Monomeric Dihydroperoxide Concentrates from Autoxidized Methyl Docosahexaenoate

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Abstract

An amount of 97% methyl docosahexaenoate (obtained from mackerel pike oil) was prepared, and then its autoxidized products were concd by a countercurrent extraction procedure. By partition chromatography, the autoxidized concs were separated into three fractions. From the first fraction, methyl docosahexaenoate monomeric dihydroperoxide concs were obtained for the first time, though the presence of the dihydroperoxide had been ascertained by the authors before. The dihydroperoxide concs were identified by determining the peroxide value, the mol wt, and UV and IR spectra. From IR data it was confirmed that weak absorptions due to α-methylene groups and C = C stretching vibrations, respectively, were present in the concs. The *trans-trans* conjugated diene and *cis* nonconjugated double bond absorptions appear as relatively strong bands, but the *cis-trans* conjugated one is weak. Therefore, the molecules of the concs may have relatively symmetrical structures. The second fraction eluted after the first one contains the products to which the dihydroperoxides have changed on the chromatographic column.

Introduction

ALTHOUGH THE HYDROPEROXIDE concs initially formed as major products of autoxidation of unsaturated fatty acid esters have been extensively studied (e.g., see reference 1), there are only the authors' reports on the hydroperoxides concs from autoxidized, highly-unsaturated esters (2~5). Unchanged hydroperoxide concs were obtained by separating autoxidized, highly-unsaturated methyl esters by a countercurrent distribution procedure (2~4), but it was difficult to separate them sufficiently. However, for the first time, it was ascertained that dihydroperoxides were present in such oils (but not separated) (2~4), a finding not previously reported. Monohydroperoxides were obtained by fractionating autoxidized, highly-unsaturated acid methyl esters by partition chromatography using silica gel as a carrier and methanol-benzene as a solvent, but in that case, dihydroperoxides were not found (5). However, the hydroperoxides changed on a chromatographic column were separated (5).

In this study, almost pure methyl docosahexaenoate obtained from mackerel pike oil was autoxidized, the oxidation products were concd by a countercurrent distribution procedure, and the concd peroxides were then fractionated by partition chromatography. The peroxide values, and UV and IR spectra were determined on the fractions. This study was made in expectation of being able to conc for the first time the unchanged monomeric dihydroperoxides, in addition to the changed hydroperoxides reported in the previous paper (5).

Experimental

Autoxidation of Methyl Docosahexaenoate

Fatty acids obtained by saponifying mackerel pike oil and by acidifying the soap, were dissolved in *n*-hexane, cooled, and the crystallized portion was removed. Then unsaturated mackerel pike oil fatty acids were prepared from the filtrate. The fatty acid methyl esters, obtained by esterifying the fatty acids with methanol using sulfuric acid as a catalyst, were treated four times by the urea adduct procedure (6). The highly unsaturated methyl esters were obtained from the fourth filtration. These highly unsaturated methyl esters were distilled in vacuum to remove a first fraction. The major second fraction was fractionally distilled in vacuum with a refluxing tube (7). Each fraction was assayed by gas chromatography (stationary phase: diethylene glycol polysuccinate, column temp: 230C), and subfractions were collected. Methyl docosahexaenoate (97%, estimated by gas chromatography) had a peroxide value of 83.9 (mEq/kg), and contained 4.2% conjugated dienes evaluated on the basis of UV spectral data.

Methyl docosahexaenoate was autoxidized by keeping 30 g of it in the dark at −2−1C in a loosely stoppered 100-ml Erlenmeyer flask. The sample was shaken every 2 hr in the daytime and left standing at night. The rate of autoxidation was slow, the methyl ester having a peroxide value of 7.18×10^2 after 21 days. Then, the product was transferred to a 200 ml Erlenmeyer flask to decrease the depth of the oil layer in order to increase the rate of autoxidation. After 33 days the methyl ester had a peroxide value of 1.56×10^3, and a specific extinction coefficient of 11.5 at 235 mμ on the basis of UV spectral data. This ester was used as the sample of autoxidized methyl docosahexaenoate.

Concentration of the Peroxides by Countercurrent Distribution (8)

The solvents were prepared by thoroughly mixing 40 parts of *n*-hexane, 40 parts of absolute ethanol, and 7 parts of distilled water, and by allowing the mixture to equilibrate and separate. A 28.2 g quantity of the autoxidized methyl docosahexaenoate was dissolved in 300 ml of the *n*-hexane portion of the equilibrium mixture. This solution was shaken with successive 150 ml portions of the ethanol portion in 10 separatory funnels. New *n*-hexane was shaken with the first portion of ethanol in the first separating funnel, and then successively with the other portions of ethanol. The fractions extracted with *n*-hexane 11 times like this were designated as the solutions containing peroxides. The portions were combined into three fractions; that in funnel No. 1, that in funnels No. 2–5, and that in funnels No. 6–10. Each fraction was diluted with water in a separating funnel, and then shaken with ether to extract peroxides. Anhydrous sodium sulfate was added to the ether extracts, and after standing overnight, the extracts were filtered. Ether was removed in vacuum at below 30C. Peroxide concs thus obtained

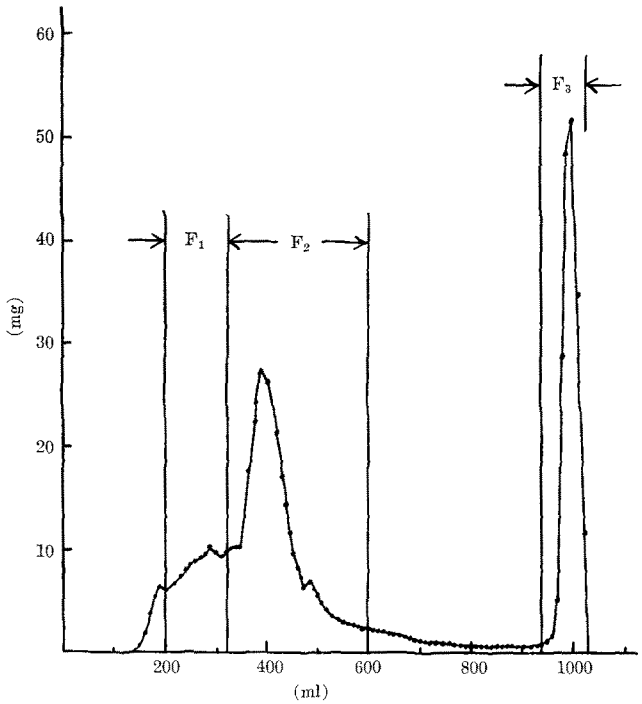

FIG. 1. Partition chromatogram of peroxide concs (I) of methyl docosahexaenoate. F_1: fraction 1; F_2: fraction 2; F_3: fraction 3.

from the three fractions of ethanol were designated (I), (II) and (III). These yields were: (I), 1.1 g; (II), 1.5 g; and (III), 1.2 g. Solvent was removed in vacuum at below 30C from the *n*-hexane layers, yielding 23.1 g of a less oxidized fraction.

Separation of Peroxide Concentrates by Partition Chromatography

The peroxide concs were separated into many 10 ml fractions by the method of Frankel (9). The solvent in the tubes was removed in vacuum at 20–25C. The weights of the peroxide concs were determined by difference, and then the chromatogram was plotted.

Experimental Results and Discussion

The partition chromatogram of peroxide concs (I) of methyl docosahexaenoate is shown in Figure 1. The partition chromatograms of peroxide concs (II), and (III) were omitted. These concs failed to give sufficient information because of the small quantities present.

The amt of peroxide concs (I) applied to the column was 690 mg, of which 94.9% was recovered. The weight per cent of the fraction 1 was 18.6%, its mol wt (cryoscopic method) was 390, its peroxide value (mEq/kg) was 9.92×10^3, and its specific extinction coefficient at 235 mμ was 24.0. Weight per cents, mol wts, peroxide values (mEq/kg), and specific extinction coefficients at 235 mμ, of fraction 2 and 3 were 43.0%, 660, 8.52×10^3, 28.9 and 26.7%, 670, 6.95×10^3, 17.1, respectively. It was ascertained that the fraction 1 of peroxide concs (I) is monomeric on the basis of mol wt. Besides, its peroxide value (mEq/kg) 9.92×10^3 is close to the theoretical peroxide value for monomeric dihydroperoxide of methyl docosahexanoate, 9.840×10^3. From the UV spectrum (Fig. 2) and the IR spectrum [5% (w) carbontetrachloride solution, 0.1 mm NaCl cell] (Fig. 3) of the fraction 1, it appears that secondary reaction products (aldehydes, etc.) are scarcely present, and there is no evidence of absorption at 1790–1740 cm^{-1} due to peracids (10,11).

If α, β-unsaturated carbonyl had been present, the absorption band at 260–280 mμ would have been evident. If aldehydes, α, β-unsaturated aldehydes, and α,β-unsaturated ketones had been present, the absorption bands at 1725 cm^{-1}, 1705–1680 cm^{-1} and 1685–1665 cm^{-1} would have been evident, in the IR spectrum. None of these absorption bands were distinctly observed.

Since peracids are not present in the fraction 1, it may be assumed that the peroxide value in this case is due to hydroperoxides only. As mentioned above, the peroxide value of the fraction 1 is almost the same as the theoretical peroxide value of the monomeric dihydroperoxide. Therefore, this fraction can be designated as dihydroperoxide conc of methyl docosahexaenoate.

The absorption at 3450 cm^{-1} due to the −OOH group (12–14) in fraction 1 is remarkably large, but not as large as that reported in previous papers (2–4). The difference may be attributed to the fact that in this study IR spectra were determined for solutions, while in the previous reports they were determined for thin films, and also that the peroxide value is smaller in this paper than in the previous ones. The absorption at 3020 cm^{-1} due to α-methylene group

$$\begin{matrix} H & & H\ H\ H \\ (-C- & \text{in} & -C=C-C-) \\ H & & H \end{matrix}$$

(12–14) is very small. As previously reported (2), absorption due to α-methylene groups may be decreased because of the disappearance of α-methylene groups due to the formation of

$$\begin{matrix} H\ H\ H\ H\ H \\ -C=C-C-C=C- \\ \quad\quad | \\ \quad\quad OOH \end{matrix}$$

or conjugated double bonds (2).

In addition, oleic acid has two α-methylene groups, but absorption due to them is absent in IR spec-

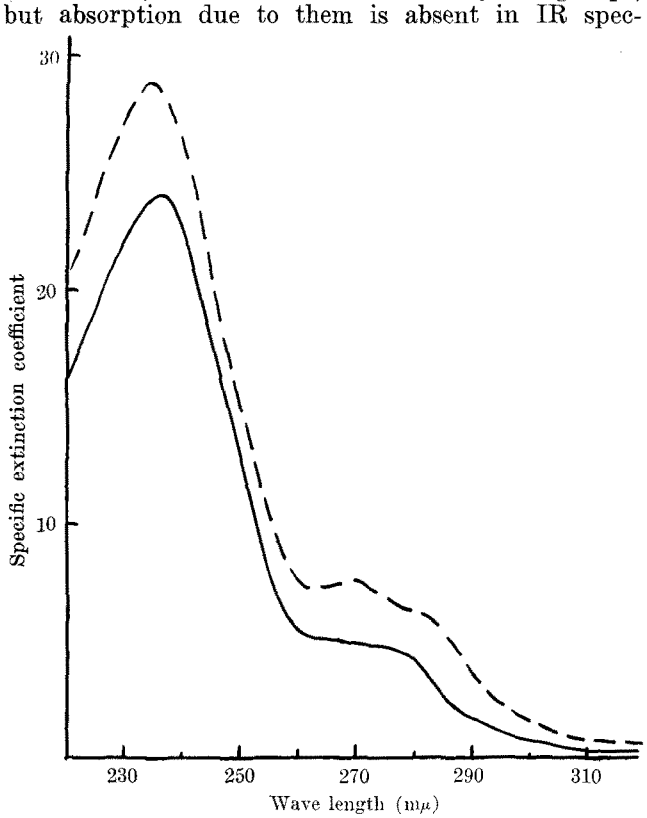

FIG. 2. UV spectra of fractions 1 and 2 of methyl docosahexaenoate peroxide concs (I)———fraction 1.— — — fraction 2.

tra with NaCl prism (2). Therefore, in the fraction 1, α-methylene groups not between two adjacent double groups (different from the $-\overset{H}{\underset{H}{C}}-$ group in $-C=C-\overset{H}{\underset{H}{C}}-C=C-$; for example, like the $-\overset{H}{\underset{H}{C}}-$ groups in $-C=C-C-\overset{H}{\underset{H}{C}}-C=C-$), may be present in large numbers.

The absorption at 1741 cm^{-1} due to the C=O stretching is almost the same as in fraction 1 and in methyl docosahexaenoate (its showing was omitted). The band at 1708 cm^{-1} due to free fatty acids (15), and the absorption at 1725 cm^{-1} due to aldehyde are not distinct, but small shoulders are present and the absorption becomes broad. The absorption band at 1660 cm^{-1}, arising from C=C stretching (15), almost disappears in the fraction 1 of conc (I). The absorption at 988 cm^{-1}, due to *trans-trans* conjugated diene (16), are quite evident. The band at 982 cm^{-1} (united with the band at 988 cm^{-1}), arising from *cis-trans* conjugated diene (16), and the characteristic absorption at 948 cm^{-1} due to *cis-trans* conjugated diene (16) are also evident. Therefore, it may be assumed that double bonds are abundant. The band due to C=C stretching almost disappears, owing to the relatively symmetrical structure (against double bonds) of fraction 1. Both the peroxide value and the absorption band at 3450 cm^{-1} due to the −OOH group of fraction 2 are smaller than those of the fraction 1. Therefore, the number of the −OOH groups of fraction 2 is smaller than that of fraction 1. The mol wt of fraction 2 is larger than that of the fraction 1, so fraction 2 contains polymers.

However, in Figure 2, the specific extinction coefficient at 235 mμ due to conjugated diene of fraction 2 is larger that of the fraction 1. The increase of conjugated dienes and the decrease of −OOH groups may be explainable, by conjugation of diene on the partition chromatogram, such as

$$-C=C-C-C=C-C-C=C- \;(H,\ OOH) \rightarrow -C-C=C-C=C-C-C=C- \;(H,\ OOH)$$

[(in the

$$-C=C-C-C=C- \;(OOH)$$

type hydroperoxides (2–4)], and by the formation of the $-C-C-C=C-C-$ groups from a part of

$$-C=C-C=C-C- \;(HOO)$$

compounds. In fraction 2, in fact, the absorption at 1100 cm^{-1} increases (19), giving tentative proof to the above-mentioned assumption. The increase of the mol wt of the fraction 2 might be due either to polymerization on the partition chromatographic column, or to concn of the polymers contained in the peroxide concs (I). However, the specific extinction coefficient at 235 mμ of the polymer is generally smaller than that of the monomer (20). But this absorption by fraction 2, containing polymers, is larger than that of fraction 1 containing no polymer. This fact may show the increase of conjugation on the above-mentioned chromatographic column. Therefore, fraction 2 probably contains substances changed on the chromatographic column. Besides, the absorption at 268 mμ due to conjugated triene is present (18) in fraction 2 shown in Figure 2.

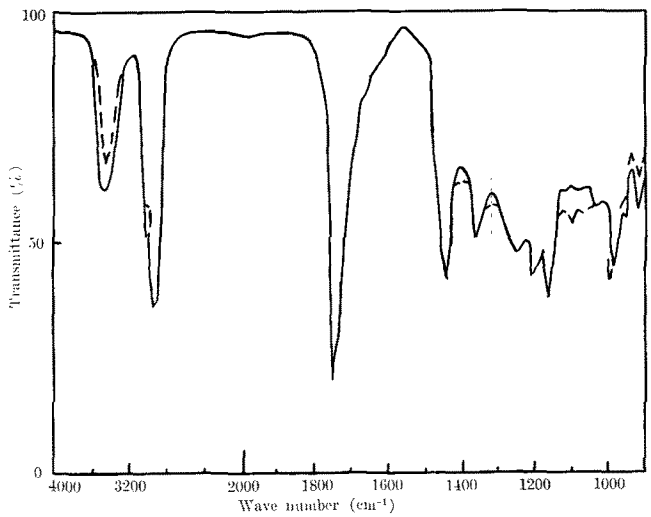

FIG. 3. IR spectra of fractions 1 and 2 of methyl docosahexaenoate peroxide concs (I).———fraction 1.— — — fraction 2.

The peroxide concs of this study contain many −OOH groups. The immobile liquid phase does not always perfectly cover the surface of silica gel. So when partition chromatography is used, a part of the silica gel and peroxides in the mobile solvent might contact each other. When the −OOH groups contact silica gel, decomposition and dehydration takes place as mentioned below. It is assumed that conjugated triene may be formed thus: $-C=C-C=C-C(OOH)(H)-C-C- \rightarrow$

$$-C=C-C=C-C(O\cdot)(H)-C-C- + \cdot OH;$$

$$-C=C-C=C-C(O\cdot)(H)-C-C- + RH \rightarrow$$

$$-C=C-C=C-C(OH)(H)-C-C- + R\cdot;$$

$$-C=C-C=C-C(OH)(H)-C-C- \rightarrow$$

$$-C=C-C=C-C=C- + H_2O.$$

The appearance of the conjugated triene in fraction 2 also takes place by the accumulation of the conjugated triene contained in the concs (I). Increase of conjugated diene, and appearance of conjugated triene

and cyclic peroxides decreases the nonconjugated *cis* double bonds. In fact, in fraction 2 (Figure 3), the absorption at 913 cm^{-1} arising from nonconjugated *cis* double bonds is considerably less than in fraction 1 (Fig. 3). It is presumed that, as mentioned above, fraction 2 contains the changed substance of fraction 1. Therefore, the separation of fraction 1 and fraction 2 is not good.

If partition chromatography is used to separate peroxide concs, monomeric dihydroperoxide concs can be isolated, as shown in this paper. But partition chromatography cannot be used to obtain unchanged monomeric dihydroperoxide concs from the autoxidized sample, because the amt of the dihydroperoxides is small. In fact, the monomeric dihydroperoxide concs found in this paper were not found previously (5).

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The Synthesis and Some Surface Active Properties of Alkylthioalkyl and Alkoxyalkyl Sulfates

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Abstract

A series of sodium alkylthio- and alkoxyalkyl sulfates was prepared to determine the effect of the presence, position and nature of the heteroatom on the critical micelle concentration (CMC), the surface activity and detergency of a surfactant. All of the compounds were linear and contained a total of 16 carbon atoms. Hexadecyl-1-sulfate was used as the reference compound.

Insertion of either a sulfur or oxygen atom into the hydrocarbon chain raised the CMC. In the oxygen series, the apparent trend was to a higher CMC as the oxygen atom was moved further away from the sulfate group whereas no trend was observed in the thioether series.

The surface activity of hexadecyl-1-sulfate was higher than either the ether or thioether series. The further the heteroatom from the sulfate group, the lower was the surface activity. This trend was more pronounced in the oxyether series.

All hetero-substituted compounds were generally inferior to hexadecyl sulfate in detergency.

Hydration of the oxygen atom in the oxyethers, but not the sulfur atom in the thioethers is proposed as the explanation for the observed trends.

Introduction

THE MAXIMUM PERFORMANCE of a surfactant molecule is attained at or near the critical micelle concentration (CMC) (1). At this concentration, the addition of fresh surfactant increases only the concentration of surfactant molecules in micellar form and not as discrete monomolecular species. The CMC of a surfactant solution is dependent, to a degree, on the solution's environment (e.g., temperature, ionic strength, etc.) but primarily on the structure of the surfactant. In a homologous series the CMC decreases with an increase in the length of the hydrophobic portion of the molecule (2), the CMC increases with branching on the chain (3,4) or if the hydrophilic group is moved toward the center of the chain (5); increases with polar substitution or unsaturation on the hydrophobe (2) and finally is influenced to a lesser extent by the nature of the hydrophilic group (2).

As Hartley indicated (4), only the single surfactant molecules are surface active and thus micelle formation competes with surface activity. He postulated that if the amphipathic property of the ion could be preserved and at the same time micelle formation inhibited, a greater surface response (in his case, interfacial tension) could be attained. He prepared and determined the CMC and interfacial tension of solutions of various sulfonated dialkyl esters of dihydric phenols and compared these values to those of a sulfonated alkyl ester of *para*-cresol. The former could not fit into the micelle as easily as the latter and thus had higher CMC values, but the latter, relatively straight chain compounds, had greater surface activity. Thus, although Hartley was able to inhibit micellization, he was unable at the same time, to preserve the amphipathic property of the ions.

This research was an attempt to inhibit micellization but *not* change the surface activity of the unsubstituted molecule. We expected to accomplish this by the insertion of either a sulfur or oxygen atom between two methylene groups on the carbon chain.

Sodium hexadecyl-1-sulfate was chosen as the unsubstituted compound, the model for the series. All of the compounds prepared resembled sodium hexadecyl-1-sulfate insofar as they were sodium salts of the sulfuric esters of linear primary alcohols containing 16 carbon atoms but differed from the model by the presence of a sulfur or oxygen heteroatom inserted in the hydrophobe chain. The position of the heteroatom was also varied to determine the effect of its position on the CMC, the surface activity and the cotton detergency of the surfactants.