[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Autoxidation of Methyl Linolenate. Isolation and Characterization of Hydroperoxides[2]

E. N. FRANKEL,[3] C. D. EVANS, D. G. McCONNELL, E. SELKE, AND H. J. DUTTON

Received January 26, 1961

Relatively pure hydroperoxides (I) were isolated by liquid partition chromatography and countercurrent distribution from methyl linolenate autoxidized at 37°. Yields were from 30 to 36% of the oxygen absorbed. I was monomeric, had three double bonds, and had a conjugated diene system with predominantly *cis,trans* and a little *trans,trans* configuration. Reduction of I gave conjugated diene hydroxylinolenate (II) which on acid dehydration yielded the conjugated methyl octadecatetraenoic ester (III). Catalytic hydrogenation of I gave a mixture of methyl 9-, 12-, 13-, and 16-hydroxystearate (IVa, b, c, d). The location of the hydroxyl was determined by boric acid dehydration of IV and by oxidative splitting of the resulting monoenoic esters (V). It is concluded that I is a mixture of methyl 9-, 12-, 13-, 16-hydroperoxyoctadecatrienoate with unsaturation in the 10, 12, 15; 9, 13, 15; 9, 11, 15; and 9, 12, 14 positions, respectively.

The early study of Farmer, *et al.*[4] on the autoxidation of ethyl linolenate showed that, initially, the absorption of one mole of oxygen was accompanied by the formation of one-half mole of conjugated diene. The mechanism suggested was similar to that advanced for methyl linoleate, which involved hydroperoxide formation on an α-methylenic carbon followed by rearrangement of double bonds. According to this mechanism, ethyl linolenate should form free-radical centers on carbons 9, 11, 12, 13, 14, and 16. Bolland and Gee[5] also assumed the mechanism of autoxidation of ethyl linolenate to be similar to that of ethyl linoleate on the basis of similar kinetics and activation energy of the over-all reaction. Autoxidation was considered as a chain reaction forming free radicals that absorb oxygen and attack an olefin to form a hydroperoxide.

More direct evidence on the mechanism of autoxidation has been difficult to obtain. Fugger *et al.*[6] fractionated autoxidized methyl linolenate with a small twenty-nine–tube countercurrent distribution apparatus and concluded that little or no monomeric hydroperoxides were present. Later, Privett *et al.*[7] reported the isolation of monomeric hydroperoxide from autoxidized methyl linolenate by a countercurrent extraction procedure, but yields of hydroperoxide relative to oxygen absorption were not given. The hydroperoxide was reduced with stannous chloride and was estimated to have about 90% *cis,trans* conjugated diene methyl monohydroxy-octadecatrienoate.

Apparently further characterization of methyl linolenate hydroperoxide has not been reported in the literature. Development of liquid-partition chromatographic procedures[8] and improvement in resolution of countercurrent distribution[9] have encouraged reexamination of this problem.

RESULTS

Fractionation of autoxidized methyl linolenate by partition chromatography resulted in complete separation of the unoxidized methyl linolenate from the oxidized products (Fig. 1). Good resolution was also obtained of the hydroperoxide fraction from secondary polar decomposition products of oxidation. These secondary products increased markedly with the level of autoxidation.

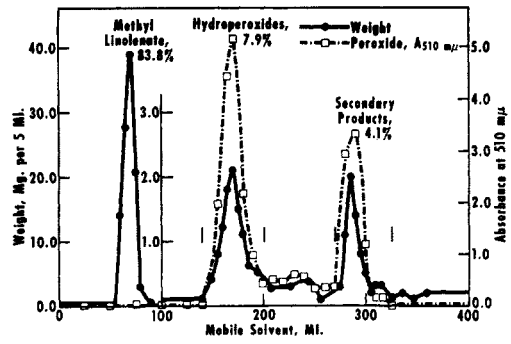


Fig. 1. Chromatographic fractionation of autoxidized methyl linolenate (peroxide value 1540)

(1) This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(2) Presented at fall meeting, American Oil Chemists' Society, New York, N. Y., October 16–19, 1960. Paper No. 42.

(3) Present address: Food Products Division, Winton Hill Technical Center, Procter and Gamble Co., Cincinnati, Ohio.

(4) H. Farmer, H. P. Koch, and D. A. Sutton, *J. Chem. Soc.*, 541 (1943); H. Farmer, *Trans. Faraday Soc.*, **42**, 228 (1946).

(5) J. L. Bolland and G. Gee, *Trans. Faraday Soc.*, **42**, 236 (1946).

(6) J. Fugger, J. A. Cannon, K. T. Zilch, and H. J. Dutton, *J. Am. Oil Chemists' Soc.*, **28**, 285 (1951).

(7) O. S. Privett, C. Nickell, W. E. Tolberg, R. F. Paschke, D. H. Wheeler, and W. O. Lundberg, *J. Am. Oil Chemists' Soc.*, **31**, 23 (1954).

(8) E. N. Frankel, C. D. Evans, H. Moser, D. McConnell, and J. C. Cowan, *J. Am. Oil Chemists' Soc.*, **38**, 130 (1961); E. N. Frankel, C. D. Evans, D. G. McConnell, and E. P. Jones, *J. Am. Oil Chemists' Soc.*, **38**, 134 (1961).

(9) H. J. Dutton, "Countercurrent Fractionation of Lipids," in *Progress in the Chemistry of Fat and Other Lipids*, R. T. Holman, Ed., Pergamon Press, Ltd., London, 1954, Vol. 2, pp. 292–325.

Countercurrent distribution of autoxidized methyl linolenate in a twenty-four-tube apparatus separated the oxidized products from unoxidized methyl linolenate as previously reported[8]; however, no resolution of hydroperoxide from secondary oxidation products was achieved. A 200-transfer fractionation (Fig. 2) completely removed unoxidized methyl linolenate but incompletely separated the hydroperoxide fraction from the secondary decomposition products. The hydroperoxide fraction (tubes 40–75) was shown by partition chromatography to be 79% pure. With the application of 600 transfers complete separation of hydroperoxide from secondary decomposition products was achieved (Fig. 3).

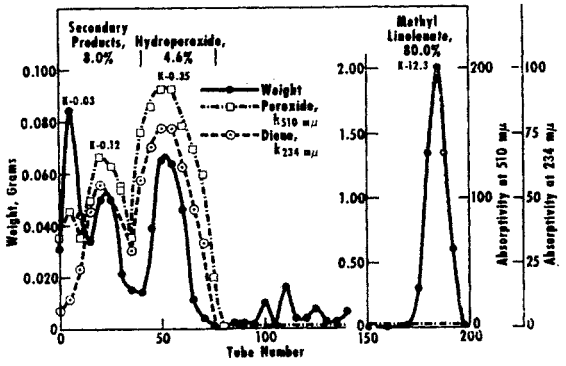


Fig. 2. Countercurrent distribution of autoxidized methyl linolenate (200 transfers)

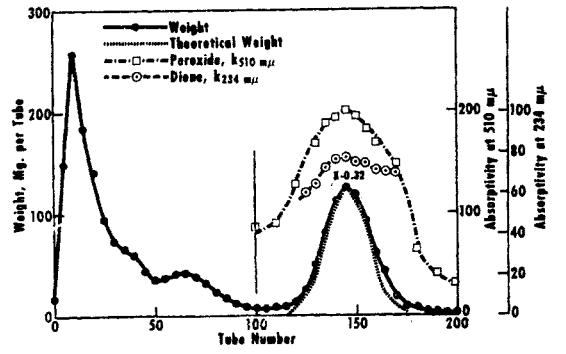


Fig. 3. Countercurrent distribution of oxidized fraction from autoxidized methyl linolenate (600 transfers)

At relatively low levels of autoxidation and at 37°, the yields of hydroperoxides reached a maximum of 36% of the oxygen absorbed. These yields are markedly lower than those reported for methyl linoleate at equivalent levels of autoxidation.[8,10] The concentration of secondary polar decomposition products was correspondingly higher in methyl linolenate than previously found in methyl oleate and methyl linoleate.[8] At higher levels of autoxidation the differences are even more pronounced.

Initially, the absorption of oxygen produced an equal molar concentration of peroxide as determined iodometrically, one-half molar concentration of diene conjugation, and about one-third molar concentration of chromatographically determined hydroperoxide. The divergence between these measurements could be accounted for by the contribution of the secondary decomposition products to the peroxide value and diene conjugation of the autoxidized methyl linolenate (Fig. 2). The relation between oxygen absorption and diene conjugation agrees well with that reported by Farmer *et al.*[4] for the autoxidation of ethyl linolenate at room temperature; however, they suggested the formation of unconjugated forms of hydroperoxide to account for the low yields of conjugated diene.

Some chemical and spectral characteristics of hydroperoxide preparations from autoxidized methyl linolenate are presented in Table I. The peroxide values and diene conjugation agree fairly well with those reported in the literature for methyl linoleate hydroperoxide[10–13] and methyl linolenate hydroperoxide.[7] The lower diene and peroxide concentrations compared to the calculated values may well reflect some decomposition during analytical manipulations, which is indicated by the presence of small concentrations of triene conjugation (1–2%). Quantitative hydrogenation indicates three double bonds. The absorption maximum for diene was at 234–245 mμ (Fig. 4) compared to 232–233 mμ for methyl linoleate hydroperoxide.[10] This difference may suggest the presence of *cis,cis* conjugation in linolenate hydroperoxide and may account for the lower diene conjugation estimated by infrared measurements than that determined by ultraviolet. Infrared analyses show that the conjugated system has a predominant *cis,trans* configuration with some *trans,trans* configuration (estimated to about 4%).[14]

The characterization of isomeric methyl linolenate hydroperoxides (Ia, b, c, d) was based on two sequences of reactions: (1) Reduction with potassium iodide or sodium borohydride to give the corresponding conjugated methyl hydroxylinolenates (IIa, b, c, d). The ultraviolet and infrared spectra of II show that its diene content corresponds to the theoretical value[10] and that it has, like I, the *cis,trans* and *trans,trans* configuration (Fig. 4). Acid dehydration of II resulted in a product containing octadecatetraenoic esters (III) shown by ultraviolet and infrared to be similar to β-parinaric acid (approximately 50%). This acid is a naturally occurring all-*trans* octadeca-9,11,13,-15-tetraenoic acid first described by Kaufmann and

(10) J. A. Cannon, K. T. Zilch, S. C. Burket, and H. J. Dutton, *J. Am. Oil Chemists' Soc.*, 29, 447 (1952).

(11) O. S. Privett, W. O. Lundberg, and C. Nickell, *J. Am. Oil Chemists' Soc.*, 30, 17 (1953).

(12) H. H. Sephton and D. A. Sutton, *J. Am. Oil Chemists' Soc.*, 33, 263 (1956).

(13) A. E. Johnston, K. T. Zilch, E. Selke, and H. J. Dutton, *J. Am. Oil Chemists' Soc.*, 38, 367 (1961).

(14) J. R. Chipault and J. M. Hawkins, *J. Am. Oil Chemists' Soc.*, 36, 535 (1959).

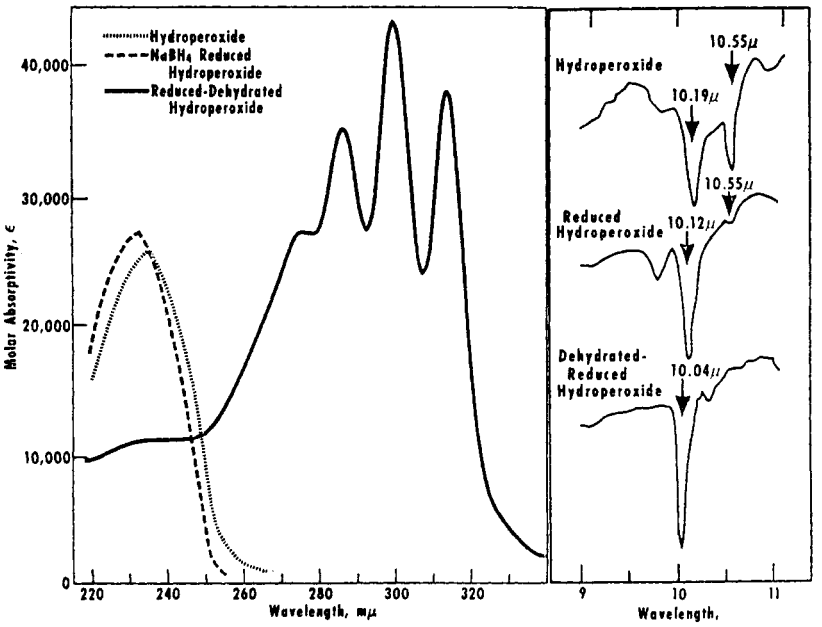


Fig. 4. Ultraviolet and infrared spectra of methyl linolenate hydroperoxides (I), reduced methyl linolenate hydroperoxides (II), and dehydrated reduced methyl linolenate hydroperoxides (III)

TABLE I

CHARACTERISTICS OF METHYL LINOLENATE HYDROPEROXIDE PREPARATIONS

Analyses	Partition Chromatography	Countercurrent Distribution	Theory
Peroxide value (me./kg.)	4,990 (3)[a]	5,040 ± 50 (6)	6,163
Unsaturation[b] (mole H_2/mole ROOH)	—	3.2 ± 0.3 (5)	3
Diene (ϵ_{234} mμ)	23,000 ± 1500 (6)	24,600 ± 500 (6)	26,400[c]
cis,trans ($\epsilon_{10.6}$ μ)	—	59.7 ± 5.1 (5)	79.5[d]
trans,trans ($\epsilon_{10.2}$ μ)	—	81.3 ± 6.0 (5)	374[e]
Molecular weight	—	340 ± 40 (6)	324
Elementary analysis ($C_{18}H_{32}O_4$)	C, 67.9 H, 9.7	—	C, 70.3 H, 10.0

[a] Numbers in parentheses represent the number of preparations analyzed. [b] Corrected for hydrogen absorption from hydroperoxide. [c] Calculated value for methyl linoleate hydroperoxide (9). [d] Conjugated *cis,trans*-methyl linoleate. [e] Conjugated *trans,trans*-methyl linoleate.

Keller.[15] Attempts to purify further this acid were unsuccessful because of its apparent great tendency to polymerize even at temperatures of −10°.

Ia. R_2—$\overset{16}{CH}$=$\overset{15}{CH}$—$\overset{14}{CH_2}$—$\overset{13}{CH}$=$\overset{12}{CH}$—$\overset{11}{CH}$=$\overset{10}{CH}$—$\overset{9}{CH}$—R_1 (OOH on 9)

Ib. R_2—$\overset{16}{CH}$=$\overset{15}{CH}$—$\overset{14}{CH_2}$—$\overset{13}{CH}$—$\overset{12}{CH}$=$\overset{11}{CH}$—$\overset{10}{CH}$=$\overset{9}{CH}$—R_1 (OOH on 13)

Ic. R_2—$\overset{16}{CH}$=$\overset{15}{CH}$—$\overset{14}{CH}$=$\overset{13}{CH}$—$\overset{12}{CH}$—$\overset{11}{CH_2}$—$\overset{10}{CH}$=$\overset{9}{CH}$—R_1 (OOH on 12)

Id. R_2—$\overset{16}{CH}$(OOH)—$\overset{15}{CH}$=$\overset{14}{CH}$—$\overset{13}{CH}$=$\overset{12}{CH}$—$\overset{11}{CH_2}$—$\overset{10}{CH}$=$\overset{9}{CH}$—R_1 (OOH on 16)

↓ KI or $NaBH_4$

IIa. R_2—CH=CH—CH_2—CH=CH—CH=CH—CH(OH)—R_1

IIb. 13-Hydroxy, $\Delta^{9,11,15}$
IIc. 12-Hydroxy, $\Delta^{9,13,15}$
IId. 16-Hydroxy, $\Delta^{9,12,14}$

↓ H_2SO_4 CH_3OH

III. R_2—CH=CH—CH=CH—CH=CH—CH=CH—R_1

R_1 = $-(CH_2)_7-COOCH_3$
R_2 = $-CH_2-CH_3$

The second sequence of reactions was: (2) Catalytic hydrogenation of I to yield a mixture of methyl hydroxystearates (IVa, b, c, d). Dehydration of IV with boric acid at 200–210° gave a mixture of monoenoic esters (Va, b, c, d) with

(15) H. P. Kaufmann and M. Keller, *Chem. Ber.*, 81, 152 (1948).

unsaturation corresponding to the position of the hydroxyl groups. The presence of 38% isolated *trans* unsaturation in V indicates that we are dealing with an equilibrium mixture of *cis* and *trans* monounsaturated esters. Oxidative fission of V with permanganate-periodate yielded a mixture of dibasic acids (VIa, b, c, d) which were analyzed by liquid partition chromatography and gas liquid partition chromatography. These analyses (Table II) show that the original methyl hydroxystearates

TABLE II

DICARBOXYLIC ACIDS FROM $KMnO_4$-KIO_4 OXIDATION OF MONOENOIC ESTERS FROM DEHYDRATED METHYL HYDROXYSTEARATES

Acids	Methyl 12-OH Stearate Found Mole %	Methyl 12-OH Stearate Adjusted Mole %	Methyl OH Stearates of Methyl Linolenate OOH Found Mole %	Methyl OH Stearates of Methyl Linolenate OOH Adjusted Mole %
Stearic acid	18.1	18.1	—	—
Dicarboxylic acids				
C_{16}, C_{15} [a]	—	—	43.0	48.1
C_{13}	2.1	2.3	3.8	4.3
C_{12}	33.8	36.5	9.9	11.0
C_{11}	36.3	39.2	4.6	5.2
C_{10}	2.2	2.4	0.6	0.7
C_9	1.5	1.6	15.0	16.7
C_8	—	—	12.0	13.5
C_7	—	—	0.4	0.5
Total	93.0	100	89.3	100

[a] Identified by gas-liquid chromatography.

consist of a mixture of 9-, 12-, 13- and 16-hydroxy isomers. Methyl 12-hydroxystearate yielded approximately equal amounts of C_{11} and C_{12} monoenes. The concentration of methylhydroxystearates of IV can be estimated as 30.2, 10.7, 9.8, and 48.1% for the 9-, 12-, 13-, and 16-hydroxystearates, respectively, assuming that an equal amount of monoenes is formed on dehydration on each side of the carbon atoms with hydroxyl groups. It is concluded that the hydroperoxide groups of I occupy the same positions as the hydroxyl groups obtained on hydrogenation.

Gas-liquid chromatography provides a means for the qualitative elucidation of the structure of methyl linolenate hydroperoxide. Direct injections of a concentrate of oxidized products from methyl linolenate into a gas chromatographic column yield a peak corresponding to methyl azelaaldehydate, confirming a previous observation.[16] Such a product is expected from thermal decomposition of Ia by scission of the C-9–C-10 bond. The aldehyde esters of the other isomeric hydroperoxides did not appear because their unsaturation would be expected to promote their thermal decomposition under the conditions of gas-liquid chromatography.

Gas-liquid chromatography of II at 190° gave a broad peak of retention volume similar to that of the tetraenoic ester III, indicating that heat dehydration occurred under the gas chromatographic conditions. Morris *et al.*[17] reports the same effect with reduced hydroperoxide of methyl linoleate giving peaks for conjugated trienoic esters. This observation has been confirmed in this laboratory. Gas-liquid chromatography of IV at 200° gave a double peak (of about equal area) the first one having the same retention time as methyl 12-hydroxystearate or methyl 9(10)-hydroxystearate. Therefore, the second half of this double peak would presumably be due to methyl 16-hydroxystearate (IVd). The monoenoic esters (Va, b, c, d) on gas-liquid chromatography gave four peaks, the first of which corresponded to methyl oleate. The other peaks could not be identified because suitable standards were lacking. Finally, gas-liquid chromatography was applied to identify the C_{15} and C_{16} dibasic acids (VId) from oxidative fission of the corresponding monoenoic acids Vd, which could not be separated or identified by liquid-partition chromatography.

Ia, b, c, d, $\xrightarrow[PtO_2]{H_2}$ IVa. $CH_3-(CH_2)_8-\underset{OH}{\overset{9}{CH}}-R_1$

IVb. Methyl 13-hydroxystearate
IVc. Methyl 12-hydroxystearate
IVd. Methyl 16-hydroxystearate

IVa $\xrightarrow[H_3BO_3]{\Delta}$ 8- and 9-monoenoic ester (Va) $\xrightarrow[IO_4^-]{MnO_4^-}$ C_8- and C_9-dicarboxylic acids VIa

IVb ⟶ 12- and 13-monoenoic ester (Vb) ⟶ C_{12}- and C_{13}-dicarboxylic acids VIb

IVc ⟶ 11- and 12-monoenoic ester (Vc) ⟶ C_{11}- and C_{12}-dicarboxylic acids VIc

IVd ⟶ 15- and 16-monoenoic ester (Vd) ⟶ C_{15}- and C_{16}-dicarboxylic acids VId

DISCUSSION

The generally accepted mechanism for the autoxidation of methyl linoleate is based on Farmer's theory[4,18] that involves a homolytic abstraction of a hydrogen atom from the pentadiene system to give a resonance hybrid of three valence-bond structures:

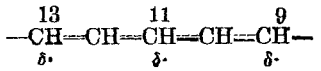
$$\left[\begin{array}{l} -\overset{13}{C}H=\overset{12}{C}H-\overset{11}{C}H-\overset{10}{C}H=\overset{9}{C}H- \\ -\dot{C}H-CH=CH-CH=CH- \\ -CH=CH-CH=CH-\dot{C}H- \end{array}\right]$$

(16) E. N. Frankel, J. Nowakowska, and C. D. Evans, *J. Am. Oil Chemists' Soc.*, **38**, 161 (1961).

(17) L. J. Morris, R. T. Holman, and K. Fontell, *J. Lipid Research*, **1**, 412 (1960).

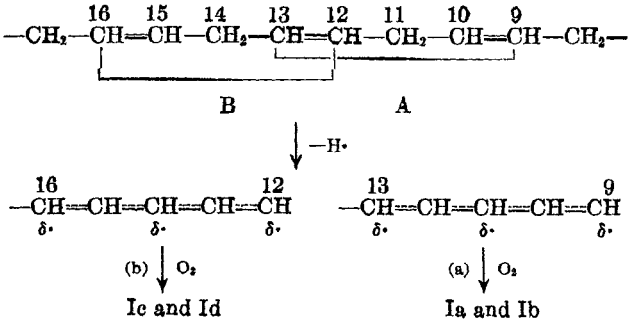
(18) E. H. Farmer, *Trans. Faraday Soc.*, **38**, 340, 356 (1942); E. H. Farmer and D. A. Sutton, *J. Chem. Soc.*, 119 (1943); 10 (1946).

which also may be written as:



The formation of conjugated hydroperoxides exclusively, which consist of about equal amounts of 9- and 13-hydroperoxides from autoxidized methyl linoleate, has been well established by various workers[10-12,19,20] and means that the hybrid radical reacts preferentially with oxygen in the 9- and 13- positions. This is not surprising since these products, and the transition states leading to them, would be resonance stabilized by a conjugated diene system.

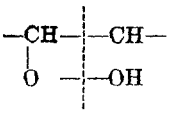
If analogous to methyl linoleate, the autoxidation of methyl linolenate, which contains two pentadiene systems A and B, should involve the hybrid radical (a) and (b) in the following scheme:



This scheme supports the previous conclusions made on the autoxidation of methyl linoleate that the reaction leads exclusively to conjugated hydroperoxides. The hybrid radical enables the conjugated system to be in either a *cis,cis, trans,trans*, or a *cis,trans* configuration. These geometric forms would also be possible for the linoleate hydroperoxides.

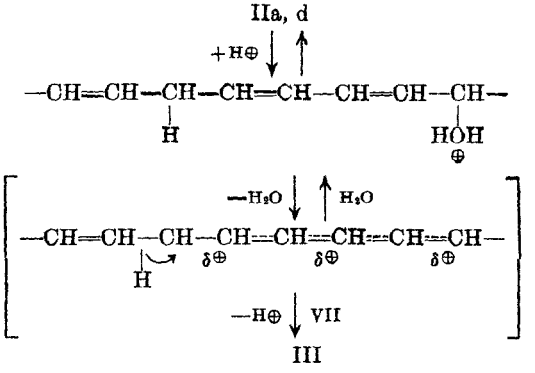
There remains the problem of explaining the significantly higher concentration obtained for 9- and 16-hydroperoxides than for 12- and 13-hydroperoxides. Either the hybrid radicals (a) and (b) prefer to react on the end positions 9 and 16 or, if the four isomeric hydroperoxides are formed in equal amounts, the 12- and 13- isomers tend to decompose more rapidly than the 9- and 16- isomers.

The presence of an active methylene group in the hydroperoxide structure Ia, b, c, d would allow a further hydrogen abstraction to occur leading to dihydroperoxides. Some of these secondary hydroperoxides would contain conjugated triene systems as observed in some of the secondary decomposition products. The great reactivity of such secondary products would account for the lower yields of monohydroperoxides from linolenate as compared to linoleate. The decomposition of the four isomeric hydroperoxides Ia, b, c, d by dismutation of the hydroperoxide group:

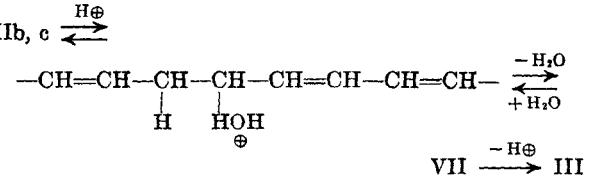


would result in formation of deca-2,4,7-trienal, hexa-3-enal, hepta-2,4-dienal and propional, respectively. Evidence for the last three aldehydes from autoxidized methyl linolenate has been reported in the literature.[21-23]

The acid dehydration of methyl hydroxylinolenates (IIa, b, c, d) to form the tetraenoic structure (III) may be explained by proton elimination[24] as follows: For *methyl 9(16) hydroxylinolenate:*



For *methyl 12(13)-hydroxylinolenate:*



(19) S. Bergstrom, *Arkiv Kemi, Mineral. Geol.*, 21A, No. 14, 1 (1945); *Nature*, 156, 717 (1945).

(20) A. Banks, J. N. Keay, and J. G. M. Smith, *Nature*, 179, 1078 (1957).

(21) F. K. Kawahara and H. J. Dutton, *J. Am. Oil Chemists' Soc.*, 29, 372 (1952).

(22) H. von Pezold, *Fette Seifen Anstrichmittel*, 61, 1018 (1959).

(23) G. Hoffmann, *J. Am. Oil Chemists' Soc.*, 38, 1 (1961).

(24) E. R. Alexander, *Principles of Ionic Organic Reactions*, John Wiley & Sons, Inc., New York, 1950, p. 105.

By this mechanism all four isomeric hydroperoxides would form the 9,11,13,15-octadecatetraenoic ester (III). The absence of measurable amounts of conjugated triene systems indicates that dehydration involves proton abstraction from the central methylene group, as indicated in the above schemes, rather than from a methylene group adjacent to the diene system of IIb,c.

EXPERIMENTAL[25]

Materials. Methyl linolenate was obtained either from The Hormel Institute (reported iodine value 260) or prepared from linseed oil methyl esters by the countercurrent distribution method of Scholfield *et al.*[26] Gas-liquid chromatography of the redistilled ester showed the presence of only one component (n_D^{30} 1.4675, iodine value, 258.7).

Autoxidation. The reaction was carried out at 37° in 25- or 50-ml. round-bottom flasks in presence of oxygen in a manometric system using a 50-ml. gas burette with magnetic stirring.

Analytical procedures. Unsaturation was determined by quantitative semimicro hydrogenation using platinum oxide as catalyst and absolute ethanol as solvent.

Peroxides were determined colorimetrically by the ferric thiocyanate method[27] to follow chromatographic and countercurrent fractionations and iodometrically[6] to characterize isolated fractions.

Molecular weights were determined cryoscopically on a microscale using cyclohexanol as the solvent[28,29] and measuring melting point depressions from capillary tubes. Considerable precaution must be exercised in this method to protect the solvent and samples from moisture.

Gas-liquid chromatography. A "Pye" instrument with radium D ionization detector was used. The column was 4 ft. long, made of ¼-in. glass tubing and packed with 10 % Craig polyester succinate on Chromosorb W 60/80.

Concentrate of oxidized products. Preliminary concentration of oxidized products from autoxidized methyl linolenate (5.2 g., 0.10 mole oxygen/mole ester) was achieved by countercurrent distribution in six 250-ml. separatory funnels containing 100 ml. each of 80% aqueous ethanol and petroleum ether (b.p. 30–60°) previously equilibrated with each other. The solvent in the combined lower and upper phases was removed under vacuum on a rotating evaporator at <50° and dried over anhydrous sodium sulfate (yield, upper layer: 4.3 g., peroxide value 45 ml./kg.; lower layer: 0.8 g., peroxide value 4790). Gas-liquid chromatography of the lower layer (174°, 43 ml./min. Argon flow) revealed one major peak of same retention time as methyl azelaaldehydate (retention time relative to methyl palmitate, 0.76).

Liquid-partition chromatography. The procedure was identical to that described previously for the determination of dimeric and polymeric acids and fat hydroperoxides.[8] Rapid preparative columns were used without a fraction collector by following peroxide value colorimetrically in a few drops of the eluate. The hydroperoxide fraction was collected in the first 100 ml. after the peroxide value of the eluate was positive.

Countercurrent distribution. A 200-tube automatic apparatus was used in which each tube contained 40 ml. of equilibrated lower layer (80% aqueous ethanol) and upper layer (petroleum ether). The sample (5 to 12 g.) was applied by distributing it in the two layers of the first five tubes in the instrument. Characteristics of combined hydroperoxide fractions (I) are given in Table I.

Reduction procedures. Hydroperoxides were reduced by potassium iodide or by sodium borohydride.[30] In the first procedure peroxide was determined iodometrically (sample, 0.0424 g. in duplicate; peroxide value 5110 $\epsilon_{234\ m\mu}$ 24,000 methanol). The reduced hydroperoxide (II) was extracted with ether and dried over sodium sulfate (product, 0.0820 g., peroxide value 50, $\epsilon_{233\ m\mu}$ 22,200, methanol).

In the second reduction procedure, 0.10 g. of hydroperoxide sample (peroxide value 5080, $\epsilon_{234\ m\mu}$ 23,200, methanol) in 5 ml. of absolute methanol was added dropwise into a 50-ml. round-bottom flask, protected from moisture, containing 0.12 g. sodium borohydride suspended in 5 ml. of methanol. The reaction mixture was stirred magnetically at room temperature for 2 hr. It was then diluted with water, about half the methanol was evaporated, and the product was extracted with ether and dried over sodium sulfate to yield 0.083 g. II (peroxide value 0, $\epsilon_{233\ m\mu}$ 21,800, methanol). This product was purified chromatographically using the same column as for the isolation of hydroperoxide, except 1% methanolic benzene was used as mobile solvent instead of 2%. II (0.077 g.) had an $\epsilon_{233\ m\mu}$ 26,500, methanol, and the infrared spectrum indicated that the conjugated system had a *cis,trans* and *trans,trans* configuration ($\epsilon_{10.6\ \mu}$ 60.4: $\epsilon_{10.2\ \mu}$ 85.5).

Dehydration of methyl hydroxylinolenate II. A procedure based on that described by Banks *et al.*[20] for the dehydration of methyl hydroxylinolenate was used. II (0.183 g., $\epsilon_{233\ m\mu}$ 25,000, methanol) was refluxed with 20 ml. of methanol containing 10% (v./v.) concd. sulfuric acid with nitrogen bubbling for 30 min. The reaction mixture was transferred into a separatory funnel with methanol and extracted three times with iso-octane. The extract was washed with water until neutral and dried over sodium sulfate. III (0.137 g.) showed the ultraviolet absorption spectrum characteristic of octadecatetraenoic ester with maxima at 287 mμ, 300 mμ (ε 42,600), and 314 mμ (iso-octane); the infrared spectrum indicated a high proportion of the all-*trans* configuration ($\epsilon_{10.0\ \mu}$ 247) (Fig. 4).

III was very unstable and an insoluble crystalline impurity was formed on standing at −10°. Attempts to purify the octadecatetraenoic acid obtained by saponification of the methyl ester through a partition chromatographic column,[8] designed to remove polymeric materials, were unsuccessful. Ahlers *et al.*[31] reported the infrared spectrum of this acid with $\epsilon_{10.04\ \mu}$ 774. Gas-liquid chromatography (190° and 50 ml./min. Argon flow) of III showed one broad peak of retention volume relative to methyl palmitate of 12.1. A similar peak was obtained under the same condition from II indicating that thermal dehydration to the tetraenoic ester occurs on the gas chromatographic column.

Catalytic hydrogenation of methyl linolenate hydroperoxide (I). A hydroperoxide sample (0.0895 g., $\epsilon_{234\ m\mu}$ 24,300, peroxide value 5060) in 5-ml. of absolute ethanol was hydrogenated at room temperature with platinum oxide catalyst to a constant hydrogen uptake measured manometrically (4.14 moles of hydrogen per mole of I). Hydrogen was then passed through the reaction mixture for another 2 hr. The catalyst was removed by filtration. The crude product

(25) Ultraviolet spectra were determined in absolute methanol or purified isooctane with either a Beckman DU spectrophotometer or a Cary 14 recording spectrophotometer. Infrared spectra were made with a Baird-Atomic KM-1 instrument using sodium chloride cells and carbon disulfide as solvent. The mention of trade names or products does not constitute endorsement by the Department of Agriculture over those not named.

(26) C. R. Scholfield, J. Nowakowska, and H. J. Dutton, *J. Am. Oil Chemists' Soc.*, **37**, 27 (1960).

(27) G. L. Hills and C. C. Thiel, *J. Dairy Research*, **14**, 340 (1946).

(28) H. N. Wilson and A. E. Heron, *J. Soc. Chem. Ind. (London)*, **60**, 168 (1941).

(29) W. O. Lundberg, J. R. Chipault, and M. J. Hendrickson, *J. Am. Oil Chemists' Soc.*, **26**, 109 (1949).

(30) M. Mattic and D. A. Sutton, *Chem. & Ind. (London)*, 666 (1953).

(31) N. H. E. Ahlers, R. A. Brett, and N. G. McTaggart, *J. Appl. Chem.*, **3**, 433 (1953).

(0.0908 g.) was purified chromatographically[8] to yield a mixture of methyl hydroxystearates (IV), m.p. 39–41°.

Anal. Calcd. for $C_{19}H_{38}O_3$: C, 72.5; H, 12.2. Found: C, 70.3; H, 11.5%.

Infrared analysis of OH gave $\epsilon_{2.8\,\mu}$ 19.5 compared to 22.0 for methyl 12-hydroxystearate. Gas-liquid chromatography of this material (200° 50 ml./min. Argon flow) showed one double peak (peak retention volume, relative to methyl palmitate 6.41 and 6.52), the first component of which coincided with methyl 12-hydroxystearate and methyl (9,10)-monohydroxystearate.

Dehydration of methyl hydroxystearates IV. IV from hydrogenated I was dehydrated by heating with boric acid[32] to determine the position of the hydroxyl group in the fatty acid chain. Methyl 12-hydroxystearate was used as control material to determine whether positional shifts of unsaturation occur through dehydration. In the dehydration procedure 10 g. of crude methyl 12-hydroxystearate (contained 12% methyl stearate as determined chromatographically) and an equal molar weight of boric acid (2.0 g.,) in a 100-ml. round-bottom flask were gradually heated on a rotating evaporator under vacuum (2 cm. mercury pressure). When the vigorous boiling occurring between 100° and 150° subsided, the temperature was raised to 200–210° and allowed to remain there for 1 hr. The cooled reaction mixture was transferred into a separatory funnel with water, extracted with ether, and dried over sodium sulfate. The product (9.3 g.) was saponified, and the acids were purified by partition chromatography[8] to yield 65% monomeric nonhydroxy acids. Infrared analyses showed the absence of hydroxyl and the presence of isolated *trans* double bond ($\epsilon_{10.4\,\mu}$ 46.1; methyl elaidate, $\epsilon_{10.4\,\mu}$ 141). The dehydration procedure was applied to 0.790 g. of IV from hydrogenated methyl linolenate hydroperoxides to yield 0.568 g. of monoenoic esters V which on partition chromatography of the acids obtained after saponification, gave 71% monomeric–nonhydroxy acids (infrared analysis: no hydroxyl; isolated *trans*, $\epsilon_{10.4\,\mu}$ 54.4).

Portions of V were remethylated with diazomethane and the methyl esters subjected to gas-liquid chromatography (temperature 173°, 33 ml./min. Argon flow rate). The esters of dehydrated methyl 12-hydroxystearate showed the presence of methyl stearate and a monoenoic ester corresponding to methyl oleate (peak retention volume relative to methyl palmitate, 2.26). The dehydrated hydroxystearates from I showed the presence of four peaks. The first and principal peak corresponded to methyl oleate (peak retention time relative to methyl palmitate, 2.22, 2.35, 2.61, 2.82, respectively; methyl oleate, 2.23). The remaining portions of V were subjected to oxidative cleavage with permanganate-periodate[33] to locate position of the unsaturation.

The short-chain monobasic acids (C_9 and lower) obtained by oxidative cleavage were removed by steam distillation. The dibasic acids VI were analyzed by liquid-partition chromatography (Table II). This method could not separate and identify dibasic acids higher than C_{13}. Therefore, a sample of the dibasic acids was methylated with methanol, hydrochloric acid, and 2,2-dimethoxypropane.[34] The methyl esters of the dibasic acids were analyzed by gas-liquid chromatography (188°, 33 ml./min. Argon flow), and identification was made by comparison with known standards. Peaks were obtained for C_8, C_9, C_{11}, C_{12}, C_{13}, C_{15}, C_{16} with retention times relative to methyl stearate of 0.34, 0.48, 0.92, 1.27, 1.77, 3.37, 4.66, respectively.

Acknowledgment. We are very grateful to Robert B. Bates (University of Illinois), B. Sreenivasan, H. M. Teeter, and J. C. Cowan for their interest and discussions of theoretical aspects of this study; to C. R. Scholfield for a generous supply of methyl linolenate; to E. P. Jones for the permanganate-periodate and chromatographic determinations of dicarboxylic acids; to A. E. Johnston for invaluable help in the molecular weight determinations; to H. M. Ven Horst for some gas chromatographic analyses; and to C. E. McGrew for elemental analyses.

PEORIA, ILL.

(32) W. Brandenberg and A. Galet, *J. Am. Chem. Soc.*, 72, 3275 (1950).

(33) E. P. Jones and J. A. Stolp, *J. Am. Oil Chemists' Soc.*, 35, 71 (1958).

(34) N. S. Radin, A. K. Hajca, and Y. Akahori, *J. Lipid Research*, 1, 250 (1960).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF UTAH]

New Synthesis of Trimethylhydroquinone[1]

W. J. BURKE, JOE A. WARBURTON, JAY L. BISHOP, AND JAMES L. BILLS

Received May 25, 1961

Condensation of 4-benzyloxyphenol with formaldehyde and morpholine, followed by treatment with concentrated hydrochloric acid and neutralization gave a high yield of 2,6-bis(morpholinomethyl)hydroquinone. Reaction of this product with equimolar quantities of morpholine and formaldehyde yielded the tris-Mannich base, which upon catalytic hydrogenation led to trimethylhydroquinone.

The wide diversity of important biological effects shown by vitamin E has stimulated considerable current interest in the tocopherols and related compounds.[2] Since vitamin E can be readily prepared from phytyl bromide and trimethylhydroquinone, much attention has been given to the synthesis of the latter.

Proposed routes to trimethylhydroquinone have involved rather complex, multistep syntheses

(1) This investigation was supported in part by a grant from the Research Corporation.

(2) H. Gebauer, *Intern. Z. Vitaminforsch., Beih.* No. 8 (1960) [*Chem. Abstr.* 54, 13297]; R. E. Olson, *Ann. Rev. Biochem.*, 28, J. M. Luck, ed., Annual Reviews, Inc., Palo Alto, Calif., 1959, p. 485; S. R. Ames, *Ann. Rev. Biochem.*, 27, J. M. Luck, ed., Annual Reviews, Inc., Palo Alto, Calif., 1958, p. 383.