Determination of Structure of Unsaturated Fatty Acid Positional Isomers

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Abstract

The methods for determining the positions of unsaturation in fatty acids are reviewed. Overoxidation and isolation problems germane to permanganate oxidations, and the formation of by-products and attack of solvents during ozonolysis are considered. The applicability of mass, nuclear magnetic and infrared spectral data to the problem of locating positions of unsaturation is discussed.

Introduction

DESPITE THE existence of many excellent methods for separating a variety of fatty acids from one another, the routine separation of positional isomers is not yet possible. As elegant and sensitive to subtle molecular changes as these separations are now and are destined to become, it appears that we will never be entirely free of the need to have to resort to methods of molecular degradation in order to identify unknown compounds, or to demonstrate occasionally to ourselves that our methods of separation are as good as we believe them to be.

In an overall view, it appears that the currently evolving methods of performing structural analysis of fatty acids are largely physical and dependent upon instrumentation, whereas useful chemical methods are modified versions of basic techniques that have long been the mainstay in this field.

The actual steps in performing a structural analysis will probably be the same whether one is dealing with a very common substance or one of the rare or unusual fatty acids with which this symposium is concerned. In these two cases, although the quantity of available material may widely vary and the interpretation of the data may require refinements, the methods, in general, will be the same.

Oxidation Methods

There appears to be no recent major change in the types of chemical degradation used to identify positional isomers of unsaturated fatty acids. A perusal of publications dealing with structure determinations of fatty acids reveals that the primary means of chemical degradation that are used are those involving either permanganate-periodate or ozone.

The very occasional use of alternative ways indicates the acceptance of these general methods. Secondary features of these general methods have been repeatedly examined and refined to the extent that an acceptable analysis can now be performed on a milligram of material. As methodology improves for means of looking at the products of these degradation reactions, it is increasingly apparent that none of the reactions involved is completely quantitative with respect to the yield of the desired products. It seems worthwhile to focus attention on some of the ways such analytical techniques may be less than straightforward.

The major complaint leveled against permanganate oxidations involves overoxidation. Even though the sample size has been reduced from the 50 g of methyl oleate and 200 g powdered potassium permanganate used some time ago by Armstrong and Hilditch (1), recent reports (2,3,4,5) reiterate the early findings that potassium permanganate causes anomalous oxidation near the site of chain cleavage. In the extreme, when a deliberate attempt is made to cause overoxidation, e.g., by use of $KMnO_4$ in refluxing acetone, saturated fatty acids have been shown to be degraded stepwise to a homologous series of shorter fatty acids (6). Interestingly, the progression of yields with respect to chain length is not regular. The quantities of successively shorter acids are not all proportionately smaller than the preceding acid. If the degradation occurred one carbon at a time in a stepwise manner, the quantity of each acid formed during the reaction would be expected to be less than that of the preceding longer-chain acid. The implication is that the oxidation does not involve a simple attack on the α-carbon followed by decarboxylation to yield an acid shorter by one carbon. One must also include a scheme that allows for the loss of two carbons at a time. This reaction of carboxylic acids and the related overoxidation processes that occur during the oxidation of olefins to carboxylic acids (7) therefore do not present a completely attractive picture from the standpoint of a desirable analytical technique. Although these difficulties have not been prevented, in some instances they have been circumvented by the device of correcting on the basis of the amount of overoxidation obtained with a standard sample (2,3).

Wishing to avoid the necessity of applying such corrections for overoxidation, many investigators have recently adopted the permanganate-periodate method of Lemieux and von Rudloff (8). This method makes use of the higher oxidation potential of periodate to regenerate permanganate from intermediate manganese ions which are formed during the oxidation process. The overall effect allows less-than-stoichiometric amounts of permanganate to be used, thereby reducing the possibility of overoxidation.

Subsequent improvements in this methods have been concerned for the most part with the isolation of products, although relative amounts of oxidants and substrate have also been varied. The relative molar amts of reactants originally prescribed were 8/.13/1 for periodate, permanganate and substrate, respectively (8). Other workers have tended to use roughly the same concns, e.g., 8.7/.14/1 (9).

In one modification (4), however, enough of the permanganate-periodate reagent was used for small samples of substrate so that the ratio was 1800/250/1. This amounted to a very large excess of permanganate –much more than necessary to complete the oxidation without the involvement of periodate at all. Relative concentrations of this order occur at the end of any routine permanganate-periodate oxidation as the amt of substrate approaches zero, of course, but by this time the reaction is nearly complete. No overoxidation was reported using these high relative concns. In light of the reported overoxidations with permanganate alone, and none when periodate is present, even

in the presence of excess permanganate, one can only speculate at this time about the relative importance of reagent concns in the reaction mixture and the relative rates of reaction of periodate and permanganate on the intermediate in the reaction sequence.

In order to use the great power of gas chromatography in solving structural problems of unsaturated fatty acids, it is necessary to isolate the reaction products and convert them into compounds that can be chromatographed without loss of any of them. Several variations have been reported of a general procedure involving destruction of excess oxidizing agent, extraction of products, esterification, and gas chromatography.

After a suitable time of 6–48 hr the excess oxidizing agent is destroyed by the addition of bisulfite (8), metabisulfite (4) or sulfur dioxide (9). Extracting the products from the reaction solution is a very critical step, since the variability of chain length and number of functional groups of the products give rise to large differences in partition coefficients. For example, assuming equilibrum and partition coefficients of 1.34 and 4.6 (moles in ether/moles in water) (10) for propionic acid and butyric acid, respectively, and using the equation for mole fraction of material left in the aqueous phase $X_n = (\frac{1}{Kr+1})^n$; (X = mole fraction, n = number of extractions, r = ratio of ether to water vol used and K = partition coefficient), it can be shown that after three extractions with equal vol of ether, about 8 mole % of the propionic acid will remain unextracted, and that about 0.5 mole % of the butyric acid would remain in the aqueous phase. Thus, for recovery of the monocarboxylic acid from an oleic oxidation, three extractions, using an equal vol of ether each time would be more than adequate to extract all of the monocarboxylic acids, but similar treatment of the products from oxidation of linolenic acid would not be adequate for quantitative recovery. In working with small quantities of materials, it is common practice to reduce the vol of the aqueous oxidation mixture before extraction. To insure that the more volatile acids are not lost during the process of water evaporation, the solution is made alkaline and subsequently acidified before extraction.

Once the acids have been satisfactorily extracted, diazomethane (4), HCl-butanol (11), 2-chloroethyl alcohol (12), and ω-bromoacetophenone (13) have been used for converting the acids into derivatives suitable for gas chromatography.

Certain products that would otherwise be of considerable value in structural analysis are destroyed during the course of the permanganate-periodate oxidation. β-Dicarbonyl compounds in general are susceptible to oxidative attack (14). Periodate itself destroys β-dicarbonyl compounds (15). Malonic acid, a product otherwise expected to result from oxidation of methylene-interrupted double bond system, is extensively destroyed by permanganate-periodate oxidation (16). The 9,11-dioxo compound obtained from sterculic acid via permanganate-periodate oxidation is itself partially oxidized (17). Oxalic acid probably is not formed during oxidation of conjugated dienes, and even if it were, its existence would be only transitory. Oxalic acid is, of course, a primary standard for determining the strength of permanganate solutions, however it is not attacked by periodate (18).

For the straight-chain unsaturated fatty acids, the identification by gas chromatography of the fragments from oxidative cleavage will be straightforward. Ambiguities attributed to the presence of substituent groups will usually be noticed during isolation and purification. A notable exception to the use of gas chromatography for cleavage products is the case of unsaturated ring systems (e.g., sterculic and chaulmoogric acids). The oxidation does not result in smaller fragments, and the products that are formed will have unexpected gas-chromatographic behavior, thus requiring other means for identification. A β-diketo fatty ester, such as the 9,11-diketo compound obtained from sterculic acid, is eluted from gas-liquid chromatography (GLC) columns as a peak with severe tailing. This reflects the propensity of such compounds for keto-enol tautomerism. The tailing of the peak is probably due to the shifting of the equilibrum of the tautomers to the species that is eluted more readily.

Ozonolysis

Ozone does not appear to be widely used as permanganate-periodate in fatty acid structure determination. Although various reports have indicated the production of undesirable overoxidation products, other work (19,20,21,22) has indicated that this does not seem to be the case. This freedom form apparent by-products is easily confirmed by gas chromatography. When thin-layer chromatography was used on the ozonides, there were indications that by-products were present (22). The undesirable products were polar materials which did not migrate on thin-layer plates, and presumably would not be converted solely to aldehydes by reduction. The yield of such polar products varied with solvent employed. Performing the ozonolysis in pentane, methylene chloride and ethyl acetate gave 1.7, 10.3 and 22.4%, respectively, of the products.

The stage in the reaction sequence in which these extraoxidation products arise is not clear. It has been postulated (22) that they might arise from the addition of a Criegee's zwitterion to a peracid formed by oxidation of the same sort of zwitterion by ozone. Cyclic ozonides were stable under conditions used in the reactions, and it is unlikely that the hydroperoxide was formed by autoxidation of the ozonide.

The choice of solvent in ozonolysis obviously plays a critical role in the course of the reaction. Carbon tetrachloride, chloroethane, fluorotrichloromethane, acetic acid and water are recommended solvents for ozonizations (23). It is interesting to note that certain solvents currently used for ozonolysis of fatty acids include some known to give peroxides or liberate halogens, e.g., pentane, ethyl acetate and methylene chloride. Most (if not all) solvents react with ozone to some extent. For example, survival of ozone passed through pentane under steady state conditions is 71%. In methanol only 27% survives. If the decomposition in these solvents were simply $2O_3 \longrightarrow 3O_2$, no problems should result. However, if free radicals or peroxides are formed in the solvent, these may lead to unexpected products during the ozonization of unsaturated centers. Even the means of delivering the ozone into the solvent can become important. An open tube is preferable to fritted glass because the latter is known to catalyze the decomposition of ozone in direct relation to surface area and contact time (24).

Thorp (24) refers to five publications of methods of quantitatively determining ozone concns by oxidation of aldehydes (25). By assuming that the Criegee mechanism for ozonolysis is essentially correct, it follows that aldehydes will always be formed

during the reaction of a simple olefins. It should not be surprising to find that reaction between free aldehydes and ozone accounts for some of the free acids and other polar materials formed during ozonolysis. The chance of side reactions may be obviated to some extent by having as short a contact time with ozone as possible. Reaction of ozone with the olefinic centers found in fatty acids appears to be instantaneous. Thus the technique (20) of introducing a sample into a solution containing ozone and then very quickly removing excess reagent should minimize overoxidation.

Acetylenic fatty acids, because of their relative scarcity, have not received as much attention as the olefinic acids. Structural work on stearolic acid has revealed the normal course of permanganate oxidation and ozonolysis (26). Controlled $KMnO_4$ oxidation in a buffered medium gave 70–80% yield of the 9, 10-diketone, the remainder of the product being carboxylic acids. Using the permanganate-periodate method the α-diketone would have been destroyed and the products form stearolic would be thus indistinguishable from those from oleic acid. It was also observed that a 3% yield of 9,10-dione was formed from ozonolysis and decomposition of the ozonide in boiling water. Work in our laboratory, however, indicates that the yield of the dione may be increased considerably. Reduction of the ozonide of methyl stearolate with triphenylphosphine gives a 67–77% yield of the 9,10-dione (gas chromatographic analysis). The remainder of the products were not eluted. Thus, determining the position of an acetylenic bond in a fatty acid by ozonolysis requires modification of the reduction methods which are useful in dealing with olefinic substances. Conversion of the acetylene to the dione may be qualitatively useful for purposes of identification, but without further work is of little value in establishing bond position.

Other Oxidative Methods

Alternative methods of oxidative cleavage of olefins involve isolation of an intermediate oxidation product. Reactions (such as hydration) which lead to mixtures of the two positional isomers ultimately yield mixtures of classes of degradation products containing homologues differing by one methylene group. Epoxidation (27), followed by ring opening to the glycol, which may be used as a substrate in a subsequent oxidative reaction, has been used (28).

Physical Methods

Physical methods will probably become increasingly important to those studying the structures of fatty acids. The availability of commercial mass spectrographs coupled with GLC, makes this a very attractive method to be considered. The work thus far, however, indicates that positional isomers cannot be distinguished from one another on the basis of mass spectra alone (29). The one exception to this appears to be the α, β-unsaturated acids (30). In explaining the inability to distinguish between positional isomers, it has been suggested that fragments may recombine to give mass peaks that are not characteristic of one particular isomer. The cracking patterns of certain derivatives of the unsaturated acids are however characteristic of the location of the substituent on the chain. Deuterium (31) or keto, hydroxy, methoxy or epoxy (32) derivatives obtained from olefins may be used in this way to determine the position of unsaturation.

Likewise, nuclear magnetic resonance spectra are being used in fatty acid structure work, but it is not possible to decide on the basis of such spectral data alone, the position of an unsaturated center. Certain arrangements of pairs of double bonds that are of interest in fatty acids may be distinguished; thus methylene-interrupted (33) and ethylene-interrupted dienes (34) can be identified and distinguished from one another.

IR spectroscopy has proved suitable for solving other specific problems concerned with locating double bond positions. In KBr pellets, the number of bands and the band progression in the region 1180–1350 cm^{-1} have been shown to be functions of the distance between the carboxyl group and the double bond in a series of octadecenoic acids (35). The number of bands is equal to $\frac{n}{2}$ for even n or $\frac{n-1}{2}$ for odd n, where n is the number of carbon atoms between the carboxyl group and the double bond including the carboxyl carbon and the first carbon of the double bond. For example, 7-octadecenoic acid would have 3 bands and the 8-isomer would have 4. The bands progress to longer wavelength in the 1180–1350 cm^{-1} region as the distance between carboxyl group and olefin increases.

Gas chromatography, although incapable of distinguishing between closely similar unsaturated positional isomers, is useful in discerning isomers involving large displacements of double bond position. In general, the closer the unsaturated center is to the carboxyl group, the shorter the retention time. This effect is more pronounced on nonpolar columns than on polar ones (36), and is accentuated on a polar column as the number of double bonds in the system increases (37). On polar columns, the effectiveness of separation of positional isomers is inversely proportional to temp (37).

In conclusion, it appears that the instrumental methods currently available cannot yet be substituted for chemical degradation methods in establishing the position of an unsaturated bond. For this use, instruments should be considered as complementing rather than competing with degradative techniques.

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Determination of Oxirane Content of Derivatives of Fats

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Abstract

Although oxirane functions react readily with a large variety of chemical reagents, no single analytical method has been universally successful in measuring the oxirane content of all epoxides. This failure is ascribable to the manner in which the chemical reactivity of the three membered cyclic ethers is modified by molecular structure and by the presence of nearby substituents.

A survey of the various types of published analytical methods is given, but major emphasis is placed upon a discussion of procedures applicable to the analysis of epoxy derivatives of fats. Most of these latter methods depend upon the addition of some reagent, HX, to the epoxide ring with simultaneous cleavage of a carbon-oxygen bond.

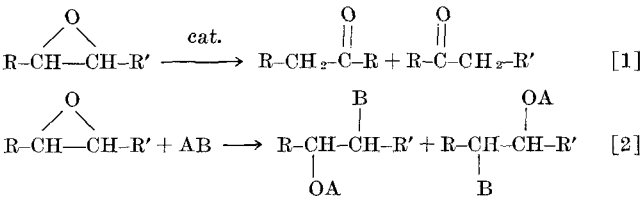
AMONG THE FUNCTIONAL groups which one might encounter among fats, oils, or their chemical derivatives, there are few which have greater chemical reactivity than the three-membered, oxygen-containing rings variously called α-epoxides, 1,2-epoxides or oxirane functions.

The chemistry of oxirane compounds has been the subject of much theoretical and practical interest in recent years and is still in the process of being unravelled. Epoxides occur naturally in some oils, have been found among the oxidation products of many unsaturated fats and oils, and are produced commercially from unsaturated fatty materials for use as chemical intermediates and as end-products. Epoxidized glycerides or their derivatives are used as plasticizers and stabilizers in plastics and resins and even as components of epoxy resins.

Because of the growing importance of oxirane compounds in research and trade, increasing demands have been placed on analytical methods which measure the amt of oxirane oxygen present in natural and in synthetic products and measure it at lower concn levels with greater accuracy. Above all, there is a demand for an analytical procedure which is highly specific, i.e., which measures only oxirane oxygen and nothing else. A high degree of specificity, however, is not easily achieved, because the chemical personality of an epoxide group changes with its surroundings so that, from a chemical reactivity viewpoint, there are several types of oxirane functions.

Any classification of epoxides into types must, of necessity, be arbitrary. For the purpose of this paper it is convenient to distinguish between three types of oxirane compounds which differ in the manner in which the cyclic ether is substituted. Terminal epoxides are those which are located at the end of a chain, so that one carbon of the oxirane group bears two hydrogens. Glycidyl esters, glycidyl ethers and epoxidized terminal olefins fall into this category. Internal epoxides are those which are located along a hydrocarbon chain, so that each carbon of the cyclic ether bears an alkyl substituent. Epoxidized natural glycerides generally fall into this classification. A third group of epoxides are those in which one of the carbons of the functional groups bears two alkyl substituents and is therefore a tertiary carbon. Tertiary epoxides, which may be terminal or internal, occur only rarely in derivatives of fats, but they are included here for the sake of completeness.

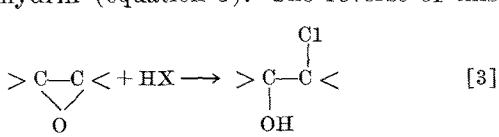
The bent or "banana" bonds of the oxirane function have a significant amount of ionic character, and the oxygen of the cyclic ether is susceptible to attack by electron-seeking reagents, such as protons, while the carbons of the group are relatively prone to attack by electron-rich reagents, such as anions. The epoxide ring may respond to chemical attack by rearranging to the isomeric carbonyl compound (equation 1), but more frequently such attack results in opening of the ring and addition of the reagent (equation 2).



Terminal and internal epoxides differ considerably in their chemical reactivity, and it is this difference which makes the achievement of a general method applicable to all epoxides difficult to attain. Terminal epoxides, for instance, are quite sensitive to attack by nucleophiles such as amines or other bases. On the other hand, internal epoxides can be subjected to saponification conditions without significant damage, but are attacked more readily by acids than are terminal epoxides. Other differences in behavior toward specific reagents will be encountered later.

Hydrohalogenation Procedures

The most frequently reported procedures for the determination of α-epoxides, and those which are most specific and generally applicable, consist of the addition of a halogen halide, HX, to the cyclic ether to form a halohydrin (equation 3). The reverse of this



reaction, namely, dehydrohalogenation with the aid

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