Stereochemistry of Olefin and Fatty Acid Oxidation. Part 3.[1] The Allylic Hydroperoxides from the Autoxidation of Methyl Oleate

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Methods have been developed, using ^{13}C n.m.r. spectroscopy and mass spectrometry, for the analysis of all eight *cis* and *trans* allylic 8-, 9-, 10-, and 11-hydroperoxides formed on autoxidation of methyl oleate.

The autoxidation of fatty acids, and their derivatives, has received much attention recently because lipid hydroperoxides have been shown to be precursors of prostaglandin-related endoperoxides,[2] and to play a role in photocarcinogenesis, [3] in the destruction of proteins and biomembranes,[4] and in chemically induced toxicity.[5] Although the autoxidation of methyl oleate (**1**) and related compounds has been the subject of many investigations (see for example[6-8]), the stereochemical course of this reaction had not been fully established prior to the studies now reported (for preliminary publication see ref. 9).

In view of the instability of the allylic hydroperoxides (**3**)—(**6**) formed on autoxidation of methyl oleate, attention was first directed to devising methods for the analysis of the mixtures of allylic alcohols (**7**)—(**10**) readily obtained by borohydride reduction of the initial autoxidation products (evidence is given later that these reductions involve no loss in stereochemistry). To develop suitable procedures for analysing such mixtures, four authentic hydroxyoctadecenoates, *cis*- and *trans*- (**11**) and (**8**), were prepared by unambiguous methods.

The *trans*-isomer of (**11**) was conveniently obtained from ricinoleic acid (**12**), *via* the keto-ester (**13**), by known procedures (see Experimental section). Reaction of the silver salt of the readily available methyl undec-10-ynoate[10,11] with heptanoyl chloride gave the expected acetylenic ketone (**14**). Reduction of the keto group with borohydride, followed by partial catalytic hydrogenation of the triple bond, gave the *cis*-isomer of (**11**).

A Grignard condensation of non-1-yne with methyl 8-formyloctanoate[12] yielded the acetylenic hydroxy-ester (**15**). Partial catalytic hydrogenation gave the *cis*-isomer of (**8**). Selective oxidation of the allylic alcohol function with pyridinium chlorochromate gave, as expected, the *trans*-keto-ester (**16**) which was reduced with borohydride to give the required *trans*-isomer of (**8**).

The stereochemistry of the model compounds was confirmed by their i.r. light absorption spectra. As expected, the *trans*-isomers of (**8**) and (**11**) exhibited absorption at 980 cm^{-1} owing to out-of-plane deformation of the olefinic C–H bonds. With the *cis*-isomers the corresponding absorptions occurred at 750 cm^{-1}. Moreover, the coupling constant between the two olefinic protons in the n.m.r. spectrum of the keto-ester (**13**), the immediate precursor of *trans*-(**11**), was 16 Hz, typical of a *trans*-disubstituted olefin. In both model series the *cis*-isomer was separated readily from the *trans* when mixtures were submitted to t.l.c. on silica gel impregnated with silver nitrate (see Experimental section).

On mass spectrometry, esters such as (**7**)—(**11**) produced no fragments of significant intensity suitable for quantitative analyses.[13] However, their trimethylsilyl (TMS) derivatives produced intense ions due to α-fission (a), and a less intense fragment assigned to (b) [formula (A)].[14] With the derivatives of the authentic allylic hydroxy esters it was found that the relative intensity of the two fragment ions varied greatly

$Me[CH_2]_7CH{=}CH[CH_2]_7CO_2Me$

(**1**) *cis*-9
(**2**) *trans*-9

$Me[CH_2]_yCH{=}CHCH(OOH)[CH_2]_xCO_2Me$

(**3**) $x = 6, y = 7$
(**4**) $x = 7, y = 6$

$Me[CH_2]_yCH(OOH)CH{=}CH[CH_2]_xCO_2Me$

(**5**) $x = 6, y = 7$
(**6**) $x = 7, y = 6$

$Me[CH_2]_yCH{=}CHCH(OH)[CH_2]_xCO_2Me$

(**7**) $x = 6, y = 7$
(**8**) $x = 7, y = 6$

$Me[CH_2]_yCH(OH)CH{=}CH[CH_2]_xCO_2Me$

(**9**) $x = 6, y = 7$
(**10**) $x = 7, y = 6$
(**11**) $x = 8, y = 5$

$Me[CH_2]_5CH(OH)CH_2CH{=}CH[CH_2]_7CO_2H$

(**12**) *cis*-9

$Me[CH_2]_5{\cdot}CO{\cdot}CH{=}CH[CH_2]_8CO_2Me$

(**13**) *trans*-10

$Me[CH_2]_5{\cdot}CO{\cdot}C{\equiv}C[CH_2]_8CO_2Me$

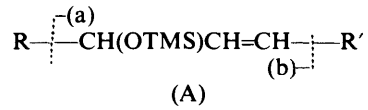
(**14**)

$Me[CH_2]_6C{\equiv}CCH(OH)[CH_2]_7CO_2Me$

(**15**)

$Me[CH_2]_6CH{=}CH{\cdot}CO{\cdot}[CH_2]_7CO_2Me$

(**16**) *trans*-10



(A)

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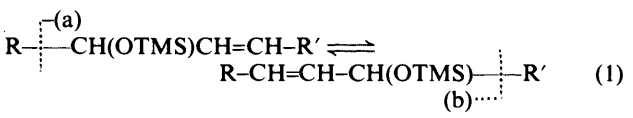
depending on the stereochemistry of the starting material, and the conditions under which the mass spectrum was run. It was therefore concluded that the minor fragment does not arise from fission of a bond between an sp^2 and an sp^3 carbon atom, but rather as the result of an initial 1,3-rearrangement of the allylic trimethylsilyl ethers under the conditions of the analysis [equation (1)]. This rearrangement clearly renders the method



(1)

unsuitable for the quantitative analysis of the positional isomers of autoxidation products. However, model experiments with *cis*- and *trans*-isomers of (**11**) revealed that mild catalytic reduction of such allylic alcohols over palladium gives the corresponding saturated hydroxy compound without selective hydrogenolysis of the allylic function. This opened up the possibility of analysing the positional isomers of the hydroperoxides formed on autoxidation after conversion into the saturated hydroxy esters. Studies with authentic samples of methyl 8-, 9-, 10-, and 11-hydroxyoctadecanoates revealed that mixtures of the type expected from the autoxidation products of methyl oleate can, after trimethylsilylation, be analysed satisfactorily (standard deviation 0.72) by direct insertion of a sample into a mass spectrometer, and using the relative abundancies of the intense lines due to both α-fissions of the trimethylsilyl ethers, or by g.c.–mass spectrometry (m.s.) under carefully controlled conditions and using the computer summation technique to avoid errors due to partial separation of the isomers.[15]

We next required a method for determining the proportion of *cis*- and *trans*-isomers for each of the allylic hydroperoxides formed on autoxidation of methyl oleate. The concentration of *trans*-isomers in these hydroperoxides, and in the mixture of allylic alcohols derived from them, has previously been estimated[7,16] by measuring the intensity of the i.r. light absorption band at 980 cm^{-1} due to the out-of-plane deformations of the C–H bonds associated with the *trans* disubstituted double bonds. This assumed that the molar absorptivity in the *trans* allylic hydroperoxides and alcohols would be the same as that in methyl elaidate (**2**). Measurements with authentic samples of methyl elaidate and of the *trans*-isomer of (**11**) showed this assumption to be invalid (136.2 and 158.4 respectively in CS_2). Moreover, background absorption in the 980 cm^{-1} region by the *cis*-allylic alcohols, and the difficulty of separating the allylic alcohols from other autoxidation products, and their derivatives, makes the traditional i.r. methods for determining the percentage of *trans*-isomers inherently unreliable for the present purpose. Estimations of the *cis*-isomers by subtraction of the *trans* percentage from 100 is particularly prone to error. A method was therefore sought, capable of analysing both sets of geometrical isomers directly.

^{13}C N.m.r. studies of authentic *cis*- and *trans*-isomers of (**8**) and (**11**) showed characteristic differences in resonance for the allylic carbon atoms bearing oxygen, and for the methylene carbon atoms α to the double bond (Table 1). Assignments were based on published values for similar carbon atoms in other long chain fatty acids.[17] For the *cis*-isomer of (**8**) the olefinic carbon band at lowest field (132.8 p.p.m.) was assigned to carbon-11 on the basis of a lanthanoid shift reagent experiment. The large difference in resonance between *cis* C–OH (67.5—67.8 p.p.m.) and *trans* C–OH (73.1—73.2 p.p.m.) suggested the possibility of utilising the peak heights and integrals for the quantitative determination of the *cis*/*trans* isomeric ratio of products from the autoxidation of methyl oleate. The reliability of this technique for the determination of *cis*:*trans* ratios was checked by employing known mixtures of the *cis*- and *trans*-isomers of (**11**) (standard deviation 1.0).

Table 1. ^{13}C N.m.r. bands (p.p.m. values) for allylic hydroxy esters[a]

Assignment	*cis*-(**11**)	*cis*-(**8**)	*trans*-(**11**)	*trans*-(**8**)
$-CO_2CH_3$	174.2	174.3	174.3	174.2
$-CH(OH)\cdot CH=CH-$	132.8	132.8	133.3	133.2
$-CH(OH)\cdot CH=CH-$	131.9	132.1	131.9	132.1
$-CH(OH)\cdot CH=CH-$	67.5	67.8	73.1	73.2
$-CO_2CH_3$	51.3	51.4	51.4	51.4
$-CH_2\cdot CH(OH)-$	37.6	37.6	37.4	37.4
$-CH_2\cdot CO_2CH_3$	34.0	34.1	34.1	34.1
$CH_3\cdot CH_2\cdot CH_2-$	31.8	31.8	31.9	31.8
$C_{12\ 15,4\ 6}$	29.6	29.8	29.2	29.2
$-CH(OH)\cdot CH=CH\cdot CH_2-$	27.6	27.7	32.1	32.2
	25.3	25.4	25.5	25.4
$-CH_2CH_2CO_2CH_3$	24.9	25.0	25.0	25.0
$CH_3\cdot CH_2-$	22.6	22.6	22.6	22.7
CH_3-	14.0	14.0	14.1	14.0

[a] Spectra were determined in deuteriochloroform at 22.63 MHz.

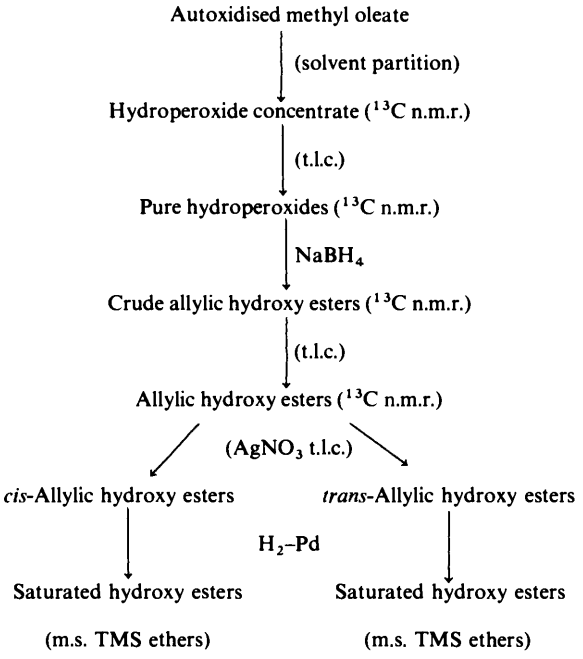
Table 2. ^{13}C N.m.r. bands (p.p.m. values) for allylic hydroperoxides obtained from the autoxidation and photosensitised oxidation of methyl oleate[a,b]

Assignment	Autoxidation (8-+9-+10-+11-OOH)	Photosensitised oxidation (9-+10-OOH)
$-CO_2CH_3$	174.3	174.3
$-CH(OOH)\cdot CH=CH-$	136.7, 136.5, 136.3, 136.2	136.7, 136.2
$-CH(OOH)\cdot CH=CH-$	129.1, 128.9, 128.7, 128.4	129.1, 128.7
$-CH(OOH)\cdot CH^t=CH-$	86.9	86.9
$-CH(OOH)\cdot CH^c=CH-$	81.1	
$-CO_2CH_3$	51.4	51.5
$-CH_2\cdot CO_2CH_3$	34.1	34.1
$-CH(OOH)\cdot CH^t=CH\cdot CH_2-$	32.6, 32.4	32.6, 32.4
$CH_3\cdot CH_2\cdot CH_2-$	31.9	31.9
$C_{12-15,4-6}$	29.1	29.1
$-CH_2CH_2CO_2CH_3$	25.2	25.3
	24.9	24.9
CH_3-	14.0	14.1

[a] See footnote *a* Table 1. [b] Hydroperoxides were purified by preparative t.l.c. (*cf.* Experimental section).

The application of the ^{13}C n.m.r. method to mixtures of oleate hydroperoxides was also investigated. The substituted allylic carbon atoms in a mixture of *cis* and *trans* hydroperoxides were found to resonate at 81.1 and 86.9 p.p.m. respectively (Table 2). No clear cut bands were apparent for the allylic methylene carbon atoms in the region 28—32 p.p.m. expected from the allylic hydroxy esters (Table 1). Although the bands at 32.4 and 32.6 p.p.m. correspond to those of the *trans* methylene carbon atoms of the allylic alcohols (**7**) and (**11**), no bands corresponding to the *cis* methylene carbon atoms, found at 27.6—27.7 p.p.m. for the alcohols, were detected in the hydroperoxide mixture. The ^{13}C n.m.r. spectrum was also obtained on a simpler mixture of the 9- and 10-hydroperoxides prepared from methyl oleate subjected to photosensitised oxidation in the presence of Methylene Blue at 0 °C.[18] In this sample there was only *trans* absorption at 86.9 p.p.m. and no *cis* absorption at 81.1 p.p.m., in keeping with the known stereochemical course of the reaction.[1]

On the basis of these model studies, the approach summarised in Scheme 1 was adopted for the analysis of the hydroperoxides formed on autoxidation of methyl oleate (**1**). Methyl oleate was stirred in oxygen, and the progress of the reaction was monitored by the determination of the peroxide values of small samples withdrawn periodically. The autoxidation pro-



Scheme 1.

ducts were concentrated by solvent partition and analysed by ^{13}C n.m.r. spectroscopy. The allylic hydroperoxides, consisting of a mixture of positional and geometrical isomers, were isolated by t.l.c. and again analysed by ^{13}C n.m.r. spectroscopy. The mixture was reduced with sodium borohydride to give the corresponding allylic alcohols which were also analysed by ^{13}C n.m.r. spectroscopy, both before and after purification by t.l.c.; the four ^{13}C n.m.r. analyses revealed no loss of stereochemistry during the preparation of the mixture of allylic alcohols from the crude hydroperoxides (Table 3). The *cis*-allylic alcohols were separated from the *trans*-isomers by t.l.c. on silica gel impregnated with silver nitrate, and the two mixtures were separately hydrogenated over palladium to give mixtures of hydroxy-octadecanoates which, after trimethylsilylation, were analysed by mass spectrometry. The percentage composition of the original mixture of allylic hydroperoxides was then calculated from the appropriate ^{13}C n.m.r. and mass spectral data (overall standard deviation, calculated [19] from the errors of the two analytical techniques, 0.65).

The results obtained from autoxidation experiments carried out at different temperatures ranging from 25 °C to 95 °C are summarised in Table 4. The percentage of *trans* products was found to increase with temperature, from 70% at 25 °C to 85% at 95 °C. At 25 °C products with the double bond in the same (Δ^9) position as the (*cis*-) starting material consisted of comparable amounts of *cis*- and *trans*-isomers, but the *trans*-isomers were favoured, at the expense of *cis*, as the temperature of the reaction was increased. Products in which the double bond was in one of the neighbouring positions consisted mainly of the *trans*-isomers, though the proportion of *cis*-isomers increased with temperature, from about 1% at 25 °C to about 3% at 75 °C. At 25 °C and 50 °C oxygen attack at C-8 and C-11, giving products with the double bond in the original (Δ^9) position, was slightly (2—4%), but consistently, greater than that at C-9 and C-10, in agreement with previous findings.[15,20,21] At 75 °C oxygen attack was approximately equal at all four carbon positions.

The results summarised in Table 4 reveal that autoxidation of methyl oleate occurs symmetrically about the double bond. Thus the 8-hydroperoxide formed closely resembles the 11-hydroperoxide, and the 9- the 10-hydroperoxide, in both yield and *cis–trans* ratio. In these respects our findings differ markedly from those of Piretti *et al.*[7,16] These discrepancies may be attributed to the limitations of some of the analytical methods used by the Italian group, and which were revealed by our studies with authentic samples.

Our results obtained with methyl oleate (**1**) can best be explained in terms of the mechanism discussed for the autoxidation of the two simple models, hex-*cis*-3-ene[22] and oct-*cis*-4-ene[23] (Scheme 2). With appropriate conformations, hydrogen abstraction from both positions α to the double bond leads to delocalised allylic radicals (**18a**), (**18b**), (**19a**), and (**19b**) which tend to lose their defined stereochemistry, particularly at elevated temperatures, to give the isomeric allylic radicals (**17a**), (**17b**), (**20a**), and (**20b**). Subsequent reaction of these eight radicals occurs at both ends of the allylic system leading to the eight hydroperoxides detected. Evidently the larger alkyl substituents in the oleate series lead to greater loss of

Table 3. ^{13}C N.m.r. analyses of products from autoxidised methyl oleate

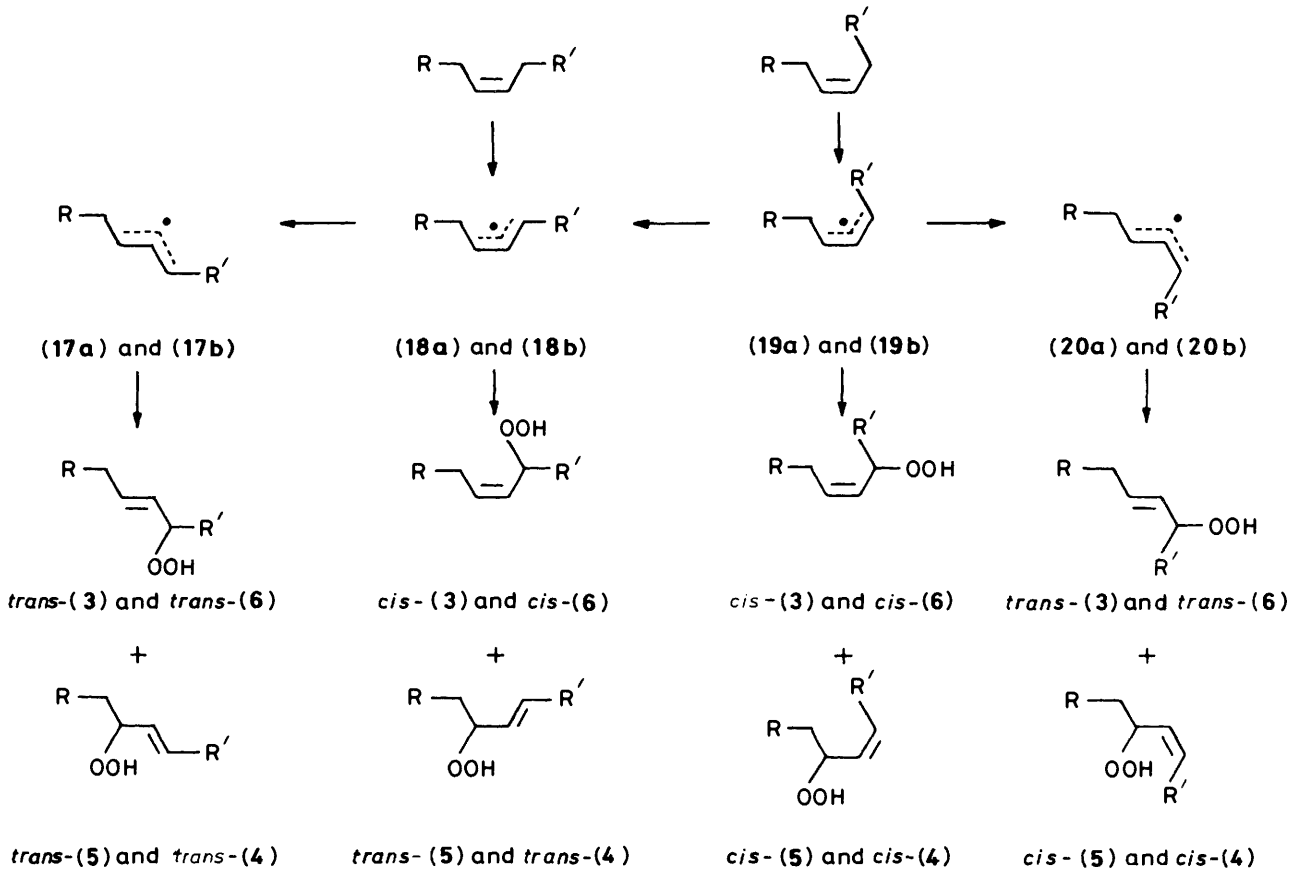
Sample [a]	% *trans*
Crude hydroperoxides	83, 82, 89
Purified hydroperoxides	83, 83
Hydroxy derivatives	84, 85

[a] See Scheme 1 for fractionation.

Table 4. Allylic hydroperoxides from autoxidation of methyl oleate

			Product (% of total)[c]			
Temp./°C	*trans*,[a] %	Conversion,[b] %	8-OOH Δ^9 (**3**)	9-OOH Δ^{10} (**4**)	10-OOH Δ^8 (**5**)	11-OOH Δ^9 (**6**)
25	70.0	5.3	26.4 (14.1, 12.3)	24.2 (1.1, 23.1)	22.8 (1.1, 21.7)	26.6 (13.7, 12.9)
40	76.0	4.9	26.6 (10.6, 16.0)	23.6 (1.6, 22.0)	23.4 (1.7, 21.7)	26.4 (10.1, 16.0)
50		14.4	26.1 (8.3, 17.8)	24.7 (2.2, 22.5)	23.5 (2.2, 21.3)	25.7 (8.3, 17.4)
75	83.0	15.2	25.1 (6.1, 19.0)	25.1 (2.7, 22.5)	24.9 (2.9, 22.0)	24.9 (5.4, 19.5)
95	85.0	19.7				

[a] By ^{13}C n.m.r. analysis of allylic alcohols. [b] Based on peroxide value of methyl oleate hydroperoxides, 6 100 mequiv. per kg. [c] By m.s. analysis of TMS ethers of saturated alcohols; *cis* and *trans*, respectively, given in parentheses.



Scheme 2. a; R = $Me[CH_2]_6$ and R′ = $[CH_2]_6CO_2Me$. b; R = $[CH_2]_6CO_2Me$ and R′ = $Me[CH_2]_6$

stereochemistry than observed with hex-*cis*-ene.[22] In recent publications Gunstone *et al.*[24] have reported the detection by e.s.r. spectroscopy of allylic radicals regarded as (**18a**) and (**18b**) during the photolysis of di-t-butyl peroxide in methyl oleate. These were found to isomerise to (**17a**) and (**17b**) during the course of the reaction, especially at elevated temperatures, and this change was shown to be irreversible. A mechanism was postulated in which the *cisoid* radicals, (**18a**) and (**18b**), and the *transoid* radicals, (**17a**) and (**17b**), were either interconverted directly (as shown in Scheme 2) or by reversible oxygen addition. However, their conditions of photolysis cannot be compared with those used in our autoxidations. Methyl *trans*-octadecenoate isomers were found under their conditions, in contrast to our observation that during autoxidation only unchanged methyl oleate remained.

The slight preference at low temperatures for reaction at C-8 and C-11 may indicate some abstraction of hydrogen from conformers of methyl oleate which do not result in a totally planar, fully delocalised radical without further rotation. Reaction with oxygen before this rotation is complete would result in the observed preference. The absence of the effect at higher temperatures, and with the simple hexene in which the smaller alkyl substituents would result in lower barriers to rotation, are consistent with this interpretation.

The methods reported in this paper to analyse the allylic hydroperoxides from methyl oleate should be applicable to those from other similar substrates. Since the ^{13}C n.m.r. spectra were determined at 22.63 MHz it is worth noting that at 100.6 MHz the signal due to the oxygenated allylic carbon atom in the mixture of the *cis* and the *trans* allylic hydroperoxides from the autoxidation of methyl oleate is resolved into seven of the expected eight peaks (Table 5). Comparison with the spectrum

Table 5. ^{13}C N.m.r. bands (p.p.m. values) at 100.6 MHz for allylic hydroperoxides[a]

	(9- + 10-OOH)[b]	(8- + 11-OOH)[b]
-*C*H(OOH)·CH′=CH-		
From autoxidation	86.89, 86.91	86.85, 86.93
From photosensitised oxidation	86.98, 87.00	86.94[c], 87.02[c]
-*C*H(OOH)·CH[c]=CH-		
From autoxidation	81.12[d]	81.09, 81.17

[a] Slight differences (0.09 p.p.m.) in chemical shift observed between the autoxidation and photosensitised oxidation products are attributed to bulk susceptibility effects. [b] On present evidence, no assignment can be made of the bands cited to the individual isomer in each pair of hydroperoxides. [c] Very weak signal attributed to traces of isomeric hydroperoxides formed by allylic rearrangement of the initial products on storage (*cf.* W. F. Brill, *J. Am. Chem. Soc.*, 1965, **87**, 3286). [d] Very weak signal.

of the allylic hydroperoxides from the photosensitised oxidation of methyl oleate reveals that two of these bands (86.89 and 86.91 p.p.m.) are associated with the 9- and 10-hydroperoxides with a *trans*-double bond, and another two (86.85 and 86.93 p.p.m.) with the corresponding *trans* 8- and 11- hydroperoxides. The band at 81.12 p.p.m. is attributed to the 9- and/or 10-hydroperoxide in the *cis*-series, in view of its low intensity, the remaining two bands (81.09 and 81.17 p.p.m.) being due to the major *cis*-isomers with hydroperoxy substituents at C-8 and C-11. These results suggest that it should be possible to estimate all isomers directly by ^{13}C n.m.r. spectroscopy when, as we have shown with methyl oleate, autoxidation occurs symmetrically

about the double bond. If the intensity of the bands were to indicate that this is not the case with a particular substrate, it would first be necessary to establish unambiguous assignments of the individual bands.

Experimental

Unless indicated to the contrary, light petroleum refers to the fraction b.p. 60—80 °C, and ether to diethyl ether.

Selected lines only are quoted for i.r. and mass spectra. T.l.c. was performed on plates of Merck Kieselgel HF 254 with eluants indicated in parentheses; spots or bands were detected either by inspection under u.v. light, or by exposure to iodine vapour.

The *cis*- and *trans*-isomers of the allylic hydroxy- and hydroperoxy-esters were separated by t.l.c. on silver nitrate-impregnated plates ($AgNO_3$ t.l.c.). For this purpose, Merck Kieselgel HF 254 + 366 (nach Stahl) (580 g) was shaken with water (2 l) in a 5 l separatory funnel. The mixture was allowed to stand for 35 min and 250 ml fractions were then collected. The first fraction was discarded. The next three were combined and most of the water was removed by centrifugation. The remaining silica was dried at 120 °C for 24 h and sieved (120 mesh). For the preparation of five 20 × 20 cm plates, a portion (47.5 g) of this silica was mixed with a solution of silver nitrate (2.5 g) in water (100 ml). The resulting gel was spread in the normal way; the plates were air dried for 35 min and finally activated at 120 °C for 2 h. Chromatograms were developed in the normal way and the plates were viewed directly under u.v. light.

12-*Oxo-octadec*-cis-9-*enoic Acid*.—(*a*) Chromic acid oxidation of 12-hydroxyoctadec-*cis*-9-enoic (ricinoleic) acid (**12**) in acetic acid, following the procedure of Nichols and Schipper,[25] gave 12-oxo-octadec-*cis*-9-enoic acid (25—30% yield provided that the reaction mixture was vigorously stirred, and the reaction time limited to 30 s). It crystallised from light petroleum and had m.p. 40—41 °C (lit.,[25] m.p. 40—40.5 °C); $\nu_{max.}$(Nujol) 3 400 and 1 700 cm^{-1}; δ 0.88 (t, *J* 6 Hz, 3 H, CH_3), 1.2—1.8 (br, 18 H), 1.8—2.6 (m, 6 H, C-2, C-8, and C-13 CH_2), 3.08—3.2 (m, 2 H, C-11 CH_2), 5.54 (m, 2 H, CH=CH), and 8.7 (br, 1 H, CO_2H).

(*b*) Chromic acid oxidation of 12-hydroxyoctadec-9-ynoic acid (prepared from ricinoleic acid) by the method of Nichols and Schipper[25] gave 12-oxo-octadec-9-ynoic acid (80%). It crystallised from light petroleum and had m.p. 62—63 °C (lit.,[25] m.p. 63—64 °C); $\nu_{max.}$(Nujol) 3 400 and 1 690 cm^{-1}; δ 0.88 (t, *J* 6 Hz, 3 H, CH_3), 1.1—1.8 (br, 18 H), 2.1—2.8 (m, 6 H, C-2, C-8, and C-13 CH_2), 3.18 (t, *J* 2 Hz, C-11 CH_2), and 10.3 (br, 1 H, CO_2H). Partial hydrogenation of the acetylenic keto-acid (1.0 g) in methanol (50 ml) over Lindlar catalyst (0.2 g) gave the required acid (85%), identical with a sample from (*a*).

12-*Oxo-octadec*-trans-10-*enoic Acid*.—A solution of the isomeric *cis*-9-enoic acid (2.5 g) in glacial acetic acid (50 ml) containing concentrated sulphuric acid (1 ml) was boiled under reflux for 5 min, then cooled and poured into ice–water (100 ml). The solid was collected, dried, and extracted with hot light petroleum. The solution was cooled to −30 °C and the solid which separated was collected at intervals. The initial fraction consisted mainly of starting material (mixed t.l.c.). The later fractions were recrystallised repeatedly from aqueous ethanol (70%) to give the required *trans*-10-enoic acid (1.0 g), m.p. 48—49 °C (lit.,[25] m.p. 50—50.5 °C); $\lambda_{max.}$(ethanol) 227 nm (lost after addition of $NaBH_4$); $\nu_{max.}$(Nujol) 3 200, 1 690, 1 630, and 980 cm^{-1}; δ 0.88 (t, *J* 6 Hz, 3 H, CH_3), 1.1—1.8 (br, 20 H), 2.1—2.7 (m, 6 H, C-2, C-9, and C-13 CH_2), 6.07 (dt, J_1 16, J_2 1.5 Hz, 1 H, C-11 CH), 7.76 (dt, J_1 16, J_2 6.5 Hz, 1 H, C-10 CH), and 10.5 (br, 1 H, CO_2H); *m/z* 296.236 ($M^{+\cdot}$. Calc. for $C_{18}H_{32}O_3$: *m/z* 296.235, 15%), 226 (12), 211 (10), 208 (17; *m** 191.4; $208^2/226$ = 191.4), 156 (13), 139 (85), and 113 (100).

Methyl 12-*Hydroxyoctadec*-trans-10-*enoate*, trans-(**11**).—(*a*) Sodium borohydride (60 mg) was added slowly to a stirred solution of the preceding keto-acid (200 mg) in diglyme (10 ml). After 1 h, the mixture was diluted with water (100 ml), and acidified with 2M-sulphuric acid. The product was isolated with ether, and preparative t.l.c. (1:1 light petroleum–ether). Crystallisation gave the hydroxy-acid (100 mg), m.p. 45—46 °C (lit.,[25] m.p. 50—50.5 °C); $\nu_{max.}$(Nujol) 3 400, 1 700, and 980 cm^{-1}; δ 0.88 (t, *J* 6 Hz, 3 H, CH_3), 1.2—1.8 (br, 20H), 1.8—2.5 (m, 4 H, C-2 and C-9 CH_2), 3.6 (m, 2 H, C-13 CH_2), 4.0 (m, 1 H, C-12 CH), 5.3—5.7 (m, 2 H, CH=CH), and 5.8 (2 H, OH and CO_2H; lost in shaking with D_2O); *m/z* 298 ($M^{+\cdot}$. Calc. for $C_{18}H_{34}O_3$: *m/z* 298, 3%), 280 (8), 213 (42), 195 (100; *m** 178.5; $195^2/213$ = 178.5), 184 (8), 177 (5; *m** 160.7; $177^2/195$ = 160.7), 166 (10; *m** 149.8; $166^2/184$ = 149.8), and 141 (30%).

The hydroxy-acid (310 mg) in ether (10 ml) was treated at 0 °C with a slight excess of ethereal diazomethane. Isolation of the product in the usual way, and preparative t.l.c. (70:30 light petroleum–ether) gave the *methyl ester* as a colourless oil (200 mg); $\nu_{max.}$(film) 3 450, 1 740, and 980 cm^{-1}; δ 0.88 (t, *J* 6 Hz, 3 H, C-CH_3), 1.1—1.8 (br, 21 H), 1.8—2.7 (m, 6 H, C-2, C-9, and C-13 CH_2), 3.64 (s, 3 H, O-CH_3), 4.0 (m, 1 H, C-12 CH), and 5.2—5.8 (m, 2 H, CH=CH); *m/z* 312.266 ($M^{+\cdot}$. $C_{19}H_{36}O_3$ requires *m/z* 312.266, 5%), 294 (8), 281 (4), 280 (4), 242 (10), 227 (100), 198 (22), 195 (60; *m** 167.5; $195^2/227$ = 167.5), 177 (4; *m** 160.7; $177^2/195$ = 160.7), 166 (30; *m** 139.2; $166^2/198$ = 139.2), and 141 (20).

(*b*) From its method of preparation, the sample described in (*a*) could conceivably have contained traces of 9-enoate isomers which have very similar t.l.c. properties. To prepare a sample where this possibility could be excluded the 'crude' material was submitted to selective allylic oxidation (see below) to give the 12-oxo-*trans*-10-enoate, which was readily separated by t.l.c. from any 12-hydroxy-9-enoates. Reduction of the 12-oxo-*trans*-10-enoate (300 mg) in methanol (20 ml) with sodium borohydride (10 mg) at 0 °C, isolation of the product in the usual way, and preparative t.l.c., gave the required 12-hydroxy-*trans*-10-enoate (260 mg) free from any 9-enoate isomers. Its spectral properties were identical with those of the sample described in (*a*). The *trimethylsilyl derivative* had *m/z* 384.305 ($M^{+\cdot}$. $C_{22}H_{44}SiO_3$ requires 384.306, 6%), 369 (4), 351 (4), 337 (3), 299 (100), 213 (8.5), and 187 (5).

Catalytic hydrogenation of the 12-hydroxyoctadec-10-enoate in ethyl acetate over 10% palladium on carbon gave methyl octadecanoate (stearate) (13%) and methyl 12-hydroxyoctadecanoate. The absence of positional isomers in the latter product was confirmed by mass spectrometry of the *trimethylsilyl derivative*, *m/z* 386.321 ($M^{+\cdot}$. $C_{22}H_{46}SiO_3$ requires 386.322, 1%), 371 (5), 353 (6), 339 (5), 301 (92), 272 (36), and 187 (100).

Methyl 12-*Oxo-octadec*-trans-10-*enoate* (**13**).—A solution of methyl 12-hydroxyoctadec-*trans*-10-enoate, *trans*-(**11**) (1.0 g), possibly containing some 9-enoate isomers, in ether (50 ml), was shaken with activated manganese dioxide (2.0 g) at 20 °C for 48 h. Isolation of the product in the usual way, and preparative t.l.c. (80:20 light petroleum–ether) gave the *keto-ester* as an oil (600 mg); $\lambda_{max.}$ 227 nm (lost on addition of $NaBH_4$); $\nu_{max.}$ 1 740, 1700, 1 680, 1 630, and 980 cm^{-1}; δ 0.88 (t, *J* 6 Hz, 3 H, C-CH_3), 1.1—1.8 (br, 20H), 2.0—2.7 (m, 6 H, C-2, C-9, and C-13 CH_2), 3.64 (s, 3 H, O-CH_3), 6.07 (dt, J_1 16, J_2 1.5 Hz, 1 H, C-11 CH), and 7.76 (dt, J_1 16, J_2 6.5 Hz, 1 H, C-10 CH); *m/z* 310.251 ($M^{+\cdot}$. $C_{19}H_{34}O_3$ requires 310.251), 279 (11%), 240 (13), 225 (5), 208 (28), 165 (51), and 139 (100).

Methyl 12-*Oxo-octadec*-10-*ynoate* (**14**).—Concentrated aqueous ammonia was added slowly to a solution of silver nitrate (17.5 g) in water (90 ml) and ethanol (40 ml) until the cloud formed initially had just cleared. The solution was cooled and stirred vigorously while methyl undec-10-ynoate[10,11] (9.1 g) was added dropwise. Stirring was continued for 1 h and the colourless solid which had formed was dissolved in carbon tetrachloride (80 ml). The organic layer was separated, washed with water (3 × 100 ml), and dried ($CaCl_2$, then molecular sieve). To the resulting pale yellow solution of the silver acetylide, heptanoyl chloride (5.5 g) was added, the mixture was boiled under reflux for 16 h and then cooled and poured onto ice (200 g). Dilute hydrochloric acid (10%; 300 ml) was added and the mixture was filtered. The filtrate was washed with aqueous potassium carbonate (5%; 100 ml), water (2 × 100 ml), and the organic solution was dried and evaporated under reduced pressure. Chromatography of the residual brown oil (14.5 g) on a column of silica gel (8:2 light petroleum–ether) gave the *acetylenic keto-ester* (8.6 g) as a pale yellow oil; $\lambda_{max.}$(ethanol) 223 nm (lost on addition of $NaBH_4$); $\nu_{max.}$(film) 2 220, 1 730, and 1 670 cm^{-1}; *m/z* 308.236 ($M^{+\cdot}$. $C_{19}H_{32}O_3$ requires 308.235), 277 (48), 276 (15), 238 (54), 233 (60), 223 (41), 215 (8), 206 (21), 191 (22), 165 (62), 164 (56), 163 (55), 152 (100), 137 (45), and 113 (100).

Methyl 12-*Hydroxyoctadec*-10-*ynoate*.—(*a*) Sodium borohydride (10 mg) was added over 20 min to a stirred solution of the preceding keto-ester (300 mg) in ethanol (20 ml). After 30 min water (50 ml) was added and the mixture acidified with 1M-sulphuric acid. Isolation of the product in the usual way with ether, and preparative t.l.c. (70:30 light petroleum–ether) gave the *acetylenic hydroxy-ester* (260 mg) as an oil; $\nu_{max.}$(film) 3 450, 2 250, and 1 740 cm^{-1}; δ 0.88 (t, *J* 6 Hz, 3 H, C-CH_3), 1.6—1.8 (br, 20H), 1.9—2.4 (m, 6 H, C-2, C-9, and C-13 CH_2), 3.64 (s, 3 H, O-CH_3), and *ca.* 4.2 (br m, 1 H, C*H*OH); *m/z* 310.251 ($M^{+\cdot}$. $C_{19}H_{34}O_3$ requires 310.251, 1.5%) 292 (1), 225 (100), 193 (73; *m** 165.6; $193^2/225$ = 165.6), 175 (3; *m** 158.7; $175^2/193$ = 158.7), 154 (3), and 139 (15).

(*b*) Undecynoic acid[10] (9.1 g) in tetrahydrofuran (25 ml) was added over 45 min to a cold (0 °C) and stirred solution of ethylmagnesium bromide (from 2.4 g of magnesium) in tetrahydrofuran (20 ml). The mixture was stirred at 20 °C for 2 h, anhydrous copper (I) cyanide (250 mg) was added, followed, after 10 min, by heptanal (2.9 g) in tetrahydrofuran (12 ml) over 15 min. The mixture was boiled under reflux for 24 h, then cooled and poured into 1M-sulphuric acid containing ice. The product was extracted with ether. The ethereal extracts were combined and washed with aqueous 2M-ammonia. The alkaline extracts were acidified with 1M-sulphuric acid and the product isolated with ether. Chromatography on a column of silica gel (light petroleum–ether) gave 12-hydroxyoctadec-10-ynoic acid (6.2 g) as a colourless liquid. Treatment with an excess of ethereal diazomethane gave, in quantitative yield, the required hydroxy-ester. Its spectral properties were identical with those of the sample described under (*a*).

Methyl 12-*Hydroxyoctadec*-cis-10-*enoate*, cis-(**11**).—A solution of the preceding acetylene (1.0 g) in ethyl acetate (50 ml) was shaken with Lindlar catalyst (0.2 g) in an atmosphere of hydrogen until absorption ceased (73 ml, 1.02 mol). Removal of catalyst and solvent, and preparative t.l.c. firstly on Kieselgel (70:30 light petroleum–ether), and then on silver nitrate-impregnated plates (80:18:2 benzene–ethyl acetate–methanol) gave the required *hydroxy-ester* as an oil; $\nu_{max.}$3 450, 1 740, and 750 cm^{-1}; δ 0.88 (t, *J* 6 Hz, 3 H, C-CH_3), 1.8 (s, 1 H, OH, exchanged with D_2O), 1.9—2.5 (m, 6 H, C-2, C-9, and C-13 CH_2), 3.64 (s, 3 H, O-CH_3), 4.4 (m, 1 H, C*H*OH), and 5.2—5.8 (m, 2 H, CH=CH); *m/z* 312.267 ($M^{+\cdot}$. $C_{19}H_{36}O_3$ requires 312.266, 4%), 294 (5), 281 (4), 280 (4), 227 (100), 198 (3), 195 (65, *m** 167.5; $195^2/227$ = 167.5), 177 (5; *m** 160.7; $177^2/195$ = 160.7), 166 (4), and 141 (15). The *trimethylsilyl derivative* had *m/z* 384.306 ($M^{+\cdot}$. $C_{22}H_{44}SiO_3$ requires 384.306, 5%) 369 (4), 353 (4), 337 (3), 299 (100), 213 (5), and 187 (7).

Catalytic hydrogenation of the hydroxy-ester in ethyl acetate over 10% palladium on carbon gave results indistinguishable from those reported above with the *trans*-isomer.

Methyl 9-*Hydroxyoctadec*-10-*ynoate* (**15**).—Methyl 8-formyloctanoate[12] (3.7 g), purified *via* the hydrogen sulphite complex,[26] was added slowly to a vigorously stirred solution of heptynylmagnesium bromide (from 2.4 g of heptyne[27]) at −30 °C. The mixture was stirred at 0 °C for 3 h, and then poured into ice–water (100 g). A slight excess of 1M-sulphuric acid was added, and the product was isolated with ether. Saturated aqueous sodium hydrogen sulphite (10 ml) was added to a solution of the crude product in ethanol (2 ml). After 8 h the mixture was filtered, the solid was washed with ether, and the aqueous filtrate was diluted with water (50 ml), and extracted with ether. The ethereal solutions were combined, washed, dried, and evaporated under reduced pressure. Preparative t.l.c. (70:30 light petroleum–ether) gave the *hydroxy-ester* (4.7 g) as a colourless oil; $\nu_{max.}$(film), 3 500, 2 250, and 1 740 cm^{-1}; δ 0.90 (t, *J* 6 Hz, 3 H, C-CH_3), 1.9 (br, 20 H), 2.0—2.8 (m, 6 H, C-2, C-8, and C-12 CH_2), 3.64 (s, 3 H, O-CH_3), 4.3 [tt, J_1 7, J_2 2 Hz, 1 H, C-9 (C*H*OH)], and 6.1 (br, 1 H, OH, exchanged with D_2O); *m/z* 310.252 ($M^{+\cdot}$. $C_{19}H_{34}O_3$ requires 310.251, 3%), 294 (4), 292 (5), 279 (4), 226 (22), 208 (7), 194 (15; *m** 166.5; $194^2/226$ = 166.5), 158 (88), 153 (22), 151 (20), 150 (21), 135 (2), 129 (16; *m** 105.3; $129^2/158$ = 105.3), 115 (42; *m** 83.7; $115^2/158$ = 83.7), 101 (22; *m** 64.6; $101^2/158$ = 64.6), 87 (100), and 74 (100).

As a further check on purity, a sample was purified *via* the acetate. Acetyl chloride (0.2 ml) was added to a stirred solution of the hydroxy-ester (500 mg) in pyridine (5 ml). The mixture was kept for 10 min and then poured into ice–water (20 ml). Isolation of the product with ether, and preparative t.l.c. (75:25 light petroleum–ether) gave the *acetoxy-ester* (460 mg) as a colourless oil; $\nu_{max.}$2 250 and 1 740 cm^{-1}; δ 0.90 (t, 3 H, C-CH_3), 1.1—1.9 (br, 20 H), 2.05 (s, 3 H, CH_3CO$_2$), 2.1—2.6 (m, 6 H, C-2, C-8, and C-12 CH_2), 3.64 (s, 3 H, O-CH_3), and 5.33 (tt, J_1 5, J_2 1.5 Hz, 1 H, C-9 C*H*OAc); *m/z* 352.261 ($M^{+\cdot}$. $C_{21}H_{36}O_4$ requires 352.261, 37%), 321 (4), 310 (4; *m** 273.01; $310^2/352$ = 273.01), 277 (16), 267 (55; *m** 229.96; $267^2/310$ = 229.96; m* 202.5; $267^2/352$ = 202.5), 260 (24), 250 (14), 237 (15), 235 (20), 226 (38), 224 (42), 209 (30), 199 (20), 195 (24), 194 (20; *m** 166.5; $194^2/226$ = 166.5), 193 (24), 185 (37), 168 (64), 167 (6), 160 (34), 153 (20), and 151 (100). Hydrolysis with 5% methanolic potassium hydroxide for 10 min gave, almost quantitatively, the hydroxy-ester with spectral properties identical with those reported above.

Methyl 9-*Hydroxyoctadec*-cis-10-*enoate*, cis-(**8**).—A solution of the preceding hydroxy-ester (1.5 g) in ethyl acetate (100 ml) was shaken in an atmosphere of hydrogen in the presence of Lindlar catalyst (200 mg) until absorption of hydrogen was complete (1.07 mol equiv.). Removal of catalyst and solvent, and preparative t.l.c. firstly on silica gel (70:30 light petroleum–ether) and then on silver nitrate-impregnated plates (80:18:2 benzene–ethyl acetate–methanol) gave the cis-10-*enoate* (1.5 g) as a colourless oil; $\nu_{max.}$(film) 3 450, 1 740, and 750 cm^{-1}; δ 0.88 (5, *J* 6 Hz, 3 H, C-CH_3), 1.1—1.8 (br, 20 H), 1.55 (br, 1 H, OH exchanged with D_2O), 1.9—2.5 (m, 6 H, C-2, C-8, and C-12 CH_2), 3.64 (s, 3 H, O-CH_3), 4.4 (m, 1 H, C*H*OH), and 5.2—5.8 (m, 2 H, CH=CH); *m/z* 312.266 ($M^{+\cdot}$. $C_{19}H_{36}O_3$ requires 312.266, 3%), 294 (12), 281 (2), 280 (2), 213 (50), 200 (6), 187 (10), 181 (33; *m** 153.8; $181^2/213$ = 153.8), 170 (8), 158 (58), 155 (100), 137 (20; *m** 121.1; $137^2/155$ = 121.1), 129 (15; *m** 105.3;

$129^2/158 = 105.3$), 115 (32; m^* 83.7; $115^2/158 = 83.7$), 101 (15; m^* 64.6; $101^2/158 = 64.6$), and 74 (100). The *trimethylsilyl derivative* had m/z 384.306 ($M^{+\cdot}$. $C_{22}H_{44}SiO_3$ requires 384.306, 4%), 369 (5), 353 (3), 337 (3), 285 (3), 259 (30) and 227 (100).

Methyl 9-*Oxo-octadec*-trans-10-*enoate* (**16**).—Pyridinium chlorochromate (400 mg) was added to a stirred solution of the preceding hydroxy-ester (500 mg) in dichloromethane (10 ml) at 20 °C, and the reaction was monitored by t.l.c. (80:20 light petroleum–ether). After 4 h the solution was decanted off, and the residue was extracted with ether. The filtrate and extracts were combined, washed with saturated aqueous sodium hydrogen carbonate, then dilute hydrochloric acid, dried, filtered through a thin layer of charcoal on Celite, and evaporated. The crude product was shown by t.l.c. to contain the *cis*- and *trans*-isomers in the proportions 1:3. When kept at 20 °C for 6 weeks nearly all the *cis*-isomer had been converted into the *trans*-isomer. The trans-10-*enoate* (330 mg) was isolated by preparative t.l.c. and had $\lambda_{max.}$(ethanol) 227 nm (lost after addition of $NaBH_4$); $\nu_{max.}$(film) 1 740, 1 700, 1 680, 1 630, and 990 cm^{-1}; m/z 310.251 ($M^{+\cdot}$. $C_{19}H_{34}O_3$ requires 310.251, 20%), 279 (20), 211 (63), 185 (45), 179 (7), 168 (50), and 153 (100).

Methyl 9-*Hydroxyoctadec*-trans-10-*enoate*, trans-(**8**).—The preceding keto-ester (200 mg) was reduced in methanol with sodium borohydride (20 mg), and the product isolated, in the manner described above for the isomeric 12-hydroxyoctadec-*trans*-10-enoate. The 9-*hydroxy-ester* had $\nu_{max.}$(film) 3 450, 1 740, and 980 cm^{-1}; δ 0.9 (t, *J* 6 Hz, 3 H, C-CH_3), 1.1—1.8 (br, 23 H, one due to OH, exchanged with D_2O), 1.8—2.5 (m, 4 H, C-2 and C-8 CH_2), 3.64 (s, 3 H, O-CH_3), 4.0 (m, 1 H, C*H*OH), and 5.2—5.8 (m, 2 H, CH=CH); m/z 312.266 ($M^{+\cdot}$. $C_{19}H_{36}O_3$ requires 312.266, 5%) 294 (12), 281 (3), 280 (4), 213 (25), 200 (12), 187 (23), 181 (21; m^* 153.8; $181^2/213 = 153.8$), 170 (15), 158 (47), 155 (100), 137 (14; m^* 121.1; $137^2/155 = 121.1$), 129 (10; m^* 105.3; $129^2/158 = 105.3$), 115 (25; m^* 83.7; $115^2/158 = 83.7$), 101 (10; m^* 64.6; $101^2/158 = 64.6$), 74 (100), and 87 (100). The *trimethylsilyl derivative* had m/z 384.305 ($M^{+\cdot}$. $C_{22}H_{44}SiO_3$ requires 384.306, 6%), 369 (4), 353 (3), 337 (1), 285 (5), 259 (8), and 227 (100).

^{13}C *N.M.R. Spectrometry*.—Spectra were recorded in $CDCl_3$ with a Brucker HFX-13 (22.63 MHz) instrument operating in the pulse mode with Fourier transform at 37 °C with proton noise decoupling. For quantitative studies involving pulsed ^{13}C n.m.r. spectroscopy, it is essential to determine both the relaxation times T_1 and the relative nuclear Overhauser effect (n.O.e.) associated with the resonance being measured. T_1 measurements of the respective allylic carbon atoms were 2.0 s for *cis*-(**11**) and 2.2 s for the *trans*-(**11**). The n.O.e. effects were also shown to be negligible. With a synthetic mixture of hydroxy-esters containing 54% of the *trans*-isomer, the experimental *trans* values obtained were 54.6% with n.O.e. and 55.6% without n.O.e. Therefore, the possibility that differential T_1 and n.O.e. might affect the resonance intensities is negligible, most likely because the chemical environments of the -*C*HX- nuclei are so similar in the *cis* and *trans* hydroxy-esters. The reliability of the ^{13}C n.m.r. technique for the determination of *cis*:*trans* ratio was checked by analysing known mixtures of *cis*- and *trans*-(**11**). The ratio of the two signals were determined from both peak heights and peak areas. The results from peak heights were in closer agreement to the known values, as shown in Table 6. The calculated standard deviation for the *trans* values determined by ^{13}C n.m.r. spectroscopy was 1.0.

Mass Spectrometry.—Spectra for allylic hydroxy-esters, after trimethylsilylation,[15] were recorded by direct inlet measurements on an AEI MS902 double focusing instrument at 70 eV (temperature 305 K, accelerating voltage 8 000 V, trap current 1 000 A, resolution 1 500). For quantitative analyses, the allylic hydroxy-esters were catalytically hydrogenated and the resulting hydroxyoctadecanoate isomers, after trimethylsilylation, were analysed quantitatively by the direct inlet method, or by the g.c.–m.s. technique previously described.[15] This latter method was calibrated with known mixtures of authentic methyl 8-, 9-, 10-, and 11-hydroxyoctadecanoate as the trimethylsilyl ether derivatives. The g.c.–m.s. method[15] was based on a computer summation of both fragments, due to α-fission on each side of the carbon bearing the TMS ether group, within all the g.c. peaks due to hydroxyoctadecanoate. By this technique a standard deviation of 0.72 was calculated for the analyses of known mixtures of authentic methyl hydroxyoctadecanoates.

Table 6.

Known % *trans*	% *trans* from Peak heights	% *trans* from Peak areas
54.0	54.0	52.0
84.7	83.5	89.0

Autoxidation.—Methyl oleate (10 g), prepared and purified as before,[15] was stirred magnetically under oxygen in a 50-ml round-bottomed flask attached to a manometric system and immersed in a constant temperature bath. Samples were withdrawn at different levels of oxidation and analysed iodometrically for peroxide value.[28] The oxidation products were isolated by partitioning between 80% ethanol (100 ml) and light petroleum (100 ml, b.p. 40—60 °C) by counter-current distribution between 6 separatory funnels.[29] The combined lower layers were concentrated with a rotating evaporator under reduced pressure. The concentrates were diluted with water and extracted with ether. The extracts were dried, and evaporated at room temperature. The hydroperoxides thus obtained were purified by t.l.c. and reduced with sodium borohydride in methanol to give the corresponding allylic hydroxy-esters which were also purified by t.l.c. (25% ether in light petroleum, b.p. 60—70 °C)

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