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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.

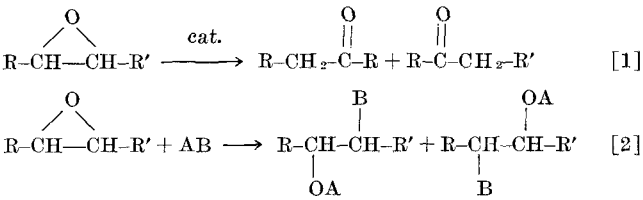
AMONG THE FUNCTIONAL groups which one might encounter among fats, oils, or their chemical derivatives, there are few which have greater chemical reactivity than the three-membered, oxygen-containing rings variously called α-epoxides, 1,2-epoxides or oxirane functions.

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.

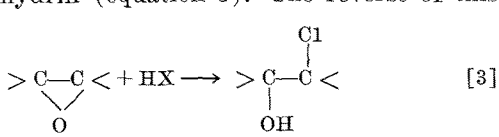
The bent or "banana" bonds of the oxirane function have a significant amount of ionic character, and the oxygen of the cyclic ether is susceptible to attack by electron-seeking reagents, such as protons, while the carbons of the group are relatively prone to attack by electron-rich reagents, such as 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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of base, has long been used as a commercial method for the preparation of some epoxides.

There are many variations of the analytical hydrohalogenation procedure, each new method being an attempt to improve on and to correct the shortcomings of earlier innovations. These methods are similar in that they all use HCl or HBr as the ultimate reagent, but they differ widely in most other details.

As early as 1930 Nicolet and Poulter (1) reported that epoxy groups in 9,10-epoxystearic acid could be measured quantitatively by treating the epoxide with excess HCl in dry ether at room temp and after 2 hr titrating the remaining acid with standard KOH. Swern and co-workers (2) tested this method on a variety of oxirane compounds and also on a number of other compounds which might reasonably be expected to be present in air-oxidized fats. Their conclusion was that, with slight modification, the HCl in ether method was quite specific for oxirane compounds, that other related compounds, especially peroxides, did not interfere and that the reagent is stable. Their results indicated that terminal epoxides were determined with somewhat less accuracy than internal epoxides, but, on the whole, agreement with calculated values was excellent. The authors warned, however, that α,β-unsaturated carbonyl compounds also absorb HCl and therefore interfere when present. In those early days of the rapid development of oxirane chemistry, the Swern method was the standard analytical procedure.

King (3) objected to the inconvenience involved in the use of a reagent composed of HCl in anhydrous ether. He suggested that a reagent prepared by pouring concentrated aqueous hydrochloric acid into purified 1,4-dioxane was much more convenient and worked equally well, as long as it was prepared immediately before use. Reaction time was decreased from 2 hr to 10 min, and the amt of reagent consumed was still determined by titration with base. Other hydrochlorination methods were proposed, mostly in answer to specific analytical needs. Recommended reagents included saturated magnesium chloride which is 0.1*N* in HCl, 0.5*N* HCl in ethanol which is saturated in magnesium chloride, 0.2*N* hydrochloric acid in methanol, pyridinium chloride in pyridine and pyridinium chloride in chloroform. Seven of these hydrochlorination methods were tested by Jungnickel and co-workers (4) and compared in their applicability. The compounds analyzed by these methods, however, were all of the terminal epoxide variety and almost all of the glycidyl ether type. Many types of organic compounds were checked for interference with the various hydrochlorination methods, but only a few functional types, e.g., strong amine bases, α,β-unsaturated carbonyl compounds, easily hydrolyzable compounds, gave trouble when present.

A further refinement in the hydrochlorination method was made by applying a differential silver nitrate titration in order to measure the amt of chloride actually absorbed. The endpoint was determined either potentiometrically (5,6) or by means of an indicator as in the Volhard method (7).

In 1956, Durbetaki (8) reported an analytical procedure by which oxirane groups can be titrated directly. Hydrogen bromide, dissolved in glacial acetic acid, is the titrant and crystal violet the indicator. The method was adopted by the AOCS within a year of its publication as AOCS Tentative Method Cd 9–56 (9), and after a recent slight amendment (10) is now known as AOCS Tentative Method Cd 9–63, Oxirane Oxygen. In determining oxirane oxygen by this procedure the sample is dissolved in some nonaqueous solvent such as benzene, chlorobenzene, methylene chloride or glacial acetic acid, a few drops of a solution of crystal violet in glacial acetic acid are added, and the sample is titrated with 0.1*N* HBr-acetic reagent. The reagent may be prepared by bubbling HBr from a cylinder through acetic acid, by purchasing the 30–32% reagent and diluting it with glacial acetic acid, or by adding bromine to a solution of phenol in glacial acetic acid. The reagent is carefully protected against moisture both during storage and during titration, and for accurate work it is standardized daily against sodium carbonate, sodium acetate or potassium acid phthalate. Storage of the reagent over prolonged periods, say 6 months or a year, may cause some detrimental changes in it and make it unsuitable for further use. Evidence for such a change is the fact that the reagent will attack the indicator and convert it to a red-brown precipitate during a prolonged titration.

The problem of HBr storage is avoided in a useful variation of the Durbetaki method, as described recently by Jay (11). In this modification HBr is prepared in situ by treatment of a quaternary ammonium halide with standard perchloric acid.

Undoubtedly the Durbetaki method is presently the most widely used procedure for the determination of oxirane oxygen. It is doubtful, however, that the limitation of this method, or of the hydrochlorination methods, is fully appreciated. The possible presence of interfering substances (12) which absorb hydrogen halide is a constant threat to accuracy, and it is often necessary to correct for such by a separate determination. For instance, it is evident that bases, such as amines, which tie up HX, will interfere with the method. However, in acetic acid even compounds such as soaps (13) act as bases and absorb HX. In certain seed oils, the presence of cyclopropenoid compounds give rise to problems in the analysis of oxirane compounds. Smith and co-workers (14) as well as Skau and associates (15,16) have proposed modifications in the Durbetaki method to overcome these difficulties.

A more subtle source of errors may be found in oxirane compounds which add less than the theoretical amount of HX. For instance, triphenyl (tolyl) ethylene oxide (17) is reported to be inert to HCl. McConnell et al. (18) discovered that 3,4-epoxy-2,2,4-trimethylpentyl isobutyrate, a tertiary epoxide, absorbs only 29% of the theoretical amt of HCl when analyzed by the HCl in the dioxane method. These authors isolated four reaction products which bear faint resemblance to the expected chlorohydrins and are, in fact, rearrangement products. Young and coworkers (19,20) reported that the epoxide of diisobutylene, also a tertiary epoxide, gave only 60–70% of the theoretical amt of HCl absorption when analyzed with HCl in ether. Among the products were found, besides the expected chlorohydrin, a quantity of an aldehyde and a chlorine-containing compound which was not a chlorohydrin. In our own laboratory it has become apparent that one of the isomers of methyl 9,10,12,13-diepoxystearate does not react quantitatively with HBr in acetic acid, thus throwing some doubt on the validity of results obtained when epoxidized polyolefinic oils are analyzed by the Durbetaki method.

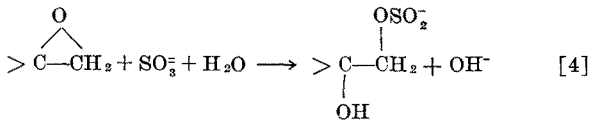
It has furthermore been our experience, that certain terminal epoxides such as glycidyl esters, are difficult to analyze by the Durbetaki method, since the endpoints fade, and results are consistently about

5% low. Durbetaki has further refined his method for special application by incorporating the use of silver nitrate to measure the amt of HBr consumed (21).

Other Chemical Methods

Let us now consider some other chemical methods by which the determination of oxirane groups has been accomplished, i.e., methods which do not involve the addition of HX.

A procedure which probably works well only on water-soluble, terminal epoxides, involves the reaction of sodium sulfite with the oxirane ring with liberation of sodium hydroxide ion (22) (equation 4). The



[4]

latter is determined by titration with acid. Since internal epoxides, such as are found in epoxidized fats and oils, are quite resistant to nucleophilic attack, in contrast to terminal epoxides, we would not expect methods similar to the sodium sulfite reaction to succeed in the determination of internal epoxides. In the same category, and subject to the same limitations, is the method of Leary (23) in which excess sodium thiosulfate is added to the epoxide, and the consumed thiosulfate is measured by a differential titration with standard iodine solution. This procedure has an advantage over that of using sodium sulfite in that amine bases, when present, do not require a correction factor. A thiosulfate addition method which is also limited to terminal epoxides, but which is carried out in ethanol, was published by Sully (24).

A novel method which gives reproducible results consists of the addition of dodecanethiol to epoxides with the aid of a basic catalyst (25). The mercaptan is used in excess and the unused portion is conveniently determined by iodometric analysis. The usefulness of this method is again limited to terminal epoxides because of the nucleophilic nature of the reagent.

The selectivity with which the basic reagents attack terminally located oxirane groups and ignore internally placed epoxide functions might well form the basis of an analytical method which measures terminal epoxides in the presence of internal oxirane compounds. Apparently such a procedure has not yet been devised.

An analytical procedure which perhaps is not of great importance in the assay of epoxidized fats, but which is of interest for its own sake, is one reported by Gunther and co-workers (26). The compound analyzed, an acaricide, is a sulfite ester of ethylene chlorohydrin and contains no epoxide group at all. However, on treatment with potassium hydroxide this material liberates ethylene oxide which in turn is treated with lepidine in diethylene glycol to form a blue dye having an absorption maximum at 610 mμ. The method is sensitive enough to measure less than 1 μg of ethylene oxide, but the procedure is slow and all reagents must be pure and freshly prepared. Other epoxides also give the blue color.

A general qualitative test for epoxides based on their reaction with periodic acid (27) has been known for almost 12 years, and, in fact, Eastham (28) had previously used dilute aqueous periodic acid to assay ethylene oxide. On paper the direct oxidation of epoxides with periodic seems to be a reaction which has potential application in the analytical field. Our own efforts to develop an analytical procedure based on periodic acid have been disappointing so far, and if others have succeeded, their success has not come to our attention.

Tertiary epoxides present a separate analytical problem since their properties differ considerably from those of other epoxides. The failure of the HBr method to give proper analyses of tertiary epoxides has been illustrated with two examples. Durbetaki (29) reported a procedure which is based on the isomeration of the epoxide to a carbonyl compound with the aid of zinc bromide catalyst followed by gravimetric determination of the 2,4-dinitrophenyl hydrazone of the carbonyl compound. The method has been successful with compounds which could not be analyzed by hydrohalogenation methods.

Many of the chemical methods of analysis which have been discussed have also been applied to analyses of oxirane groups in epoxy resins (30–32). The chemical reactions involved in the analysis of oxirane groups in resin are the same as those in other epoxides, but special means must be employed to deliver the reagent to the reactive site in a cross-linked resin.

Infrared Spectroscopy

Numerous authors have reported the measurement of oxirane content by IR absorption spectra, and such methods may well find application if the concn of a known epoxide or group of epoxides is to be determined repeatedly. However, different epoxides absorb at various wavelengths, and the exact location and intensity of absorption maxima depends very much on the structure of the epoxide in question. Characteristic bands for epoxides have been reported over the entire range from 10.5 to 12.5 μ (33–35). Goddu (36) has reported that terminal epoxides have sharp bands in the 1.65–2.20 μ region in the near-infrared, while Henbest and co-workers (37) have discussed the detection of partially alkylated ethylene oxides of moderate mol wt from characteristic C–H stretching bands in the 3.3–3.4 μ region. Some authors (38,39) reported success with IR absorption methods for the determination of unreacted oxirane groups in resins.

The foregoing has been a summary of some of the analytical methods now available for the determination of oxirane compounds. No effort has been made to provide a complete list of published methods, although the literature to mid-1963 has been scanned. Rather it has been the intention to place various prominent methods in their proper perspective, to point out the deficiencies and pitfalls in the several procedures in common use, and to emphasize the need for a method which is both generally applicable and specific for epoxides.

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Analysis of Cyclopropenoid and Cyclopropanoid Acids in Fats and Oils

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Abstract

The analysis of cyclopropenoid acids may be considered, from a historical standpoint, to have started with the discovery of the Halphen test. Although this test as originally conceived was utilized as a means of detecting adulteration of premium edible oils with cottonseed oil, it has since been shown to be a characteristic test for cyclopropenoid fatty acids and has been adapted with various modifications as a quantitative colorimetric test for these substances. More recently, spectrophotometric methods particularly in the IR region have been applied to the analysis of these substances. The 9.8 μ band, characteristic of the cyclopropane, and the 9.91 μ band, characteristic of the cyclopropene group, as well as the 11.0 μ band, characteristic of some of the noncyclic degradation derivatives, have been utilized. Gas-liquid chromatography (GLC) has been applied to the methyl esters of cyclopropanoid and hydrogenated cyclopropenoid acids. The reactivity of the cyclopropene ring toward hydrohalogens has been the basis of several analytical methods developed for use with cyclopropene acid-containing oils. Both aqueous and nonaqueous solutions of hydrohalogens have been employed. The hydrohalogenation methods are the most precise methods currently available for these analyses but only GLC has the inherent potential of identifying the specific cyclopropenoid or cyclopropenoids involved.

Introduction

THE OBSERVATIONS of the last decade (11,12,13,15, 23,26,31,41) which show that the occurrence of cyclopropenoid and cyclopropanoid fatty acid moieties in natural products is not as uncommon as once believed, and the current interest in their biological significance have focused attention on the need for reliable methods of detection and estimation. In this discussion the methods have been arbitrarily grouped in two categories: Chemical Methods and Instrumental Methods. Cyclopropanoid analytical methodology will not be considered separately because of the very limited work reported in this area, but will be discussed when appropriate along with cyclopropenoid methods.

The determination of cyclopropenoid fatty acids may be considered from a historical standpoint to date back to 1897 with the disclosure of the Halphen test (19). This colorimetric test was originally believed to be specific to cottonseed oil and was employed to detect the presence of cottonseed oil as an adulterant in premiumn-type edible oils. This test has more recently been observed with numerous other oils derived from seeds or fruits of the *Malvaceae, Sterculiaceae, Tiliaceae,* and *Bombacaceae* families (5, 11, 38a). Faure (15) showed that a positive Halphen test was observed with sterculic acid, the predominant constituent acid moiety in *Sterculia foetida* oil. This acid was characterized by Nunn (36) as a C_{19} acid containing a cyclopropenyl group involving the ninth and tenth carbon atoms of the aliphatic chain. The analogous cyclopropanyl derivative, dihydrosterculic acid, does not give a Halphen test (4a,9,41a). These facts established that the Halphen reaction is characteristic of the cyclopropenyl group in this acid. Other naturally occurring acids of this type which give a positive Halphen test are malvalic and bombacic acids (9, 31, 40, 41).

Chemical Methods

Halphen Test

The conventional Halphen color test (38) calls for heating for several minutes at 75-80C a mixture consisting of two parts of the oil under examination, one part of amyl alcohol, and one part of a 1% solution of sulfur in carbon disulfide followed by a ½ hr heating period at 110-115C. The color developed at low cyclopropenoid levels, 0.1 to 1% malvalic acid, may range from orange to red (26), frequently even in replicate tests on the same oil (34). Variation of the color intensity is also a frequent occurrence. Although these variances do not pose any problem in a qualitative test, they make the method highly unreliable, as Mehlenbacher (34) showed, in quantitative applications. The color developed with high cyclopropenoid concns, on the other hand, is so intense that visual differentiation is virtually impossible unless a dilution approach is used with the color-reaction product. Such a method, reported by Shenstone et al. (41), involved an adjusted dilution

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