An Electron Spin Resonance Study of Fatty Acids and Esters. Part 1. Hydrogen Abstraction from Olefinic and Acetylenic Long-chain Esters

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The radicals generated from unsaturated fatty acid esters by hydrogen abstraction with photochemically formed t-butoxyl radicals have been investigated by e.s.r. spectroscopy. The substituted allyl and pentadienyl radicals generated from monoenoic and dienoic esters were conformationally stable under the conditions of the experiments. The main species observed on hydrogen-abstraction from monoynoic esters were substituted propynyl radicals, and the same radical type was generated from diynoic esters with more than one methylene unit separating the triple bonds. Substituted penta-1,4-diynyl and penta-1,3-diynyl radicals were identified on hydrogen-abstraction from methylene-interrupted and conjugated diynoic esters. Hydrogen abstraction from triacylglycerols containing one or more double bonds was also examined. Most of the unsaturated esters also gave rise to secondary radicals produced by hydrogen-abstraction from the chain methylene groups. From the measured concentrations of the secondary and delocalised radicals the following approximate relative rates of hydrogen-abstraction by t-butoxyl radicals from the different sites were obtained: secondary : propynylic : allylic : bisallylic = 1 : 18 : 36 : 116 at 293 K.

Lipid peroxidation may occur by a controlled enzymic oxidation leading to biologically important molecules [1] whose physiological roles include blood pressure regulation,[2] inflammation,[3] platelet aggregation,[4] and anaphylaxis.[5] Alternatively, lipid peroxides can be formed by random free radical reactions.[6] The random free radical autoxidation of polyunsaturated fatty acids appears to be an important process *in vivo*,[7] as exemplified by the expiration of pentane and ethane by organisms under free-radical stress.[8]

In vivo lipid peroxidation has been identified as one of the major free radical deteriorative reactions associated with cell membranes.[9] Among the pathological events associated with lipid peroxidation are oxidative damage to cells [10] and lungs induced by atmospheric pollution,[11] certain phases of atherosclerosis,[12] chlorinated hydrocarbon hepatoxicity,[13] oxygen toxicity,[14] ethanol-induced liver injury,[15] cataract formation,[16] paraquat toxicity,[17] the ageing process,[18] and cancer.[19] Free radical damage to biological membranes and foodstuffs has been the subject of several reviews.[20]

Although the free radical nature of lipid autoxidation is undisputed, the evidence for this relies on indirect observations such as oxygen consumption [21] and the formation of peroxides [22] and conjugated dienes from polyene acids.[21b,23] Much effort has been expended in trying to detect free radicals from fatty acids by e.s.r. spectroscopy [24-31] and signals have been recorded from ozonised linoleic acid,[24] from u.v.-irradiated unsaturated methyl esters [25] and triglycerides,[27] from γ-ray-irradiated triglycerides [28] and from the reaction of nitrogen dioxide with unsaturated fatty acids and phospholipids.[29] In all cases however the signals obtained were broad and without any resolved hyperfine splittings. Hence no structural or conformational information about the radical intermediates could be obtained.

Other research groups [30-32] used the spin trap technique to obtain information about the environment of the unpaired electron. De Groot *et al.*[30] studied the soybean lipoxygenase catalysed oxidation of linoleic acid in this way and, by use of deuterium labelled analogues, deduced that the main trapped species was the substituted pentadienyl radical (1a). Similar observations were made for the oxidation of linoleic acid with potato lipoxygenase.[31] Recently Chiba *et al.*[32] observed spin adducts from u.v.-irradiated solutions of methyl linoleate in the presence of deuteriated nitrosodurene as the spin trap.

In a preliminary report we showed that the pentadienyl radicals obtained on hydrogen abstraction from polyene acids by photochemically generated t-butoxyl radicals can be directly detected by e.s.r. spectroscopy.[33] Similarly, we observed allyl-type radicals generated from monoenoic acids such as oleic. We now report details of an e.s.r. spectral study of a variety of delocalised radicals obtained on hydrogen abstraction from methyl esters of long-chain acids with one or more double or triple bonds. The results provide additional insight into the mechanism of lipid autoxidation.

(1) (1a)

$R^1 = CH_3(CH_2)_4$, $R^2 = (CH_2)_7CO_2Me$ or $(CH_2)_7CO_2H$

Results and Discussion

E.s.r. spectra of the fatty ester radicals were obtained by photolysis of degassed solutions of the freshly purified ester and di-t-butyl peroxide in the cavity of the spectrometer. Hydrocarbon solvents could be used, particularly for low temperature work, but the most intense spectra were generally obtained with neat di-t-butyl peroxide and temperatures around ambient. Sample lifetimes were usually of the order of 15 min owing to the build-up of polymer on the walls of the tubes. In a few cases, where relatively large quantities of esters were available, a flow system was employed which increased the effective sample lifetimes.

Monoenoic Fatty Acid Esters.—A series of isomeric methyl octadecenoates was studied in which the double bond varied from the Δ^2 to the Δ^{17} positions. In most cases the spectra were very weak and/or poorly resolved and could not be analysed. The exceptions were methyl elaidate (18 : 1 Δ^{9t}) † and methyl oleate (18 : 1 Δ^{9c}), and the well resolved double septet spectrum obtained from the former has already been published. Hydrogen abstraction from the two allylic sites in

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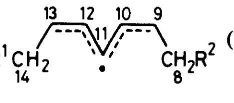
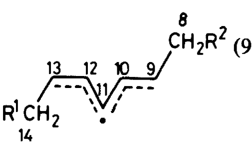
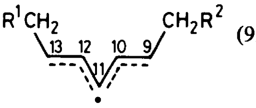
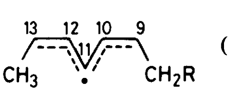
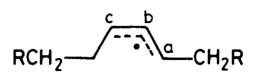
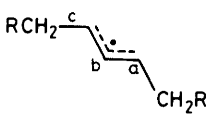
† The symbol 18 : 1 indicates a straight-chain C_{18} acid or ester with one unsaturated centre the position and configuration of which may be designated by further symbols.

Table 1. E.s.r. parameters of substituted allyl and pentadienyl radicals [a]

Radical	Precursor methyl ester	T/K	a/mT	
	18 : 2 (9Z,12Z)	290	H (10,12) CH_2(8,14 *endo*) H (9,13 *exo*) H (11)	0.32 0.78 0.98 1.11
	18 : 2 (9E,12Z) [b]	315	H (10,12) CH_2(*endo*) CH_2(*exo*) H (9 *endo*) H (13 *exo*) H (11)	0.32 0.78 0.90 0.90 0.98 1.11
	18 : 2 (9E,12E)	310	H (10,12) 2CH_2(*exo*) H (9,13 *endo*) H (11)	0.31 0.90 0.90 1.11
	14 : 2 (9Z,12Z)	270	H (10,12) CH_2(8 *endo*) H (9,13 *exo*) H (11) CH_3(14 *endo*)	0.32 0.78 0.98 1.11 0.90
	18 : 1 (9Z)	230	H (b) 2CH_2(*exo*,*endo*) H (a *endo*) H (c *exo*)	0.39 1.24 1.24 1.43
	18 : 1 (9E)	270	H (b) H (a, c *endo*) 2CH_2(*exo*)	0.37 1.29 1.29

[a] All h.f.s. were checked by computer simulation of the spectra. [b] 18 : 2 (9Z,12E) also present in the sample examined gives an identical spectrum.

methyl elaidate produced two isomeric *transoid* radicals (2b) but, as would be expected, these were spectroscopically indistinguishable.

Methyl oleate, oleic acid, and *cis*-octadec-9-ene (obtained by reduction of the carboxylate group) all gave identical spectra. These were readily analysed and the hyperfine splittings (h.f.s.) were similar to those of allyl [34,35] and alkyl substituted allyl radicals [35–38] and the assignments to specific hydrogens, made by comparison with these model compounds, are in Table 1.

The spectrum obtained from methyl oleate can be assigned to the *cisoid* allyl type radical (2a). On prolonged photolysis, especially at higher temperatures, *i.e. ca.* 330 K, the spectra became more complex showing the presence of a second allyl radical; eventually the spectrum became identical to that obtained from methyl elaidate. On cooling the sample the spectrum of the *transoid* radical persisted, *i.e.* the change was irreversible. After photolysis the contents of the e.s.r. tube were analysed by capillary g.l.c. and it was found that virtually all the remaining fatty acid ester had been converted to *trans*-isomers. Capillary g.l.c. showed peaks for 8*c* (very small), 9*c* (small), and 9*t* (very large) esters. This last peak may also contain the 8*t*, 10*t*, and 10*c* isomers and we would expect the minor 8*c* ester to be accompanied by a similar proportion of the 10*c* ester.[39]

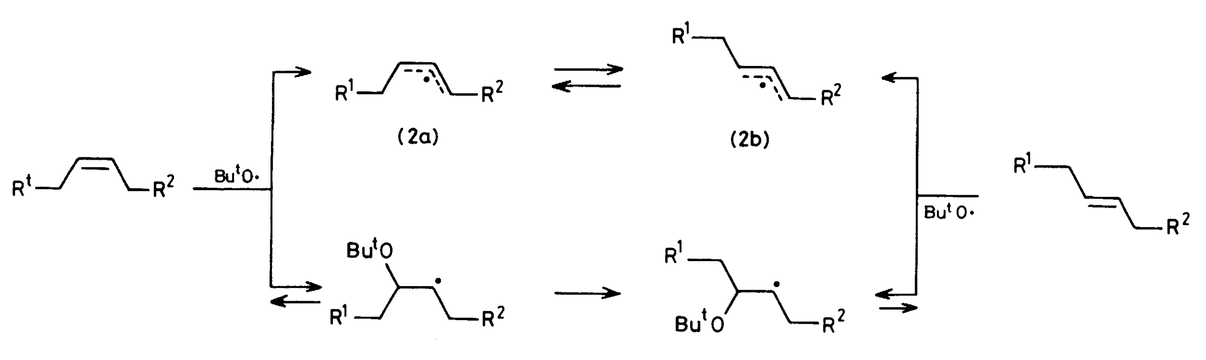
The initial observation of radical (2a) followed by (2b) on prolonged reaction *might* be explained by a direct isomerisation of (2a) to (2b) *via* rotation about the partial double bond (see Scheme 1). However, the process should then be reversible, *i.e.* on cooling the sample the spectrum of the *cisoid* radical (2a) should reappear, contrary to the experimental observations. Furthermore, the barriers to rotation about the partial double bonds in allyl (66 kJ mol^{-1}) [40] and substituted allyl radicals [41] suggest that direct isomerisation of the radicals would not occur at ambient temperatures. Alternatively, isomerisation could occur by reversible addition of $Bu^tO\cdot$ radicals to the oleate double bond (Scheme 1). The presence of methyl *trans*-octadecenoates after photolysis, though consistent with this explanation, could also be explained by the direct interconversion scheme if radicals (2b) abstract hydrogen from substrate to give elaidate.

The conversion of (2a) to the *transoid* conformer (2b) has important implications for oleate autoxidation. The classical mechanism of oleate autoxidation involves allylic hydrogen abstraction to give (2a) followed by oxygen entrapment to give the corresponding peroxyl radicals which subsequently give hydroperoxides on hydrogen abstraction.[39] Experimentally it is found that *trans*-hydroperoxides predominate and that the proportion of *trans* product increases with the temperature of autoxidation.[42–45] Oxygen entrapment by conformer (2a) does not provide an adequate explanation for the uneven distribution observed for both geometrical and positional isomers of oleate hydroperoxides.[42–47] The results clearly parallel our e.s.r. observations on the reaction of oleate with t-butoxyl radicals. The increase in relative concentration of *trans-* to *cis*-hydroperoxides with increasing temperature observed in the autoxidations can be explained by a similar argument, namely that radical (2a), which can give rise to both *cis-* and *trans*-hydroperoxides, is converted into the *transoid* radical (2b) which leads exclusively to *trans*-hydroperoxides. Our results do not give a definite indication of whether this conversion occurs directly or by reversible oxygen addition.[48]

The small proportion of hydroperoxides with *cis* unsaturation at Δ^8 and Δ^{10} imply that radical (2c) must also participate.[39] This radical was not detected in the e.s.r. experiments, but the signal to noise ratio was such that only the main radicals would be observed and the participation of minor amounts of (2c) is not excluded.

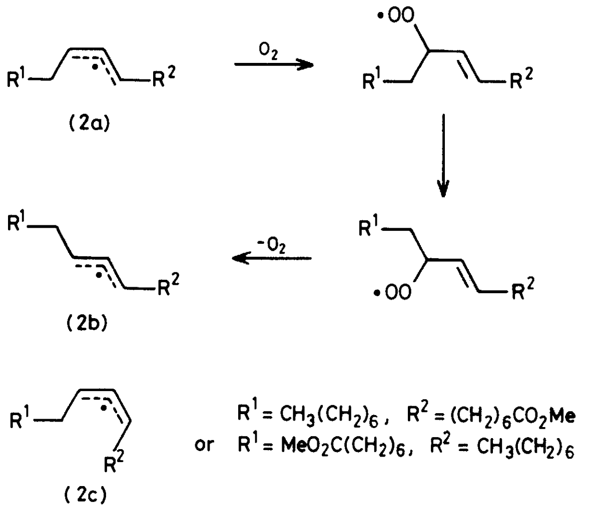
The e.s.r. spectra from both methyl oleate and methyl elaidate showed additional weak signals from secondary radicals produced by hydrogen abstraction from non-allylic chain methylenes. The inner lines of the secondary radicals were unobservable because of extensive overlap by the main spectrum of the allylic radicals, but the outer lines were clearly distinguishable. Very weak, but distinct, signals could also be observed for the radicals produced by hydrogen abstraction from the methylene adjacent to the carboxylate group. Distinct signals were also expected for the secondary radicals formed on hydrogen abstraction from the methylene adjacent to the terminal methyl group because of the extra β-hydrogen but these radicals were too weak for observation in the spectra from methyl elaidate. They were, however, observed on hydrogen abstraction from saturated fatty acids.

The relative concentration of allylic (A) and secondary alkyl (S) radicals was estimated by double integration of suitable peaks in the spectra. Accurate values were difficult to obtain in the static system because of poor signal intensities and the build-up of broad signals from further products. Spectra were also run using a flow system and a signal averager which gave higher accuracy. From the flow system



$R^1 = CH_3(CH_2)_6$, $R^2 = (CH_2)_6CO_2Me$,
or $R^1 = (CH_2)_6CO_2Me$, $R^2 = CH_3(CH_2)_6$,

Scheme 1.



$R^1 = CH_3(CH_2)_6$, $R^2 = (CH_2)_6CO_2Me$
or $R^1 = MeO_2C(CH_2)_6$, $R^2 = CH_3(CH_2)_6$

data we find [A]/[S] = 7.2 ± 1.6 at 293 K. There are four allylic hydrogens in methyl elaidate and 20 chain methylene hydrogens (excluding the methylene groups adjacent to the carboxylate and terminal methyl groups which give separate signals too weak to measure), hence the relative rate of allylic to secondary hydrogen abstraction for t-butoxyl radicals is *ca.* 36 ± 8 : 1 on a per hydrogen basis (see Table 2). Small *et al.*[49] measured the absolute rates of hydrogen abstraction from fatty acids by t-butoxyl radicals using a laser flash photolysis method and obtained a value of *k*(rel) = 7.2 with stearic and oleic acids. Our e.s.r. results thus indicate greater selectivity for t-butoxyl radicals and the reasons for this discrepancy are not clear. The calculation of *k*(rel) from the measured concentrations of the two radical types in the e.s.r. method depends on the assumption of equal termination rates for the secondary and allylic radicals. The termination rates are likely to be very similar, because they will be diffusion controlled, but a somewhat greater termination rate for secondary radicals could account for part of the difference.

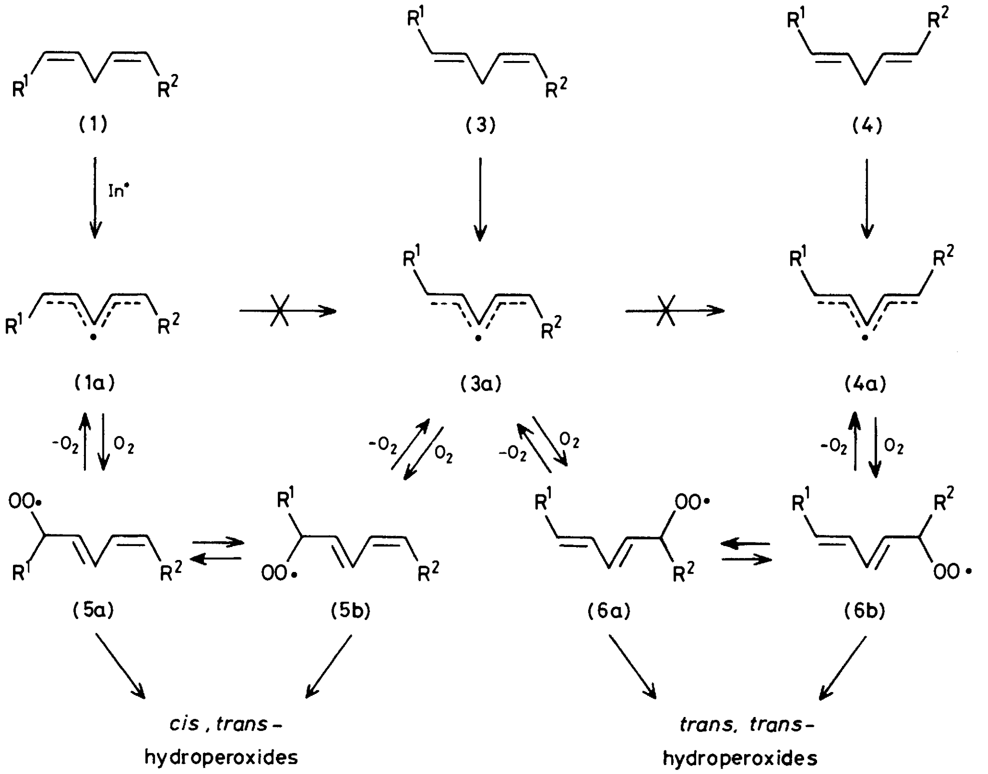
Our results, and those of Small *et al.*[49] show that hydrogen abstraction by t-butoxyl radicals from non-allylic positions in unsaturated acids and esters amounts to a significant proportion of the total. This contrasts with autoxidation studies where only hydroperoxides derived from attack on the allylic positions are detected. In autoxidation reactions hydrogen is abstracted by peroxyl radicals which are probably more selective than t-butoxyl radicals, and this helps to explain the difference in the product distribution. The results with t-butoxyl radicals suggest however that minor amounts of hydroperoxides derived from attack at the non-allylic methylene groups should be formed in the autoxidation of unsaturated fatty acids. This could be more significant at higher temperatures because alkoxyl radicals formed by thermal cleavage of hydroperoxides are present. Hydroperoxides attached to secondary carbon atoms are as yet undetected but their degradation products could be important in influencing the flavour and smell of stored fats and oils.

Table 2. Relative rates of hydrogen abstraction from unsaturated C_{18} esters by t-butoxyl radicals at ambient temperature

Site	Secondary H	Propynylic H	Allylic H	Bisallylic H
k(rel) (298 K)	1.0	18 ± 5	36 ± 8	116 ± 25
D(C–H)/kJ mol^{-1}	397	365 [a]	345 [b]	322 [c]

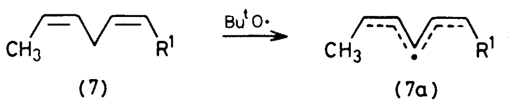
[a] From $CH_3CH_2C{\equiv}CCH_3$.[61] [b] From $CH_3CH_2CH{=}CH_2$.[62] [c] From $CH_2{=}CHCH_2CH{=}CH_2$.[62,63]

Di-, Tri-, and Tetra-enoic Fatty Acid Esters.—Hydrogen abstraction from methyl linoleate (1), the isomeric *cis,trans*-mixture (3), and the *trans,trans*-ester (4) gave rise to e.s.r. spectra which were assigned to the pentadienyl radicals (1a), (3a), and (4a) respectively (Scheme 2). The three spectra were distinct from each other and the h.f.s. were assigned to specific hydrogens by comparison with those of alkyl-substituted pentadienyl radicals [50–52] (Table 1). Pentadienyl radicals with e.s.r. parameters identical to those of (1a) were observed on hydrogen abstraction from the hydrocarbon (octadeca-9*Z*,-12*Z*-diene) produced by reduction of the carboxylate group of (1). It is seen that similarly sited hydrogens in the three radicals have identical h.f.s., *e.g.* all the *endo*-hydrogens have the same h.f.s. This internal consistency which supports the assignments has also been reported in alkyl-substituted pentadienyl radicals.[52] Several conformations are conceivable for the radicals (1a), (3a), and (4a) but molecular models indicate that the conformations illustrated will be the most stable with least steric crowding and we have assumed that other conformations are unimportant.



$R^1 = CH_3(CH_2)_4$, $R^2 = (CH_2)_7CO_2Me$,
or $R^1 = (CH_2)_7CO_2Me$, $R^2 = CH_3(CH_2)_4$,

Scheme 2.



$R^1 = (CH_2)_7CO_2Me$

Photolysis of methyl tetradeca-9*Z*,12*Z*-dienoate (7) in neat di-t-butyl peroxide gave the pentadienyl radical (7a). The h.f.s. constants have been unequivocally assigned to the hydrogens of the pentadienyl system in the *cis,cis* conformation and are given in Table 1. The terminal methyl group has little effect on the h.f.s. constants which are in excellent agreement with our parameters for methyl linoleate.

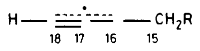
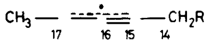
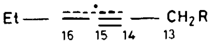
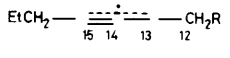
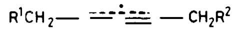
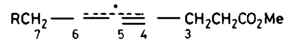
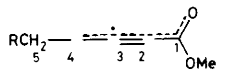
Neither secondary nor allyl radicals could be detected on the spectra obtained from methyl linoleate in static or flow systems. Secondary radicals were observable when mixtures of linoleic and methyl palmitate were examined and the relative concentrations of the bisallylic (BA) and secondary (S) radicals were measured. From the concentrations of the two radical types the rate of abstraction of the bisallylic hydrogens relative to the rate of abstraction of secondary hydrogens on a per hydrogen basis [*k*(rel)] was calculated. Reasonable agreement in the *k*(rel) values was obtained for the three concentration ratios and the average is in Table 2.

[18 : 2]/[16 : 0]	0.069	0.347	0.442
[BA]/[S]	0.603	2.51	3.95
k(rel) (293 K)	109	104	135

The spectrum of radical (1a) generated from methyl linoleate did not change with time or temperature in the range 250—330 K, *i.e.* the *cis,cis*-radicals do not convert to the *trans,cis*- (3a) or *trans,trans*-conformers (4a) under the conditions of the e.s.r. experiments. It is generally accepted that autoxidation of methyl linoleate (1) proceeds by hydrogen abstraction at the bisallylic site [C(11)] to give the pentadienyl radical (1a) and our direct e.s.r. identification of (1a) as the principal radical from (1) confirms this assumption and agrees with spin-trapping results of De Groot *et al.*[30] Oxygen entrapment by (1a) gives the peroxyl radicals (5a and b) (Scheme 2) which can only lead to the formation of *cis,trans*-hydroperoxides. Experimentally it has been found that both *cis,trans*- and *trans,trans*-hydroperoxides are formed in this autoxidation. To account for this Porter[53] and others[46] proposed that the oxygen addition step must be reversible, so that the *cis-trans*-radical (3a) can be generated from (1a) *via* (5a and b). Oxygen addition to C(9) or C(13) in (3a) can then give peroxyl radicals (6a) and hence (6b) which are responsible for *trans,trans*-hydroperoxides. The direct interconversion of (1a), (3a), and (4a) was discounted on energetic grounds and by analogy with the pentadienyl radical itself.[52] Our e.s.r. observations confirm that direct interconversion does not occur, and lend support to the indirect route.

We note with interest that the spin density at the central carbon of the pentadienyl system is higher than at C(9) and C(13). Experimentally, autoxidation of methyl linoleate has been reported to give exclusively conjugated diene hydroperoxides resulting from oxygen addition at C(9) and C(13). Oxygen entrapment at the central carbon of the pentadienyl system C(11) would give a non-conjugated hydroperoxide. However, if the product distribution is under thermodynamic

Table 3. E.s.r. parameters for substituted propynylic radicals derived from methyl octadecaynoates. Relative rates of hydrogen abstraction by t-butoxyl radicals

Radical	Methyl ester	*T*/K	H.f.s.[a] (mT)	[P]/[S]	*k*(rel)[b]
	17-yne	280	H(18) 1.12, CH_2(15) 1.80, H(16) 1.80, CH_2(14) 0.05	—	—
	16-yne	280	CH_3(18) 1.16, H(15) 1.74, CH_2(14) 1.74, CH_2(13) 0.05	—	—
	15-yne	280	CH_2(17) 1.11, H(14) 1.77, CH_2(13) 1.77	—	—
	15-yne	280	CH_2(14) 1.21, H(17) 1.80, CH_3(18) 1.85	—	—
	14-yne	295	CH_2(13) 1.11, H(16) 1.86, CH_2(17) 1.86	1.7 ± 0.5	17 ± 5
	14-yne	295	CH_2(16) 1.11, H(13) 1.69, CH_2(12) 1.69	—	—
	13-yne[c]	270	(2 H) 1.11, (3 H) 1.77	3.6 ± 1	18 ± 5
″	12-yne[c]	270	(2 H) 1.11, (3 H) 1.77	3.7 ± 1	18.5 ± 5
″	9-yne[c]	280	(2 H) 1.12, (3 H) 1.77	—	—
″	8-yne[c]	280	(2 H) 1.12, (3 H) 1.77	3.8 ± 1	19 ± 5
″	6-yne[c]	280	(2 H) 1.12, (3 H) 1.77	—	—
″	5-yne[c]	270	(2 H) 1.12, (3 H) 1.79	3.6 ± 1	18 ± 5
	4-yne	295	H(6) 1.79, CH_2(7) 1.79, CH_2(3) 1.12	2.8 ± 1	14 ± 5
	2-yne	290	H(4) 1.78, CH_2(5) 1.78	0.8 ± 1	12 ± 4

[a] All h.f.s. checked by computer simulations. [b] Rate of abstraction of propynylic hydrogens relative to secondary hydrogens on a per hydrogen basis, *i.e.* statistically corrected for the number of each type of hydrogen. [c] Two indistinguishable radicals are present, see text.


(8)


(9)

$R^1 = CH_3(CH_2)_4$, $R^2 = (CH_2)_4CO_2Me$, $R^3 = (CH_2)_3CO_2Me$

control as opposed to kinetic control then the 11-hydroperoxide might rearrange, *via* reversible oxygen addition, to the thermodynamically more stable conjugated dienehydroperoxides. It is conceivable that at low tempeatures the 11-hydroperoxide may be an isolable autoxidation product.

Hydrogen abstraction from the two bisallylic positions in methyl γ-linolenate (8) would also be expected to produce pentadienyl radicals. We found that t-butoxyl radicals abstract hydrogen specifically from the bisallylic sites and that the two pentadienyl radicals are indistinguishable from each other and from (1a) by e.s.r. spectroscopy. Similarly, the pentadienyl radicals observed on hydrogen abstraction from the three bisallylic methylenes of the tetraenoic ester (9) (methyl arachidonate) are indistinguishable. The e.s.r. parameters of these radicals are the same, within experimental error, as those given for (1a) in Table 1.

Monoynoic Fatty Acid Esters.—A series of isomeric methyl octadecynoates in which the position of the triple bond varied from C(2) to C(17) was also studied. In contrast to the methyl octadecenoates well resolved spectra with good signal intensity were obtained for most of the isomers. The main radical in each case was readily identified as the corresponding substituted propynylic (allenic) radical. Similar spectra, consisting of a quartet of triplets, were obtained for the methyl octadec-4- through -13-ynoates; the spectrum from methyl octadec-8-ynoate is shown in Figure 1. The h.f.s. were assigned to specific hydrogens by comparison with the parameters for propynyl [54] and alkyl-substituted propynyl radicals,[55] and the results are in Table 3. Two different propynyl radicals can be formed by hydrogen abstraction from the propynylic sites (Scheme 3) but for the 5-yne through 13-yne esters the two radicals were spectroscopically indistinguishable. Two propynylic radicals with different h.f.s. but the same *g* factors were observed for the 14-yne and 15-yne esters. For the 16-yne ester abstraction of the primary hydrogens of the terminal methyl group was not observed and only one propynyl radical was detected; in this and the single propynylic radical obtained from methyl octadec-17-ynoate triplet hyperfine splittings from the methylene groups γ to the triple bond were resolved. Two distinct propynylic radicals were expected for the 4-yne ester (by analogy with the 14-yne ester) but only one was detected. It is possible that the hydrogens β to the carboxylate group are deactivated so that the radical generated at this site is too weak for detection (see below).

Although the main radical from each acetylenic ester was

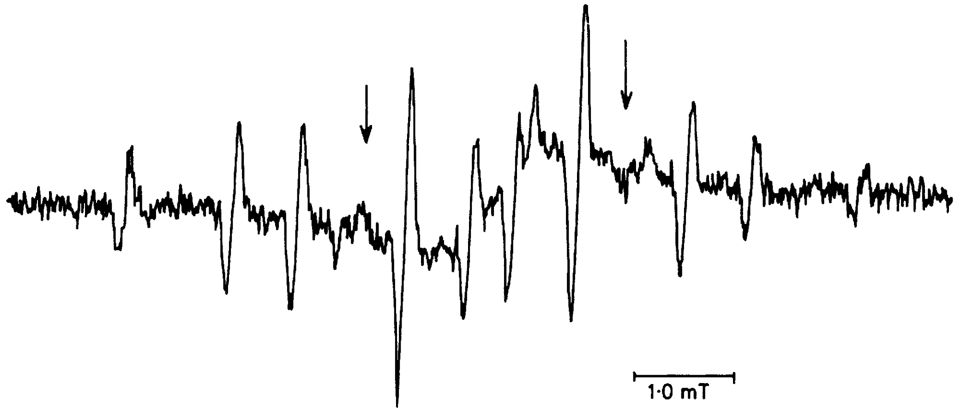
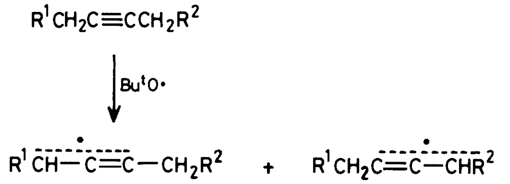


Figure 1. 9.4 GHz E.s.r. spectrum of the propynyl radical derived from methyl octadec-8-ynoate. Arrows indicate the main lines of the secondary radicals derived from hydrogen-abstraction at chain methylenes



Scheme 3.

obtained by abstraction of the propynylic hydrogens, signals from secondary radicals generated by hydrogen abstraction from the chain methylenes were also observable in most of the spectra (see Figure 1). The acetylenic esters were not available in sufficient quantities to permit the use of the flow technique but the ratio of the concentrations of the propynylic (P) to secondary (S) radicals was determined from the intensities of the signals in static runs, for a number of the isomers, and the results are given in Table 3. The methyl octadecynoates studied contained four propynylic hydrogens (except for the 14-yne isomer where the concentration ratio was calculated from only one of the two propynylic radicals) and 20 secondary hydrogens (excluding those adjacent to the terminal methyl and carboxylate groups) and the *k*(rel) values (Table 3) were calculated on this assumption. The average *k*(rel) is given in Table 2 but the results for the 4-yne and 2-yne esters have been excluded. The lower *k*(rel) values obtained for these two isomers may be due to deactivation of the propynylic methylenes by the carboxylate group. Tedder and his co-workers have shown that carboxylate and other electron-withdrawing substituents deactivate both α- and β-hydrogens to abstraction by halogen atoms.[56] In the case of the 4-yne ester one set of propynylic hydrogens is β to the carboxylate group. The low *k*(rel) for this isomer could be a consequence of similar deactivation towards hydrogen abstraction by t-butoxyl radicals. It is possible that the C(3) hydrogens in this isomer are sufficiently deactivated that the propynylic radical which would be generated by their abstraction is too weak to detect. This would explain the absence of the two distinct propynylic radicals in the spectra from this isomer. Similarly, the deactivating effect of the electron-withdrawing carboxylate substituent would be transmitted by the triple bond in the 2-yne ester thus accounting for the lower rate of hydrogen-abstraction observed for the C(4) propynylic hydrogens of this ester.

The relative rates of hydrogen abstraction from the various sites in the fatty acid esters are collected in Table 2. The order of reactivity of the hydrogen bonds towards Bu^{t}O· radicals is bisallylic > allylic > propynylic > secondary. This order is in agreement with the results of Small *et al.*[49] but our e.s.r. data indicate a greater difference in reactivity of allylic and secondary hydrogens and a smaller difference in reactivity between allylic and bisallylic hydrogens. The *overall* rate constants for reaction of hydroxyl radicals with propane, propene, propyne, and related molecules have been determined.[57-60] The rate constants include addition of HO· to the double bond as well as hydrogen abstraction, but they do suggest that the order of reactivity is the same for HO· and Bu^{t}O· radicals. The C–H bond dissociation energies from model compounds are compared with the *k*(rel) values in Table 2,[61-63] which shows that the rate of hydrogen-abstraction increases monotonically with decrease in *D*(C–H). The relative rates of abstraction of primary, secondary, and tertiary hydrogen by t-butoxyl radicals are[64] 1 : 84 : 44. A plot of log[*k*(rel)] against the bond dissociation energies is shown in Figure 2, where our data (Table 2) is combined with the relative rates for primary and tertiary hydrogens. For a closely related series of reactions the Evans–Polanyi relation $\{E = \alpha[D(\text{C–H})] + C'\}$ predicts a linear correlation of the activation energy of hydrogen abstraction with the strength of the C–H bond. If the *A* factors do not change very much from one reaction to another, and often the changes are small for a series of similar abstractions, then log[*k*(rel)] will be propor-

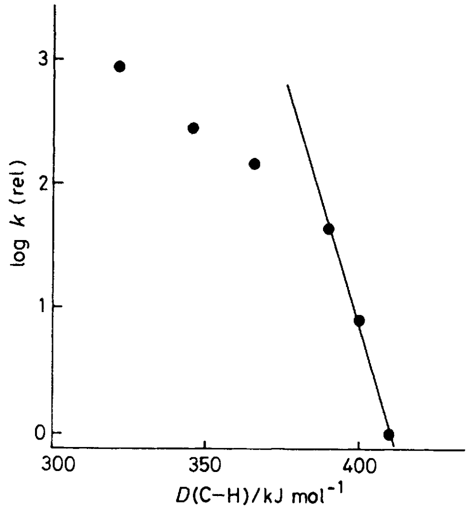
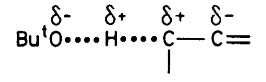
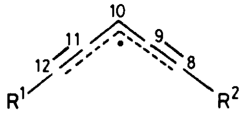


Figure 2. Plot of log *k*(rel) *versus* *D*(C–H) for the experimental data of Table 2



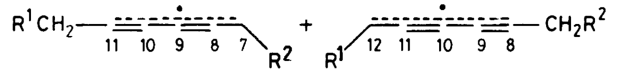
$\overset{\delta-}{\mathrm{Bu^tO}}\cdots\overset{\delta+}{\mathrm{H}}\cdots\overset{\delta+}{\underset{|}{\mathrm{C}}}-\overset{\delta-}{\mathrm{C}}\equiv$

(10)



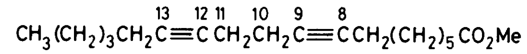
(11)

$R^1 = CH_3(CH_2)_8$, $R^2 = (CH_2)_6CO_2Me$



(12)

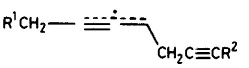
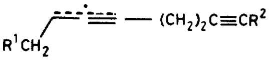
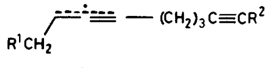
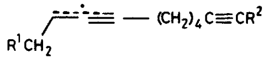
$R^1 = CH_3(CH_2)_5$, $R^2 = (CH_2)_5CO_2Me$



$\mathrm{CH_3(CH_2)_3CH_2\overset{13}{C}{\equiv}\overset{12}{C}\,\overset{11}{C}H_2\overset{10}{C}H_2\overset{9}{C}{\equiv}\overset{8}{C}CH_2(CH_2)_5CO_2Me}$

(13)

Table 4. E.s.r. parameters for radicals generated from diacetylenic fatty acid esters and methyl octadeca-9*E*-en-12-ynoate

Radical	Methyl ester	T/K	H.f.s.[a] (mT)
(11)	8,11-diyne	300	H(10) 1.64, $CH_2(7)$ 0.87, $CH_2(13)$ 0.87
(12)	8,10-diyne [b]	270	(3 H) 1.53, (2 H) 0.64
	8,12-diyne [c]	290	(2 H) 1.12, (1 H) 1.78, (2 H) 1.95
			(2 H) 1.12, (3 H) 1.77
	7,12-diyne	290	(2 H) 1.12, (3 H) 1.77
	6,12-diyne	295	(2 H) 1.12, (3 H) 1.77
(14)	9*E*-en-12-yne	275	H(10), 0.36 H(11) 1.38, H(9) 1.01, $CH_2(8)$ 1.01, $CH_2(14)$ 0.74

[a] All h.f.s. checked by computer simulations. [b] Two indistinguishable radicals are present. [c] Four indistinguishable radicals are present.

tional to the activation energy. The points for primary, secondary, and tertiary hydrogen abstraction lie on a straight line, but the remaining points for hydrogen-abstraction adjacent to one or more unsaturated bonds lie below this line. Thus, hydrogen-abstraction by Bu^{t}O· radicals from these sites is less rapid than would be expected on bond dissociation energy grounds alone. This may partly be explained by the presence of an unfavourable polar effect in the reactions adjacent to unsaturated bonds. Approach of the electron-attracting t-butoxyl radical will be retarded by the electron-attracting multiple bond, *i.e.* there will be an unfavourable charge distribution in the transition state (10).

Diynoic and Enynoic Fatty Acid Esters.—The methylene-interrupted compound, methyl henicosa-8,11-diynoate, was difficult to purify because of rapid deterioration in air. The material was chromatographed and used immediately, after blowing off the solvent in a stream of dry nitrogen. The e.s.r. spectrum in neat di-t-butyl peroxide was very short lived due to polymer build-up, but it consisted of a double quintet and the h.f.s. are given in Table 4. The splitting pattern and the magnitude of the h.f.s., in comparison with those of unsubstituted penta-1,4-diynyl radicals,[65] enable the spectrum to be assigned to the substituted penta-1,4-diynyl radicals (11). The 1.64 mT h.f.s. from H(10) in (11) is close to that of the analogous hydrogen in penta-1,4-diynyl (1.66) but is considerably larger than a[H(11)] in pentadienyl radicals such as (1a), *i.e.* 1.11 mT (Table 1). Similarly the h.f.s. from the methylene hydrogens at the ends of the delocalised system in (11) are smaller than the h.f.s. of the analogous hydrogens in pentadienyl radicals. This evidence corroborates the previous conclusion[65] that triple bonds are less effective at delocalising spin density than double bonds. No other radicals were visible in the spectra from the diynoic ester so we can estimate that the concentration of (11) must be at least three times that of the propynylic radicals which would be formed by hydrogen abstraction from C(7) or C(13), and also at least three times that of the secondary radicals generated by abstraction of the chain methylene hydrogens. Hydrogen abstraction from the conjugated diyne ester, methyl octadeca-8,10-diynoate, gave a radical with a spectrum consisting of a quartet of triplets; no other radicals were detectable. We attribute this spectrum to penta-1,3-diynyl radicals (12) formed by t-butoxyl radical attack at the C(7) or C(12) methylenes. As expected, the two radicals were spectroscopically indistinguishable. This type of delocalised radical has not been observed previously by e.s.r. but comparison of the h.f.s. of (12) (Table 4) with those of the propynyl radicals (Table 3) and penta-1,4-diynyl radicals strongly supports the identification.

Other methyl octadecadiynoates containing 2—4 methylene groups between the triple bonds were also examined. Intense spectra due to substituted propynylic radicals, similar to the spectra from monoacetylenic esters, were obtained. In principle four different propynylic radicals can be formed by hydrogen abstraction from the four different sets of propynylic hydrogens. For the diacetylenic esters with more than two methylene groups between the triple bonds a single e.s.r. spectrum was obtained, *i.e.* the four radicals gave indistinguishable spectra. However, for methyl octadeca-8,12-diynoate (13) with only two methylene groups between the triple bonds two different spectra from two types of propynylic radical were obtained in approximately equal amounts. It is probable that the radicals formed by hydrogen-abstraction from the 'outer' propynylic methylenes [C(7) and C(14)] are indistinguishable from each other and correspond to the spectrum identical to that obtained from the monoacetylenic esters. Hydrogen-abstraction from the 'inner' propynylic

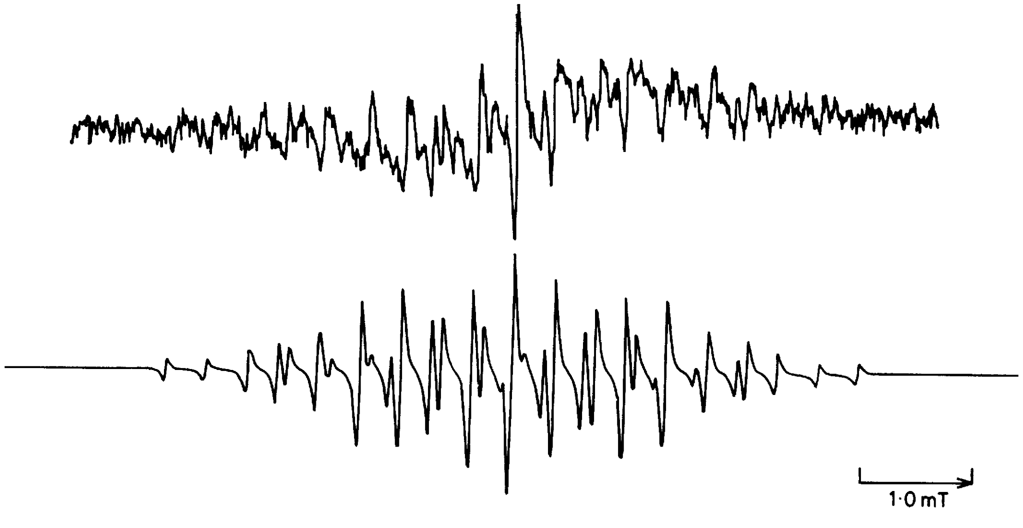
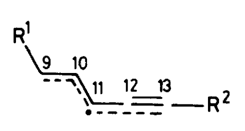


Figure 3. 9.4 GHz E.s.r. spectrum of pent-1-en-4-ynyl radicals (14) in neat di-t-butyl peroxide. Upper trace, experimental spectrum; lower trace, computer simulation



(14)

$R^1 = MeO_2C(CH_2)_7$, $R^2 = (CH_2)_4CH_3$

methylenes [C(10) and C(11)] will give two radicals indistinguishable from each other, but different from the ' outer ' radicals because the h.f.s. of the ' inner ' hydrogens will be perturbed by the proximity of the second triple bond (see Table 4). The spectrum obtained from methyl octadeca-7,12-diynoate was exceptionally intense and showed traces of secondary radicals generated from the chain methylenes. The ratio of the concentrations of the propynylic to secondary radicals was found to be 12 ± 3. Since there are eight propynylic hydrogens and twelve hydrogens which can give radicals contributing to the secondary signal, the rate of abstraction of propynylic hydrogen relative to secondary hydrogen, *k*(rel) = 18 ± 4. This value is in good agreement with results from the monoacetylenic esters (Table 3) and corroborates the average value used in Table 2.

The e.s.r. spectrum obtained on hydrogen-abstraction from methyl octadeca-9*E*-en-12-ynoate is shown in Figure 3. Hydrogen-abstraction is expected to occur at the doubly activated site [C(11)] to give the substituted pent-1-en-4-ynyl radicals (14). The spectrum was analysed as shown in Table 4 and the computer simulation is also shown in Figure 2. The h.f.s. are similar to those of pent-1-en-4-ynyl radicals,[65] which supports the assignment of the spectrum to radical (14). There are two possible conformations of radical (14), the *transoid*-form as shown and the *cisoid*-form which could be obtained by a 180° rotation about the C(10)–C(11) bond. For the unsubstituted pent-1-en-4-ynyl radicals both conformers were observed, but the *transoid* form predominated at temperatures above ambient. Radical (14) is probably mainly in the *transoid* conformation because this will be the least sterically hindered form. In addition, the h.f.s. of H(11) (1.38 mT) is identical to the h.f.s. of the analogous hydrogen in the *transoid* conformer of pent-1-en-4-ynyl radicals and significantly different from the h.f.s. of the same hydrogen in the *cisoid*-conformer. The quality of the spectrum was such that minor amounts of the *cisoid*-conformer of (14) would not have been detectable (see Figure 3).

Triacylglycerols.—E.s.r. spectra of allyl radicals derived from triacylglycerols were also observed on photolysis of mixtures containing di-t-butyl peroxide and a triacylglycerol having one or more oleate or elaidate units. The e.s.r. spectrum from 1-palmitoyl-2,3-dielaidoyl-*rac*-glycerol is similar to that obtained from methyl elaidate,[33] but, as expected, the proportion of hydrogen abstraction occurring from non-activated methylenes is larger, since one of the three fatty acids in the triacylglycerol has a fully saturated chain. Quantitative determination of the relative amount of hydrogen abstraction from the allylic sites (A) to hydrogen abstraction from non-activated methylenes (S) gave a value of [A]/[S] = 4.6 ± 0.9 [*k*(rel) = 36.4 ± 7], in excellent agreement with that obtained from methyl elaidate.

The e.s.r. spectrum obtained from 1-palmitoyl-2-stearoyl-3-oleoyl-*rac*-glycerol showed signals due to allyl radicals in the *cisoid*-conformation and also signals due to secondary radicals. Values of [A]/[S] and *k*(rel) were 2.3 ± 0.5 and 40 ± 8, respectively.

The much larger triacylglycerol radicals tumble less efficiently than those from the fatty acids and line broadening is evident and probably arises from the incomplete averaging of the anisotropic dipolar coupling. It is unlikely that hydrogen abstraction from the glycerol backbone occurs to any significant extent since attempts to obtain an e.s.r. spectrum from a photolysed solution of triacetin in di-t-butyl peroxide were unsuccessful.

Experimental

E.s.r. spectra were recorded with a Bruker ER200D spectrometer. Di-t-butyl peroxide was passed through a column of alumina and distilled under reduced pressure. Substrates dissolved in di-t-butyl peroxide (*ca.* 1 : 20) were placed in a quartz tube and degassed with at least three freeze–pump–thaw cycles. The samples were photolysed directly in the cavity of the spectrometer unless otherwise stated.

The flow cell used for some experiments consisted of a motor driven syringe which smoothly pumped the substrate in di-t-butyl peroxide through a quartz tube in the cavity of the spectrometer where it was photolysed. Flow rates were typically of the order 3—5 ml min^{-1}. In this case samples were de-aerated by purging the solution with nitrogen.

For the determination of *k*(rel) for methyl linoleate, this ester was added to methyl palmitate in accurately known amounts. The mixture was then photolysed as described above and spectra were accumulated using a signal averager. Values of *k*(rel) were corrected for the amount of methyl palmitate added.

General procedures were the same as those used previously.[66]

Methyl oleate, linoleate, and γ-linolenate were isolated from olive oil, sunflower oil, and evening primrose oil by reported procedures.[67] Monoacetylenic, diacetylenic, and dienoic acids, available from previous research programmes,[68] were esterified using 2% MeOH in H_2SO_4. Methyl arachidonate was commercially available and had a purity in excess of 99.9% by g.l.c. Great care was taken to ensure that no autoxidised product was present before the e.s.r. experiment was carried out and to this end all samples were subjected to column or thin layer chromatography and used within 16 h. Unless stated otherwise all samples had a g.l.c. purity >99.5% and were also judged pure by analytical t.l.c.

Methyl Octadeca-9E,12E-, *and* -9E(Z),12Z(E)-*dienoates*.—To methyl linoleate (99.5%, 5.0 g, 1.67 mmol) dissolved in ether (100 ml) was added an ice-cold mixture of aqueous sodium nitrite (2M, 70 ml) and nitric acid (6M, 45 ml), and the solution shaken vigorously for 20 min. The ethereal layer, separated from the aqueous phase and washed with aqueous sodium thiosulphite solution (5%, 2 × 30 ml) and saturated brine (2 × 30 ml), gave the isomerised product (4.8 g). As required this was separated by preparative, silver ion t.l.c. using 10% ether in petroleum (PE 10), as eluent,[66] furnishing three bands at high R_F(A–C) together with a small amount of oxidised material at low R_F.

Band A, octadeca-9*E*,12*E*-dienoate, had ^{1}H, ^{13}C n.m.r., i.r., and mass spectral properties identical with previously published data.[69,70] G.l.c. analysis (SP 2300) showed only one peak at equivalent chain length (e.c.l.) 18.6. Capillary g.l.c. (Carbowax) showed only one peak with a shorter retention time than either the *cis*, *trans*- or *cis,cis*-dienoate isomers. T.l.c. showed one spot in PE 10, R_F 0.83.

Band B was a mixture of octadeca-9*E*-12*Z*- and octadeca-9*Z*,12*E*-dienoates. ^{1}H, ^{13}C N.m.r., i.r., and mass spectral data were identical to those given in the literature.[69,70] G.l.c. showed only a single peak (SP 2300) at e.c.l. 18.6. Capillary g.l.c. (Carbowax) showed two completely resolved peaks in approximately equal amounts. Their retention times were longer than the *trans-trans*-ester but shorter than the *cis,cis*-ester. T.l.c. showed only one spot in PE 10, R_F 0.83.

Band C was identified as unchanged methyl linoleate by comparison with authentic material.

Octadeca-9Z,12Z-*diene*.—Methyl octadeca-9*Z*,12*Z*-dienoate (300 mg, 1 mmol) in dry diethyl ether (10 ml) was added dropwise to a vigorously stirred solution of lithium aluminium hydride (60 mg) in dry ether (25 ml) at such a rate as to maintain gentle reflux. This took 30 min and the mixture was stirred for a further 30 min before adding 'wet ether' (20 ml), water (20 ml), and finally dilute sulphuric acid (10%, 15 ml). Extraction of the organic phase gave octadeca-9*Z*,12*Z*-dien-1-ol, >98.5% pure as judged by t.l.c. and g.l.c., δ_H 5.31 (4 H, m, olefinic), 3.55 (2 H, t, *J* 6.0 Hz, CH_2OH), 3.05br (1 H, s, OH), 2.72 (2 H, m, double allylic CH_2, s), 2.00 (4 H, m, allylic CH_2), 1.30br (20 H, s, chain CH_2), and 0.88 (3 H, t, *J* 5.4 Hz, CH_3); δ_C 129.82 (C-13), 128.66 (C-9), 127.82 (C-10), 126.66 (C-12), 62.27 (C-1), 32.57 (C-3), 31.39 (C-16), 29.55 (C-15), 29.39 (C-7, -5), 29.19 (C-6, -4), 27.07 (C-8, -14), 25.72 (C-11), 25.49 (C-2), 22.43 (C-17), and 13.84 p.p.m. (C-18). $\nu_{max.}$ 3 300 (O–H) stretch) and 3 005 cm^{-1} (HC=CH, C–H stretch). The mass spectrum showed no diagnostic fragments as expected from aliphatic polyene primary alcohols. Peaks of general formula C_nH_{2n-5} and C_nH_{2n-1} predominated. The base peak was *m/e* 55 ($C_4H_7^{+\cdot}$).

The alcohol was stirred at 0 °C with dry pyridine (2 ml) and freshly distilled methanesulphonyl chloride (0.5 ml) for 4 h, after which time ice-cold 2M-HCl was added and the organic product extracted with hexane to give 1-methylsulphonyloxyoctadeca-9*Z*,12*Z*-diene (302 mg, 85%). The i.r. spectrum showed loss of hydroxy and new absorption bands at 1 350, 1 170, and 940 cm^{-1}. Lithium aluminium hydride reduction of the methanesulphonate gave the desired octadeca-9*Z*,12*Z*-diene (200 mg, 79% for the three steps), δ_H 5.33 (4 H, m, olefinic), 2.72 (2 H, m, doubly allylic CH_2), 2.05 (4 H, m, allylic CH_2), 1.30br (20 H, s, chain CH_2), and 0.88 (6 H, t, *J* 5.4 Hz, CH_3), $\nu_{max.}$ 3 007 cm^{-1} (HC=CH, C–H stretch), absence of OH, C=O, and HC=CH (*trans*) stretching or bending frequencies. The mass spectrum showed an $M^+ - 18$ peak at 250 (10%) and peaks associated with sequential loss of methylene units.

Octadeca-9Z-*ene*.—The procedure described for octadeca-9*Z*,12*Z*-diene was followed using methyl octadeca-9*Z*-enoate as substrate to furnish the desired octadeca-9*Z*-ene (191 mg, 76% net yield).

Spectral data for octadeca-9*Z*-en-1-ol was as follows: δ_H 5.33 (2 H, t, *J* 4.6 Hz, olefinic), 3.56 (2 H, t, *J* 6.0 Hz, CH_2OH), 2.50br (1 H, s, OH), 1.98 (4 H, m, allylic CH_2), 1.25br (24 H, s, chain CH_2), and 0.85 (3 H, t, *J* 5.4 Hz, CH_3), δ_C 129.68 (C-10), 129.60 (C-9), 62.64 (C-1), 32.57 (C-3), 31.72 (C-16), 29.57 (C-7, -12), 29.30 (C-6, -13, -15),* 29.12 (C-4, -5, -14),* 27.01 (C-8, -11), 25.60 (C-2), 22.47 (C-17), and 13.84 p.p.m. (C-18), $\nu_{max.}$ 3 340 (OH stretch) and 3 000 cm^{-1} (HC=CH, C–H stretch), *m/e* 250 ($M^+ - 18$), 222 (250 – C_2H_4), 208 (250 – C_3H_8), 194 (250 – C_4H_{10}), 180 (250 – C_5H_{12}), 166, 152 *etc.*, due to sequential loss of CH_2 units.

The following spectroscopic data was recorded for octadeca-9*Z*-ene: δ_H 5.37 (2 H, t, *J* 4.6 Hz, olefinic), 1.97 (4 H, m, allylic CH_2), 1.25br (24 H, s, chain CH_2), and 0.86 (6 H, t, *J* 5.4 Hz, CH_3), $\nu_{max.}$ 3 005 cm^{-1} (HC=CH), C–H stretching, absence of OH, C=O, and HC=CH (*trans*) absorptions. The mass spectrum showed an M^+ peak at 252 (8.5%).

Methyl Elaidate from Oleic Acid.—The procedure described for the stereomutation of octadeca-9*Z*,12*Z*-enoate was carried out on oleic acid to furnish a mixture of elaidic and oleic acid (*ca.* 7 : 1 by capillary g.l.c.). Four recrystallisations from petroleum furnished pure elaidic acid, m.p. 45—45.5 °C. The acid was esterified using 2% MeOH–H_2SO_4 to give methyl elaidate as an oil. Spectra were in agreement with the literature.[69,70]

Methyl Octadec-9,12-*diynoate*.—The free acid was available from a previous research programme.[68] The acid was esterified with 2% MeOH–H_2SO_4. Column chromatography gave a product which still had a yellow taint and showed small amounts of polar material, presumably oxidation products. We were unable to obtain an analytically pure sample from even repeated column chromatography. T.l.c. was even less successful. Due to its extreme liability to autoxidation the ester was chromatographed, the solvent removed, and the product used directly, minimising air contact at all stages. G.l.c. showed only one peak.

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* Some uncertainty exists about these assignments.

Acknowledgements

We thank Dr. C. M. Scrimgeour for synthesising the triacylglycerols and one of us (E. B.) thanks Unilever for financial support.

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Received 13*th July* 1982; *Paper* 2/1190