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Oxidation of Oxa and Thia Fatty Acids and Related Compounds Catalysed by 5- and 15-Lipoxygenase

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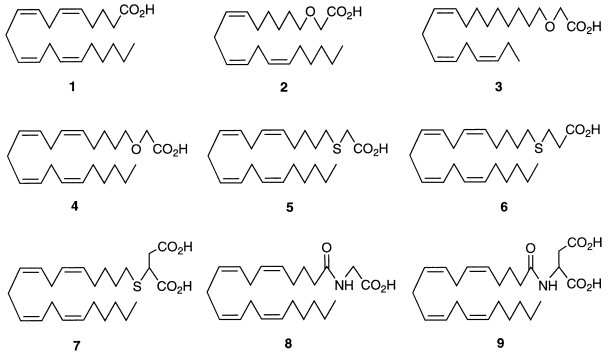
Abstract—The modified fatty acids, (*Z,Z,Z*)-(octadeca-6,9,12-trienyloxy)acetic acid, (*Z,Z,Z*)-(octadeca-9,12,15-trienyloxy)acetic acid, (all-*Z*)-(eicosa-5,8,11,14-tetraenyloxy)acetic acid, (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)acetic acid, 3-[(all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)]propionic acid, (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)succinic acid, *N*-[(all-*Z*)-(eicosa-5,8,11,14-tetraenoyl)]glycine and *N*-[(all-*Z*)-(eicosa-5,8,11,14-tetraenoyl)]aspartic acid, all react with soybean 15-lipoxygenase. The products were treated with triphenylphosphine to give alcohols, which were isolated using HPLC. Analysis of the alcohols using negative ion tandem electrospray mass spectrometry, and by comparison with compounds obtained by autoxidation of arachidonic acid, shows that each enzyme-catalysed oxidation occurs at the ω-6 position of the substrate. In a similar fashion, it has been found that (*Z,Z,Z*)-(octadeca-6,9,12-trienyloxy)acetic acid, (*Z,Z,Z*)-(octadeca-9,12,15-trienyloxy)acetic acid, (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)acetic acid and 3-[(all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)]propionic acid each undergoes regioselective oxidation at the carboxyl end of the polyene moiety on treatment with potato 5-lipoxygenase. Neither (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)succinic acid nor *N*-[(all-*Z*)-(eicosa-5,8,11,14-tetraenoyl)]aspartic acid reacts in the presence of this enzyme, while *N*-[(all-*Z*)-(eicosa-5,8,11,14-tetraenoyl)]glycine affords the C11′ oxidation product. The alcohol derived from (*Z,Z,Z*)-(octadeca-6,9,12-trienyloxy)acetic acid using the 15-lipoxygenase reacts at the C6′ position with the 5-lipoxygenase.

Introduction

Polyunsaturated fatty acids (PUFAs) have been used to address a wide range of plant and mammalian physiological conditions, including allergic and autoimmune diseases.[1] However, they have not achieved the expected level of efficacy, and this can be attributed, at least in part, to their instability in vivo due to β-oxidation.[2–4] In order to avoid this, thia[5–8] and oxa[5,9] PUFAs and other heteroatom-substituted analogues[10] have been prepared, but to understand the physiological behaviour of these compounds[10–13] it is necessary to understand how they interact with enzymes that metabolise the natural PUFAs. Lipoxygenases catalyse the synthesis of hydroperoxides from PUFAs, for example as part of the biosynthesis of eicosanoids such as leukotrienes and lipoxins.[14] We now report on the interaction of the modified fatty acids **2**–**9** with soybean 15-lipoxygenase and potato 5-lipoxygenase.



Results and Discussion

Reactions of arachidonic acid (**1**) and other methylene-interrupted polyenoic fatty acids with lipoxygenases can be monitored using ultraviolet spectroscopy and the increase in absorbance at 234 nm, which is characteristic of formation of the conjugated *E,Z*-diene moiety of the hydroperoxides **10a**–**15a** and the corresponding alcohols **10b**–**15b**.[15–17] The molar extinction coefficients of such

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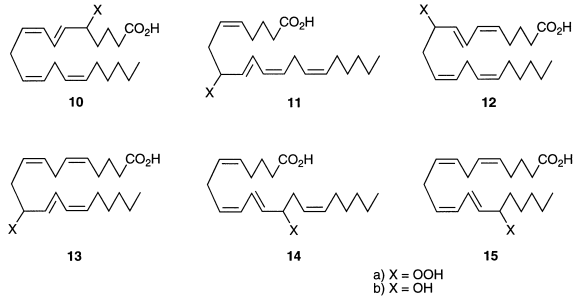
compounds are all similar and near 25,000–30,000 M^{-1} cm^{-1}, and the relative increases in absorbance observed with different substrates on treatment with a lipoxygenase provide a good approximation of their relative extents of reaction.[18–20] When each of the fatty acids **1**–**9** was treated with soybean 15-lipoxygenase, the total absorbance change observed over 10 min for compounds **2**–**9** was 94, 66, 78, 33, 72, 19, 44 and 5%, respectively, of that seen with arachidonic acid (**1**). Clearly, each of the compounds **2**–**9** is a substrate of the enzyme, though the activity observed with the aspartate derivative **9** is relatively low. With potato 5-lipoxygenase, the total absorbance change observed over 5 min for the fatty acids **2**, **3**, **5**, **6** and **8** was 20, 6, 8, 30 and 140%, respectively, of that seen with arachidonic acid (**1**), while no absorbance change was detected with either of the fatty acids **7** or **9**. It is therefore apparent that neither **7** nor **9** is a substrate of this enzyme and compounds **3** and **5** show only little activity, but the acids **2**, **6** and **8** are oxidised effectively by the 5-lipoxygenase.



To be able to characterise the products of the reactions of the modified fatty acids **2**–**9** catalysed by the 5- and 15-lipoxygenases, methods were first developed using compounds derived from arachidonic acid (**1**). Thin-film autoxidation of arachidonic acid (**1**) under an atmosphere of oxygen and in the presence of α-tocopherol afforded a mixture of approximately equal proportions of the hydroperoxides **10a**–**15a**, as determined by HPLC.[16,19,21,22] α-Tocopherol is used in this procedure principally as a hydrogen atom donor to trap the peroxy radical precursors of the hydroperoxides **10a**–**15a**, before they undergo alternative reactions. The hydroperoxides **10a**–**15a** are unstable, so for analysis they were reduced to the corresponding alcohols **10b**–**15b** by treatment with either triphenylphosphine or sodium borohydride.[16,23] The alcohols **10b**–**15b** were distinguished using analytical HPLC on the basis of their chromatographic behaviour.[16,24] Samples of the individual alcohols **11b**–**15b** were obtained using preparative HPLC but it was not practical to isolate the isomer **10b** in this manner because it tended to lactonise.[25] Instead, the (*S*)-enantiomer of the alcohol **10b** was prepared by treating arachidonic acid (**1**) with 5-lipoxygenase, followed by reduction of the product hydroperoxide (*S*)-**10a** with sodium borohydride.[26] In a similar manner a sample of the (*S*)-enantiomer of the alcohol **15b** was prepared from arachidonic acid (**1**) using 15-lipoxygenase.[27]

The ^{1}H NMR spectra of the alcohols (*S*)-**10b** and **11b**–**15b** each show resonances characteristic of the conjugated *E,Z*-diene moiety,[23,28] but they do not provide a way to distinguish the regioisomers. Previously this distinction has been made by preparing derivatives and analysing them using single sector mass spectrometric techniques,[16,29,30] or by analysing the alcohols **10b**–**15b** directly using either liquid secondary ion tandem mass spectrometry[31] or low energy fast atom bombardment tandem mass spectrometry.[32,33] In the present work, the alcohols (*S*)-**10b** and **11b**–**15b** were analysed using negative ion tandem electrospray mass spectrometry. In each case the dominant fragment ions correspond to loss of water [$(M-H)^- - H_2O$] from the molecular ion [$(M-H)^-$] and cleavage of either of the carbon–carbon bonds adjacent to the hydroxy group. The latter are characteristic for each regioisomer and are illustrated in Figure 1. The descriptors R^-, $HX\text{-}CO_2^-$ and $HC(O)X\text{-}CO_2^-$ are used to designate the corresponding fragment ions in the Experimental.

The methodology outlined above was used to characterise the products of reactions of the modified fatty acids **2**–**9**. Each substrate was incubated with the 15-lipoxygenase and the crude products were treated with triphenylphosphine. Subsequent analysis using HPLC and monitoring for materials absorbing at 234 nm indicated that a single *E,Z*-diene had formed in each case. These materials were isolated by HPLC and shown to be the alcohols **16**–**23**, using ^{1}H NMR spectroscopy and negative ion tandem electrospray mass spectrometry. The stereochemistry of the alcohols **16**–**23** was not determined, but 15-lipoxygenase-catalysed oxidation of natural PUFAs normally gives (*S*)-enantiomers.[14] Oxidation of the sulfide **7** at C15′ and sulfur to give the sulfoxide **21** was apparent by ^{1}H NMR and mass spectrometry. The NMR resonance for the C2 hydrogen of the sulfoxide **21** was observed at δ 3.96, 0.32 ppm downfield from the corresponding signal of the sulfide **7**. The sulfide **7** may be oxidised at both C15′ and sulfur through interaction with the lipoxygenase. Alternatively, the C15′ hydroperoxide of the sulfide **7** may oxidise the thioether moiety.[34–36] Either way it is not clear why the other sulfides **5** and **6** are not oxidised at sulfur, but the results are reproducible. Characterisation of the alcohols **16**–**23** establishes that each of the substrates **2**–**9** underwent 15-lipoxygenase-catalysed oxidation regioselectively at the ω-6 position. A similar selectivity was reported by Hamberg and Samuelson[37] for the oxidation of a range of natural PUFAs by this enzyme, indicating it is the aliphatic termini of PUFAs and their modified forms that are recognised by this lipoxygenase.

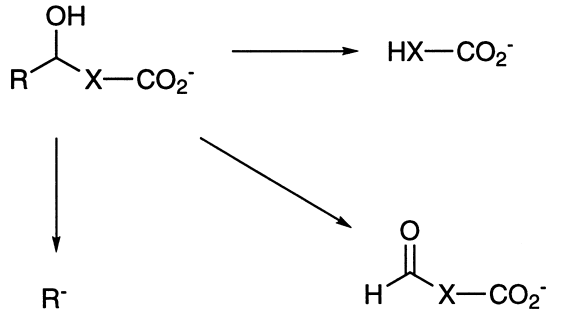
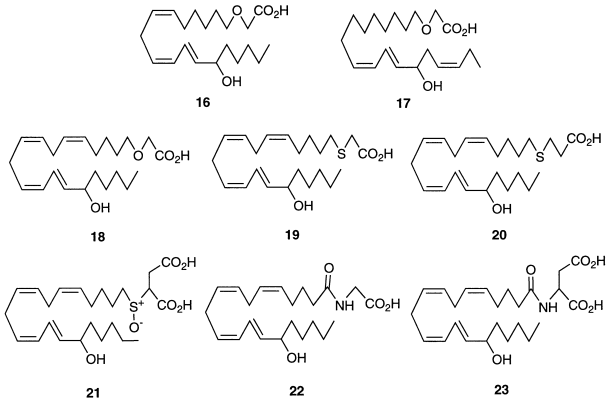
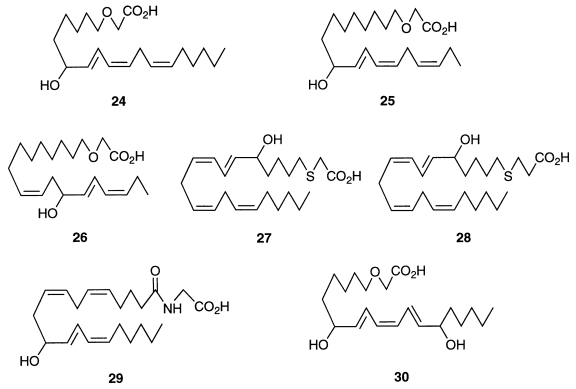


Figure 1. Fragmentation patterns displayed by fatty acid alcohols in negative ion tandem electrospray mass spectrometry.



It was more difficult to obtain products of the reactions catalysed by the 5-lipoxygenase, due to loss of enzyme activity during the course of the experiments. However, reactions of the modified fatty acids **2**, **3**, **5** and **6** with the 5-lipoxygenase, followed by treatment with triphenylphosphine, gave small quantities of the alcohols **24**, **25**, **27** and **28**, respectively, as the principal oxidation products. The alcohols **17** and **26** were also identified as minor products from reaction of the β-oxa fatty acid **3**, with the combined yield of these species being approximately 10% of that of the regioisomer **25**, as determined by HPLC of the crude product mixture. No other hydroxydienes were observed in the reactions of the other substrates **2**, **5** and **6**. The regiochemical oxidation of the fatty acids **2**, **3**, **5** and **6**, each at the carboxyl end of their polyene moiety, is consistent with the selectivity of reactions of natural PUFAs with the 5-lipoxygenase, as is the reaction of the alcohol **16** with the 5-lipoxygenase which gave, after reduction with sodium borohydride, the diol **30** from oxidation at C6′. By contrast, the glycine derivative **8** afforded only the alcohol **29** from oxidation at C11′. This regioselectivity and the particular reactivity of the substrate **8** with the enzyme, being greater than that of arachidonic acid (**1**), are not easily explained.



Experimental

Ultraviolet spectra were recorded on either a Varian Cary 1E spectrophotometer or a Shimadzu UV 160 spectrophotometer. ^{1}H NMR spectra were recorded on either a Gemini 300 MHz spectrometer or a Varian VXR 300 (300 MHz) spectrometer, in deuterochloroform with tetramethylsilane as the internal standard. High resolution mass spectra were recorded using a Fisons Instruments Autospec mass spectrometer. Negative ion tandem electrospray mass spectra were recorded on a Fisons Instruments Quattro II mass spectrometer. HPLC was performed using a Waters system equipped with a differential refractometer operating at 234 nm. Analytical and preparative HPLC was performed using Waters μPorasil silica columns (5 μm silica), of dimensions 4.6×250 mm and 19×300 mm, respectively. The columns were eluted with hexane, isopropanol and acetic acid in the ratios specified. Retention times (R_t) refer to analytical HPLC. Chromatography on silica refers to the use of Merck silica gel 60 (230–400 mesh).

Arachidonic acid (**1**) was purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). The modified fatty acids, (*Z,Z,Z*)-(octadeca-6,9,12-trienyloxy)acetic acid (**2**), (*Z,Z,Z*)-(octadeca-9,12,15-trienyloxy)acetic acid (**3**), (all-*Z*)-(eicosa-5,8,11,14-tetraenyloxy)acetic acid (**4**), (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)acetic acid (**5**), 3-[(all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)]propionic acid (**6**) and (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)succinic acid (**7**), were prepared as reported previously,[8,9] while the amino acid extended fatty acids, *N*-[(all-*Z*)-(eicosa-5,8,11,14-tetraenoyl)]glycine (**8**) and *N*-[(all-*Z*)-(eicosa-5,8,11,14-tetraenoyl)]aspartic acid (**9**), were gifts from Peptech Ltd. Soybean 15-lipoxygenase (type I-B) and Brij® 58 were purchased from Sigma Chemical Co. Potato 5-lipoxygenase extract was obtained as reported previously.[26] Tween® 20 was purchased from Aldrich Chemical Co.

Oxidation of the fatty acids 1–9 catalysed by 15-lipoxygenase and monitored using ultraviolet spectroscopy. Soybean 15-lipoxygenase (2 μg, ca. 30 units) in 0.1 M potassium phosphate buffer at pH 9.0 (30 μL) was added to solutions each containing one of the fatty acids **1**–**9** (50 nmol) in the buffer (2.47 mL), maintained at 303 K, and the changes in absorbance at 234 nm were monitored for 10 min. The total absorbance changes observed during this time for the solutions containing the fatty acids **2**–**9** were 94, 66, 78, 33, 72, 19, 44 and 5%, respectively, of that seen with the mixture containing arachidonic acid (**1**).

Oxidation of the fatty acids 1–3 and 5–9 catalysed by 5-lipoxygenase and monitored using ultraviolet spectroscopy. Potato 5-lipoxygenase extract (50 μL, ca. 100 units) was added to solutions each containing one of the fatty acids **1**–**3** and **5**–**9** (0.125 μmol) in 0.1 M potassium phosphate buffer at pH 5.5 (2.45 mL), with 0.005% Tween® 20, saturated with oxygen, and maintained at 298 K, and the changes in absorbance at 234 nm were monitored for 5 min. The total absorbance changes observed during this time for the solutions containing the fatty acids **2**, **3**, **5**, **6** and **8** were 20, 6, 8, 30 and 140%, respectively, of that seen with the mixture containing arachidonic acid (**1**). No absorbance change was detected with the mixtures containing either of the fatty acids **7** or **9**.

Analysis of the products of autoxidation of arachidonic acid (1). A thin film of a mixture of arachidonic acid (**1**) (42 mg, 138 μmol) and α-tocopherol (5 mg, 12% w/w) on the inside surface of a 250 mL round-bottom flask

was stored under oxygen in darkness at room temperature for 42 h, then it was dissolved in ethanol (5 mL) and treated with sodium borohydride (5 mg, 132 μmol). After 1 h, the mixture was concentrated under reduced pressure and the residue was dissolved in dichloromethane. The organic solution was washed with dilute aqueous hydrochloric acid and water, then it was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was subjected to analytical HPLC (hexane/isopropanol/acetic acid, 985/13/1, v/v/v, flow rate 0.6 mL min^{-1}) which showed the presence of the six alcohols **10b**–**15b**.[16,23,24] A sample of approximately 1 mg of each of the alcohols **11b**–**15b** was isolated from the mixture using preparative HPLC (hexane/isopropanol/acetic acid, 920/80/1, v/v/v, flow rate 9 mL min^{-1}). It was not possible to isolate a sample of the alcohol **10b** from the mixture because it was unstable.[25] Instead, a sample of the (*S*)-enantiomer of the alcohol **10b** was obtained by incubating arachidonic acid (**1**) with potato 5-lipoxygenase extract, then reducing the product with sodium borohydride.[26] In a similar manner, a sample of the (*S*)-enantiomer of the alcohol **15b** was obtained from arachidonic acid (**1**) using soybean 15-lipoxygenase.[27] The alcohols (*S*)-**10b** and **11b**–**15b** had physical and spectral properties consistent with those reported,[15–17,21,28] while the location of the hydroxy group in each of these regioisomers was confirmed using negative ion tandem electrospray mass spectrometry.

(*S*)-(*E,Z,Z,Z*)-5-Hydroxyeicosa-6,8,11,14-tetraenoic acid (*S*)-(**10b**): HPLC R_t 55 min; ESI MS/MS *m/z* (%): 319 (100) [$(M-H)^-$], 301 (88) [$(M-H)^- - H_2O$], 257 (42) [$(M-H)^- - H_2O - CO_2^-$], 203 (40) [R^-], 177 (46), 141 (70), 115 (52) [$HC(O)X\text{-}CO_2^-$], 59 (72%).

(*Z,E,Z,Z*)-8-Hydroxyeicosa-5,9,11,14-tetraenoic acid (**11b**): HPLC R_t 32.5 min; ESI MS/MS *m/z* (%): 319 (100) [$(M-H)^-$], 301 (85) [$(M-H)^- - H_2O$], 257 (13) [$(M-H)^- - H_2O - CO_2^-$], 163 (78) [R^-], 155 (79) [$HC(O)X\text{-}CO_2^-$], 127 (18%) [$HX\text{-}CO_2^-$].

(*Z,E,Z,Z*)-9-Hydroxyeicosa-5,7,11,14-tetraenoic acid (**12b**): HPLC R_t 26.8 min; ESI MS/MS *m/z* (%): 319 (100) [$(M-H)^-$], 301 (23) [$(M\text{-}H)^- - H_2O$], 179 (24), 167 (46) [$HC(O)X\text{-}CO_2^-$], 151 (46) [R^-], 123 (35%).

(*Z,Z,E,Z*)-11-Hydroxyeicosa-5,8,12,14-tetraenoic acid (**13b**): HPLC R_t 18.9 min; ESI MS/MS *m/z* (%): 319 (100) [$(M-H)^-$], 301 (4) [$(M-H)^- - H_2O$], 167 (92) [$HX\text{-}CO_2^-$], 149 (4), 59 (7%).

(*Z,Z,E,Z*)-12-Hydroxyeicosa-5,8,10,14-tetraenoic acid (**14b**): HPLC R_t 12.5 min; ESI MS/MS *m/z* (%): 319 (100) [$(M-H)^-$], 301 (57) [$(M-H)^- - H_2O$], 257 (17) [$(M-H)^- - H_2O - CO_2^-$], 208 (24) [$HX\text{-}CO_2^-$], 179 (53), 59 (60%).

(*Z,Z,Z,E*)-15-Hydroxyeicosa-5,8,11,13-tetraenoic acid (**15b**): HPLC R_t 13.6 min; ESI MS/MS *m/z* (%): 319 (100) [$(M-H)^-$], 301 (44) [$(M-H)^- - H_2O$], 257 (7) [$(M-H)^- - H_2O - CO_2^-$], 219 (20) [$HX\text{-}CO_2^-$], 175 (9%).

Analysis of the products of reactions of the modified fatty acids 2–9 with soybean 15-lipoxygenase. In a typical procedure, soybean 15-lipoxygenase (15 mg) was added to a solution containing one of the modified fatty acids (ca. 50 mg) in 0.1 M potassium phosphate buffer at pH 9.0 (200 mL), stirred and maintained at 303 K. A stream of oxygen was passed through the mixture for 10 min, then a solution of triphenylphosphine (50 mg) in ice-cooled dichloromethane (200 mL) was added, followed by 0.2 M hydrochloric acid (20 mL), and the mixture was stirred on ice for 45 min. The organic layer was then separated and the aqueous layer was extracted with dichloromethane. The combined organic solutions were dried ($MgSO_4$) and concentrated under reduced pressure. Analytical HPLC showed the presence of a single product which was isolated by chromatography on silica.

(*Z,Z,E*)-(13-Hydroxyoctadeca-6,9,11-trienyloxy)acetic acid (**16**): reaction of (*Z,Z,Z*)-(octadeca-6,9,12-trienyloxy) acetic acid (**2**) (50 mg) afforded, after chromatography (ether/hexane/acetic acid, 80/20/0.1, v/v/v), the alcohol **16** (12 mg, 23%); HPLC (hexane/isopropanol/acetic acid, 960/40/1, v/v/v, flow rate 0.6 mL min^{-1}) R_t 15 min; ^1H NMR δ 0.89 (3H, t, $J = 6.8$ Hz, C18′-H_3), 1.35 (10H, m), 1.66 (2H, m, C2′-H_2), 2.06 (4H, m, C5′-H_2, C14′-H_2), 2.81 (2H, m, C8′-H_2), 3.56 (2H, t, $J = 6.5$ Hz, C1′-H_2), 4.10 (2H, s, C2-H_2), 4.20 (1H, dt, $J = 6.6$ and 6.0 Hz, C13′-H), 5.37 (3H, m, C6′-H, C7′-H, C9′-H), 5.70 (1H, dd, $J = 6.6$ and 15.2 Hz, C12′-H), 5.99 (1H, dd, $J = 10.9$ and 11.0 Hz, C10′-H), 6.55 (1H, dd, $J = 10.9$ and 15.2 Hz, C11′-H); ESI MS/MS *m/z* (%): 337 (100) [$(M-H)^-$], 319 (81) [$(M-H)^- - H_2O$], 237 (52%) [$HX\text{-}CO_2^-$]; HRMS calcd for $C_{20}H_{34}NaO_4$ *m/z* 361.2355 ($M + Na^+$), found 361.2361.

(*Z,E,Z*)-(13-Hydroxyoctadeca-9,11,15-trienyloxy)acetic acid (**17**): reaction of (*Z,Z,Z*)-(octadeca-9,12,15-trienyloxy)acetic acid (**3**) (50 mg) afforded, after chromatography (ethyl acetate/hexane/acetic acid, 80/20/0.1, v/v/v), the alcohol **17** (15 mg, 29%); HPLC (hexane/isopropanol/acetic acid, 960/40/1, v/v/v, flow rate 1.0 mL min^{-1}) R_t 12 min; ^1H NMR δ 0.95 (3H, t, $J = 7.6$ Hz, C18′-H_3), 1.28 (10H, m), 1.55 (2H, m, C2′-H_2), 2.06 (2H, m), 2.18 (2H, m), 2.32 (2H, m), 3.42 (2H, t, $J = 6.5$ Hz, C1′-H_2), 3.86 (2H, s, C2-H_2), 4.23 (1H, dt, $J = 6.5$ and 6.0 Hz, C13′-H), 5.45 (3H, m, C9′-H, C15′-H, C16′-H), 5.67 (1H, dd, $J = 6.5$ and 15.0 Hz, C12′-H), 5.95 (1H, apparent t, $J = 11.0$ Hz, C10′-H), 6.49 (1H, dd, $J = 11.0$ and 15.0 Hz, C11′-H); ESI MS/MS *m/z* (%): 337 (100) [$(M-H)^-$], 319 (19) [$(M-H)^- - H_2O$], 267 (33), 239 (79%) [$HX\text{-}CO_2^-$]; HRMS calcd for $C_{20}H_{34}NaO_4$ *m/z* 361.2355 ($M + Na^+$), found 361.2345.

(*Z,Z,Z,E*)-(15-Hydroxyeicosa-5,8,11,13-tetraenyloxy) acetic acid (**18**): reaction of (all-*Z*)-(eicosa-5,8,11,14-tetraenyloxy)acetic acid (**4**) (50 mg) afforded, after chromatography (ether/hexane/acetic acid, 80/20/0.1, v/v/v), the alcohol **18** (14 mg, 27%); HPLC (hexane/isopropanol/acetic acid, 960/40/1, v/v/v, flow rate 0.6 mL min^{-1}) R_t 14 min; ^1H NMR δ 0.88 (3H, t, $J = 6.8$ Hz, C20′-H_3), 1.39 (9H, m), 1.64 (3H, m), 2.10 (2H, m), 2.82 (2H, m), 2.92 (2H, m), 3.55 (2H, t, $J = 6.6$ Hz, C1′-H_2), 4.10 (2H, s, C2-H_2), 4.20 (1H, dt, $J = 6.4$ and 6.2 Hz, C15′-H), 5.39 (5H, m, C5′-H, C6′-H, C8′-H, C9′-H, C11′-H), 5.70 (1H, dd, $J = 6.4$ and 15.0 Hz, C14′-H), 6.00 (1H, dd, $J = 10.9$ and 11.0 Hz, C12′-H), 6.53 (1H, dd, $J = 11.0$ and

15.0 Hz, C13′-H); ESI MS/MS *m/z* (%): 363 (100) [(M−H)$^-$], 345 (11) [(M−H)$^-$−H_2O], 265 (35%) [HX-CO_2^-]; HRMS calcd for $C_{22}H_{36}NaO_4$ *m/z* 387.2511 (M + Na^+), found 387.2518.

(*Z*,*Z*,*Z*,*E*)-(15-Hydroxyeicosa-5,8,11,13-tetraenylthio) acetic acid (**19**): reaction of (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)acetic acid (**5**) (43 mg) afforded, after chromatography (ether/hexane/acetic acid, 60/40/2, v/v/v), the alcohol **19** (8 mg, 18%); HPLC (hexane/isopropanol/acetic acid, 940/60/1, v/v/v, flow rate 1.0 mL min^{-1}) R_t 10 min; ^{1}H NMR δ 0.82 (3H, t, *J* = 6.9 Hz, C20′-H_3), 1.28 (8H, m), 1.47 (4H, m), 1.99 (2H, m), 2.57 (2H, m), 2.75 (2H, t, *J* = 7.2 Hz, C1′-H_2), 2.89 (2H, m), 3.17 (2H, s, C2-H_2), 4.15 (1H, dt, *J* = 6.0 and 6.2 Hz, C15′-H), 5.31 (5H, m, C5′-H, C6′-H, C8′-H, C9′-H, C11′-H), 5.60 (1H, dd, *J* = 6.0 and 15.3 Hz, C14′-H), 5.93 (1H, dd, *J* = 10.9 and 11.0 Hz, C12′-H), 6.48 (1H, dd, *J* = 11.0 and 15.3 Hz, C13′-H); ESI MS/MS *m/z* (%): 379 (100) [(M−H)$^-$], 361 (21) [(M−H)$^-$−H_2O], 335 (29) [(M−H)$^-$−CO_2], 317 (39) [(M−H)$^-$−H_2O−CO_2], 235 (74%) [HX-CO_2^-]; HRMS calcd for $C_{22}H_{36}NaO_3S$ *m/z* 403.2283 (M + Na^+), found 403.2290.

3-[(*Z*,*Z*,*Z*,*E*)-(15-Hydroxyeicosa-5,8,11,13-tetraenylthio)] propionic acid (**20**): reaction of 3-[(all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)]propionic acid (**6**) (10 mg) afforded, after chromatography (ethyl acetate/hexane/acetic acid, 80/20/0.1, v/v/v), the alcohol **20** (2.5 mg, 24%); HPLC (hexane/isopropanol/acetic acid, 940/60/1, v/v/v, flow rate 1.0 mL min^{-1}) R_t 10 min; ^{1}H NMR δ 0.82 (3H, t, *J* = 6.8 Hz, C20′-H_3), 1.24 (8H, m), 1.42 (4H, m), 1.84 (2H, m), 2.03 (2H, m), 2.64 (4H, m), 2.76 (2H, m), 2.94 (2H, m), 4.10 (1H, dt, *J* = 6.2 and 6.6 Hz, C15′-H), 5.29 (5H, m, C5′-H, C6′-H, C8′-H, C9′-H, C11′-H), 5.63 (1H, dd, *J* = 6.6 and 15.3 Hz, C14′-H), 5.93 (1H, dd, *J* = 10.7 and 10.9 Hz, C12′-H), 6.46 (1H, dd, *J* = 10.9 and 15.3 Hz, C13′-H); ESI MS/MS *m/z* (%): 393 (100) [(M−H)$^-$], 375 (10) [(M−H)$^-$−H_2O], 321 (23) [(M−H)$^-$−$C_3H_5O_2$], 221 (82%) [HX-CO_2^-−$C_3H_5O_2$]; HRMS calcd for $C_{23}H_{38}NaO_3S$ *m/z* 417.2439 (M + Na^+), found 417.2465.

(*Z*,*Z*,*Z*,*E*)-(15-Hydroxyeicosa-5,8,11,13-tetraenylsulfinyl) succinic acid (**21**): reaction of (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)succinic acid (**7**) (42 mg) afforded, after chromatography (ether/hexane/acetic acid, 10/10/2, v/v/v), the alcohol **21** (16 mg, 35%); HPLC (hexane/isopropanol/acetic acid, 900/100/1, v/v/v, flow rate 1.0 mL min^{-1}) R_t 29 min; ^{1}H NMR δ 0.81 (3H, t, *J* = 6.7 Hz, C20′-H_3), 1.28 (8H, m), 1.47 (4H, m), 1.73 (2H, m), 2.07 (4H, m), 2.75 (2H, m), 2.89 (2H, m), 3.96 (1H, m), 4.12 (1H, dt, *J* = 6.0 and 6.5 Hz, C15′-H), 5.29 (5H, m, C5′-H, C6′-H, C8′-H, C9′-H, C11′-H), 5.61 (1H, dd, *J* = 6.5 and 15.1 Hz, C14′-H), 5.93 (1H, dd, *J* = 10.9 and 11.2 Hz, C12′-H), 6.45 (1H, dd, *J* = 11.2 and 15.1 Hz, C13′-H); ESI MS/MS *m/z* (%): 453 (47) [(M−H)$^-$], 409 (100) [(M−H)$^-$−CO_2], 319 (34) [(M−H)$^-$−H_2O−$C_4H_4O_4$], 301 (31), 255 (26), 219 (32) [HX-CO_2^-−H_2O−$C_4H_4O_4$], 173 (62%) [HX-CO_2^-−$C_5H_8O_5S$]; HRMS calcd for $C_{24}H_{38}NaO_6S$ *m/z* 477.2287 (M + Na^+), found 477.2282.

N-[(*Z*,*Z*,*Z*,*E*)-(15-Hydroxyeicosa-5,8,11,13-tetraenoyl)] glycine (**22**): reaction of *N*-[(all-*Z*)-(eicosa-5,8,11,14-tetraenoyl)]glycine (**8**) (25 mg) afforded, after chromatography (ether/hexane/acetic acid, 90/10/5, v/v/v), the alcohol **22** (9 mg, 40%); HPLC (hexane/isopropanol/acetic acid, 820/180/1, v/v/v, flow rate 0.6 mL min^{-1}) R_t 15 min; ^{1}H NMR δ 0.81 (3H, t, *J* = 6.8 Hz, C20′-H_3), 1.12 (8H, m), 1.67 (2H, m), 2.04 (2H, m), 2.16 (2H, m), 2.74 (2H, m), 2.90 (2H, m), 3.98 (2H, s, C2-H_2), 4.22 (1H, dt, *J* = 6.0 and 6.2 Hz, C15′-H), 5.31 (5H, m, C5′-H, C6′-H, C8′-H, C9′-H, C11′-H), 5.63 (1H, dd, *J* = 6.0 and 15.2 Hz, C14′-H), 5.93 (1H, dd, *J* = 10.9 and 11.1 Hz, C12′-H), 6.51 (1H, dd, *J* = 11.1 and 15.2 Hz, C13′-H); ESI MS/MS *m/z* (%): 376 (100) [(M−H)$^-$], 358 (79) [(M−H)$^-$−H_2O], 276 (61%) [HX-CO_2^-]; HRMS calcd for $C_{22}H_{35}NNaO_4$ *m/z* 400.2464 (M + Na^+), found 400.2468.

N-[(*Z*,*Z*,*Z*,*E*)-(15-Hydroxyeicosa-5,8,11,13-tetraenoyl)] aspartic acid (**23**): reaction of *N*-[(all-*Z*)-eicosa-5,8,11,13-tetraenoyl)]aspartic acid (**9**) (36 mg) afforded, after chromatography (ethyl acetate/acetic acid, 100/5, v/v), the alcohol **23** (6 mg, 39%); HPLC (hexane/isopropanol/acetic acid, 800/200/5, v/v/v, flow rate 0.6 mL min^{-1}) R_t 13 min; ^{1}H NMR δ 0.81 (3H, t, *J* = 7.0 Hz, C20′-H_3), 1.45 (10H, m), 1.97 (2H, m), 2.20 (4H, m), 2.80 (4H, m), 4.10 (1H, m), 4.16 (1H, dt, *J* = 6.2 and 6.4 Hz, C15′-H), 5.30 (5H, m, C5′-H, C6′-H, C8′-H, C9′-H, C11′-H), 5.62 (1H, dd, *J* = 6.2 and 15.0 Hz, C14′-H), 5.93 (1H, dd, *J* = 11.0 and 11.2 Hz, C12′-H), 6.47 (1H, dd, *J* = 11.2 and 15.0 Hz, C13′-H); ESI MS/MS *m/z* (%): 434 (100) [(M−H)$^-$], 390 (21) [(M−H)$^-$−CO_2], 319 (24) [(M−H)$^-$−$C_4H_3O_4$], 300 (19), 257 (17), 219 (11%) [HX-CO_2^-−$C_4H_3O_3$]; HRMS calcd for $C_{24}H_{37}NNaO_6$ *m/z* 458.2519 (M + Na^+), found 458.2508.

Analysis of the products of reactions of the modified fatty acids 2, 3, 5, 6 and 8 with potato 5-lipoxygenase. In a typical procedure, potato 5-lipoxygenase extract (200 μL, ca. 400 units) was added to a solution containing one of the modified fatty acids (ca. 1 mg) in 0.04 M potassium phosphate buffer at pH 6.0 (2.5 mL), containing 0.01% Brij® 58, and stirred and maintained at 298 K. After 20 min sodium borohydride (0.5 mg) was added and the mixture was stirred for 30 min, then it was acidified and extracted with dichloromethane. The extract was concentrated under reduced pressure and the residue was subjected to HPLC, to analyse and isolate products.

(*E*,*Z*,*Z*)-(6-Hydroxyoctadeca-7,9,12-trienyloxy)acetic acid (**24**): reaction of (*Z*,*Z*,*Z*)-(octadeca-6,9,12-trienyloxy) acetic acid (**2**) afforded the alcohol **24**; HPLC (hexane/isopropanol/acetic acid, 900/100/1, v/v/v, flow rate 1.0 mL min^{-1}) R_t 21 min; ESI MS/MS *m/z* (%): 337 (100) [(M−H)$^-$], 319 (26) [(M−H)$^-$−H_2O], 173 (13) [HC(O)X-CO_2^-], 163 (10%) [R$^-$]; HRMS calcd for $C_{20}H_{34}NaO_4$ *m/z* 361.2355 (M + Na^+), found 361.2342.

(*E*,*Z*,*Z*)-(9-Hydroxyoctadeca-10,12,15-trienyloxy)acetic acid (**25**): reaction of (*Z*,*Z*,*Z*)-(octadeca-9,12,15-trienyloxy)acetic acid (**3**) afforded the alcohol **25**; HPLC (hexane/isopropanol/acetic acid, 960/40/1, v/v/v, flow rate 1.0 mL min^{-1}) R_t 14 min; ESI MS/MS *m/z* (%): 337 (100) [(M−H)$^-$], 319 (82) [(M−H)$^-$−H_2O], 215 (38) [HC(O)X-CO_2^-], 121 (49%) [R$^-$]; HRMS calcd for

$C_{20}H_{34}NaO_4$ m/z 361.2355 ($M+Na^+$), found 361.2348. The reaction also afforded trace amounts of a mixture of the alcohol **17** and (*Z,E,Z*)-(12-hydroxyoctadeca-9,13,15-trienyloxy)acetic acid (**26**) (ratio **25**:**17**+**26**, ca. 10:1); ESI MS/MS m/z (%): 337 (100) [$(M-H)^-$], 319 (26) [$(M-H)^- - H_2O$], 267 (33) [**17**:HC(O)X-CO_2^-], 255 (54) [**26**:HC (O)X-CO_2^-], 239 (18) [**17**:HX-CO_2^-], 227 (15%) [**26**: HX-CO_2^-].

(*E,Z,Z,Z*) - (5 - Hydroxyeicosa - 6,8,11,14 - tetraenylthio) acetic acid (**27**): reaction of (all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)acetic acid (**5**) afforded the alcohol **27**; HPLC (hexane/isopropanol/acetic acid, 960/40/1, v/v/v, flow rate 1.0 mL min^{-1}) R_t 19 min; ESI MS/MS m/z (%): 379 (100) [$(M-H)^-$], 361 (33) [$(M-H)^- - H_2O$], 317 (61) [$(M-H)^- - H_2O - CO_2^-$], 203 (91%) [HC(O)X-CO_2^-]; HR-MS calcd for $C_{22}H_{36}NaO_3S$ m/z 403.2283 ($M+Na^+$), found 403.2279.

3-[(*E,Z,Z,Z*)-(5-Hydroxyeicosa-6,8,11,13-tetraenylthio)] propionic acid (**28**): reaction of 3-[(all-*Z*)-(eicosa-5,8,11,14-tetraenylthio)]propionic acid (**6**) afforded the alcohol **28**; HPLC (hexane/isopropanol/acetic acid, 960/40/1, v/v/v, flow rate 1.0 mL min^{-1}) R_t 26 min; ESI MS/MS m/z (%): 393 (100) [$(M-H)^-$], 321 (19), 303 (26), 203 (96%) [R^-]; HRMS calcd for $C_{22}H_{38}NaO_3S$ m/z 417.2439 ($M+Na^+$), found 417.2442.

N-[(*Z,Z,E,Z*)-(11-Hydroxyeicosa-5,8,12,14-tetraenoyl)] glycine (**29**): reaction of *N*-[(all-*Z*)-(eicosa-5,8,11,14-tetraenoyl)]glycine (**8**) afforded the alcohol **29**; HPLC (hexane/isopropanol/acetic acid, 800/200/15, v/v/v, flow rate 1.0 mL min^{-1}) R_t 28 min; ESI MS/MS m/z (%): 376 (100) [$(M-H)^-$], 224 (76%) [HX-CO_2^-]; HRMS calcd for $C_{22}H_{35}NNaO_4$ m/z 400.2464 ($M+Na^+$), found 400.2452.

(*E,Z,E*)-(6,13-Dihydroxyoctadeca-7,9,11-trienyloxy) acetic acid (30). Treatment of (*Z,Z,E*)-(13-hydroxyoctadeca-6,9,11-trienyloxy)acetic acid (**16**) with the potato 5-lipoxygenase then sodium borohydride, as described above for the reactions of **2**, **3**, **5**, **6** and **8**, afforded the diol **30**; HPLC (hexane/isopropanol/acetic acid, 900/100/1, v/v/v, flow rate 0.6 mL min^{-1}) R_t 23 min; ESI MS/MS m/z (%): 353 (100) [$(M-H)^-$], 335 (8) [$(M-H)^- - H_2O$], 253 (26) [C12–C13 cleavage, HX-CO_2^-], 179 (8) [C6–C7 cleavage, R^-], 173 (65%) [C6–C7 cleavage, HX-CO_2^-]; HRMS calcd for $C_{20}H_{34}NaO_5$ m/z 377.2304 ($M+Na^+$), found 377.2303.

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