Structure of Monohydroperoxides Formed by Chlorophyll Photo-Sensitized Oxidation of Methyl Linoleate[1]

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Abstract

The structures of the hydroperoxides prepared by photochemical oxidation of methyl linoleate in the presence of chlorophyll were determined by gas liquid chromatography-mass spectrometry (GLC-MS) of the trimethylsilyl (TMS) derivatives of the hydroxyesters. The position of the double bonds were deduced via hydroxylation with OsO_4, followed by GLC-MS analysis of the TMS derivatives. Structures of the original hydroperoxides were elucidated from these data.

Introduction

The autoxidation of olefins and, in particular, of esters of unsaturated fatty acids is known to proceed by a free radical mechanism giving hydroperoxide groups linked to a carbon atom beta to the original olefinic bond (1–4). The photochemical autoxidation of unsaturated esters in presence of chlorophyll gives hydroperoxides. Shown by many authors (5–10), but not well understood, are the mechanism and the structures of the products; Cobern et al. (11) have proved the position of the hydroperoxide groups in the monohydroperoxides derived from methyl oleate and linoleate but they did not give any structure for the products.

It is the purpose of our work to present further evidence on the chemical nature of the hydroperoxides formed in the photochemical oxidation of methyl linoleate catalized by chlorophyll.

Materials and Methods

Gas liquid chromatography (GLC) and mass spectrometry (MS) were performed with a Perkin-Elmer, model 270. Condition: GLC column, 2 m × 2 mm, SE 30, 1.5% on Chromosorb W (HDMS); column temperature, 210 C; accelerating voltage 70 eV. The IR analyses were made with a Perkin-Elmer Infracord. The spectral analyses were made with an Optica CF4 (Optica, Milan).

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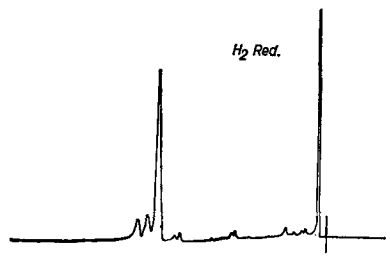


FIG. 1. Products formed by catalytic hydrogenation of the hydroperoxides.

Methyl linoleate was purchased from the Hormel Institute and shown by UV, IR and GLC to be free from isomers; the solvent used in the autoxidation procedure, *n*-heptane, was purified by concentrated sulfuric acid treatment, washed with water and distilled. The chlorophyll was obtained from fresh spinach leaves and purified according to the method of Khan and Lundberg (12). Plates 20 × 20 cm and 0.3 mm thick of Kieselgel G (E. Merck, Darmstradt, Germany) were used for thin layer chromatography (TLC) fractionation; the plates were prewashed by benzene elution followed by reactivation at 80 C for 20 min.

Methyl linoleate, 10 g, was dissolved in 96 ml of *n*-heptane and mixed with 4 ml of a solution of chlorophyll in *n*-heptane, 4 mg/ml. The solution was placed in a cold water bath, 1 ± 0.1 C, and air was bubbled at 6 ml/min. A blank sample without chlorophyll was also run. After 18 hr bubbling, the blank sample had a peroxide valve (PV) 65.6 meq/Kg; the chlorophyll sample, PV 1955; at 233 mμ this second sample showed $E^{1\%}_{1cm}$ 4340. The crude product was purified by TLC, eluting with a mixture ethyl ether-skellysolve F-acetic acid 1:1:0, 02 (V/V). The recovered hydroperoxide fraction had a PV 6320 meq/Kg. The hydrogenation in methanol of the purified hydroperoxides in presence of Lindlar catalyst gave the theoretical absorption of hydrogen (two double bonds and a hydroperoxy group). The GLC and MS analyses were done on the trimethyl silyl (TMS) derivatives of the hydrogenated product, prepared according to standard procedures. The re-

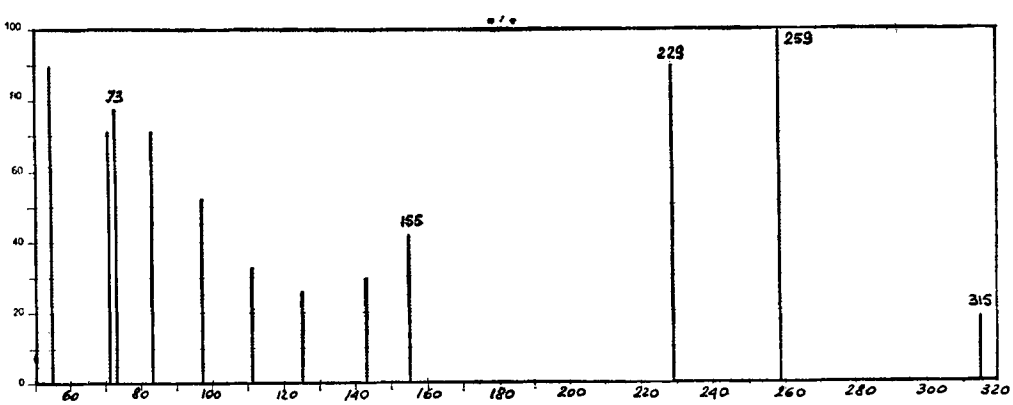


FIG. 2. Mass spectrum of the trimethyl silyl derivative of the hydrogenated hydroperoxides.

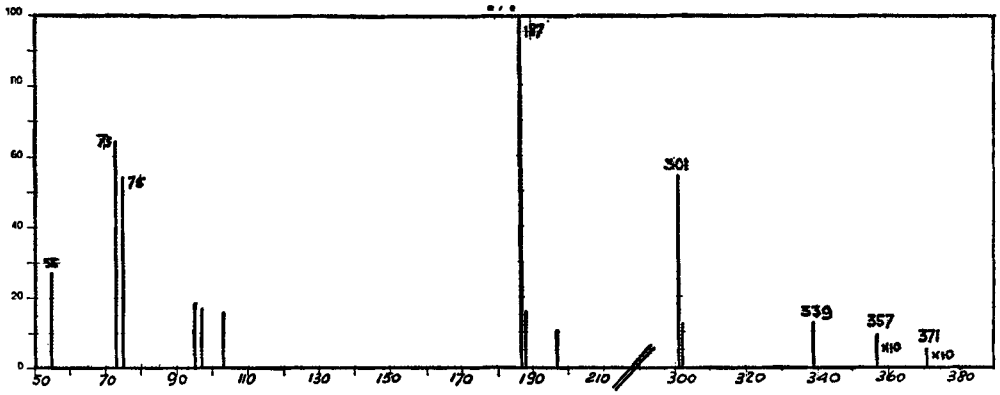


FIG. 3. Mass spectrum of the trimethyl silyl derivative of methyl-12-hydroxy stearate.

duction of the purified hydroperoxides by stannous chloride at room temperature was accomplished according to the procedure of Privett et al. (13); the mixture of hydroxy compounds was purified by TLC and recovered. The procedure described by McCloskey and McLelland (14) was used for the hydroxilation of the double bonds present in the recovered compounds. The products from the reaction were analyzed by GLC-MS.

Results and Discussion

As described in Methods and Materials, the hydroperoxides were prepared by reaction of methyl linoleate with oxygen at low temperature in the presence of UV light and chlorophyll. The mixture was fractionated by preparative TLC and the recovered hydroperoxide fraction was further purified by the same procedure.

The pure hydroperoxide fraction was catalytically reduced to the saturated hydroxyester and analyzed by GLC-MS as the TMS (Fig. 1) whose MS spectrum is shown in Figure 2; two fragments m/e 259 and m/e 229 can be noted in the spectrum as the most prominent.

The MS spectrum of the TMS of methyl 12-hydroxy stearate, obtained by catalytic hydrogenation of pure methyl ricinoleate, is shown in Figure 3. According to this the fragmentation mechanism of monohydroxy esters must be that described in the scheme below: two main fragments, m/e 301 and m/e 187, are derived by the rupture of the bonds around the tertiary carbon atom.

$$CH_3-CH_2-\underset{\displaystyle (CH_2)_{10}-COOCH_3}{\underset{|}{CH}}-OTMS \longrightarrow CH_3-CH_2-\overset{+\cdot}{C}H-OTMS + \underset{\displaystyle (CH_2)_{10}-COOCH_3}{\underset{|}{CH}}-OTMS$$

Applying the same mechanism to the hydroxy ester derived by the catalytic hydrogenation of the hydroperoxide from methyl linoleate, the two fragments shown above (Fig. 2) would be indicative of a methyl 9-hydroxystearate. We must conclude therefore that the main product formed in the chlorophyll catalyzed autoxidation of methyl linoleate is the 9-hydroperoxide.

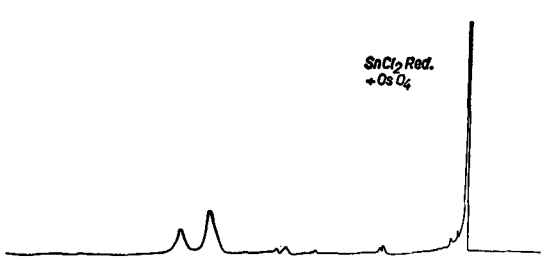
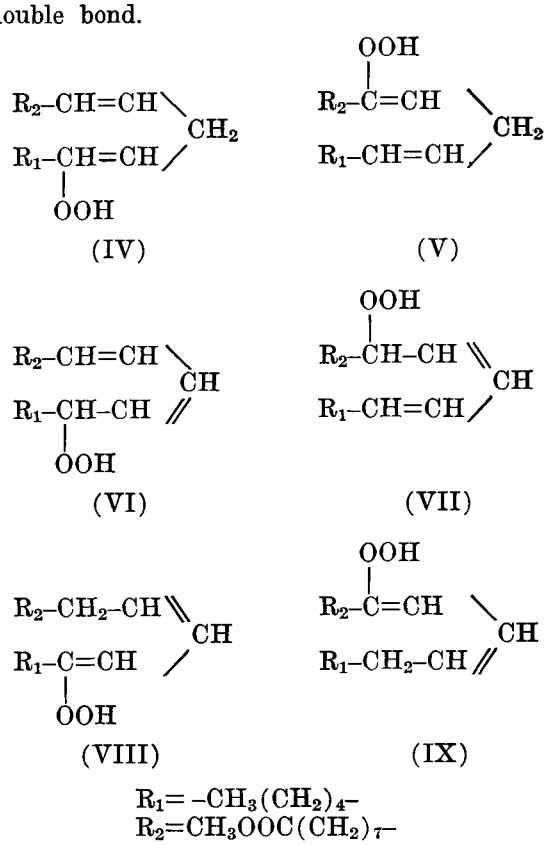


FIG. 4. Products formed from the hydroperoxides by reduction ($SnCl_2$) and hydroxylation (O_sO_4); trimethyl silyl derivatives.

The second product, present in the GLC of the hydrogenated product (Fig. 1), analyzed by MS showed two fragments at m/e 315 and m/e 173 indicating the presence of a 13-hydroperoxide. This finding proves that the hydroperoxide group introduced in the autoxidation of methyl linoleate, catalyzed by chlorophyll, is linked to the carbons 9 or 13 initially involved in the formation of a double bond, the 9-hydroperoxide form predominating. Therefore the photochemical autoxidation of fatty acid methyl esters catalyzed by chlorophyll is different from the normal autoxidation mechanism, as shown by Cobern et al. (11), and Khan et al. (8,9), which is known to attach the beta carbons atoms to a double bond.

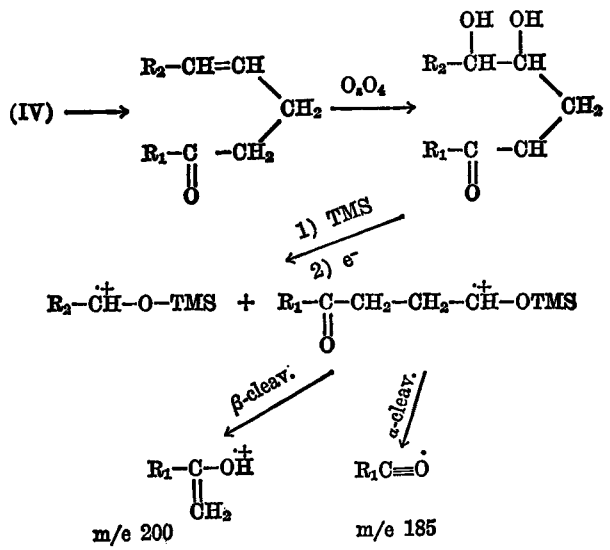


$R_1 = -CH_3(CH_2)_4-$
$R_2 = CH_3OOC(CH_2)_7-$

Several structures derived from methyl linoleate, having a hydroperoxide group in position 9 or 13

are possible (see above scheme); structures IV and V only do not contain any conjugated double bond. $SnCl_2$ reduction would give from structures IV, V, VIII and IX the corresponding keto esters of which the reduced structures are enolic forms. The compounds VI and VII would give instead hydroxy esters.

The hydroxylation by osmium tetroxide of the products derived from the stannous chloride reduction would give in the first case a dihydroxyderivative and in the case of structures VI and VII a tetrahydroxyderivative; the reaction scheme and a possible MS fragmentation mechanism are outlined for structure IV in the scheme below. The fragments which would be outlined from the various structures as TMS derivatives are shown in Table I.



Stannous chloride reduction followed by osmium tetroxide hydroxylation were applied to the purified hydroperoxide and the mixture analyzed by GLC and MS; GLC of the TMS derivatives (Fig. 4) showed the presence of a predominating compound with a minor amount of a longer retention time product.

TABLE I

Fragments Outlined From Structures at Trimethyl Silyl Derivatives

Compound	m/e of fragments A	m/e of fragments B	α Cleavage	β Cleavage
IV	315	173	185	200
V	259	229	99	114
VI	259	173	.....	.....
VII	259	173	.....	.....
VIII	301	187	185	200
IX	273	215	99	114

Two main fragments were present in the mass spectrum of the main compound, one having m/e 173 and the other, m/e 259; the two fragments are indicative for structures VI and VII.

The second product had a similar mass spectrum, and we must conclude that the structures of the hydroperoxides derived by the photochemical oxidation of methyl linoleate are, respectively, VI and VII, form VI predominating over VII.

REFERENCES

1. Farmer, E.H., Trans. Faraday Soc. 42: 228 (1946).
2. Farmer, E.H., G.F. Bloomfield, A. Sundralingam and B.A. Sutton, Ibid. 38: 348 (1942).
3. Farmer, E.H., and A. Sundralingam, J. Chem. Soc. 1942: 121.
4. Farmer, E.H., and D.A. Sutton, Ibid. 1943: 119.
5. Khan, N.A., Biochem. Biophys. Acta 16: 159 (1955).
6. Khan, N.A., J. Indian Chem. Soc. 36: 529 (1959).
7. Khan, N.A., Oleagineux 14: 231 (1959).
8. Khan, N.A., W.O. Lundberg and R.T. Holman, J. Amer. Chem. Soc. 76: 1779 (1954).
9. Khan, N.A., W.E. Tolberg, D.H. Wheeler and W.O. Lundberg, JAOCS 31: 460 (1954).
10. Lundberg, W.O., Oleagineux 4: 85 (1949).
11. Cobern, D., J.S. Hobbs, R.A. Lucas and Mackenzie, J. Chem. Soc. 1966: 1897.
12. Khan, N.A., and W.O. Lundberg, JAOCS 31: 460 (1954).
13. Privett, O.S., W.O. Lundberg, N.A. Khan, W.E. Tolberg and D.M. Wheeler, Ibid. 30: 61 (1953).
14. McCloskey, J.A., and M.J. McClelland, J. Amer. Chem. Soc. 87: 5090 (1965).

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