Quantitative Determination of Methyl Octadecadienoate Hydroperoxides by Infrared Spectroscopy[1]

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ABSTRACT

The determination of peroxide values has been used for many years to estimate the concentration of the -OOH group in hydroperoxides formed during autoxidation of unsaturated fatty acid esters. Generally a considerable amount of a sample is required for this determination. The thiobarbituric acid test for estimating oxidative deterioration in various fats has also been used but mainly to determine malonaldehyde in an oxidized sample. From the IR spectra of fatty acid methyl esters, -OOH groups can be detected, but not estimated in general, because of association of the -OOH groups by hydrogen bonds. The present study shows that the -OOH group, even in small amounts of intact methyl octadecadienoate hydroperoxides, can be quantitatively determined by IR spectroscopy using a 1 cm cell (100 times the depth of usual cells) and a solution usually of concentration 1:100. Thus, a new quantative determination method for the -OOH group by IR spectroscopy has been developed.

INTRODUCTION

Polyenes such as diene, triene or others more highly unsaturated play the leading part in the autoxidation of lipids. The rate of autoxidation of monoenes is extremely slow at room temperature but if dienes are present in lipids, the rate of autoxidation is very fast. Therefore, when studying the autoxidized product of lipids, dienes should be selected as the standard substrate.

In order to estimate the -OOH group in hydroperoxides forming during the autoxidation of unsaturated fatty acid esters, the peroxide value is usually determined. Generally a considerable amount of sample is required for this estimation. The thiobarbituric acid method permits estimation with small amounts of a sample, but mainly malonaldehyde is determined. In order to develop a method for the quantitative determination of the -OOH group in lipids using a small amount of an intact sample, the estimation of the -OOH group in methyl octadecadienoate hydroperoxides has been made by IR spectroscopy. Detection of the -OOH group in lipids by IR spectroscopy has been reported (1-10), but its quantitative determination has never been presented. A study of a method to quantitatively determine the -OH group concentration in methyl ricinoleate by IR spectroscopy has been reported (11). The detection of the -OOH group in t-butyl hydroperoxide, which is not a lipid but has steric hindrance, was carried out by IR spectroscopy (12).

[1]Presented at Ninth ISF Congress, Rotterdam, September 1968.

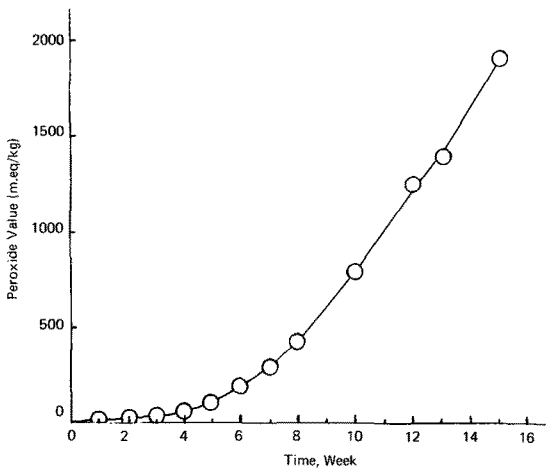

FIG. 1. Change with lapse of time for peroxide value of autoxidized methyl linoleate.

EXPERIMENTAL PROCEDURES

Materials

To prepare the methyl linoleate, safflower oil fatty acid methyl esters were obtained by the saponification of safflower oil followed by the esterification of safflower oil fatty acids with 2% sulfuric acid in methanol. Purified methyl linoleate was prepared by urea adduct separation of the methyl esters at room temperature (methyl linoleate was present in the filtrate), and further at -20 C (methyl linoleate existed in the urea adduct and the natural antioxidants were removed). This ester was further treated by elution chromatography (adsorbent: Mallinckrodt silica gel, 100 mesh, dried at 90 C for 3 hr; elution solvent, n-hexane). The purity of methyl linoleate prepared as above was 99% by gas liquid chromatography (GLC).

Autoxidation

Methyl linoleate, placed in 20 glass dishes (diameter 2.8 cm) to an oil depth of 2 mm was autoxidized at -3 to 0 C in a refrigerator. Infra-red spectra (cell, 0.1 mm, NaCl; solvent, carbon tetrachloride), UV spectra (for the determination of conjugated diene content) and peroxide values were generally determined at intervals of one week.

The paper on the concentration of hydroperoxides from autoxidized methyl linoleate (13) served as a reference for selecting the experimental conditions for a countercurrent distribution method to concentrate the hydroperoxides in this study. Methyl octadecadienoate, 25.2 g with a peroxide value of 870 (meq/kg), was dissolved in 300 ml of n-hexane, and the solution obtained was mixed by shaking successively with each 150 ml of ethanol in 10 separatory funnels. To prepare the n-hexane and ethanol, n-hexane, ethanol and water (40:40:7) were shaken to obtain a state of equilibrium and then separated. New n-hexane was shaken with the first ethanol layer in the first separatory funnel, and successively with the other ethanol layers. The ethanol layers successively shaken with n-hexane layers 11 times in this manner were used as the solutions containing the hydroperoxides. The ethanol layers were divided into three fractions: fraction 1, the ethanol layer in separatory funnel 1; fraction 2, the layers in separatory funnels 2-5; and fraction 3, the layers in separatory funnels 6-10. Water was added to the fractions and hydroperoxides were extracted from them with ethyl ether. After dehydrating the ethyl ether extracts with anhydrous sodium sulfate, ethyl ether was removed at below 30 C under vacuum. The weights of hydroperoxide concentrates (A, B and C)

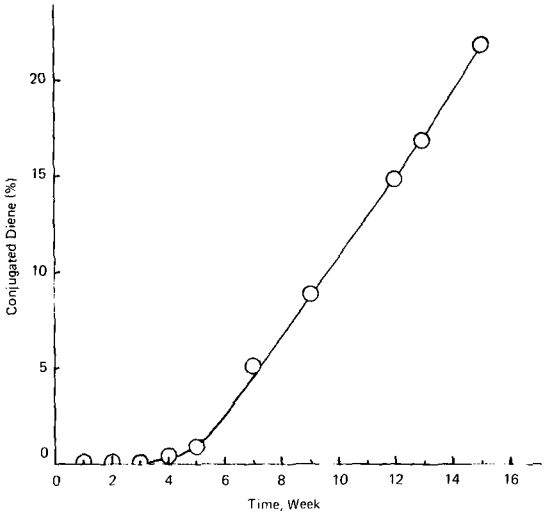

FIG. 2. Change with lapse of time for conjugated diene content of autoxidized methyl linoleate.

obtained from the three fractions mentioned above were 1.0 g, 1.1 g and 1.3 g respectively.

Separation of Hydroperoxides

The paper on the separation of hydroperoxides from autoxidized methyl linoleate (14) was used as reference. The peroxide concentrate (A), 0.87 g, was treated by liquid-partition chromatography. About 120 g of 100 mesh Mallinckrodt silica gel were dried at 119-120 C for 14 hr. The gel was divided into six portions. To each portion 16 ml of immobile solvent, 20% methanol in benzene (v/v), were added and thoroughly mixed in a mortar. These treated silica gel portions were united. A volume of 200 ml of mobile solvent, 2% methanol in benzene (v/v), was added to slurry the silica gel. The slurried mixture was poured into a 53 mm i.d. column previously containing mobile solvent. The sample, 0.8 g, was dissolved in 5 ml mobile solvent and added to the column. It was developed and eluted with 600 ml mobile solvent and finally eluted with 200 ml ethyl ether. From the 10 ml fractions obtained in receiving tubes, solvents were removed at 20-25 C under vacuum. The partition chromatogram was obtained from the weight difference of each receiving tube. In this chromatography the recovery ratio of the sample was 94.8%. Fractions 1, 2 and 3 were designated for eluting solvents 280-320 ml, 350-440 ml, and 670-690 ml respectively. The weight per cents of fractions 1, 2 and 3 not containing solvents were 27, 65 and 7% respectively.

Quantitative Determination of Methyl Octadecadienoate Hydroperoxides

Infra-red spectra were determined of methyl octadecadienoate hydroperoxides obtained from autoxidized methyl linoleate (at -3 to 0 C) by countercurrent distribution and liquid-partition chromatography. The conditions were as follows: IR spectrophotometer, Shimazu IR-27 type; cell, 0.997 cm NaCl; solution, 0.5-1.5 g/liter CCl_4.

RESULTS AND DISCUSSION

Changes with lapse of time for peroxide value and conjugated diene content of autoxidized methyl linoleate are shown in Figures 1 and 2. Chromatogram obtained by liquid-partition chromatography following the countercurrent distribution about methyl octadecadienoate hydroperoxides is given in Figure 3.

The peroxide value for fraction 2 in Figure 2 was 6,110 meq/kg. The calculated peroxide value for methyl octadecadienoate monomeric monohydroperoxide is 6,107

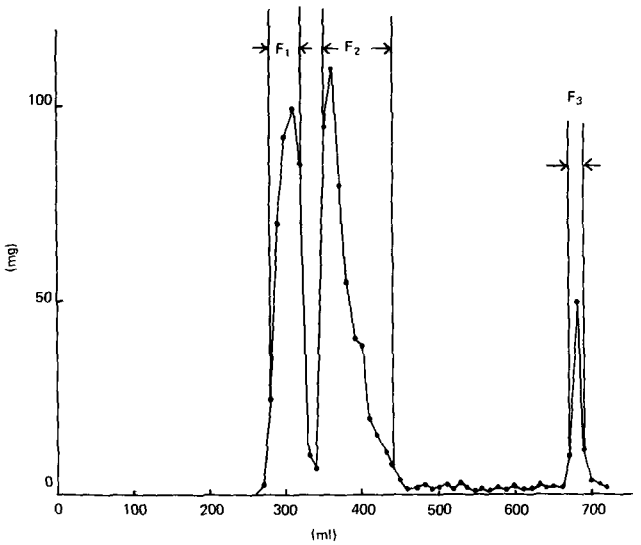

FIG. 3. Liquid-partition chromatogram for methyl octadecadienoate hydroperoxides concentrate (A). (F_1 = fraction 1; F_2 = fraction 2; F_3 = fraction 3).

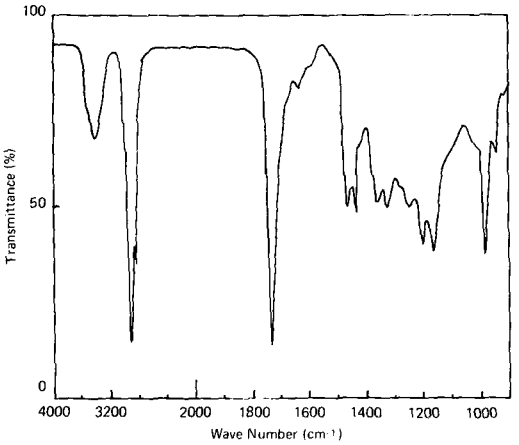

FIG. 4. Infrared spectrum for fraction 2 in Figure 3.

meq/kg. The IR spectrum of fraction 2 (cell, 0.1 mm NaCl; solvent, CCl_4) is given in Figure 4. The -OOH group absorbs at 3450 cm^{-1} (1-3). This band is shifted to the lower wave number by the hydrogen bonding of the -OOH groups.

All compounds containing ester carbonyl groups show a characteristic absorption band at 1741 cm^{-1} of the IR spectrum. If saturated aldehydes and ketones are contained in these compounds, new absorption bands due to these carbonyl groups appear at 1725 cm^{-1} (15) and 1710 cm^{-1} (16) respectively. Therefore compounds containing not only esters but also aldehydes and ketones show a band at 1741 cm^{-1} which is broad in its right side, or sometimes new shoulders or bands appearing at 1725 cm^{-1} and 1710 cm^{-1} respectively. The absorption band at 1741 cm^{-1} for the hydroperoxide sample in Figure 4, which is attributed to ester carbonyl groups, is almost the same in the broadness in its right side as that for methyl linoleate. The band is unaccompanied by shoulder appearing in 1725-1710 cm^{-1}. That is, the absorption bands at 1725 cm^{-1} and 1710 cm^{-1} due to aldehydes and ketones respectively were practically nonexistent. Methyl linoleate, autoxidized at about 0 C in the dark, with a peroxide value of 870 (meq/kg), was carefully kept from heat, air and light in subsequent treatments. The hydroperoxides fraction thus obtained contains almost no aldehydes and ketones, as mentioned above. But, in general, autoxidized samples may often contain aldehydes and ketones. The region of an absorption band attributed to the hydroperoxy groups is

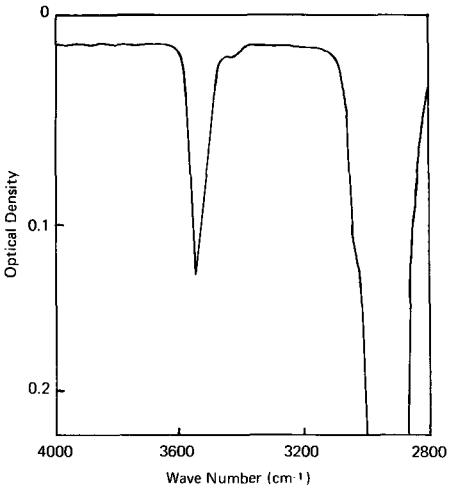

FIG. 5. Infrared partial spectrum for methyl octadecadienoate monomeric monohydroperoxide in a 0.997 cm NaCl cell in 1.5 g/liter carbon tetrachloride solution.

far from that due to aldehydes and ketones. Though a large quantity of ester carbonyl groups exists under these conditions for the IR spectrum determination, there is almost no hydrogen bonding between hydroperoxy and ester carbonyl groups, as mentioned below and shown in Figure 5. Therefore a large quantity of ester carbonyl group has no effect on the determination of hydroperoxy groups in this experimental condition. Even if aldehydes and ketones, which are similar to ester carbonyl groups in electron-attracting power, exist, no hydrogen bonding between hydroperoxy groups and aldehydes or ketones may be formed under these experimental conditions. Consequently the existence of aldehyde and ketone groups may have no effect on this determination of hydroperoxy groups.

As mentioned above, for fraction 2 the peroxide value is equal to the calculation value, and the secondary oxidation products such as aldehydes and ketones are almost undetectable. Therefore fraction 2 is recognized as methyl octadecadienoate monomeric monohydroperoxide. In the hydroperoxide, absorption bands appear near 988 cm^{-1} (the band at 988 cm^{-1} due to *trans, trans* conjugated diene (δ_{C-H}) (17)) and at 948 cm^{-1} (the bands at 982 cm and 948 cm^{-1} ascribed to *cis, trans* conjugated diene (δ_{C-H}) (17)). In our case, as shown in Figure 4, the absorption bands at 982 cm^{-1} and 988 cm^{-1} overlap one another (18) and the overlap band is stronger than the band due to *cis, trans* conjugated diene alone (18). So not only *cis, trans* conjugated diene, but also *trans, trans* conjugated diene exists in considerable quantity (18). Moreover, a very weak absorption band at 913 cm^{-1} ascribed to nonconjugated *cis* double bonds (17) is detectable. The object of this study is the determination of the -OOH group, therefore conjugated dienes and the like are not further discussed.

Quantitative Determination for Methyl Octadecadienoate Hydroperoxides by IR Spectroscopy

Infra-red spectrum for methyl ricinoleate in a 1 cm NaCl cell in carbon tetrachloride solution (2.33 g/liter) showed a sharp absorption band at 3625 cm^{-1} due to monomeric -OH group (11). Under similar conditions in t-butyl hydroperoxide a sharp absorption band at 3554 cm^{-1} ascribed to monomeric -OOH group (12) appeared. But in this case, in the range of concentration above 1.8 g/liter the Beer-Lambert law does not apply. In the carbon tetrachloride solution of methyl octadecadienoate hydroperoxide, a sharp absorption band at 3550 cm^{-1} did not shift in a 0.997 cm^{-1} NaCl cell in the concentration range 0.5-1.5 g/liter. No new absorption bands appeared at other wave numbers.

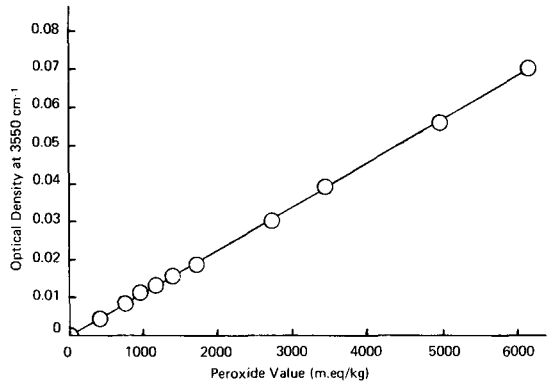

FIG. 6. Relation between optical densities at 3550 cm^{-1} and peroxide values for the mixture of methyl octadecadienoate monomeric monohydroperoxide and methyl linoleate. (Cell, 0.997 cm NaCl; solution, 0.8 g/liter carbon tetrachloride).

A very weak absorption band at 3450 cm^{-1} was detectable. Therefore the -OOH group does not appreciably associate by hydrogen bonding in concentrations at least below 1.5 g/liter. The very weak absorption band at 3450 cm^{-1} is ascribed to a very small quantity of associated -OOH groups by hydrogen bonding. But, as mentioned, the linear relation between optical densities at 3550 cm^{-1} and peroxide values was recognized. The very weak absorption band at 3450 cm^{-1} did not hinder the practical analysis. The sharp absorption band at 3550 cm^{-1} is recognized as the monomeric -OOH group in lipids. Besides, it was confirmed that in the concentration range below 1.5 g/liter in the determination, the Beer-Lambert law (the linear relation between optical density and concentration of the solution) is valid. The IR partial spectrum for methyl octadecadienoate monomeric monohydroperoxide in a 0.997 cm NaCl cell in 1.5 g/liter carbon tetrachloride solution is given in Figure 5. The relation between the optical density at 3550 cm^{-1} in 0.997 cm NaCl cell in 0.8 g/liter carbon tetrachloride solution and the peroxide values for the samples (the mixture of methyl octadecadienoate monomeric monohydroperoxide and methyl linoleate) is shown in Figure 6.

The linear relation between the optical densities and the peroxide values has been demonstrated. In addition, the validity of the Beer-Lambert law in the concentration below 1.5 g/liter has been confirmed.

By substituting the optical density, 0.132_3, for methyl octadecadienoate monomeric monohydroperoxide (peroxide value, 6,110 meq/kg; calculated, 6,107 meq/kg), the concentration 1.5 g/liter, and the depth of the cell 0.997 cm, for the Beer-Lambert relationship a=kcl (a, optical density; k, specific absorption coefficient; c, concentration of sample, g/liter; l, depth of cell, cm), k=0.0885 was obtained. Therefore, the peroxide value for the sample having the specific absorption coefficient k' at 3550 cm^{-1} in IR spectrum (cell, 1.000 cm; concentration, below 1.5 g/liter CCl_4) can be expressed by the following relation:

$$\text{Peroxide value} = \frac{k'}{0.0885} \times 6{,}107 \text{ (meq/kg)} \qquad \text{(I)}$$

The hydroperoxy group content can be obtained from the relation:

$$\text{-OOH group content} = \frac{k' \times 10.08}{0.0885} \text{ (wt \%)} \qquad \text{(II)}$$

By determining the IR absorption spectrum for a sample under conditions mentioned above, the hydroperoxide value (meq/kg) and the hydroperoxy group content (-OOH, wt %) can be obtained from the relation (I) and the relation (II) respectively (in either case, number of significant

figures should be generally three).

Lipids containing hydroperoxides are readily autoxidized. In different experimental laboratories, even in the same original sample, the properties of these samples—for instance, quantity of hydroperoxy groups—are generally different from one another.

The specific extinction coefficient of hydroxyl groups in pure methyl ricinoleate at 3625 cm^{-1}, determined with the apparatus mentioned above, is 0.0680, almost equal to the value reported in the literature (11). But it is probable that the value of the specific extinction coefficient of hydroperoxy groups in a sample changes if the same IR spectrometer and experimental condition are not used. Accordingly, the specific extinction coefficient of hydroxyl groups in pure methyl ricinoleate at 3625 cm^{-1} (k_o) should be determined by the same apparatus and under the same experimental conditions as the coefficient of hydroperoxy groups in a sample. And then the value obtained from relation (I) or (II) should be multiplied by the following correction coefficient:

$$\frac{0.0680}{k_o}$$

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[Received June 1, 1971]