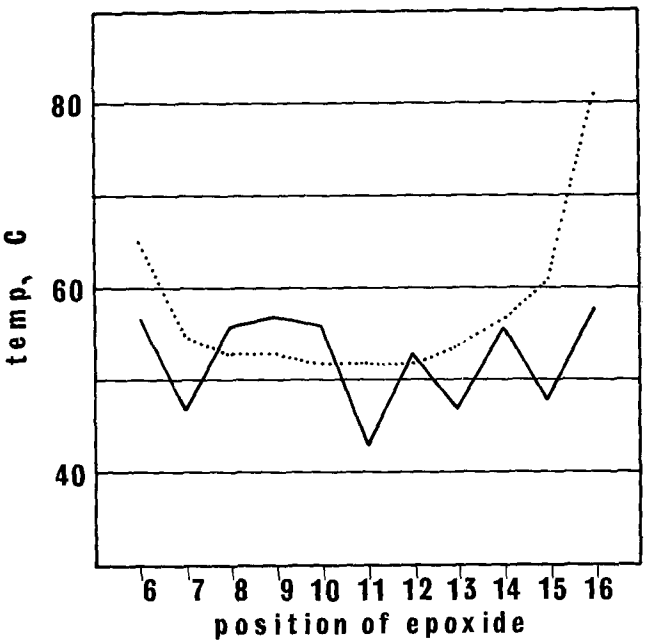


FIG. 2. Melting points of *cis* (———) and *trans* (· · · ·) epoxyoctadecanoic acids. Each isomer is indicated by a single number, e.g. 2,3-epoxyoctadecanoic acid by 2, etc.

these, but it will not be possible to do justice to these related studies. Therefore, I ask forgiveness for this egotistical outlook.

SYNTHETIC STUDIES

Synthesis

In the course of our work we have prepared three groups of unsaturated C_{18} acids (1-4): (a) All the *cis* and *trans* octadecenoic acids and all the octadecynoic acids (47 isomers); (b) all the 9,12-diunsaturated C_{18} acids (9 isomers); (c) a series of C_{18} acids (the *cis cis* and *trans trans* dienes and the diynes) with unsaturation at 5,12; 6,12; 7,12; 8,12; 9,12; 10,12; 6,11; 6,10; 6,9; and 6,8 (30 isomers). The dienes contain the structural unit $-CH{=}CH(CH_2)_nCH{=}CH-$ where n ranges from 0 to 5.

In addition, we have converted most of the alkenoic and alkadienoic acids to the corresponding epoxides (5) and to the cyclopropane derivatives (6-8).

These syntheses have been achieved mainly by taking advantage of the special properties of acetylene: its ability to undergo alkylation and stereospecific semihydrogenation to *cis* or *trans* alkenes. We have also prepared some of these acids by manipulation of more-or-less readily available natural products. For example, we used methyl vernolate (*cis*-12,13-epoxyoleate) to produce the 9*c*12*t*, 9*a*12*t*, and 9*a*12*c* diunsaturated C_{18} acids by suitable combination of three reactions: (a) the stereospecific conversion of *cis* epoxides to *threo* or *erythro* diols, (b) the conversion of an alkene to an alkyne, and (c) the conversion of *threo* and *erythro* diols to *trans* and *cis* alkenes respectively (4).

Melting Point

The melting points of the monounsaturated C_{18} acids are summarized in Figure 1 (3). Among the alkenoic acids the *cis* isomers have the lower melting points, with oleic acid the lowest of all. Both *cis* and *trans* isomers show alternation of melting point when the double bond is in the central region, and this effect is superimposed on a tendency for the melting point to rise as the double bond approaches either end of the molecule. In contrast, the acetylenic acids do not show this alternation though it has been reported to occur among alkynoic acids with an *odd* number of carbon atoms in their molecules (9).

We have also observed some interesting results with the melting points of the *cis* and *trans* epoxystearic acids (5) (Fig. 2). Alternation is apparent among the *cis* members but not among the *trans*, so that sometimes the *cis* epoxide and sometimes its *trans* isomer has the higher melting point. The regular alternation of melting point among the *cis* epoxy acids is broken by one value which appears to be too high; but, since this refers to *cis*-9,10-epoxystearic acid and has been reported from several laboratories, there can be no doubt about the correctness of this observation.

Spectroscopy

IR spectroscopy has been used for many years to detect *trans* unsaturation, but there is no satisfactory IR procedure for the recognition of *cis* unsaturation or acetylenic unsaturation. Using our synthetic esters, Davies and Hodge (10) showed that these compounds display C=C or C≡C stretching bands in their Raman spectra, which can be used easily for diagnostic purposes. Using carbon tetrachloride solutions, these bands are observed at 1656±1 (vs) and 1670±1 (vs) for the *cis* and *trans* alkenoates respectively and at 2232±1 (s) and 2291±2 cm^{-1} (m) for the alkynoates.

Our earlier studies of the NMR spectra of our synthetic esters on 60 and 100 MHz spectrometers (4-8,11,12) have been repeated on a 220 MHz instrument in collaboration with Frost (Unilever, Vlaardingen). Methyl oleate shows distinct signals at 9.12 ($C\underline{H}_3CH_2$-), 8.75 ($C\underline{H}_2)_n$, 8.43 ($-C\underline{H}_2CH_2COOCH_3$), 8.01 ($=CHC\underline{H}_2$-), 7.79 ($-C\underline{H}_2COOCH_3$), and 4.72$\tau$ ($-C\underline{H}{=}C\underline{H}-$). From our studies of the various isomeric compounds, we have determined the long range deshielding effects of several groups, have shown that these are additive, and consider that it is possible to distinguish by NMR spectroscopy alone unsaturation in positions ω1-5 and Δ2-7. This means that for the methyl *cis*-octadecenoates only the Δ8-12 isomers are difficult to distinguish, and Frost claims that even this is possible, mainly on the basis of the shape of the $(CH_2)_n$ signal (13). The identification of polyenoic esters is usually simpler.

Chromatography

It is chromatography—particularly thin layer chromatography (TLC) and gas liquid chromatography (GLC)—which has most changed the behavior pattern of lipid chemists and biochemists in recent years, and we have used our series of synthetic esters to probe these techniques further. While confirming that isomeric *cis* and *trans* alkenoates can be separated from each other, we have also shown that there are minor differences, especially among the *cis*-octadecenoates, depending upon the position of the double bond (Fig. 3). This effect is also apparent in the series of isomeric *cis*-octadecenals and *cis*-octadecenyl acetates but not in the *cis*-octadecenols or *cis*-octadecenes (4,5,14-17).

GLC now is used routinely in lipid research, though sufficient care is not always taken in making structural

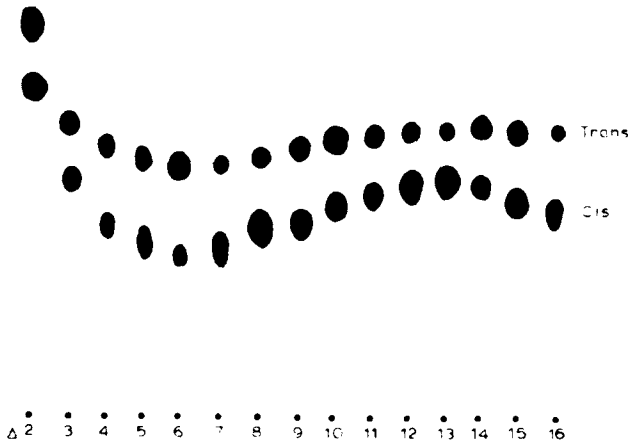


FIG. 3. Thin layer chromatogram of methyl *cis* and *trans* octadecenoates (Δ2-Δ16). With the Δ2 esters the *cis* isomer has the higher Rf value; in all other cases the *trans* isomer has the higher Rf value. Experimental conditions: silica layers (250 mμ) containing silver nitrate (15%), activated by heating at 105 C for 75 min, developed with dibutyl ether and hexane (40:60), made visible by heating with a glassblower's torch.

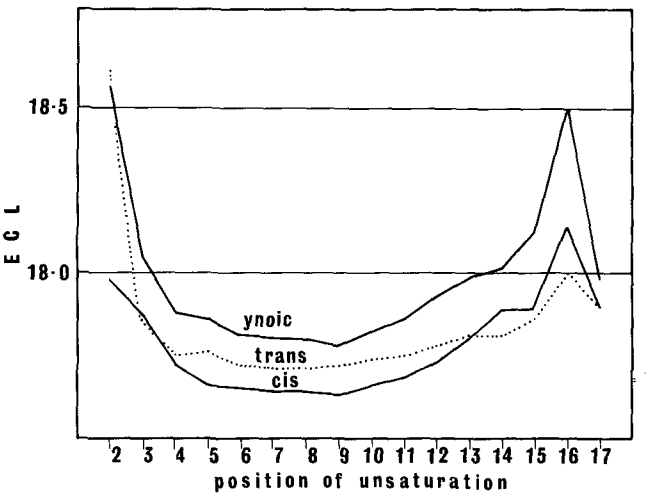


FIG. 4. Retention behavior (ECL) of isomeric methyl octadecenoates (*cis* and *trans*) and methyl octadecynoates on an ApL column.

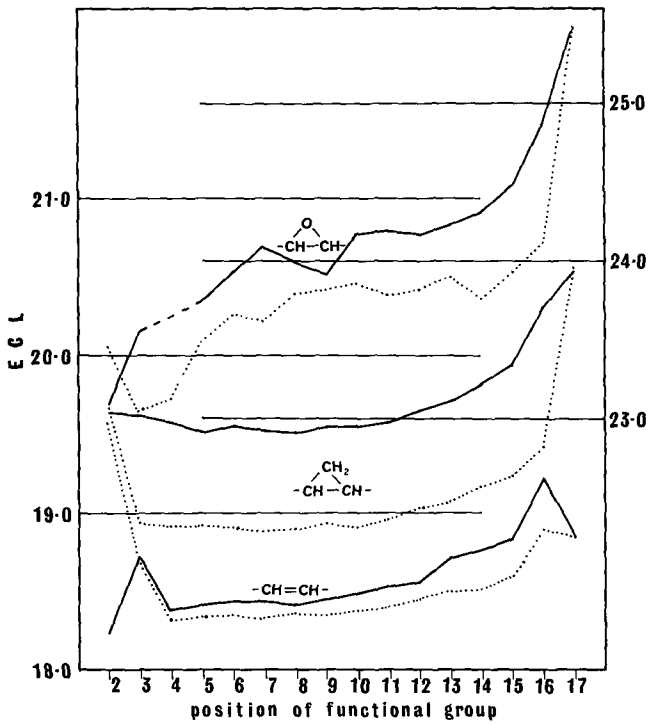


FIG. 5. Retention behavior of isomeric *cis* (———) and *trans* (• • • •) methyl octadecenoates (18.2-19.6), methyl methyleneoctadecanoates (18.9-20.6), and methyl epoxyoctadecanoates (23.0-25.5) on DEGS columns. Each isomer is indicated by a single number, e.g. methyl 2,3-epoxyoctadecanoate by 2, etc.

assignments to GLC peaks. We have investigated the GLC behavior of our synthetic esters on a variety of columns, mainly on ApL and DEGS, and some of our results are presented here (5-7,14-17). On an ApL column the *cis*-olefinic, *trans*-olefinic, and acetylenic esters behave slightly differently from each other, though for most isomers useful separations can be observed only on capillary columns. There are also small differences in retention behavior between esters differing only in the position of unsaturation. This is particularly true in the Δ2-5 and the Δ10-17 isomers (Fig. 4). The relative changes in retention behavior, observed first on ApL columns, are largely reproduced on more polar columns (XE60, NPGS, DEGS).

The difference between *cis* and *trans* isomers on ApL or DEGS columns is too small to be useful, and Emken (18) has described a chromatographic procedure for the determination of *cis* and *trans* alkenoates after quantitative epoxidation. This is based upon the more efficient chromatographic separation of *cis* and *trans* epoxides, at least over the range of double bond position expected in most hydrogenated fats. Our results (5,6,8) suggest that the cyclopropane derivatives would be more useful over a wider range of double bond position; but unfortunately the conversion of alkenoates to cyclopropanes is not so simple experimentally as epoxidation, nor does the reaction proceed in such good yield (Fig. 5).

We have also tried to predict retention behavior, expressed in terms of equivalent chain lengths (ECL), by the use of the concept of fractional chain length (FCL), and this has been developed by Ackman and his colleagues (19). We find that, in the important series of methylene-interrupted polyunsaturated esters, the FCL is not simply additive, and an additional correction factor has to be invoked (17) (Table I). Since this correction is fairly consistent through a range of compounds, it can be incorporated into the calculation. Though we cannot calculate ECL with a high degree of accuracy, it is possible to predict the order of elution of a series of closely related isomers, such as the 8 stereoisomers of methyl octadeca-9,12,15-trienoate. These results are most satisfactory on

TABLE I

Equivalent Chain Lengths and Fractional Chain Lengths of some Polyunsaturated C_{18} Esters on ApL Columns

Ester	ECL[a] (obs)	FCL[b]	ECL (calc)[c]	Δ(x100)
18:1 (6*c*)	17.65	-0.35	—	—
18:1 (9*c*)	17.63	-0.37	—	—
18:1 (12*c*)	17.73	-0.27	—	—
18:1 (15*c*)	17.89	-0.11	—	—
18:2 (6*c*9*c*)	17.42	—	17.28	14
18:2 (9*c*12*c*)	17.50	—	17.36	14
18:3 (6*c*9*c*12*c*)	17.27	—	17.01	26
18:3 (9*c*12*c*15*c*)	17.49	—	17.25	24
18:4 (6*c*9*c*12*c*15*c*)	17.26	—	16.90	36

[a]ECL = Equivalent chain lengths.

[b]FCL = Fractional chain lengths.

[c]ECL (calc) for methyl linoleate is 18.00 + (-0.37) + (-0.27) = 17.36 and the remaining values are calculated in a similar way.

ApL columns where retention behavior is reproducible both within and between laboratories. Polar columns present added difficulties, because ECL is less reproducible on such columns.

Biological Studies

The study of some biological properties (and of some other physical properties) of our synthetic acids has been undertaken collaboratively between ourselves and other laboratories in Europe and North America (20). I have selected for discussion here the work carried out by W.E.M. Lands and his colleagues (Michigan). This work seeks to discover the influence of fatty acid structure on the esterification reaction occurring between lysolecithins and acyl-CoA in the presence of acyltransferases derived from rat or pig liver microsomes. The enzymic acylation of 1-acylGPC and of 2-acylGPC has been examined with the four series of C_{18}-CoA esters derived from the *cis*-octadecenoic acids, the *trans*-octadecenoic acids, the octadecynoic acids, and the *cis*-methyleneoctadecanoic acids (21). Briefly, their results lead to the following conclusions: (a) The enzyme(s) responsible for acylation at the 2 position responds to an unsaturated center present at the Δ5, Δ9, and Δ12 positions independently of whether this is *cis*-olefinic, *trans*-olefinic, or acetylenic. (b) The enzyme(s) responsible for acylation at the 1 position responds to the *conformation* of the acyl group, so that there is little difference in reactivity between the *cis*-alkene and the similarly shaped *cis*-cyclopropane CoA esters. There is, however, a marked difference between the *cis*-alkenoates on the one hand and the *trans*-alkenoates and the alkynoates on the other. Lands has called attention to the alternating selectivity between the acetylenic and the *cis*-olefinic compounds, so that, for example, the 9*c* ester behaves like the 10*t* and the 10*a* esters, and the 10*c* ester behaves like the 11*t* and 11*a* esters. This is particularly true in the Δ9 to Δ13 region.

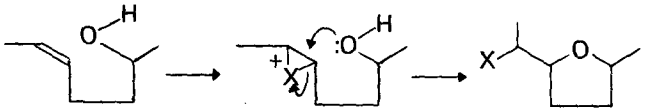
CHEMICAL REACTIONS

In the reactions of a molecule with at least two functional groups, there is always the possibility that reaction at one functional group may be influenced by the second. This influence will depend upon the nature of the second functional group and on its position with respect to the first. For example, in the reactions of long chain esters containing the hydroxy alkene system -CH=CH(CH_2)$_n$CH(OH)- we have discovered how reaction at the double bond may be influenced by the nearby hydroxyl group and how reaction at the hydroxyl group can be affected by the double bond and how, in both cases, the interaction depends markedly upon the number of methylene groups between the double bond and the hydroxyl group and sometimes on the configuration of the

double bond. These studies have led us to new types of fatty acid derivatives and to reactions of potential value for analytical and diagnostic purposes.

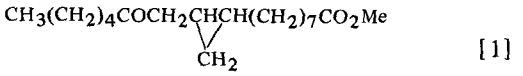
We have, for example, discovered some reactions of methyl vernolate and of methyl ricinoleate which result in the formation of cyclopropane compounds and which can be explained in terms of the resonance known to occur in homoallylic carbenium ions:

Also when a hydroxyl group becomes involved in reactions occurring at a double bond, intramolecular formation of a cyclic ether often occurs. We have observed this during epoxidation, halogenation, and oxymercuration of appropriate hydroxy alkenes:



Cyclopropane Esters from β-Hydroxyalkenes and βγ-Epoxyalkenes via a Homoallylic Rearrangement

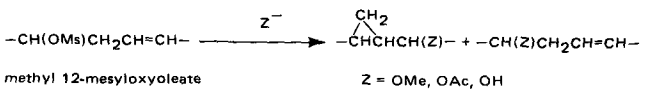
We chanced upon this type of reaction when looking for a reagent which would convert methyl vernolate to methyl coriolate. We found that this rearrangement could be effected with lithium diethylamide ($LiNEt_2$) (22) but not until we had investigated several other reagents, including boron trifluoride. As expected (23), this converted the epoxy ester mainly to oxo esters, including, surprisingly, some saturated compounds. In benzene solution these were formed in 34% yield and consisted mainly of the *cis* and *trans* isomers of the cyclopropane oxo ester [1]. We explained this observation in terms of the homoallylic



[1]

resonance which has been extensively studied among compounds of lower mol wt (24). Our results were confirmed by the independent observations of an Italian group (25).

It then seemed to us that similar reactions should occur with suitable derivatives of methyl ricinoleate, and we showed that methyl 12-mesyloxyoleate can be converted in good yield (30-60%) to cyclopropane compounds so long as the reaction solution is kept alkaline (26). In acidic solution the cyclopropane compounds are unstable, and the

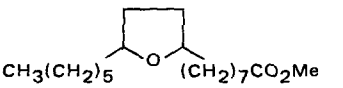


methyl 12-mesyloxyoleate Z = OMe, OAc, OH

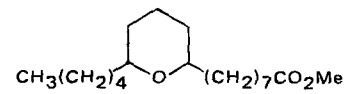
major product is a substituted octadecenoate in which the double bond is mainly *trans*. Similar results have been reported by Ucciani and Naudet (27) with methyl 12-tosyloxyelaidate. This reaction is confined to compounds which can furnish a homoallylic carbenium ion and is not observed, for example, in similar reactions of methyl 9-mesyloxyoctadec-12-enoate (a γ-hydroxy alkene).

1,4- (and 1,5-) C_{18} Epoxides

We recently have become interested in a new type of epoxystearate ([2] and [3]), containing a five- or six-membered heterocyclic ring system, which is formed easily in several reactions of appropriate hydroxyalkenoates.

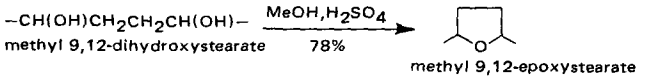


methyl 9,12-epoxystearate [2]



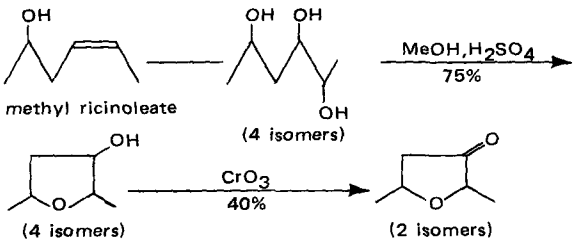
methyl 9,13-epoxystearate [3]

We first produced such compounds when treating methyl linoleate fairly vigorously under acidic conditions (28). The main product (48%) was a mixture of epoxystearates (mainly 9,12 and 10,13 with a little 9,13), and we confirmed this by an independent synthesis of the 9,12-epoxide by acid-catalyzed cyclization of the already known methyl 9,12-dihydroxystearate. This reaction proceeded so



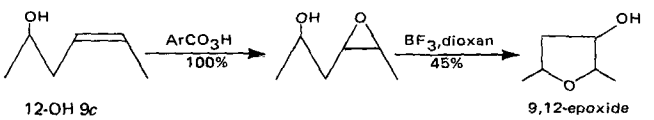
methyl 9,12-dihydroxystearate 78% methyl 9,12-epoxystearate

smoothly that we examined the cyclization of the methyl 9,10,12- and 9,12,13-trihydroxystearates which can be prepared from methyl ricinoleate and methyl 9-hydroxyoctadec-12-enoate respectively. Our studies led to an interesting investigation of the stereochemistry of the trihydroxy acids and of the hydroxy epoxides and oxo epoxides derived from them by cyclization and by oxidation (29). For example:

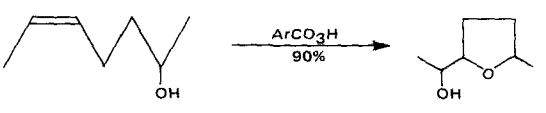


methyl ricinoleate (4 isomers) 75% (4 isomers) 40% (2 isomers)

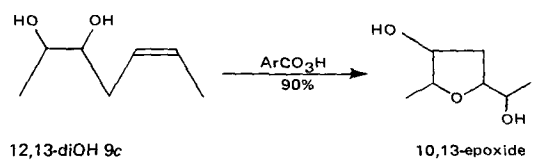
1,4-Epoxides can also be obtained from appropriate 1,2-epoxides (30). The 9,10-epoxide from the β-hydroxyalkene, methyl ricinoleate, for example, rearranges to a 9,12-epoxide when treated with boron trifluoride. In contrast, the γ-hydroxyalkene, methyl 9-hydroxyoctadec-*cis*-12-enoate, did not give an isolable 12,13-epoxide but furnished the 9,12-epoxide directly. We believe, though we have not checked this, that epoxidation of methyl ricinelaidate also might lead directly to the 9,12-epoxide. Methyl 12,13-dihydroxyoleate is both a β- and a γ-hydroxy alkene, but on epoxidation it reacts as the latter and gives the 10,13-epoxide directly.



12-OH 9c 100% 45% 9,12-epoxide



9-OH 12c 90% 9,12-epoxide



12,13-diOH 9c 90% 10,13-epoxide

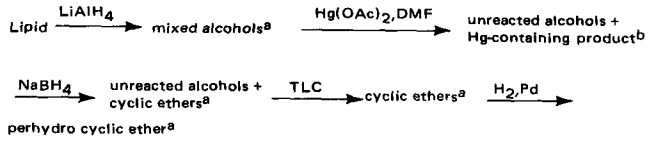
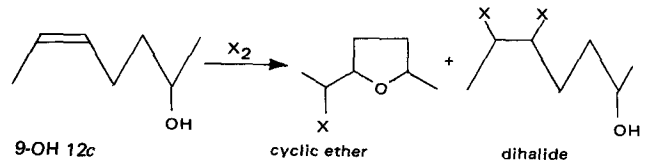
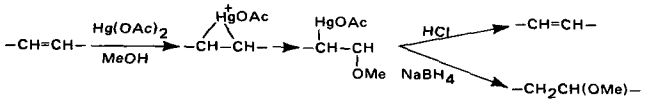


FIG. 6. Procedure for the examination of lipids containing fatty acids having unsaturation at Δ3 (*trans*), Δ4, or Δ5. a = Product may be examined by gas liquid chromatography at these stages. b = Reaction product may be placed on a chromatographic column at this stage and eluted with ether to remove the unreacted alcohols and then with methanol containing hydrochloric acid to elute the mercury-containing product as regenerated alcohols having unsaturation at Δ3 (*trans*), Δ4, or Δ5.

We have observed similar results during bromination and iodochlorination of hydroxyalkenoates and find the products to be a mixture of cyclic ethers and dihalides (31). Cyclic ethers are best obtained with γ-hydroxyalkenes and are formed in greater proportion during iodochlorination (~80%) than during bromination (~40%).



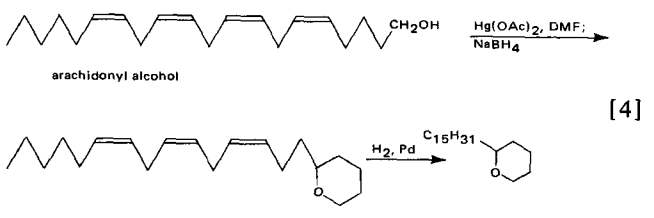
Of wider interest is the oxymercuration reaction which has been known for some time but which we now put to new use. In simple form this reaction occurs thus:



Methyl oleate, for example, by oxymercuration and demercuration, gives methyl 9(10)-methoxystearate in high yield (98%) and ethoxystearates (ethanol, 95%), acetoxystearates (acetic acid, 82%), and hydroxystearates (water, 77%) when methanol is replaced by the appropriate nucleophilic solvent (32).

The reaction proceeds on an intramolecular basis whenever the hydroxyl group and the double bond are disposed appropriately (33). β-Hydroxyalkenes form cyclic ethers only when the double bond has the *trans* configuration. γ-Hydroxyalkenes in either *cis* or *trans* configuration readily form tetrahydrofurans and δ-hydroxyalkenes (*cis* or *trans*) form tetrahydropyrans in virtually quantitative yield. These reactions can be carried out in dimethylformamide solution but even when conducted in methanol intramolecular cyclization predominates, and no methoxy ethers are observed. Hydroxyalkenes are not common among the fatty acids, but there exist some important acids with unsaturation at Δ3*t* or Δ4 or Δ5 which can be reduced to alkenols and then converted easily to cyclic ethers. This observation can be employed as a means of identification or analysis.

Methyl arachidonate, for example, can be converted to the tetrahydropyran [4], and other acids with Δ5 unsaturation will behave in a similar way. Acids with Δ4 unsaturation (common among the C_{22}-polyenoic acids) produce the corresponding tetrahydrofurans.



[4]

The cyclic ethers are much less polar than the alcohols and are separated readily by TLC from those alcohols which do not form cyclic ethers. It is even possible to separate the alkyl and alkenyl tetrahydrofurans from the alkyl and alkenyl tetrahydropyrans and thereafter to separate each ether class into components of differing unsaturation by Ag^+ TLC. Unreacted alcohols can be separated from the acetoxymercury compounds after mercuration and before demercuration; and Δ3, Δ4, and Δ5 alkenols can be regenerated from the latter by reaction with hydrochloric acid.

We have proposed a procedure for examining lipids containing acids with unsaturation at Δ3*t*, Δ4, or Δ5 which can be employed for analytical or isolation purposes and have applied this to tall oil, rat liver lipids, and to fish oils both before and after partial hydrogenation (34) (Fig. 6). By this procedure, for example, we showed the alcohols from pilchard oil to give cyclic ethers (19%) of which the major components were those arising from the Δ5 alcohol 20:5ω3 (12.7%) and the Δ4 alcohol 22:6ω3 (4.1%).

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