Synthesis, Characterisation, and Transformations of a Lipid Cyclic Peroxide

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Photosensitised oxidation of (9*E*,11*E*)-methyl octadeca-9,11-dienoate gave an unsaturated cyclic peroxide (epidioxide) in high yield. This was characterised spectroscopically. The peroxide underwent facile rearrangement to a furanoid ester under a variety of reaction conditions. Catalytic reduction of the unsaturated peroxide cleaved the O–O bond. Bromination and epoxidation gave dibromo and epoxy esters respectively with the peroxide group still intact.

The role of singlet molecular oxygen ($^1O_2$) in enzyme-mediated peroxidation reactions *in vivo* has aroused considerable interest.[1–5]

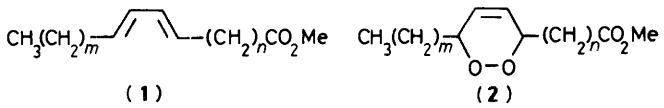
The products of the photosensitised oxidation of methyl oleate and of methylene-interrupted polyene esters have been identified and quantified by a variety of techniques.[6–10] Singlet oxygen reacts with such alkyl substituted olefins in an ene type reaction to give allylic hydroperoxides with a shifted double bond.[11–15] In contrast, reaction with conjugated dienes furnish cyclic peroxides (epidioxides) by 1,4-cycloaddition.[16]

Growing interest in the cyclic peroxides of unsaturated fatty acids is apparent in reports on the isolation of hydroperoxy epidioxides from both enzymic[17,18] and non-enzymic[19–22] oxidations of polyene acids. Singlet oxygen may also be involved in the biosynthesis of prostaglandins[23a,b] and of furano terpenes.[24a,b]

We report here the synthesis, reactions, and spectroscopic properties of an epidioxide produced by the photo-oxidation of a long-chain conjugated dionoic ester.

Results and Discussion

Preparation of the Epidioxide.—In their study of long-chain furanoid acids, Gunstone *et al.*[25] converted the (8*E*,10*E*)-diene ester (**1a**) into the epidioxide (**2a**) in a five-day photo-oxidation in methanol using Methylene Blue as sensitiser. We have now prepared the cyclic peroxide (**2b**) from the (9*E*,11*E*)-diene ester (**1b**) which we obtained from methyl ricinoleate.[26]



(**1**) (**2**)

a; $m = 6, n = 6$
b; $m = 5, n = 7$

The lifetime of singlet oxygen can vary about 1000-fold depending on the solvent in which it is generated. It has a lifetime of only 7 μs in methanol, but more than 600 μs in tetrachloromethane and Freon 11. We therefore used a mixture of tetrachloromethane and methanol (95:5) as solvent and a reaction time of only 16 h compared with the 5 days used previously. Using Methylene Blue as sensitiser and operating on a gram scale a >80% yield of epidioxide [based on the content of (*E*,*E*)-diene in the starting material] was obtained after purification by column chromatography.

The purified product showed a single gas chromatogram peak on a SP2300 column with the same retention time as that of the furanoid ester (**7**) and a sample of material recovered from the g.l.c. eluate had the same ^1H n.m.r. spectrum as the authentic furanoid ester. This change probably results from thermal dehydration on the column.[27]

Chromatographic analysis of a sample of the cyclic peroxide kept for 12 months at −20 °C showed less than 2% of more polar material.

The i.r. spectrum of the cyclic peroxide showed only the absorptions expected of a long-chain ester including an olefinic C–H stretching absorption at 3020 cm^{-1}.

The ^1H n.m.r. spectrum contained signals of special interest at δ 5.84 (2 H, d, *J* 0.8 Hz, olefinic protons), 4.37 (2 H, br, peroxy methines), and 1.47 (4 H, br, methylenes α to the peroxy methines). These assignments are based on decoupling experiments.

The ^{13}C n.m.r. spectrum showed one signal for the two olefinic carbon atoms (127.65 p.p.m.) and one for the two peroxidic carbon atoms (78.35 p.p.m.). No splitting of these signals was observed, even at −20 °C.

The mass spectrum displayed peaks characteristic of the corresponding furanoid ester and we assume that this is formed under the conditions existing in the mass spectrometer. Particularly significant are the peaks at M^+, 165, 95, and 81.

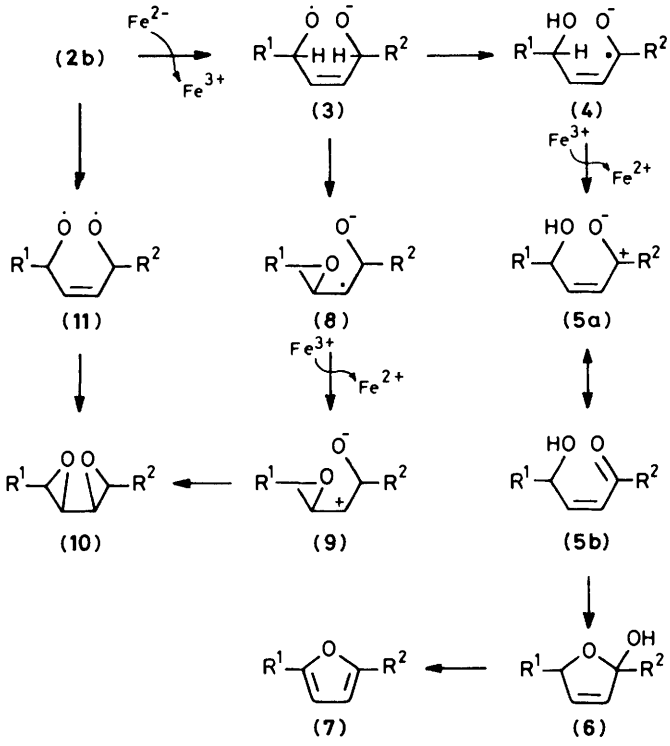
Fe^{2+} *Induced Decomposition of the Peroxide* (**2b**).—With ferrous ion in aqueous tetrahydrofuran the unsaturated cyclic peroxide (**2b**) is converted into the furanoid ester (**7**) and two minor products identified as the isomeric bis(epoxides) (**10**) by i.r., mass, and ^1H n.m.r. spectroscopy (Scheme 1).

The formation of the furanoid ester (**7**) probably follows the route shown by analogy with similar reactions in the phellandrene series.[28] Cleavage of the O–O bond, as a result of one-electron transfer to Fe^{2+}, gives the radical anion (**3**) which is converted into (**4**) by 1,5-hydrogen migration. This furnishes, in turn, the hydroxy ketone (**5b**), the hemiacetal (**6**), and the furan (**7**). Dehydration is probably catalysed either by the Lewis acid Fe^{3+} or by a proton within the system. The reaction is worked up by extraction of an ethereal solution of the product mixture with dilute acid to facilitate the removal of Fe^{3+}. However dehydration of compound (**6**) does not occur at this stage since t.l.c. showed the presence of furan prior to work up, confirming similar observations by Turner *et al.*[29]

The rearrangement of the peroxide (**2b**) to the bis(epoxides) (**10**) may occur *via* the radical anion (**3**) and the monoepoxides (**8**) and (**9**) or *via* the diradical (**11**) (Scheme 1).

We were unable to determine the exact stereochemistry of the bis(epoxides), but a related study suggests that they have the *trans* configuration.[30]

Thermolysis of the Peroxide (**2b**).—Heating the peroxide (**2b**) in various solvents at reflux temperature or at 80 °C where this is lower than the b.p. sometimes gave an appreciable amount of the furan (**7**) along with unidentified minor products (<5%) (Table 1). Separate experiments showed that there was no significant decomposition of the peroxide in boiling toluene even after 24 h and that longer reaction times in butanol gave >90%



Scheme 1. $R^1 = Me(CH_2)_5$, $R^2 = (CH_2)_7CO_2Me$

of the furanoid ester. Our results suggest that a solvent of high dielectric constant (*E*) is required before reaction occurs. This is in marked contrast to bicyclic peroxides which undergo thermolysis readily in toluene.[31]

Bromination of the Peroxide (**2b**).—Bromination of the unsaturated peroxide in dichloromethane solution was complete within 5 min and almost quantitative yields of dibromide were obtained. Useful spectroscopic data are reported in the Experimental section.

Epoxidation of the Peroxide (**2b**).—Using a two-phase system of dichloromethane and aqueous sodium hydrogen carbonate, the unsaturated cyclic peroxide (**2b**) was epoxidised at room temperature for 16 h with 3-chloroperoxybenzoic acid and the product isolated in high yield by preparative t.l.c. The unsaturated cyclic peroxide can exist in two half-chair conformations and epoxidation can occur from either the *exo*-or the *endo*-face of the ring; however molecular models suggest that *endo* attack would be less sterically hindered.

The ^1H n.m.r. spectrum of the epoxy cyclic peroxide showed a two-proton multiplet at δ 4.22 corresponding to the peroxide methines and two one-proton multiplets at δ 3.35 and 3.16 corresponding to epoxidic methines. The ^{13}C n.m.r. spectrum showed only one resonance for the epoxide carbons at 52.25 p.p.m. The proton-coupled spectrum split the resonance into a doublet (*J* 38.25 Hz). Our results do not allow us to conclude whether both *exo*- and *endo*-epoxides are formed or whether *endo*-epoxide formation is stereospecifically favoured.

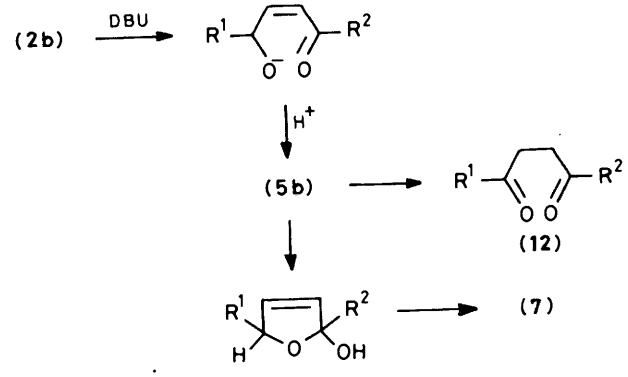
The mass spectrum of the epoxide at 70 eV was uninformative, but more intense peaks were observed at lower ionising potentials (15 eV). The most significant high mass fragments were observed at *m/z* 257 and 225 at 15eV. The symmetrical stretching or ring breathing frequency of the epoxy ring is known to occur at *ca.* 1 250 cm^{-1} in the i.r. spectrum. Although an absorption was observed it must be remembered that methyl esters absorb near 1 250, 1 205, and 1 175 cm^{-1} and the absorption at 825 cm^{-1} is more diagnostic for epoxides.

Table 1. Conversion of the peroxide (**2b**) into the furan (**7**) by heating in various solvents for 5 h

Solvent	*E*/Debye (°C)	Furan[a] (%)
Hexane	1.89(20)	none
Toluene	2.38(25)	none
Chloroform	4.38(20)	5
Methanol	32.6 (25)	27
Butan-1-ol	17.8 (20)	33
DMF		36

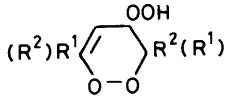
[a] Determined by ^1H n.m.r. spectroscopy.

Reaction of the Peroxide (**2b**) with 1,8-*Diazabicyclo*[5.4.0]-*undec-7-ene* (*DBU*).—The cyclic peroxide (**2b**) is stable in dilute acid solution, being recovered unchanged from a solution of methanolic sulphuric acid (2M) at room temperature after 36 h. In contrast it undergoes a series of changes when stirred with DBU in hexane at room temperature (Scheme 2). The product contained the furan ester (**7**) (4%), methyl 9,12-dioxostearate (**12**) (19%), (10*Z*)-methyl 9(12)-hydroxy-12(9)-oxo-octadec-10-enoates (**5b**) (74%), and an unidentified polar product (3%). These were identified by spectroscopic procedures and are probably formed according to the reaction sequence shown in the Scheme. The instability of the ketol mixture complicates its recovery and identification. Its mass spectrum is similar to that of the furan (**7**) into which it is probably converted in the mass spectrometer. On standing, even at −20 °C, it rearranged in part to furanoid (*ca.* 20%) and diketo esters (*ca.* 5%) and on gas chromatographic analysis gave three peaks of which the largest (70%) is that associated with the furanoid ester. On standing longer with DBU the dioxo ester (**12**) was the major product (80—85%). It is known that in an acidic solution, the 1,4-dioxo and the furanoid esters are in equilibrium.[32] Similar observations on bicyclic peroxides have been reported by Hagenbuch *et al.*[33]



Scheme 2. $R^1 = Me(CH_2)_5$, $R^2 = (CH_2)_7CO_2Me$

Attempted Further Oxidation of the Peroxide (**2b**) *with Singlet Oxygen.*—An attempt to oxidise the unsaturated peroxide (**2b**) to hydroperoxy peroxides such as (**13**) by further reaction with singlet oxygen gave only unchanged starting material after 5 days.



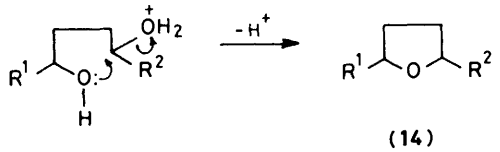
(**13**)

$R^1 = Me(CH_2)_5$, $R^2 = (CH_2)_7CO_2Me$

Reduction of the Peroxide (**2b**).—(i) Attempts to reduce the cyclic peroxide (**2b**) with sodium borohydride were unsuccessful. The stability of the dialkyl peroxide to this reagent is in contrast to that of hydroperoxides which are smoothly reduced to the corresponding hydroxy derivative.

(ii) Catalytic hydrogenation of the cyclic peroxide over a Pd/C catalyst (10%) under hydrogen for *ca.* 90 min gave mainly methyl 9,12-epoxystearate (**14**) as a mixture of *cis*- and *trans*-isomers together with methyl stearate, methyl 9- and 12-hydroxystearates, their corresponding keto derivatives, and small amounts of methyl 9,12-dihydroxystearate. Shorter reaction times gave more 9,12-dihydroxystearate and less epoxystearate.

We consider that this depends on the acidic nature of the catalyst support. The dihydroxystearate formed first undergoes facile cyclisation thus:

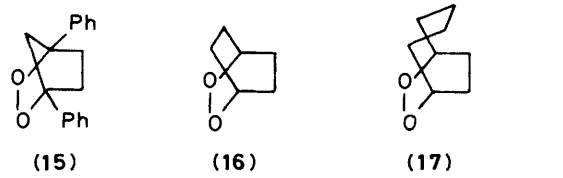


(14)

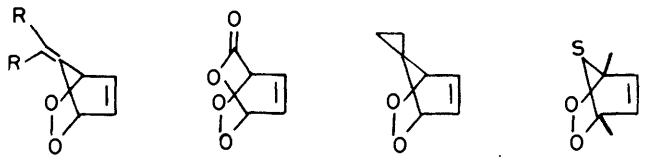
$R^1 = Me(CH_2)_5, R^2 = (CH_2)_7CO_2Me$

Support for the formation of the dihydroxystearate as an intermediate was obtained by the catalytic hydrogenation of an authentic sample of methyl 9,12-dihydroxystearate.[34] Amongst the products was methyl 9,12-epoxystearate (**14**) as a mixture of *cis*- and *trans*-isomers. Monohydroxy and monoketo esters result from the hydrogenolysis of the dihydroxystearate.

(iii) Some unsaturated bicyclic peroxides such as ascaridole have been selectively reduced by catalytic hydrogenation to saturated peroxides, but complete reduction of unsaturated peroxides to saturated diols is more usual.[35] The discovery that di-imide[36] reduces the double bond of singlet oxygen–diene adducts while leaving the peroxide linkage intact opened up a general route to the saturated bicyclic peroxides (**15**)—(**17**).[36–38] This reduction is usually effected in methanol solution using di-imide generated *in situ* from dipotassium azodicarboxylate and acetic acid. For more labile compounds such as (**18**)—(**21**) it is desirable to use dichloromethane as



(15) (16) (17)



(18) (19) (20) (21)

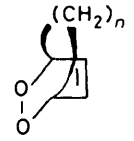
solvent and to employ a little less than the required amount of acetic acid.[39–41] Addition of hydrogen occurs stereospecifically *cis–exo* as a result of steric hindrance to *endo* attack due to the non-bonding electron pairs on oxygen, and also as a result of the unsymmetrical π-bond which has greater electron density on the *exo*-face.[42]

Attempts to reduce compound (**2b**) with di-imide were unsuccessful in dry methanol or dichloromethane even after the reaction had been repeated using a 15-fold excess of dipotassium azodicarboxylate. Conducting the experiment under nitrogen or in air made no difference. No examples of the di-imide reduction of monocyclic peroxides are to be found in the literature.

Table 2. Effects of ring strain on the reduction of bicyclic systems with di-imide

Cyclic peroxide (**22**)	% Reduction	Ref.
$n = 1$	30	41
$n = 2$	40—48[a]	37
$n = 3$	20—38[a]	38
$n = 4$	19	38

[a] We have obtained similar results for these two compounds.



(22) $n = 1-4$

As the ring strain is reduced in the bicyclic ring systems, *i.e.* with an increase in n, then di-imide reduction becomes increasingly difficult (Table 2). However, it is unlikely that ring strain is the overriding consideration since acyclic olefinic systems can be reduced. For example, methyl linoleate has been reduced with a two-fold excess of di-imide to a mixture containing monoenoic (40.8%) and fully saturated ester (11.4%). Reductions of many other cyclic compounds have also been reported.[43] A more likely explanation is that the approach of di-imide to the cyclic peroxide (**2b**) is hindered through an unfavourable electronic interaction between di-imide and the non-bonded electron pairs on oxygen. In bicyclic systems (**22**) with $n = 1,2$, or 3 the preferred conformation of the six-membered ring is such that the C–O–O–C moiety is co-planar, *i.e.* in the boat conformation. However in a non-strained system such as (**2b**) the preferred conformation of the six-membered ring will be half-chair conformers in which electron repulsion will be experienced on approach of di-imide from either above or below the plane of the double bond. With large values of n in bicyclic peroxides, the conformation of the six-membered ring will tend to the half-chair conformation, hence making reduction more difficult.

Experimental

Thin layer chromatography (t.l.c.) was carried out on thin layers of silica gel-G (0.5 mm for preparative purposes, 0.25 mm for analytical purposes). The developing solvent was light petroleum–ether, symbols such as PE30 indicating a 70:30 mixture by volume of light petroleum and ether. Light petroleum refers to the fraction with b.p. 40—60 °C, and ether to diethyl ether. Peroxidic material was apparent after spraying with either a freshly prepared methanolic solution of 4-amino-*N*,*N*-dimethylaniline hydrochloride (2%) or a freshly decolourised solution of ammonium thiocyanate and ferric chloride in pentan-1-ol.[44]

Gas liquid chromatography (g.l.c.) was carried out on a Pye series 104 chromatograph equipped with a flame ionisation detector and fitted with a glass column (5 ft × ¼ in) packed with SP2300 (10%) as the stationary phase on Chromosorb WAW (100—120 mesh) operating at 220 °C. Carrier gas (nitrogen) was used at a flow rate of 55—65 ml/min. Retention charac-

teristics are reported as equivalent chain lengths (E.C.L.) using saturated esters as standards.

I.r. spectra were run on a Perkin-Elmer 257 grating spectrophotometer using thin films between sodium chloride discs. Only absorption frequencies of diagnostic value are quoted. ^{1}H N.m.r. spectra were recorded at 80 MHz on a Bruker WP80 instrument, using dilute solutions in deuteriochloroform. Chemical shifts are given in p.p.m. relative to the chloroform lock signal at δ 7.27. ^{13}C N.m.r. spectra were recorded at 20 MHz on a Varian CFT-20 ^{13}C n.m.r. instrument, using dilute (0.5—0.55M) solutions in deuteriochloroform. Mass spectra were obtained following the direct probe insertion of samples into the source of the mass spectrometer (AEI MS902). The source pressure was 2.7 × 10^{-5} Pa, source temperature 200 °C, and the ionisation voltage 70 eV unless otherwise stated.

Preparation of Methyl Octadeca-9,11-dienoate (**16**)—Methyl ricinoleate (12-hydroxyoctadec-9-enoate) was isolated from castor oil esters by chromatography on a column of silica using PE25 as developing solvent, or by preparative h.p.l.c. using light petroleum–isopropyl alcohol (98.5:1.5) as eluant on normal phase chromatography (R_t 9 min). The hydroxy ester (4.5 g, 14 mmol) was stirred with redistilled methanesulphonyl chloride (4 ml, 45 mmol) and pyridine (30 ml) for 2 h at 0 °C and then for 2 h at 5—10 °C. Ice-cold hydrochloric acid (2M; 150 ml) was then added slowly with further cooling. Methyl 12-mesyloxyoleate (4.7 g) was obtained as a straw-coloured oil by extraction with ether (3 × 50 ml).

A mixture of methyl 12-mesyloxyoleate (4.7 g, 1.2 mmol), toluene (20 ml), and 1,8-diazabicyclo[5.4.0]undec-7-ene (8.0 ml) was refluxed for 4 h. The resulting solution was cooled, neutralised with acetic acid, diluted with water (40 ml), and extracted with ether (3 × 50 ml) to yield a product (2.9 g). G.l.c. analysis showed three peaks due to the (9*Z*,12*E*) (6%), (9*Z*,11*E*) (73%), and (9*E*,11*E*) (21%) octadecadienoates.

Isomerisation of this mixture with a crystal of iodine in hexane (20 ml) illuminated by a 100-W tungsten lamp for 4—6 h gave a product (2.9 g), after washing with 0.1M-sodium thiosulphate solution (50 ml), containing the (9*Z*,12*E*) (6%), (9*Z*,11*E*) (22%), and (9*E*,11*E*) (72%) octadecadienoates.

Photosensitised Oxidation of (9E,11E)-*Methyl Octadeca-9,11-dienoate*.—The conjugated diene (**1b**) (1.00 g, 3.3 mmol) was placed in a photochemical reactor with an inner and outer cooling jacket. Methylene Blue (50 mg), dissolved in dry tetrachloromethane–methanol (95:5 v/v, 250 ml) was added and oxygen was bubbled through the solution at a flow rate of 50—60 ml/min for 16 h. The flask was illuminated with tungsten light bulbs (3 × 150 W; 6 in from the flask). The cyclic peroxide [6-hexyl-3-(7-methoxycarbonylheptyl)-3,6-dihydro-1,2-dioxine] (**2b**) was isolated by column chromatography on a silica column (30 g) eluting with a PE gradient from 5—20. The pure cyclic peroxide (**2b**) (560 mg) was eluted after the unchanged starting material: $\nu_{max.}$ 3 020 cm^{-1} (olefinic C–H stretch); δ_H 5.84 (2 H, d, *J* 0.8 Hz, HC=CH), 4.37 (2 H, m, HCO), 3.62 (3 H, s, CO_2CH_3), 2.32 (2 H, asym. t, CH_2CO_2Me), 1.47 (shoulder, 4 H, 8- and 13-methylenes), 1.35 (18 H, br s, chain methylenes), and 0.8 (3 H, asym. t, CH_3); δ_C 174.16 (C-1), 127.65 (C-10 and -11), 78.35 (C-9 and -12), 51.37 (OCH_3), 34.08 (C-2), 33.04 (C-8 and -13), 31.74 (C-16), 29.10 (C-4 to -6, C-15), 25.38 (C-7 and -14), 24.94 (C-3), 22.58 (C-17), and 14.05 p.p.m. (C-18); *m/z* 309 (0.3%, $M^+ + 1$), 308 (3, M^+), 277 (1), 165 (47), 152 (100), 95 (63), 81 (100), and 74 (79) and other unlisted values.

Table 3 gives accurate mass numbers for the significant cleavage fragments from compound (**2b**). The assignments are compatible with the suggestion that the product is converted into the furanoid ester (7) in the mass spectrometer (see following section).

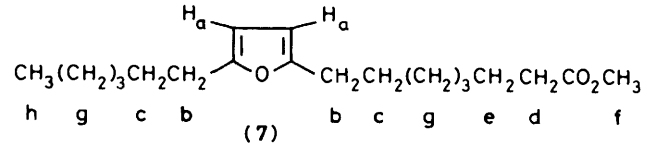
Table 3. Accurate mass analysis for the significant cleavage fragments from compound (**2b**)

	Residual mass		
m/z	Calc.	Found	Assignment[a]
308	0.235 131	0.234 774	$C_{19}H_{32}O_3$ (M^+)
277	0.216 743	0.217 605	$C_{18}H_{29}O_2$ ($M^+ - 31$)
237	0.149 060	0.148 316	$C_{14}H_{21}O_3$
165	0.127 933	0.127 177	$C_{11}H_{17}O$
95	0.048 916	0.049 687	C_6H_7O
81	0.033 884	0.034 037	C_5H_5O

[a] For structures, see discussion of the spectrum of the furan ester (7).

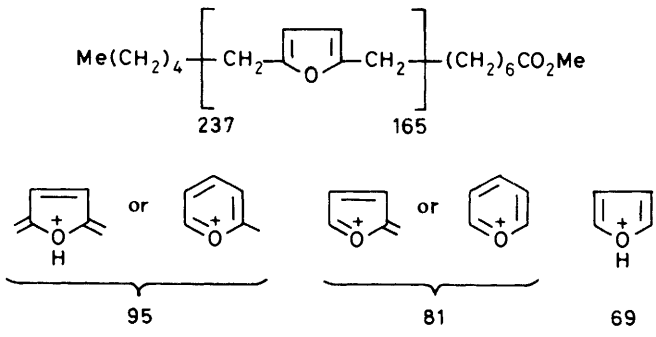
Reaction with Ferrous Sulphate.—A solution of ferrous sulphate (40 mg) in distilled water (0.6 ml) was added to a solution of compound (**2b**) (40 mg) in redistilled tetrahydrofuran (0.6 ml) and stirred at room temperature for 5 h. After removal of the solvent at 10 °C, the mixture was diluted with distilled water (10 ml), acidified with hydrochloric acid (2M) to facilitate the removal of the ferric sulphate, and extracted with ether (3 × 15 ml). G.l.c. analysis of the product (36.4 mg) showed only one peak, but preparative t.l.c. gave five bands on elution with PE20.

Band A (16.8 mg, 50%; E.L.C. 20.4, R_F 0.71 in PE20) was identified as methyl 9,12-epoxyoctadeca-9,11-dienoate (7) on the basis of the following spectroscopic data: $\lambda_{max.}$ 224; $\nu_{max.}$ 3 145 (C–H furan ring stretching), 1 745 (C=O stretching), 1 683 (C=C in furan stretching), and 1 012 cm^{-1} (ring breathing); δ_H 5.83 (2 H, s, H_a), 3.68 (3 H, s, H_f), 2.58 (4 H, asym. t, H_b, *J* 7.0 Hz), 2.30 (2 H, asym. t, Hd, *J* 7.0 Hz), 1.63 (6 H, br m, H_c and



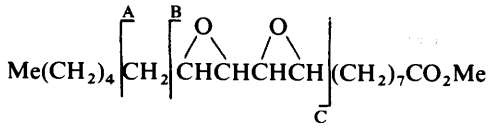
(7)

H_e), 1.20 (12 H, br s, H_g) and 0.88 (3 H, asym. t, *J* 6.2 Hz, H_h); *m/z* 308 (4%, M^+), 277 (2, $M^+ - 31$), 237 (5), 205 (4, 237 − 32), 165 (66), 95 (76), 81 (69), 74 (44, McClafferty rearrangement), 69 (48), 55 (base peak, $C_4H_7^+$) and other unlisted values.



Band B (4.6 mg, 14%, E.C.L. 20.4, R_F 0.56) was unchanged peroxide.

Band C (4.3 mg, 13%, no peak on g.l.c., R_F 0.29) was a mixture of diastereoisomeric methyl 9,10:11,12-bisepoxyoctadecanoates: δ_H 3.68 (3 H, s, CO_2CH_3), 2.85 and 2.60 (4 H, both m, epoxidic H), 2.30 (2 H, asym. t, $CH_2CO_2CH_3$), 1.55 and 1.35 (chain methylenes), and 0.88 (3 H, asym. t, CH_3); *m/z* 241 (0.3%, fragment B), 225 (0.3, A − 30), 223 (0.4, A − 32), 209 (2, B − 32), 181 (2, A − 74), 169 (2, C), 55 (100), and other unlisted values.



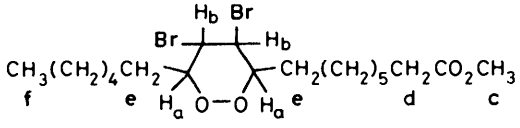
Band D (2.9 mg, 9%, no peak on g.l.c., R_F 0.24) gave ^{1}H n.m.r. and mass spectra identical with those obtained for band C.

Band E (4.8 mg, 14%; R_F <0.1) was not identified.

Thermolysis of Compound (**2b**).—(*a*) The peroxide (**2b**) (50 mg, 0.15 mmol) was heated to 80 °C for 5 h with butan-1-ol (2 ml). After removal of the solvent under reduced pressure (2 mmHg) the residue was analysed by g.l.c., t.l.c., and ^{1}H n.m.r. spectroscopy. In some experiments with other solvents the reaction temperature was that of the refluxing solvent (Table 1). The product was a mixture of unchanged peroxide (**2b**), the furan (**7**), and unidentified minor products (<5%).

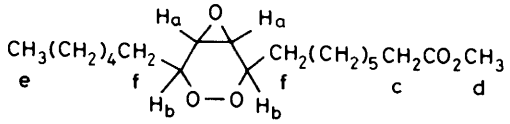
(*b*) When the peroxide (50 mg) was refluxed with toluene for 24 h, the product showed one major spot on t.l.c. (>96%) and a single peak of E.C.L. 20.4 on g.l.c. Its spectroscopic properties were identical with those of the starting cyclic peroxide.

Bromination of the Peroxide (**2b**).—Bromine (60 mg, 0.33 mmol) was added in the dark to a solution of the peroxide (**2b**) (100 mg, 0.3 mmol) in dichloromethane (5 ml) in a vial kept at 0 °C. After 5 min the solvent was removed under a stream of dry nitrogen and the residue, purified by preparative t.l.c. (R_F 0.80, PE10), gave 4,5-dibromo-6-hexyl-3-(7-methoxycarbonylheptyl)-1,2-dioxine in almost quantitative yield: $\nu_{max.}$ absence of



3 030 (C=C), 733 and 909 cm^{-1} (C–Br$_{eq}$ stretch); δ_H 4.52—4.10 (2 H, m, H_a), 4.23 (2 H, dd, *J* 14.0 and 8.0 Hz, H_b), 3.64 (3 H, s, H_c), 2.28 (2 H, asym. t, H_d), 1.50—1.25 (4 H, m, H_e), 1.30 (18 H, br s, chain CH_2), and 0.88 (3 H, asym. t, H_f); δ_C 173.95 (C-1), 86.84 and 88.58 (C-9 and -12), 55.73 and 52.72 (C-10 and -11), 51.15 (OCH_3), 33.81 (C-2), 31.73 (C-16), 31.46 and 31.33 (C-8 and -13), 28.74, 26.74 (C-4 to -6 and -15), 25.27, 25.09 (C-14 and -7), 24.66 (C-3), 22.32 (C-17), and 13.79 p.p.m. (C-18); *m/z* 389 and 387 (3, M^+ – Br – H_2O), 357 and 355 (3, 389 and 387 – MeOH), 308 [25, M^+ – 2Br – H_2O *i.e.* furan ester (**10**)], 275 and 277 (5), 261 and 259 [8, fragment C – HBr – CH_2=C(OH)OCH_3], 249 and 247 (3, B′ – HBr), 245 and 243 (13, A′ – HBr – H_2O), 237 (5), 209 (8), 185 (60), 181 (15), 165 (44), 155 (60, B – 2 H), 143 (15, A), 113 (61) and other unlisted values.

Epoxidation of the Peroxide (**2b**).—A mixture of the cyclic peroxide (**2b**) (95 mg, 0.29 mmol) in dichloromethane (2 ml), 3-chloroperoxybenzoic acid (80%, 70 mg, 0.32 mmol) in the same solvent (3 ml), and saturated aqueous sodium hydrogen carbonate (5 ml) was stirred vigorously for 16 h. The organic layer was separated and washed with sodium hydrogen carbonate solution (2 × 5 ml), brine (5 ml), and water (3 ml). All the aqueous washings were re-extracted with trichloromethane (2 ml) and the organic layers were combined. Preparative t.l.c. (PE25) gave three bands. The major band (83 mg, 90%, R_F 0.57) was identified as 4,5-epoxy-6-hexyl-3-(7-methoxycarbonylheptyl)-1,2-dioxane on the basis of the following

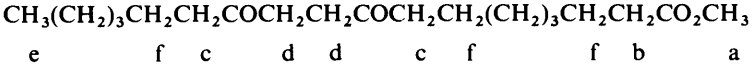


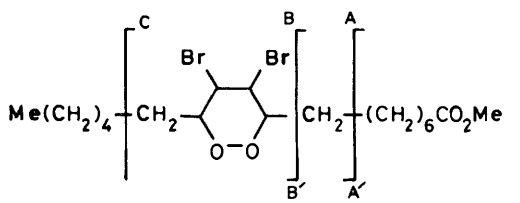
spectroscopic evidence: $\nu_{max.}$ 813 cm^{-1}; δ_H 4.22 (2 H, m, H_b), 3.63 (3 H, s, H_d), 3.35 (1 H, apparent dd, H_a), 3.16 (1 H, apparent t, H_a), 2.30 (2 H, asym. t, *J* 7.0 Hz, H_c), 1.80—1.50 (4 H, shoulder, H_f), 1.30 (br s, chain CH_2), and 0.88 (3 H, asym. t, *J* 5.5 Hz, H_e); δ_C 78.02 and 77.42 (C-9 and -12), 52.25 (C-10 and -11), 51.25 (OCH_3), 33.91 (C-2), 31.59 (C-16), 30.19, 28.90, and 25.06 (C-4 to -7 and -12 to -15), 24.76 (C-3), 22.38 (C-17), and 13.86 p.p.m. (C-18); *m/z* (15 eV) 257 (3, M – C_6H_{13}), 225 (27, M – C_6H_{13} – 32), 155 [55, M – $(CH_2)_7CO_2Me$ – 2], 86 (100), 74 (55, McClafferty rearrangement), and other unlisted values.

Reaction with DBU.—DBU (25 mg, 0.16 mmol) was added to a solution of the peroxide (**2b**) (50 mg, 0.15 mmol) in hexane (3 ml) and the mixture was stirred at room temperature until t.l.c. showed the complete consumption of the peroxide. After the removal of the solvent under a stream of dry nitrogen, the residue was separated into four fractions (net recovery 36.1 mg, 72%) by preparative t.l.c. (PE30).

Band A (1.3 mg, 4%, R_F 0.7 in PE20) gave a single peak on g.l.c. and was identified as the furanoid ester (**7**) by comparison with an authentic sample.

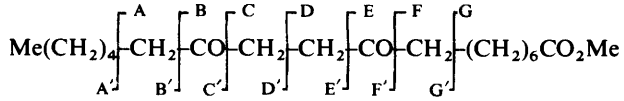
Band B (6.8 mg, 18.8%, R_F 0.52 in PE30) was identified as methyl 9,12-dioxostearate on the following spectral evidence: $\nu_{max.}$ 1 740 (ester carbonyl stretch) and 1 700 cm^{-1} (ketone carbonyl stretch); δ_H 3.68 (3 H, s, H_a), 2.65 (4 H, s, H_d), 2.44 (4 H, t, *J* 7.0 Hz, H_c), 2.29 (2 H, t, *J* 7.0 Hz, H_b), 1.55—1.30 (6 H, shoulder, H_f), 1.25 (12 H, br s, chain CH_2), and 0.88 (3 H, asym. t, *J* 5.4 Hz, H_e); *m/z* 326 (3%, M^+), 308 (0.5, M^+ – 18), 295 (1, M^+ – 31), 256 (10, fragment A + 1), 241 (1, B), 213 (7, C), 184 (18, G′ + 1), 183 (18, G′), 169 (20, F′), 141 (20, E′), 127 (19, D′), 114 (38, C′ + 1), 113 (37, C′), 85 (33, B′), 71 (49, A′), 55 (100), and other unlisted values.

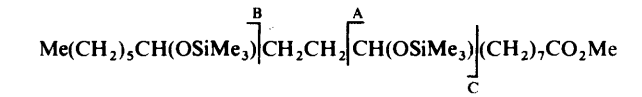




The peaks at *m/z* 185 $[CO(CH_2)_7CO_2Me]^{+\cdot}$, 155 (B – 2 H)$^{+\cdot}$, 143 $[(CH_2)_6CO_2Me]^{+\cdot}$, and 113 (155 – C_3H_6)$^{+\cdot}$ are common to 9,12-dioxygenated methyl esters, and those at 277, 237, 209, 181, and 165 are derived from the furan ester (**7**).

Sodium borohydride reduction of band B followed by trimethylsilylation afforded methyl 9,12-bis(trimethylsilyloxy)-stearate, identified by comparison with an authentic sample on g.l.c. and by its characteristic mass spectrum fragmentation pattern which showed peaks at *m/z* 259 (15%, fragment A), 227 (12, A – 32 and/or C – 90), 187 (27, B), 155 (15, A – Me_3Si – OMe), 57 (100), and other unlisted values.





Independent synthesis of 9,12-dioxostearic acid[45] followed by esterification gave a product spectroscopically identical with the material in band B.

Band C (27 mg, 75%, R_F 0.17 in PE30) was shown to be a mixture of (10*Z*)-methyl 9(12)-hydroxy-12(9)-oxo-octadeca-10-enoates (**5b**) on the basis of the following spectroscopic results: $\nu_{max.}$ 3 600—3 200 (O–H stretching) and 1 740, 1 670, and 1 628 cm^{-1} (C=O stretch); δ_H 6.80 (1 H, dd, J_{dc} 16.0 Hz, J_{cb} 1.4 Hz, H_c), 4.50—4.13 (1 H, br m, H_b), 3.61 (3 H, s, CO_2CH_3), 2.50 (2 H, asym. t, *J* 7.2 Hz, $CH_2CO_2CH_3$), 2.25 (2 H, asym. t, *J* 7.2 Hz, H_a), 1.77—1.40 (shoulder), 1.27 (br s, chain methylene), and 0.81 (3 H, t, *J* 5.5 Hz, CH_3); *m/z* (see text for explanation of the fragmentation pattern) 308 (27%), 277 (12), 237 (18), 165 (100), 95 (50), 81 (32), 74 (21), 69 (32), and other unlisted values.

$$(R^2)R^1CH_2COCH{=}CHCH(OH)R^2(R^1)$$

a d c b

$R^1 = Me(CH_2)_4$ $R^2 = (CH_2)_7CO_2Me$

Band D (1.0 mg, 3%, R_F 0.05—0.00 in PE30) was not identified.

Reaction with Methanolic Sulphuric Acid.—The cyclic peroxide (**2b**) (20 mg) was stirred with 10% methanolic sulphuric acid (5 ml) for 36 h at room temperature. The starting material was recovered unchanged in quantitative yield.

Attempted Further Oxidation with Singlet Oxygen.—The cyclic peroxide (**2b**) (100 mg) and methyl palmitate (5 mg) were placed in a photolytic cell with tetrachloromethane (238 ml), methanol (12 ml), and Methylene Blue (50 mg) and irradiated (3 × 150-W tungsten bulbs) for 5 days. Aliquots (*ca.* 0.1 ml) were withdrawn at 12 h intervals and examined by g.l.c. There was no change in the relative size of the two peaks. T.l.c. examination of the product after 5 days also indicated that the peroxide was unchanged.

Attempted Reduction with Sodium Borohydride.—A solution of sodium borohydride (20 mg) in dimethylformamide (0.3 ml) was added to the cyclic peroxide (**2b**) in the same solvent (1 ml) and stirred for 1 h. After the addition of water (2 ml) and extraction with ether (3 × 5 ml), the product (18.5 mg) was shown to be unchanged starting material on the basis of its g.l.c. (single peak at E.C.L. 20.49) and t.l.c. behaviour. A similar result was obtained when the reaction was attempted in water–tetrahydrofuran (1:1).

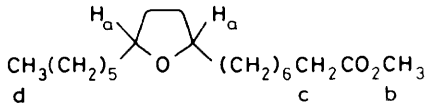
Attempted Reduction with Di-imide.—A mixture of the peroxide (**2b**) (100 mg, 9.3 mmol) and dipotassium azodicarboxylate (870 mg, 4.5 mmol) in super-dry methanol (10 ml) was kept at 0 °C while a 30% solution of acetic acid in methanol (5 ml) was added dropwise during 30 min. After 3—5 h the solvent was evaporated at room temperature and the residue, dissolved in water (20 ml), was extracted with light petroleum (2 × 25 ml). After washing with saturated sodium hydrogen carbonate (2 × 15 ml), the product was examined by ^1H n.m.r. spectroscopy to determine the ratio of the size of the signals at δ 5.84 (olefinic hydrogens) and 4.37 (peroxy methines) as an indication of the extent of reduction. No reduction was observed in five separate experiments.

The experiment was repeated without success (i) using dichloromethane as solvent in place of methanol, (ii) under nitrogen using each of the solvents used above, and (iii) repeating the reduction procedure three times on the same sample.

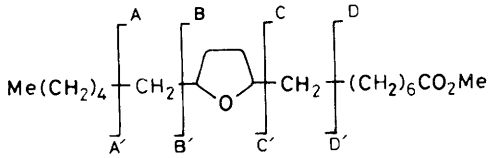
Catalytic Hydrogenation.—A mixture of 10% palladium–charcoal (20 mg), methanol (5 ml), and cyclic peroxide (20 mg) was stirred at room temperature for 90 min under hydrogen. The charcoal was filtered off and washed with methanol (2 × 5 ml). The combined washings were evaporated at room temperature to furnish an organic product (16 mg). Preparative t.l.c. (PE30) gave seven bands.

Band A (E.C.L. 18.0, R_F 0.63 in PE20) was identified as methyl stearate from its E.C.L., R_F value, and mass spectrographic comparison with authentic material.

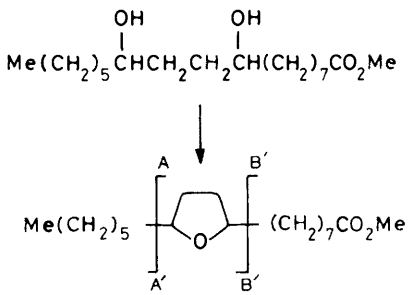
Band B (E.C.L. 20.5 (65%) and 20.7 (35%), R_F 0.5 in PE20) was identified as a mixture of *cis*- and *trans*-methyl 9,12-epoxyoctadecanoates on the following spectral evidence: δ_H 3.80 (2 H, br s, H_a), 3.68 (3 H, s, H_b), 2.30 (2 H, asym. t, H_c), 1.30 (br s, chain CH_2), and 0.88 (3 H, asym. t, H_d); *m/z* 313 (1%, $M^+ + 1$), 312 (1, M^+), 294 (0.8, $M^+ - 18$), 281 (2, $M^+ - 31$),



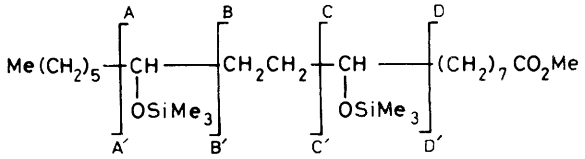
277 (48, fragment B), 209 (15, B − 18), 195 (51, B − 32), 177 (14, B − 18 − 32) 155 (100, C′ and/or C − 2), 137 (55, A − 18), and other unlisted values.



Bands C and D were shown to be methyl 9-and 12-oxostearates, and bands E and F methyl 9- and 12-hydroxystearates, by g.l.c. and mass spectroscopic comparison with authentic material.



Band G was identified as methyl 9,12-dihydroxystearate based on the following spectral evidence: *m/z* 312 (1, $M - 18$), 227 (27, fragment A), 209 (9, 227 − 18), 195 (33, 227 − 32), 177 (11, 209 − 32), 155 (100, B′ and/or B − 2), 137 (30, B′ − 18), 85 (33, A′), 55 (100), and other unlisted values.



The mass spectrum of the bis(trimethylsilyl) ether showed *m/z* 369 (11, A − 30), 283 (8, A − 104), 259 (13, C), 227 (13, C − 32), 187 (13, B′), 155 (10, C − 104 and/or D − 2), 57 (100), and other unlisted values.

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