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Location of Hydroperoxide Groups in Monohydroperoxides formed by Chlorophyll-photosensitised Oxidation of Unsaturated Esters

By D. Cobern, J. S. Hobbs, R. A. Lucas, and D. J. Mackenzie

Monohydroperoxides were isolated from chlorophyll-photosensitised oxidation of methyl oleate, methyl linoleate, methyl linolenate, and ethyl arachidonate. The positions of the hydroperoxide substituent were determined by two methods of analysis of the saturated hydroxy-esters formed by catalytic hydrogenation. Gas–liquid chromatographic analysis of degradation products and mass spectrometric analysis of the corresponding keto-esters both proved that the hydroperoxide groups are located at each of the carbon atoms which originally formed part of a double bond.

It was established by Farmer [1] and others that autoxidation of simple olefins proceeds by a free-radical mechanism resulting in the formation of isomeric hydroperoxides, in which the hydroperoxide groups are attached to carbon atoms adjacent to an olefinic bond. Yet there is little direct evidence for the structures of the hydroperoxides.

The formation of hydroperoxides of unsaturated fatty esters by chlorophyll-catalysed photo-oxidation has long been known,[2-4] but it is not known whether the hydroperoxides are the same as those formed by uncatalysed (normal) autoxidation.

We have now proved the positions of the hydroperoxide groups in the monohydroperoxides formed by chlorophyll-photosensitised oxidation of methyl oleate, methyl linoleate, methyl linolenate, and ethyl arachidonate.

The hydroperoxides were prepared by reaction of the unsaturated compounds with oxygen at room temperature in the presence of light and chlorophyll. Though the mechanism of this reaction is obscure, the chief products are hydroperoxides,[3,4] which decompose only very slowly under these conditions.

The reaction is much faster than normal autoxidation, and after only a few hours the mixtures contained high concentrations of hydroperoxides, as shown by determination of the peroxide value and by thin-layer chromatography (t.l.c.) which showed spots or groups of spots attributable to (*a*) unchanged ester, (*b*) monohydroperoxide isomers, and (*c*) di- and higher hydroperoxides. This pattern is characteristic of autoxidised unsaturated fatty acids and esters,[5] and the above assignments were verified by isolation of each of the three classes from the reaction mixtures by preparative t.l.c. Unchanged ester (*a*) was identified by its infrared spectrum; monohydroperoxides (*b*) were characterised by peroxide value, infrared spectrum, and by catalytic hydrogenation to the saturated hydroxy-esters, which were characterised by elemental analysis, infrared spectrum, and chromatography. The di- and higher hydroperoxides (*c*) had infrared spectra which closely resembled those of the monohydroperoxides (*b*), but the peroxide values of (*c*) were 2—3 times greater. A by-product (about 15%) of catalytic reduction of the monohydroperoxides was isolated and characterised as saturated keto-esters by their infrared spectra, elemental analyses, and chromatographic properties. A similar by-product has been reported from catalytic reduction of methyl oleate epoxide.[6]

The positions of the hydroperoxide group in the mixtures of isomeric monohydroperoxides were proved by determining the positions of the hydroxyl group in the mixture of isomeric hydroxy-esters formed by catalytic reduction.

Degradation Method.[7]—The hydroxy-esters were oxidised to the corresponding keto-esters by chromic acid in acetone.[8] The oximes were prepared and heated with concentrated sulphuric acid, whereupon they underwent the Beckmann rearrangement. The resulting amides were hydrolysed by alkali, and the hydrolysate was separated into acidic and basic portions. Each original hydroxy-ester isomer gives a characteristic amine, amino-acid, and mono- and di-carboxylic acid; determination of the chain-length of any of these four products gives unambiguously the position of the original hydroxyl group.

Degradation products were identified by their gas–liquid chromatography (g.l.c.) retention times on two different stationary phases. The amines were in the basic fraction and were analysed directly by g.l.c. The mono- and di-carboxylic fraction was methylated and then analysed by g.l.c., but the complicated mixtures of esters from linolenate and arachidonate gave chromatograms difficult to interpret because of overlapping of mono- and di-ester peaks. This problem was overcome by separating the acidic portion of the amide hydrolysate into mono- and di-acid fractions by chromatography on silicic acid. A useful feature of this method is that the acids within each of the two fractions emerge from the

[1] E. H. Farmer and A. Sundralingam, *J. Chem. Soc.*, 1942, 121; E. H. Farmer, G. F. Bloomfield, A. Sundralingam, and D. A. Sutton, *Trans. Faraday Soc.*, 1942, **38**, 348; E. H. Farmer and D. A. Sutton, *J. Chem. Soc.*, 1943, 119; E. H. Farmer, *Trans. Faraday Soc.*, 1946, **42**, 228.

[2] N. A. Kahn, *Biochim. Biophys. Acta*, 1955, **16**, 159; *J. Indian Chem. Soc.*, 1959, **36**, 529; *Oleagineaux*, 1959, **14**, 231; R. O. Carter, U.S.P. 2,727,857.

[3] N. A. Khan, W. O. Lundberg, and R. T. Holman, *J. Amer. Chem. Soc.*, 1954, **76**, 1779.

[4] N. A. Khan, *Canad. J. Chem.*, 1954, **32**, 1149.

[5] E. Schauenstein and H. Esterbauer, *Monatsh.*, 1963, **94**, 164.

[6] D. R. Howton and R. W. Kaiser, *J. Org. Chem.*, 1964, **29**, 2420.

[7] J. Ross, A. I. Gebhart, and J. F. Gerecht, *J. Amer. Chem. Soc.*, 1949, **71**, 282.

[8] K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 1946, 39.

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silicic acid column in order of *decreasing* molecular weight. There is therefore no possibility of ambiguity about the end of the mono-acid fraction and the start of the di-acid fraction, because at that point, g.l.c. of the methylated sub-fractions shows new peaks of much longer retention time, because the esters emerge from the column in order of *increasing* molecular weight.

Precautions must be taken at every step to prevent the intrusion of any impurities or by-products which might give a peak on the gas–liquid chromatogram. Model experiments on the degradation of methyl 12-hydroxystearate emphasised the need to work with carefully redistilled solvents throughout, and to purify the intermediate ketones and oximes by column chromatography.

Mass Spectrometry.—Mixtures of isomeric keto-esters were obtained from the hydroperoxides as above, or were isolated chromatographically after chromic acid oxidation of the crude hydroperoxide hydrogenation product. The keto-esters were fractionated by preparative t.l.c. to give fractions sufficiently simple to be analysed by mass spectrometry. It was necessary to compare these mass spectra with the spectra of all the possible keto-ester isomers, *i.e.*, the isomeric ethyl 4- to 16-oxoarachidates, which were synthesised from the appropriate ω-ethoxycarbonylacyl chlorides and dialkylcadmium compounds.[9] The isomeric methyl 2- to 17-oxostearates were kindly supplied by Dr. L. J. Morris.

Comparison of the R_f value of each t.l.c. band with those of the pure synthetic keto-ester isomers restricted the number of possible isomers for which the spectrum of the mixture had to be examined. The relative proportions of each constituent in the mixture could be approximately determined from the weights of the eluted bands and the intensities of certain peaks in the mass spectra.

The mass spectra of the methyl oxostearates have been reported [10] and the oxoarachidate spectra exhibit many of the same features. Each keto-ester gives a characteristic mass spectrum with preferential cleavage α and β to the keto-group. The peaks corresponding to these fragments are much larger than those of the same mass which arise by random fragmentation of other isomeric keto-esters. The intensities of a few peaks in the spectrum of a mixture of isomeric keto-esters can thus be used to determine the presence of each keto-ester which could possibly be present, the limitations indicated by t.l.c. R_f values being borne in mind.

The major peaks in the mass spectra of oxoarachidates are due to breakdown by 6 modes as shown in Table 1.

For the ethyl 5- to 10-oxoarachidates these fragments gave peaks as listed in Table 2.

Each isomer gave several characteristic peaks which indicated its presence in a mixture. Sometimes a fragment of given mass was formed from two different isomers by different fragmentation modes, for example isomer 6, mode I and isomer 9, mode III both give a fragment of mass 157. If both isomers are present this peak will be intense since the peak heights are additive. This duplication must be considered when estimating the proportions of the isomers present.

TABLE 1

Fragmentation of oxoarachidates, general formula $CH_3{\cdot}[CH_2]_n{\cdot}CO[CH_2]_m{\cdot}CO_2C_2H_5$, where $n + m = 17$

Mode of breakdown	Fragment formed
I	$CO{\cdot}[CH_2]_m{\cdot}CO_2C_2H_5^+$
II	$(CO{\cdot}[CH_2]_m{\cdot}CO{-}H)^+$
III	$(CH_2)_{m-1}CO_2C_2H_5^+$
IV	$(CH_2CO{\cdot}[CH_2]_m{\cdot}CO_2C_2H_5 + H)^+$
V	$CH_2CO{\cdot}[CH_2]_m{\cdot}CO^+$
VI	$(CH_3{\cdot}[CH_2]_n{\cdot}COCH_2 + H)^+$

TABLE 2

Mass spectral peaks of oxoarachidates from breakdown by modes I—VI

Position of keto-group	I	II	III	IV	V	VI	Other prominent peaks
5	143	97	101	158	112	254	239, 281
6	157	111	115	172	126	240	225
7	171	125	129	186	140	226	168, 211
8	185	139	143	200	154	212	197
9	199	153	157	214	168	198	183
10	213	167	171	228	182	184	169

RESULTS

Both the degradation and mass spectrometric methods gave results which prove the positions of the hydroperoxide groups to be those listed in Table 3.

TABLE 3

Positions of hydroperoxide group in monohydroperoxides

Unsaturated ester	Positions of hydroperoxide groups
Methyl oleate	9, 10
Methyl linoleate	9, 10, 12, 13
Methyl linolenate	9, 10, 12, 13, 15, 16
Ethyl arachidonate	5, 6, 8, 9, 11, 12, 14, 15

Analyses of degradation products on which these conclusions were based are in Table 4.

These chain-lengths can be compared with those predicted to be formed from hydroxystearate and hydroxyarachidate isomers, given in Table 5.

All the expected products (and no others) were detected from oleate and linoleate. Arachidonate gave all the expected mono- and di-acids and amines except the C_{14} and C_{15} amines. Linolenate gave the expected mono- and di-acids except the C_3 and C_4 monoacids (the amines were not analysed). Since the analysis of any one of these classes is sufficient indication of the original hydroperoxides present, these results provide much confirmatory evidence of the correctness of the conclusions in Table 3. The missing acids were probably lost by volatilisation during work-up. The missing high-boiling amines were probably absorbed by the column packing during g.l.c.

The results of analysis by mass spectrometry also give

[9] J. Cason, *J. Amer. Chem. Soc.*, 1946, **68**, 2078.
[10] R. Ryhage and E. Stenhagen, *Arkiv Kemi*, 1960, **15**, 545.

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TABLE 4

Gas–liquid chromatographic analysis of monohydroperoxide degradation products

	Chain-length of degradation products		
Unsaturated ester	Mono-acids	Di-acids	Amines
Methyl oleate	C_9,C_{10}	C_9,C_{10}	C_8,C_9
Methyl linoleate	C_6,C_7,C_9,C_{10}	C_9,C_{10},C_{12},C_{13}	C_5,C_6,C_8,C_9
Methyl linolenate	C_6,C_7,C_9,C_{10}	$C_9,C_{10},C_{12},C_{13},C_{15},C_{16}$	—
Ethyl arachidonate	$C_6,C_7,C_9,C_{10},C_{12},C_{13},C_{15},C_{16}$	$C_5,C_6,C_8,C_9,C_{11},C_{12},C_{14},C_{15}$	$C_5,C_6,C_8,C_9,C_{11},C_{12}$

the positions of hydroperoxide groups as in Table 3, and the isomers are estimated to be present in approximately equal proportions.

TABLE 5

Expected products from degradation of x-hydroxystearates and x-hydroxyarachidates

	Chain-lengths of degradation products		
	Monoacid	Diacid	Amine
Stearates	$19 - x$	x	$18 - x$
Arachidates	$21 - x$	x	$20 - x$

TABLE 6

Postulated structures of monohydroperoxides from chlorophyll-catalysed oxidation

Unsaturated ester	Posn. of hydroperoxide group	Positions of double bonds
Methyl oleate	9	10
	10	8
Methyl linoleate	9	10, 12
	10	8, 12
	12	9, 13
	13	9, 11
Methyl linolenate	9	10, 12, 15
	10	8, 12, 15
	12	9, 13, 15
	13	9, 11, 15
	15	9, 12, 16
	16	9, 12, 14
Ethyl arachidonate	5	6, 8, 11, 14
	6	4, 8, 11, 14
	8	5, 9, 11, 14
	9	5, 7 11, 14
	11	5, 8, 12, 14
	12	5, 8, 10, 14
	14	5, 8, 11, 15
	15	5, 8, 11 13

Both methods of determining positions of hydroperoxide groups have shown that each unsaturated ester gave monohydroperoxide positional isomers in which the hydroperoxide groups are located at each of the carbon atoms which originally formed part of a double bond. No other positions are substituted. Chlorophyll-catalysed oxidation of linoleate does not give any 11-hydroperoxylinoleate as suggested by Khan *et al.*[3] The chlorophyll-photosensitised oxidation therefore gives quite different results from those expected from normal autoxidation, in which it is now generally accepted [11] that initial free-radical formation at a methylene group adjacent to a double bond may be followed by an allylic shift of one electron, thus transferring the radical site and conjugating the double bonds. According to this theory, oleate would give the 8-, 9-, 10-, and 11-hydroperoxide isomers; linoleate, the 9- and 13-isomers; linolenate, the 9-, 12-, 13-, and 16-isomers; and arachidonate, the 5-, 8-, 9-, 11-, 12-, and 15-isomers. This has been demonstrated by degradation experiments for autoxidation of oleate,[7] linoleate,[12] and linolenate.[13] Clearly photosensitised oxidation has a different mechanism.

Although the present study gives no direct information about positions of double bonds, the results could be interpreted in terms of a mechanism similar to that suggested for normal autoxidation, except that migration of a double bond *invariably* occurs,[14] to give hydroperoxides containing both conjugated and non-conjugated diene structures. Thus the structures of hydroperoxides formed by chlorophyll-photosensitised oxidation can be postulated as in Table 6.

A recent study [15] of the hydroperoxides formed by chlorophyll-catalysed oxidation of methyl oleate and methyl linoleate has shown that their n.m.r. spectra are in complete agreement with the structures postulated in Table 6.

EXPERIMENTAL

Light petroleum had b. p. 40—60° Thin-layer chromatography was done on 0·3 mm. layers of Kieselgel G (Merck) using ether–light petroleum (2 : 3 v/v) as eluant, unless otherwise stated. Spots were detected by exposure to iodine vapour. Plates for preparative t.l.c. were pre-washed by elution with chloroform followed by reactivation at 100° for 30 min. Infrared spectra were obtained on a Unicam S.P. 200 spectrophotometer. Ultraviolet spectra were obtained on a Unicam S.P. 700 recording spectrophotometer. Silicic acid was Mallinckrodt (AR, 100 mesh). Alumina was Hopkins and Williams, 8% deactivated with water.

G.l.c. Analysis.—Gas–liquid chromatography was done using an F and M 720 instrument, with helium as carrier gas at a flow rate of 40 ml./min. The columns were 2 ft. silicon gum rubber (20% on silanised Celite), ("SGR"), and 6 ft. phenyldiethanolamine succinate (10% on silanised Celite), ("PDS"), isothermally or with temperature programming as stated.

Unsaturated Esters.—Methyl oleate, linoleate, and linolenate were all better than 95% purity as determined by g.l.c. Ethyl arachidonate, 85% pure, supplied by Hoffmann La Roche, was freed from antioxidant immediately before use by filtration through alumina with ether–light petroleum (1 : 20).

Formation and Characterisation of Hydroperoxides.—

[11] D. Swern, "Autoxidation and Antioxidants," ed. W. O. Lundberg, Interscience, New York, 1961, 8—21.

[12] H. H. Sephton and D. A. Sutton, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 263.

[13] E. N. Frankel, C. D. Evans, D. G. McConnel, E. Selke, and H. J. Dutton, *J. Org. Chem.*, 1961, **26**, 4663.

[14] G. O. Schenk, H. Eggert, and W. Denk, *Annalen*, 1953, **584**, 177; G. O. Schenk, *Angew. Chem.*, 1957, **69**, 579.

[15] G. E. Hall and D. G. Roberts, *J. Chem. Soc.* (*B*), 1966, in the press.

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Photosensitised oxidations were carried out in a water-cooled (18°) oxygenation tube. Oxygen was passed through it and the sample was illuminated by two 250 w lamps. 0·2 ml. of chlorophyll solution (0·3% in hexane) was added at 30 min. intervals. The progress of the oxidation was followed by determination [16] of peroxide value and by t.l.c. and the reaction was stopped when a high concentration of hydroperoxides was present.

The fresh oxidation mixtures were immediately chromatographed on a column of silicic acid. Elution with light petroleum gave unchanged ester and then elution with ether–light petroleum mixtures gave monohydroperoxides. Composition of fractions was determined by t.l.c. and solvent was removed at room temperature using a rotary evaporator. (Further elution of the column gave dihydroperoxides.) Characteristics of the isolated monohydroperoxides are in Table 7.

TABLE 7

Monohydroperoxides of unsaturated esters

Ester	Typical yield (%)	Peroxide value (mmole/kg.) (Found)	(Calc.)
Methyl oleate	80	3800	3050
Methyl linoleate	44	4000	3070
Methyl linolenate	36	4020	3090
Ethyl arachidonate	29	3400	2750

Hydrogenation of Hydroperoxides.—The freshly isolated monohydroperoxides were hydrogenated in ethanol over Adams catalyst at atmospheric pressure and room temperature until hydrogen uptake ceased (2—5 hr.). After removal of the catalyst and solvent, the product was chromatographed on a silicic acid column.

Oleate. The hydrogenation product (11·5 g.) was chromatographed on a 175 g. column. Elution with ether–light petroleum (1 : 20 v/v; 900 ml.) gave methyl stearate (2·8 g.); with ether–light petroleum (1 : 9 v/v; 300 ml. followed by 1 : 6 v/v; 800 ml.), methyl oxostearates (2·0 g.); and with ether–light petroleum (1 : 3 v/v; 1500 ml.), methyl hydroxystearates (6·2 g.).

Linoleate. The hydrogenation product (5·0 g.) was chromatographed on a 150 g. column. Elution with ether–light petroleum (1 : 9 v/v; 300 ml.) gave methyl stearate (1·4 g.). Further elution (1200 ml.) gave methyl oxostearates (1·4 g.), followed by methyl hydroxystearates (1·4 g.).

Linolenate. The hydrogenation product (15·9 g.) was chromatographed on a 120 g. column, eluted with ether–light petroleum (1 : 20 v/v; 300 ml.), to give methyl stearate (4·2 g.). Continued elution (300 ml.) gave methyl oxostearates (1·6 g.). Elution with ether–light petroleum (1 : 9 v/v; 600 ml.) gave a mixture (1·6 g.) of oxo- and hydroxy-stearates, and with ether–light petroleum (1 : 4 v/v; 800 ml.), methyl hydroxystearates (4·1 g.). A final impure hydroxystearate fraction (2·2 g.) was also collected.

Arachidonate. The hydrogenation product (7·0 g.) was chromatographed on a 180 g. column. Elution with ether–light petroleum (1 : 9 v/v; 250 ml.) gave ethyl arachidate (0·9 g.). Further elution (800 ml.) gave ethyl oxoarachidates (1·1 g.). Subsequent elution with ether–light petroleum (up to 1 : 3 v/v; 800 ml.) gave ethyl hydroxyarachidates (4·0 g.).

Characterisation of Hydrogenation Products.—*Oleate.* (*a*) Methyl stearate separated from methanol as plates, m. p. and mixed m. p. 35—37°. The infrared spectrum (in Nujol) was identical with that of an authentic specimen.

(*b*) Methyl oxostearates separated from methanol as plates, m. p. 41·5—42·5°, $\nu_{max.}$ (in Nujol) 1710 (ketone) and 1750 cm.$^{-1}$ (ester) (Found: C, 72·85; H, 11·5. Calc. for $C_{19}H_{36}O_3$: C, 73·1; H, 11·5%). On t.l.c. the product behaved as methyl 12-oxostearate. Hydrolysis with 10% aqueous methanolic potassium hydroxide (refluxed for 30 min.) gave the keto-acids, which separated from ethanol as crystals, m. p. 70—72° (Found: C, 72·05; H, 11·3. Calc. for $C_{18}H_{34}O_3$: C, 72·5; H, 11·4%).

(*c*) Methyl hydroxystearates separated from methanol as plates, m. p. 45·5—46·5°, $\nu_{max.}$ (in Nujol) 3320, 3230 (hydroxyl), and 1750 cm.$^{-1}$ (ester) (Found: C, 73·2; H, 11·8. Calc. for $C_{19}H_{38}O_3$: C, 72·6; H, 12·1%); t.l.c. behaviour was similar to that of methyl 12-hydroxystearate.

Linoleate. (*a*) Methyl stearate was identified as above.

(*b*) Methyl oxostearates had similar t.l.c. properties to those from oleate; $\nu_{max.}$ (in Nujol) 1710 and 1750 cm.$^{-1}$ (keto-ester).

(*c*) Methyl hydroxystearates, further purified by chromatography on an alumina column and eluted in benzene–light petroleum (9 : 11 v/v), had m. p. 44·5—46·5°, $\nu_{max.}$ (in Nujol) 3300 (hydroxyl) and 1750 cm.$^{-1}$ (ester) (Found: C, 72·7; H, 12·2. Calc. for $C_{19}H_{38}O_3$: C, 72·6; H, 12·1%); t.l.c. behaviour was similar to that of the analogous fraction from oleate.

Linolenate. (*a*) Methyl stearate was identified as above.

(*b*) Methyl oxostearates crystallised from methanol as plates, m. p. 34—36°; $\nu_{max.}$ (Nujol) 1740 (ester carbonyl) and 1715 cm.$^{-1}$ (ketone) (Found: C, 72·8; H, 11·3. Calc. for $C_{19}H_{36}O_3$: C, 73·0; H, 11·5%).

(*c*) Methyl hydroxystearates were recrystallised from methanol to give plates, m. p. 48—50° (Found: C, 72·1; H, 12·0. Calc. for $C_{19}H_{38}O_3$: C, 72·6; H, 12·1%); $\nu_{max.}$ (Nujol), 3450 (hydroxyl) and 1738 cm.$^{-1}$ (ester carbonyl).

Arachidonate. (*a*) Ethyl arachidate was identified by its g.l.c. retention time and its infrared spectrum.

(*b*) Ethyl oxoarachidates were purified further by column chromatography on alumina (Found: C, 74·55; H, 11·9. Calc. for $C_{22}H_{42}O_3$: C, 74·55; H, 11·9%); $\nu_{max.}$ (Nujol) 1740 and 1710 (shoulder) cm.$^{-1}$ (keto-ester); t.l.c. behaviour was the same as that of authentic oxoarachidates.

(*c*) Ethyl hydroxyarachidates separated from aqueous ethanol as needles, m. p. 49—52° [Found: OEt, 10·55%; *M* (Rast), 302. Calc. for $C_{22}H_{42}O_3$: 1 OEt, 12·65%; *M*, 336]; $\nu_{max.}$ (in Nujol) 3300 (hydroxyl) and 1740 cm.$^{-1}$ (ester). Hydrolysis with aqueous methanolic potassium hydroxide gave hydroxy-acids, which separated from aqueous ethanol as prisms, m. p. 75—79° (Found: C, 73·0; H, 12·65; OEt, 3·9. Calc. for $C_{22}H_{40}O_3$: C, 73·1; H, 12·4; OEt, 0·0%).

Determination of the Structure of Hydroperoxides.—*Degradation method.*—(*a*) *Degradation of methyl* 12-*hydroxystearate* (*model compound*). Methyl 12-hydroxystearate (10 g.) in acetone (75 ml.) was treated dropwise with 4N-chromium trioxide in 5N-sulphuric acid (7·5 ml.). After 15 min. at room temperature, the mixture was diluted with excess of water and extracted with ether. The ether solution was washed with saturated sodium hydrogen carbonate solution, dried ($MgSO_4$), and evaporated, and the residue filtered through a short column of silicic acid in ether–light petroleum (1 : 5 v/v). Removal of the solvent gave methyl 12-oxostearate (9·0 g.), identified by t.l.c.

[16] L. K. Dahle and R. T. Holman, *Analyt. Chem.*, 1961, **33**, 1960.

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and m. p. (crystals, from methanol, m. p. 44—45·5°; Perrotte[17] reports m. p. 44·5°).

The keto-ester (6·0 g.), hydroxylamine hydrochloride (3·6 g.), and sodium acetate (4·8 g.) were dissolved in aqueous ethanol (1 : 4 v/v; 75 ml.) and boiled under reflux for 3·5 hr. The cooled mixture was diluted with excess of water and extracted with ether. Evaporation of the washed and dried ether solution afforded 6·1 g. of an oil, which was purified on a column of silicic acid (700 g.). Elution with light petroleum (1000 ml.) removed non-polar impurities. Elution with ether–light petroleum (1 : 20 v/v; 1200 ml.) removed traces of unchanged oxostearate. Elution with ether–light petroleum (1 : 9 v/v; 1200 ml.) afforded the oxime (4·8 g.) (Found: N, 4·4. Calc. for $C_{19}H_{37}NO_3$: N, 4·3%).

The oxime (1·9 g.) was heated with concentrated sulphuric acid (18 ml.) at 100° for 1 hr. The cooled mixture was diluted with excess of water and repeatedly extracted with ether. Evaporation of the washed and dried ether solution gave the solid amide (1·9 g.).

The amide (1·8 g.) was hydrolysed by aqueous potassium hydroxide (20% w/v; 6 ml.) in a sealed tube at 200° for 3 hr. The product was separated into alkali-soluble and acid-soluble fractions in the usual way. G.l.c. of the acid-soluble fraction on the SGR column at 75° showed a single peak, identified as hexylamine by comparison with an authentic sample.

A portion (10 mg.) of the alkali-soluble product was boiled under reflux with a solution (10 ml.) of boron trifluoride in methanol (15% w/v) for 10 min. The mixture was diluted with excess of water and extracted with ether. The ether layer was washed with sodium hydrogen carbonate solution and water, and dried. Evaporation of the solvent gave the methyl esters. G.l.c. on the SGR column (50—200° at 15° per min.) gave two peaks only, identified as methyl n-heptanoate and dimethyl dodecanedioate by comparison of their retention times with those of known standards.

A further portion (0·11 g.) of the alkali-soluble product was chromatographed on a column of silicic acid (25 g.). Elution with ether–light petroleum–acetic acid (15 : 85 : 1; 200 ml.) gave the monoacid only; further elution gave the diacid only. Each fraction was methylated as above and shown by g.l.c. to be >95% pure.

(*b*) *Degradation of hydroxystearates from methyl oleate.* The methyl hydroxystearates (3·5 g.) were oxidised with chromic acid solution (3 ml.) as described above. Chromatography afforded methyl oxostearates (1·8 g.) and unchanged methyl hydroxystearates (1·0 g.). Methyl oxostearates (2·6 g.) were converted into the oximes and purified by chromatography as described above to give the oximes as an oil (2·5 g.). The oximes (2·5 g.) were heated with concentrated sulphuric acid (12·5 ml.) at 100° for 2 hr. The product (amides), isolated as above, was a solid (2·2 g.).

The amides (2·0 g.) were hydrolysed by alkali at 200° in a sealed tube and the product was separated into acidic and basic fractions. G.l.c. of the basic fraction on the PDS column gave only two peaks, identified by comparison of the retention times at 100° with known standards, as octylamine and nonylamine.

A portion of the acidic fraction was methylated in the usual way. G.l.c. on the SGR column (50—200° at 15° per min.) showed only 4 peaks, with the same retention times as methyl nonanoate, methyl decanoate, dimethyl decanedioate and dimethyl nonanedioate.

6 K

(*c*) *Degradation of hydroxystearates from methyl linoleate.* The methyl hydroxystearates were oxidised and the product purified as before. Conversion into the oximes gave, after purification, an oil, $\nu_{max.}$ (liquid film) 1600 (oxime) and 1740 cm.$^{-1}$ (acid). Beckmann rearrangement in the usual way gave a solid, $\nu_{max.}$ (in Nujol) 1545, 1640 (amide), 1700, and 950 cm.$^{-1}$ (carboxylic acid). The product of alkaline hydrolysis was separated into acidic and basic fractions as before.

The basic fraction gave, on g.l.c. on the PDS column, only 4 peaks, identified as pentylamine, hexylamine, octylamine, and nonylamine, by comparison of retention times at 100° with those of known standards.

A portion of the acidic fraction was methylated and analysed by g.l.c. on the SGR column (50—200° at 15° per min.). Peaks were obtained with the same retention times as methyl hexanoate, heptanoate, nonanoate, and decanoate, and dimethyl nonanedioate, decanedioate, dodecanedioate, and tridecanedioate.

A portion (0·66 g.) of the acidic fraction was chromatographed on a column of silicic acid (65 g.). Elution with ether–light petroleum–acetic acid (15 : 85 : 1 v/v; 400 ml.) gave an oil which was methylated. G.l.c. showed only the first four monoester peaks above, whose identity was confirmed by comparison with known standards on two different stationary phases (SGR and PDS at 150°). Further elution of the column afforded the four di-esters, identified similarly (SGR and PDS columns at 200°).

(*d*) *Degradation of hydroxystearates from methyl linolenate.* The hydroxystearates (2·0 g.) were oxidised with chromic acid solution as before, and the keto-esters (1·1 g.) were purified by chromatography on silicic acid. The oxostearates (1·0 g.) gave oximes (0·6 g.) after purification as above. The amides (0·6 g.) from Beckmann rearrangement were heated with 20% aqueous potassium hydroxide (10 ml.) in a sealed tube at 200° for 3 hr. From the hydrolysate, acidic, and basic fractions were isolated in the usual way.

A portion of the acidic fraction was separated by preparative t.l.c. (eluant, ether–light petroleum–acetic acid, 40 : 60 : 1 v/v). The positions of the mono- and di-acid bands were identified by reference to marker spots. The acids were recovered from the plates, methylated, and examined by g.l.c. on both SGR and PDS columns. Four monoester peaks were obtained, having the same retention times (on SGR and PDS, 50—200° at 15° per min.) as methyl hexanoate, heptanoate, nonanoate, and decanoate. Six diester peaks were recorded, corresponding to dimethyl nonanedioate, decanedioate, dodecanedioate, tridecanedioate, pentadecanedioate, and hexadecanedioate (SGR and PDS columns at 200°).

(*e*) *Degradation of hydroxyarachidates from ethyl arachidonate.* The ethyl hydroxyarachidates (3·9 g.) were oxidised with chromic acid solution as before, and the product chromatographed on a column of silicic acid (150 g.). Elution with ether–light petroleum (1 : 20 v/v; 1700 ml.) gave pure ethyl oxoarachidates (3·0 g.). The oxoarachidates (1·4 g.) were converted into the oximes in the usual way. The purified oximes (1·1 g.), after Beckmann rearrangement, gave amides (1·0 g.). Hydrolysis with alkali gave basic and acidic fractions as before.

The basic fraction gave, on g.l.c. (on the PDS column at 100°), peaks corresponding in retention time to pentylamine, hexylamine, octylamine, and nonylamine only. At 200°,

[17] R. Perrotte, *Compt. rend.*, 1935, **200**, 746.

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g.l.c. gave the above amines (largely unresolved) together with undecylamine and dodecylamine.

A portion of the acidic fraction was methylated and run on g.l.c. About 15 peaks could be distinguished on both the SGR and PDS columns (50—200° at 15° per min.), but no certain assignment could be made because the mono- and di-ester peaks overlapped.

A portion (0·37 g.) of the acidic fraction was chromatographed on a column of silicic acid (75 g.) to give two fractions. Fraction 1 was eluted in ether–light petroleum–acetic acid (15 : 85 : 1 v/v; 600 ml.), and fraction 2 in solvent mixtures containing increasing concentrations of ether. The solvent was removed and the residues methylated using boron trifluoride–methanol. G.l.c. of fraction 1 gave 8 peaks attributable to the following monoesters: methyl hexanoate, heptanoate, nonanoate, decanoate, dodecanoate, tridecanoate, pentadecanoate, and hexadecanoate, identified from their retention times on both the SGR and PDS stationary phases (at 150° and 200°). Also present was a peak which could not be attributed to a normal monoester (C_{13}–C_{15} but *not* C_{14}). G.l.c. of fraction 2 gave 8 peaks corresponding to the following diesters: dimethyl pentanedioate, hexanedioate, octanedioate, nonanedioate, undecanedioate, dodecanedioate, tetradecanedioate, and pentadecanedioate. They were identified by their retention times on both the SGR (200°) and PDS (200°) columns. Also present was a peak which could not be attributed to a normal diester (C_9–C_{11}, but not C_{10}).

Mass spectrometric method. The spectra were obtained on an AEI MS12 instrument with voltage scanning. The samples were introduced through an all-glass inlet system at 200°.

Ethyl Oxoarachidates.—Ethyl 4- to 16-oxoarachidates (Table 8) were synthesised by Cason's "general procedure A." [9] The intermediate ω-ethoxycarbonyl-acids (Table 9) were prepared [18] from the corresponding diacids and converted into ω-ethoxycarbonylacyl chlorides with thionyl chloride.

TABLE 8

Ethyl oxoarachidates

Postn. of oxo-group	M. p.	Yield (%)	Found (%) C	Found (%) H
4	49—50°	72	74·7	12·0
5	58—59	71	74·5	11·9
6	45—46	67	74·9	12·2
7	47—48	72	74·7	11·9
8	44—45	87	74·8	11·9
9	49—50	75	74·9	11·9
10	50—51	96	74·6	11·9
11	47—48	60	74·4	11·8
12	50—51	71	74·5	11·8
13	47—48	74	74·6	12·0
14	43—45	82	74·7	11·9
15	48—49	51	75·2	11·9
16	49—51	96	75·1	12·0

TABLE 9

ω-Ethoxycarbonyl-acids, $C_2H_5OCO\cdot[CH_2]_n\cdot CO_2H$

n	B. p./mm.	M. p.	Yield (%)	Acyl chloride (b. p./mm.)
2	120—123°/3·0	—	51	63—64°/1·5
3	107/0·6	—	42	74—75/1·5
4	117/0·8	—	40	85/1·5
5	135—138/1·0	—	71	100/1·5
6	140—142/1·0	—	43	108—110/1·5
7	145—147/1·0	—	61	119—120/1·5
8	147/0·5	34—35°	58	135/2·0
9	165—167/0·5	40—41	53	142—144/2·0
10	174—176/0·5	48—49	51	156—158/2·0
11	175—177/0·5	55—56	53	163/1·25
12	178—180/0·5	59—60	48	171/1·25
13	184—186/0·5	64—65	62	169—170/0·5
14	196—198/0·5	64—65	54	174—177/0·5

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[18] S. Swann, R. Oehler, and R. J. Buswell, *Org. Synth.*, Coll. vol. II, 1943, 276.

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