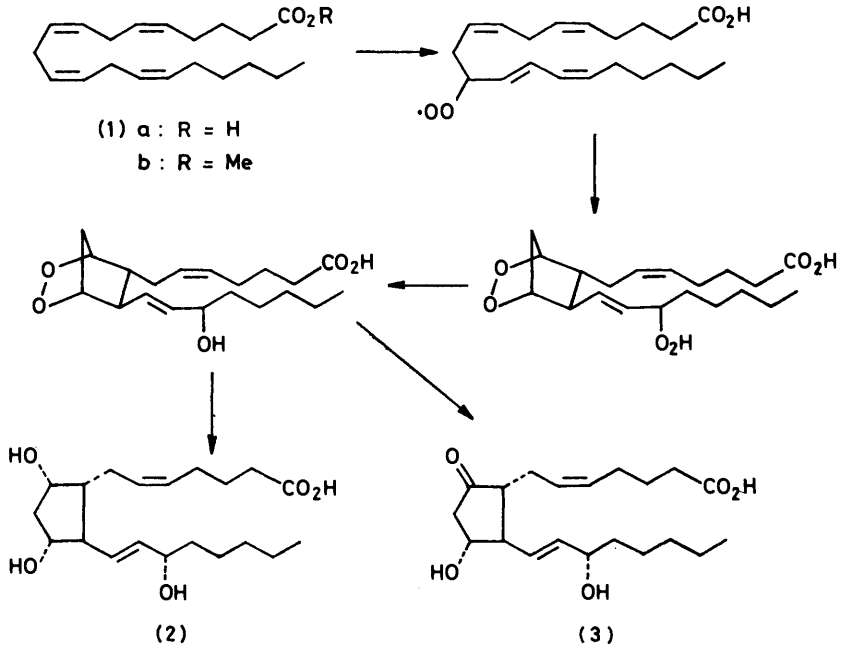
Synthesis from Arachidonic Acid of Potential Prostaglandin Precursors

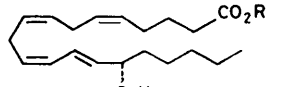
By **Jack E. Baldwin,*** Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, U.S.A.
David I. Davies * and **Lyn Hughes**, Department of Chemistry, King's College, Strand, London WC2R 2LS
Norman J. A. Gutteridge, Lilly Research Centre Limited, Erl Wood Manor, Windlesham, Surrey GU20 6PH

Arachidonic acid has been converted by aerobic oxidation with lipoxygenase (*ex* soybean) as catalyst and in the presence of sodium borohydride in a one-pot procedure (1 g scale) into (5*Z*,8*Z*,11*Z*,13*E*)(15*S*)-15-hydroxyeicosa-5,8,11,13-tetraenoic acid. The methyl (15*S*)-15-hydroxyeicosatetraenoate or its *p*-nitrobenzoate derivative may be epoxidised with *m*-chloroperbenzoic acid to afford mixtures of the 5,6-, 8,9-, 11,12-, and 13,14-monoepoxides together with a mixture of bisepoxides. The methyl (15*S*)-15-(*p*-nitrobenzoyloxy)eicosatetraenoate epoxides can be separated by preparative t.l.c. The methyl (15*S*)-15-(mercaptoacetoxy)eicosatetraenoate has been prepared from the chloroacetate derivative of the methyl (15*S*)-15-hydroxyeicosatetraenoate by reaction with potassium thioacetate followed by selective hydrolysis of the thioester function.

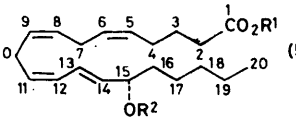
THE biosynthetic conversion of arachidonic acid (1a) into the prostaglandins $PGF_{2\alpha}$ (2) and PGE_2 (3) by prostaglandin synthetase-catalysed aerobic oxidation involves the introduction of several chiral centres and the formation of a *trans*-double bond.[1,2] A peroxyl radical cyclisation mechanism (Scheme 1) has been proposed for the biosynthesis,[3] and the intermediacy of *endo*-peroxides has been established by the isolation of two such compounds,[4] and their identification as intermediates in prostaglandin formation.[5,6] Arachidonic acid (1a) is also a substrate for the enzyme lipoxygenase, which catalyses the aerobic oxidation of (1a) to afford (5*Z*,8*Z*,11*Z*,13*E*)(15*S*)-15-hydroperoxyeicosa-5,8,11,12-tetraenoic acid (4a) having the same chirality and 13,14-*E*-double bond as are found in the natural prostaglandins.[7-9] Reduction of (4a) with sodium borohydride affords (5*Z*,8*Z*,11*Z*,13*E*)(15*S*)-15-hydroxyeicosa-5,8,11,13-tetraenoic acid (5a). Because of the relationship of (4a) and (5a) to the natural prostaglandins it was considered desirable to develop the preparation of (4a) and (5a) to a scale sufficient to permit their investigation as intermediates for prostaglandin synthesis. This could lead to a synthesis from arachidonic acid [10] of natural prostaglandins in which the oxygen functions would be introduced in the opposite order to that envisaged in the biosynthetic scheme (Scheme 1).[3] The initial investigators, Hamberg and Samuelsson,[7,9] had carried out small-



SCHEME 1



(4) a; R = H
b; R = Me



(5) a; $R^1 = R^2 = H$
b; R^1 = Me, R^2 = H
c; R^1 = Me, R^2 = *p*-$NO_2C_6H_4CO$
d; R^1 = Me, R^2 = $SiMe_3$
e; R^1 = Me, R^2 = $COCH_2Cl$
f; R^1 = Me, R^2 = $COCH_2SCOCH_3$
g; R^1 = Me, R^2 = $COCH_2SH$

scale reactions using arachidonic acid (1a) (4 mg) which was converted into its ammonium salt and then incubated with lipoxygenase (20 000 units per mg, *ex* soybean) at pH 9 (borate buffer) in the presence of oxygen. The hydroperoxide (4a) was then isolated by extraction with ether, and reduced to (5a) with sodium borohydride in ethanol. In the present work a careful reinvestigation of the reaction at various concentrations and ratios of arachidonic acid (1a) and lipoxygenase was carried out by monitoring the u.v. absorption of the conjugated diene unit of (4a) ($\lambda_{max.}$ 236 nm, ε 2.7 $\times$ 10^4) to devise optimum reaction conditions. The best balance between practicability and efficiency of reaction in the production of (4a) was found to be a ratio of (1a) to lipoxygenase of 10 : 1 (w/w) (*i.e.* 1 g : 100 mg) with the concentration of (1a) 1.6 $\times$ 10^{-3}M (*e.g.* 1 g in 2 l). Reaction was carried out in borate buffer at pH 9.0 with acidification to pH 5.5 prior to extraction of the hydroperoxide (4a). When isolated in this way (4a) could be immediately reduced with an excess of sodium borohydride in ethanol to yield the more stable hydroxy-acid (5a) (45%).

Borohydride is reported [11] to decompose relatively slowly in an aqueous medium at approximate neutrality, and it was found that if an excess of borohydride was added at the beginning of the lipoxygenase reaction sufficient borohydride was present throughout the reaction period to reduce all the hydroperoxide (4a) to the hydroxy-acid (5a) *in situ*. The presence of the sodium borohydride increased the overall yield and, somewhat surprisingly, reduced the reaction time for the aerobic oxidation. The optimised reaction conditions were found to be a ratio of (1a) to lipoxygenase of 6.5 : 1 (w/w) (*i.e.* 1 g : 150 mg) with the concentration of (1a) 1.6 $\times$ 10^{-3}M (*e.g.* 1 g in 2 l). The reaction was carried out in borate buffer at pH 9.0, with acidification to pH 4.1 prior to extraction of the product (5a). The hydroxy-acid (5a) was converted into the hydroxy-methyl ester (5b) on treatment with diazomethane. The preferred procedure for purification of (5b) was column chromatography using silicic acid containing 1% fluorescent indicator (F_{254}). Ester (5b) was applied to the column and eluted with a volume of solvent (1 : 3 diethyl ether–hexane) equal to the retention volume of the column. The adsorbent was then carefully extruded *en bloc*, dissected longitudinally, and viewed under u.v. light. The central band contained the hydroxy-methyl ester (5b) (purity >90%). A yield, from arachidonic acid (1a) (1 g), in the region of 50% could be obtained consistently. The hydroxy-acid (5a) could be similarly purified by column chromatography using 1 : 2.5 diethyl ether–hexane as eluant.

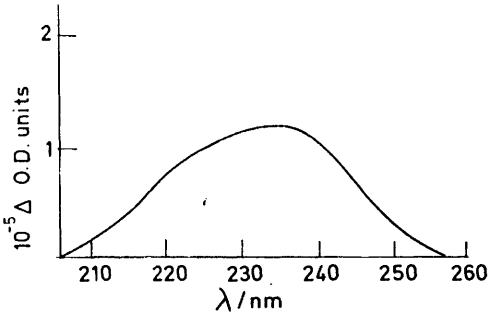
Hamberg and Samuelsson [7,9] proved the structure of (4a), (5a), and (5b) by conversion into methyl 15-oxo-arachidate. The identification was based on the mass spectral fragmentation pattern which we have confirmed. Further support for the structure of (5a) was obtained from the mass spectrum of its methyl ester trimethylsilyl ether derivative (5d), which exhibited peaks at *m/e* 406 $M^{+\cdot}$, 391 $[M - 15]^+$, 335 $[M - 71]^+$, 316 $[M - 90]^{+\cdot}$, 305 $[M - 101]^+$, 225, and 173. The structure for (5b) is further supported by n.m.r. data (at 90 MHz). Although the spectrum was not fully resolved, spin-decoupling techniques allowed the chemical shift values, together with certain coupling constants (Table 1), to be determined.

Because the hydroxy-methyl ester (5b) has a chromophore in the vicinity of an asymmetric centre it should exhibit a Cotton effect in the o.r.d. and c.d. spectra. This was observed, but owing to the small specific rotation of the molecule {$[\alpha]_{589}$ (hexane) + 3.04, $[\alpha]_{578}$ + 3.18, and $[\alpha]_{546}$ + 3.77° mol^{-1} dm^{-1}} the Cotton effect is small. The c.d. spectrum, which exhibits a positive Cotton effect of the order of 10^{-4} Δ O.D. units, is shown in the Figure.

TABLE 1

N.m.r. data (90 MHz) for the hydroxy-methyl ester (5b)

Location of proton	2	4	7	10	11	12	13	14	15	16	3, 17, 18, 19, 20
Chemical shift (δ)	2.35	2.08	2.84	2.98	5.51	6.02	6.56	5.7	4.16	1.47	0.9—2.3
J	6, 7	7, 8	9,10	10, 11	11, 12	12, 13	13, 14	14, 15	15, 16	10, 12	13, 15
Hz	6	6	6	6	11	11	14.5	6.8	6.8	1	1
OCH_3	δ 3.69 (s)										

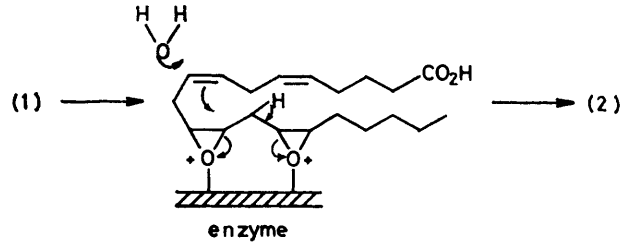


Circular dichroism spectrum of methyl (5*Z*,8*Z*,11*Z*,13*E*)(15*S*)-15-hydroxyeicosa-5,8,11,13-tetraenoate (5b) in 2-methylheptane

The extinction coefficient at 236 nm ($\lambda_{max.}$ for the conjugated diene unit) obtained from the u.v. spectrum of the hydroxy-methyl ester (5b) in iso-octane was 27 200, a similar value being obtained in ethanolic solution. These values are consistent with the generally accepted value (27 000) [12] and that (30 000) recorded by Hamberg and Samuelsson.[7]

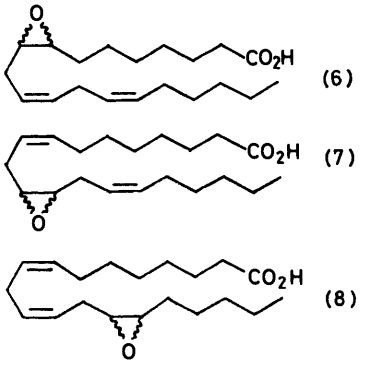
Gunstone [13] suggested in 1966 that epoxides might be involved in prostaglandin biosynthesis. He visualised a mechanism (Scheme 2) for the biosynthetic conversion of arachidonic acid (1a) into $PGF_{2\alpha}$(2) involving the

formation of an 11,12:14,15-bisepoxide. However, the evidence to date has not been in support of these proposals. Hamberg and Samuelsson [4,14] isolated the



SCHEME 2

endoperoxide PGG_2 from the biosynthesis of PGE_2 and showed that it was not formed from epoxides. The epoxides (6)—(8) were prepared by Chung and Scott [15]



who found that they could not be converted into prostaglandins by incubation with ovine seminal vesicle microsomes. A similar negative result was observed by Sood, Nagasawa, and Sih [16] using the epoxide (8) and bovine seminal vesicle microsomes. However, in spite of these negative results in respect of epoxides as *biosynthetic* precursors of the prostaglandins, it was thought worthwhile to see if suitable epoxides could be precursors in a *chemical synthesis* of prostaglandins. It was therefore decided to see if appropriate epoxides could be obtained *via* epoxidation of the hydroxy-methyl ester (5b), particularly as (5b) already has a 13,14-*E*-double bond and a C-15 hydroxy group of the same stereochemistry as in the natural prostaglandins. The chosen reaction conditions for epoxidation were those devised by Anderson and Veysoglu [17] using *m*-chloroperbenzoic acid. This reagent in a dichloromethane–aqueous sodium hydrogen carbonate biphasic system causes minimal deleterious effect on acid-sensitive olefinic or epoxide functions. Application of this oxidant to the epoxidation of (5b) gave a mixture of at least seven components. G.l.c.–mass spectrometric analysis of the mixture after trimethylsilylation revealed the presence of material having a molecular weight equivalent to that of the starting material plus one atom of oxygen. The mixture was separated by preparative t.l.c. but this proved to be unsatisfactory since the recovery of material was only 25%.

The *p*-nitrobenzoate derivative (5c) of the hydroxy-methyl ester (5b) could be prepared in 85% yield by reaction of (5b) with *p*-nitrobenzoyl chloride in pyridine. It was considered possible that the *p*-nitrobenzoate group, being quite bulky, would hinder attack at the 13,14-double bond and improve the statistical chances of attack on the 11,12-double bond. The epoxidation of (5c), however, gave a mixture containing the same number of components as in the epoxidation of (5b), although the intensities of the t.l.c. spots indicated that product proportions were different. Separation of the mixture with a total recovery of 75%, into six fractions was achieved by preparative t.l.c. on low activity silica using benzene–ethyl acetate (19 : 1) as eluant. Later, hexane–diethyl ether (1 : 1) containing 1% of triethylamine was found to be a suitable alternative eluant. R_F Values and product proportions are given in Table 2.

Each fraction was analysed by mass spectrometry (direct insertion at 70 eV) and n.m.r., i.r., and u.v. spectroscopy. Fraction 1 consisted of unchanged starting material. The mass spectra of the main components of fractions 2—5 indicated that reaction of (5c) with *m*-chloroperbenzoic acid had given a mixture of epoxides. Ions of *m/e* 499 $M^{+\cdot}$, 483 $[M - 17]^+$, 469 $[M - 30]^{+\cdot}$, 428 $[M - 71]^+$, 398 $[M - 101]^+$, and 332 were common to all epoxides in fractions 2—5. However, the presence of additional ions characteristic of the fragmentation of the epoxides [18] indicated the position of the epoxide group. Ions of *m/e* 288 and 318 were assigned to the 13,14-epoxide (10b), whilst that of *m/e* 249 was characteristic of the 11,12-epoxide (9b). Similarly, ions of *m/e* 314 and 188, and 355 were characteristic of the 8,9-epoxide (11b) and the 5,6-epoxide (12b), respectively. Fraction 6 was a mixture of bisepoxides with ions at *m/e* 515 $[M^{+\cdot}]$ in the mass spectrum.

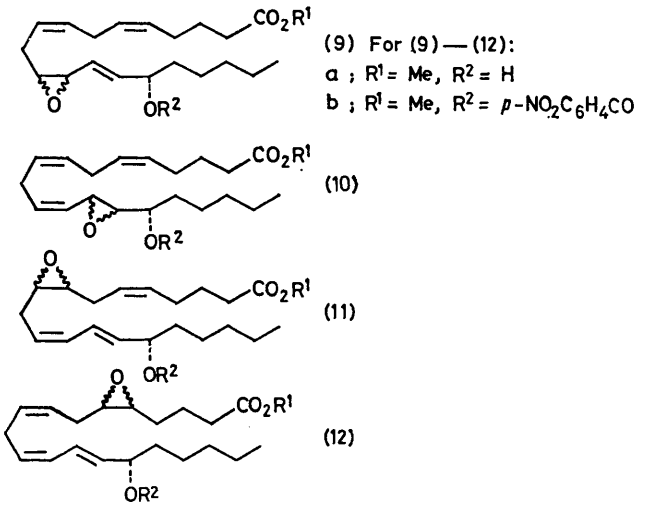
The n.m.r. spectra of fractions 2 and 3 confirmed their assignment to (9b) and (10b). Integration in the region

TABLE 2

Separation of epoxides (9)—(12)

Fraction no.	R_F	Compound	Yield (%) (5b)→(5c)→(9b)—(12b)	Yield (%) (5b)→(9a—12a)→(9b)—(12b)
1	0.5	5c	30	10
2	0.37	9b	3	20
3	0.30	10b	11.5	11.5
4	0.24	11b	22	31
5	0.20	12b	19	26.5
6	0.10 0.08	Bisepoxides	14.5	

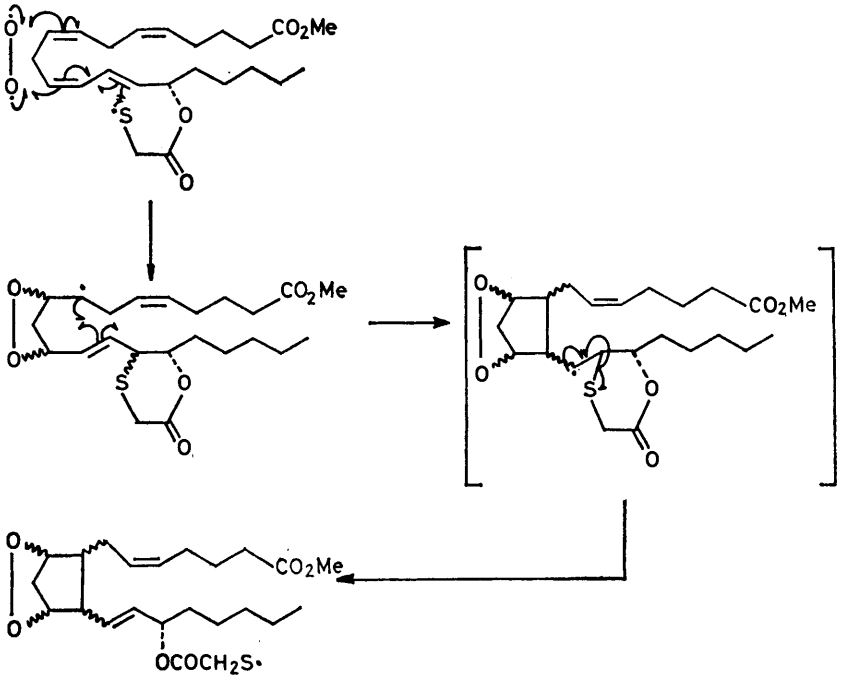
of δ 2.8 (methylene group between two double bonds) corresponds to two protons in (9b) and four protons in



(10b). In the u.v. spectra of both fragments there was no maximum at *ca.* 236 nm corresponding to a conjugated diene system, although a maximum in the region of 260 nm, characteristic of the *p*-nitrobenzoate group, was present. Both fractions 4 and 5 exhibited u.v. spectra with maxima at *ca.* 336 and 260 nm corresponding to the conjugated diene system and *p*-nitrobenzoate group, respectively. Fractions 4 and 5 could be assigned to (11b) and (12b), respectively, since in (11b) separate resonances were observed for C–4 and C-16 protons at δ 2.04 and 1.75, respectively, whereas in (12b) the 5,6-epoxide group shifts the C-4 proton signal to δ 1.7, the same position as that of the C-16 protons.

When the epoxide mixture (9a)—(12a) derived from the hydroxy-methyl ester (5b) was converted into the *p*-nitrobenzoate mixture (9b)—(12b) and separated, the product proportions (Table 2) were different from those resulting from the direct epoxidation of the *p*-nitrobenzoate methyl ester (5c). It is clear that the *p*-nitrobenzoate group in (5c) reduces attack on the 11,12-double bond compared with attack on this bond in (5b). Also the yield of epoxide mixture [70% from (5c)] is increased [90% from (5b)].

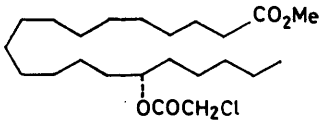
Thiols are known to catalyse the autoxidation of olefins.[19] A possible precursor to a prostaglandin type molecule is the mercapto-methyl ester (5g) in which the mercapto group is conveniently situated for direct oxidation at C-10 as depicted in Scheme 3. It has now been found possible to prepare (5g) from (5b) using the following procedure. Reaction of (5b) with chloroacetic anhydride in pyridine–ether affords the chloroacetate (5e) having consistent n.m.r., u.v., and i.r. spectral properties. It was not possible to obtain g.l.c.–mass spectral data for the chloroacetate (5e) because it was unstable under the experimental conditions. A direct insertion mass spectrum confirmed the molecular weight as 410/412, and the base peak had *m/e* 316 corresponding to loss of chloroacetic acid. Further confirmation of structure was obtained by hydrogenation of (5e). The mass spectrum of the product, *viz.* ions at *m/e* 418 ($M^{+\cdot}$), 347, 341, 325, 324, 293, and 292, was characteristic of the saturated derivative (13).



SCHEME 3

The chloroacetate (5e) could be converted to the thioacetate (5f) by treatment with potassium thioacetate in aqueous ethanol. Hydrolysis of (5f) with ethanolic ammonium hydroxide at 60 °C was found to hydrolyse

completely the *S*-ester function and leave the *O*-ester function intact, thus resulting in the formation of the



(13)

thiol (5g). This preference for *S*-ester hydrolysis with ammoniacal bases is consistent with the observations of Connors and Bender.[20] The mass spectrum of (5g) exhibited ions at *m/e* 408 ($M^{+\cdot}$), 316, 245, 180, 175, in support of its structure.

This preliminary report covers the preparation of a number of possible precursors for use in a prostaglandin synthesis from arachidonic acid. We believe the acid-catalysed opening of the epoxide (9) followed by cyclisation of the intermediate carbocation is a synthetic route of some potential. We are exploring this as well as trying to increase the proportion of (9) produced in the epoxidation of (5a—c). The intramolecular oxidative cyclisation of (5g) is also being examined. An associated paper [21] reports on the basic heterocyclic system (Scheme 3) which could result from the intramolecular free radical reactions of (5g).

EXPERIMENTAL

(5Z,8Z,11Z,13E)(15S)-15-*Hydroperoxyeicosa*-5,8,11,13-*tetraenoic Acid* (4a).—Arachidonic acid (1 g) dissolved in the minimum of aqueous ammonia (s. g. 0.88) was added to borate buffer (2 l) at pH 9.0 [prepared from aqueous sodium tetraborate (1 700 ml; 0.05M) plus hydrochloric acid (300 ml; 0.1M)]. The pH of the resultant solution was then re-adjusted to pH 9.0 with concentrated hydrochloric acid. Lipoxygenase *ex* soybean (Fluka 100 mg; 50 000 units mg^{-1}) was dissolved in a small volume of buffer and added to the reaction mixture, which was then stirred under a stream of oxygen, arranged to produce a minimum of foaming, for 30 min. The reaction was stopped by the addition of ethanol (300 ml) and water (700 ml), acidified to pH 5.5 with concentrated hydrochloric acid, and then extracted with diethyl ether (4 × 400 ml). The combined extracts were evaporated to dryness and the residue redissolved in diethyl ether. The solution was washed with water, dried with sodium or magnesium sulphate, filtered and evaporated to afford the crude hydroperoxide (4a) (1.1 g).

(5Z,8Z,11Z,13E)(15S)-15-*Hydroxyeicosa*-5,8,11,13-*tetraenoic Acid* (5a).—(*a*) The crude hydroperoxide (4a) (1.1 g) was dissolved in absolute ethanol (15 ml) and the solution cooled in an ice-bath. An excess of sodium borohydride (600 mg) was added and the mixture stirred for 15 min in an ice-bath and for a further 45 min at room temperature, after which the mixture was acidified with glacial acetic acid until effervescence ceased. The mixture was then diluted with water (30 ml), and extracted with diethyl ether (3 × 25 ml). The extract was washed with water, dried with sodium sulphate, filtered, and evaporated to afford the crude alcohol (5a) (1.1 g).

(*b*) Ammonium arachidonate (1 g), borate buffer (2 l; pH 9.0) and lipoxygenase (150 mg; 50 000 units mg^{-1}) were prepared and mixed as in the preparation of (4a). Sodium borohydride (600 mg) was then added and the mixture stirred under a stream of oxygen for 30 min. Ethanol (300 ml) and water (700 ml) were then added and the solution acidified to pH 4.1 with concentrated hydrochloric acid. The mixture was then extracted with diethyl ether (4 × 400 ml or better by continuous extraction for 4—6 h), and the combined extracts were evaporated to dryness. The residue was redissolved in diethyl ether (30 ml), and the resulting solution was washed with water (3 × 20 ml) and dried (sodium sulphate). After filtration the solution was evaporated to afford the crude alcohol (5a) (1.2 g). This was dissolved in a small volume of hexane–diethyl ether (2.5 : 1 v/v) and applied to the top of a column (12.5 cm × 5 cm. diam.) of silicic acid containing 1% of fluorescent indicator (F_{254}) and then eluted with the hexane–diethyl ether (2.5 : 1 v/v). The column of silicic acid was then extruded intact, dissected longitudinally and viewed under 254 nm u.v. light. The central band, R_F *ca.* 0.5, was removed and extracted with warm methanol (3 × 150 ml). The extracts were combined and evaporated. The residue was dissolved in methylene chloride (10 ml) and the solution filtered through Celite. Evaporation afforded the hydroxy-acid (5a) (0.47 g).

Methyl (5Z,8Z,11Z,13E)(15S)-15-*Hydroxyeicosa*-5,8,11,13-*tetraenoate*.—The crude acid (5a) could be methylated with diazomethane in ether solution to give a quantitative conversion into the crude hydroxy-methyl ester (5b). This was purified as for (5a) with the exception that hexane–diethyl ether (3 : 1 v/v) was used as eluant, to afford *ca.* 0.47 g of (5b) (Found: C, 75.35; H, 10.15%; $M^{+\cdot}$, 334.251 1. Calc. for $C_{21}H_{34}O_3$: C, 75.45; H, 10.1%; *M*, 334.250 8). Spectroscopic and optical data are given in the text.

Epoxidations with m-*Chloroperbenzoic Acid*.—*m*-Chloroperbenzoic acid (1 mol. equiv.) was added slowly, in portions, to a stirred solution of olefin (1 mol. equiv.) in a mixture of dichloromethane (1 ml per 20 mg of peracid) and aqueous sodium hydrogen carbonate (0.5M; 1 ml per 3 ml of dichloromethane). The solution was stirred vigorously for 2 h. The organic layer was separated and washed with aqueous sodium hydroxide (0.1M) and water. It was then dried (Na_2SO_4), filtered through Celite, and evaporated to afford the crude epoxide (*ca.* 94%).

Derivatisations with p-*Nitrobenzoyl Chloride*.—The alcohol or epoxy-alcohol mixture (1 mol. equiv.) was dissolved in dry pyridine (1 ml per 50 mg of alcohol) and *p*-nitrobenzoyl chloride (1.3 mol. equiv.) was added with stirring. After stirring for a further 1.75 h the mixture was diluted with aqueous sodium hydrogen carbonate (5% w/v) and then extracted with diethyl ether. The extract was washed with water, dried (Na_2SO_4), and filtered through Celite. Evaporation afforded the *p*-nitrobenzoate (85—90%). Derivatisation of (5b) afforded *methyl* (5Z,8Z,11Z,13Z)(15S)-15-(*p-nitrobenzoyloxy*)*eicosa*-5,8,11,13-*tetraenoate* (5c); $M^{+\cdot}$ 483 with a base peak corresponding to the loss of the *p*-nitrobenzoyloxy moiety; δ(60 MHz; $CDCl_3$) 0.9—2.5 (H-2, -3, -4, -16, -17, -18, -19, and 20), 2.6—3.1 (H-7 and -10), 3.6 (s, OCH_3), 4.2 (H-15), 5.0—6.2 (H-5, -6, -8, -9, -11, -12, -13, and -14), and 9.0 (aromatic); $\nu_{max.}$ (CCl_4) 3 100—2 800 (C–H) and 1 753 cm^{-1} (C=O).

Methyl (15S)-15-(p-*Nitrobenzoyloxy*)*monoepoxyeicosatrienoates* (9b)—(12b).—Mixtures of epoxides (9b)—(12b) were prepared by two routes: (*a*) epoxidation of the methyl hydroxy-ester (5b) followed by derivatisation with *p*-nitrobenzoyl chloride and (*b*) epoxidation of the *p*-nitrobenzoyloxy derivative (5c).

The isomers were separated by preparative layer chromatography using silica (low activity) and benzene–ethyl

acetate (19 : 1 v/v) or, better, hexane–diethyl ether (1 : 1) containing 1% triethylamine as eluant. (R_F Values and product proportions are given in Table 2, and mass spectral and n.m.r. details in text.)

The 11,12-*epoxide* (9) was obtained as crystals, m.p. >60° (decomp.) (from hexane), $[\alpha]^{\circ}_{589}$ −3.846°, $[\alpha]^{\circ}_{546}$ −3.076° and $[\alpha]^{\circ}_{436}$ +3.653° mol^{-1} dm^{-1}; δ 8.26 (ArH), 6—5.2 (H-5, -6, -8, -9, -13, and -14), 4.22 (H-15), 3.67 (OCH_3), 2.98, 3.54 (H-11 and -12), 2.77 (H-7), 2.34 (H-2), 2.08 (H-4 and -10), 1.68 (H-16), 1.25, and 0.88 (aliphatic H); $\lambda_{max.}$ 260 nm (ε 10 000) (Found: $M^{+\cdot}$, 499.256 4. $C_{28}H_{37}NO_7$ requires M, 499.256 8).

The 13,14-*epoxide* (10b) was a pale yellow semi-solid, δ 8.26 (ArH), 6.1—5.37 (H-5, -6, -8, -9, -11, and -12), 4.2 (H-15), 3.67 (OCH_3), 3.46, 3,23 (H-13 and -14), 2.97 (H-7 and -10), 2.37 (H-2), 2.11 (H-4), 1.76 (H-16), 1.3, and 0.9 (aliphatic H), $\lambda_{max.}$ 260 nm (ε 9 500) (Found: $M^{+\cdot}$, 499.256 8. $C_{28}H_{37}NO_7$ requires M, 499.256 8).

The 8,9-epoxide (11b) was a pale yellow semi-solid, δ 8.20 (ArH), 6.66—5.33 (H-5, -6, -11, -12, -13, and -14), 4.04 (H-15), 3.66 (OCH_3), 3.6, 3.13 (H-8 and -9), 2.8 (H-10), 2.23 (H-2 and -7), 2.04 (H-4), 1.75 (H-16), 1.38, and 0.96 (aliphatic H), $\lambda_{max.}$ 235 (ε 22 500) and 260 nm (11 300).

The 5,6-*epoxide* (12b) was a pale yellow semi-solid, δ 8.20 (ArH), 7—5.3 (H-8, -9, -11, -12, -13, and -14), 4.00 (H-15), 3.72 (OCH_3), 3.45, 3.08 (H-5, 6), 2.83 (H-10), 2.29 (H-2 and -7), 1.7 (H-4 and -16), 1.38, 0.96 (aliphatic H), $\lambda_{max.}$ 237 (ε 25 700) and 260 nm (13 600).

A mixture of (11b) and (12b) showed $M^{+\cdot}$ 499.256 6 ($C_{28}H_{37}NO_7$ requires M, 499.256 8).

A mixture of the bisepoxides of (5c) was obtained as a pale yellow semi-solid, δ 8.23 (ArH), 7.08—5.33 (olefinic H), 4.05 (H-15), 3.66 (OCH_3), 3.33, 2.95 (epoxide H), 2.83 ($-CH_2-$ between double bonds), 2.3 (H-2), 1.66 (H-16), 1.4, and 0.98 (aliphatic H), $\lambda_{max.}$ 235 (ε 9 600) and 260 nm (10 600), $M^{+\cdot}$ 515.

The i.r. spectra of the epoxides (9b)—(12b) and the mixture of bisepoxides of (5c) were broadly similar and exhibited $\nu_{max.}$ ($CHCl_3$) *ca.* 3 010, 2 950, 2 860 (C–H stretch), 1 730 (C=O), 1 560 (aromatic), 1 275 and 1 110 (C–O), and 1 205—1 250 (epoxide).

Methyl (5Z,8Z,11Z,13E)(15S)-15-(*Chloroacetoxy*)*eicosa*-5,-8,11,13-*tetraenoate* (5e).—Methyl (15*S*)-15-hydroxyeicosatetraenoate (5b) (1 mol. equiv.) dissolved in dry diethyl ether (1 ml per 100 mg of ester) was added to dry pyridine (1.3 mol. equiv.). The mixture was cooled in an ice-bath and a solution of chloroacetic anhydride (1.3 mol. equiv.) in dry diethyl ether (1 ml per 40 mg of ester) was added dropwise with stirring. After several minutes the flask was removed from the ice-bath and stirred at room temperature for *ca.* 1.5 h; t.l.c. on silicic acid using hexane–diethyl ether (1 : 1 v/v) as eluant then indicated complete consumption of (5b). The mixture was then diluted with diethyl ether and washed with water. The ether solution was dried (Na_2SO_4), filtered through Celite, and evaporated to afford the *chloroacetoxy-derivative* (5e) 85% as a viscous liquid, δ(60 MHz; CCl_4) 0.9—2.4 (H-2, -3, -4, -16, -17, -18, -19, and -20), 2.65—3.1 (H-7 and -10), 3.6 (s, OCH_3), 3.95 (s, $OCOCH_2Cl$), 4.1—4.3 (H-15), 5.0—6.8 (H-5, -6, -8, -9, -11, -12, -13, and -14), $\nu_{max.}$ (CCl_4) 3 100—2 800 (C–H) and 1 735—1 740 cm^{-1} (C=O), $\lambda_{max.}$ (ethanol) 236 nm (ε 27 000), $[\alpha]_{589}$ −2.10, $[\alpha]_{578}$ −2.07, and $[\alpha]_{546}$ −2.41° mol^{-1} dm^{-1}, $M^{+\cdot}$ 410/412.

Methyl 15-(*Chloroacetoxy*)*eicosanoate* (13).—Adams catalyst (PtO_2) (15 mg) was added to methanol (2 ml) and the mixture shaken in hydrogen. A solution of the methyl chloroacetoxyester (5e) (15 mg) in ethanol (2 ml) was introduced, and the mixture shaken for 3 h. T.l.c. analysis (silicic acid), using hexane–diethyl ether (1 : 1 v/v) as eluant, then showed reaction was complete. The catalyst was removed and the solution evaporated to afford *methyl* (15S)-15-(*chloroacetoxy*)*eicosanoate* (13) (15 mg) as a yellow oil, δ(60 MHz; CCl_4) 0.9—2.4 (saturated aliphatic protons), 3.6 (s, OCH_3), 3.95 (s, $OCOCH_2Cl$), $\nu_{max.}$ (CCl_4) 3 000—2 800 (C–H) and 1 735 cm^{-1} (C=O), $M^{+\cdot}$ 418/420.

Potassium Thioacetate.—Thioacetic acid (1.6 g) was dissolved in absolute ethanol (10 ml) and the solution adjusted to pH 9—10 by adding, dropwise with stirring and cooling to below 50 °C saturated aqueous potassium hydroxide. The resultant solution of potassium thioacetate was used immediately.

Methyl (5Z,8Z,11Z,13E)(15S)-15-[(*Acetylthio*)*acetoxy*]-*eicosa*-5,8,11,13-*tetraenoate* (5f).—The methyl chloroacetoxyester (5e) (1 mol. equiv.) was dissolved in absolute ethanol (1 ml per 100 mg of ester) and aqueous potassium thioacetate (1.3 mol. equiv.) was added slowly with stirring at room temperature. The mixture was stirred for 1.5 h; t.l.c. on silicic acid using hexane–diethyl ether (3 : 1 v/v) as eluant then showed reaction to be complete. The mixture was diluted with dichloromethane and washed with water. After drying (Na_2SO_4) the solution was filtered through Celite and was evaporated to afford the ester (5f) (87%) as a pale yellow viscous liquid, δ(60 MHz; CCl_4) 0.9—2.6 (H-3, -4, -16, -17, -18, -19, and -20), 2.35 (s, CH_3CO), 2.6—3.1 (H-7 and -10), 3.6 (s, CH_3O and $OCOCH_2S$), 4.0—4.4 (H-15), and 5.0—6.8 (H-5, -6, -8, -9, -11, -12, -13, and -14), $\nu_{max.}$ (CCl_4) 3 100—2 800 (C–H), 1 735—1 740 (C=O), and 1 700 cm^{-1} (C=O), $\lambda_{max.}$ (ethanol) 236 nm (ε 27 000), $M^{+\cdot}$ 450.

Methyl (5Z,8Z,11Z,13Z)(15S)-15-(*Mercaptoacetoxy*)*eicosa*-5,8,11,13-*tetraenoate* (5g).—The thioacetate (5f) (370 mg, 0.82 mmol) was dissolved in absolute ethanol (6 ml) and aqueous ammonia (s.g. 0.88; 6 ml) was added. The mixture was stirred at *ca.* 60 °C under nitrogen for 45 min. It was then acidified to pH 4.0 with hydrochloric acid (water–conc. HCl 1 : 1) and extracted with diethyl ether. The extracts were combined and evaporated to dryness. The residue dissolved in diethyl ether was washed with water, dried (Na_2SO_4), and filtered. Evaporation afforded the *thiol* (5g) (292 mg), δ(60 MHz; $CDCl_3$) 0.9—2.6 (H-2, -3, -4, -16, -17, -18, -19, and -20, and -SH), 2.6—3.1 (H-7 and -10), 3.55 (d, *J* 8 Hz, $OCOCH_2SH$), 3.6 (s, OCH_3), 4.0—4.4 (H-15), and 5.0—6.8 (H-5, -6, -8, -9, -11, -12, -13, and -14), $\lambda_{max.}$ (ethanol) 237 nm (ε 27 000), $\nu_{max.}$ (CCl_4) 3 100—2 800 (C–H) and 1 735 cm^{-1} (C=O), $M^{+\cdot}$ 408.

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