A simple preparation of ω-hydroxydienoic fatty acids with double-bond positional isomerism

Igor V. Ivanov,*[a,b] Galina I. Myagkova[a] and Hartmut Kühn[b]

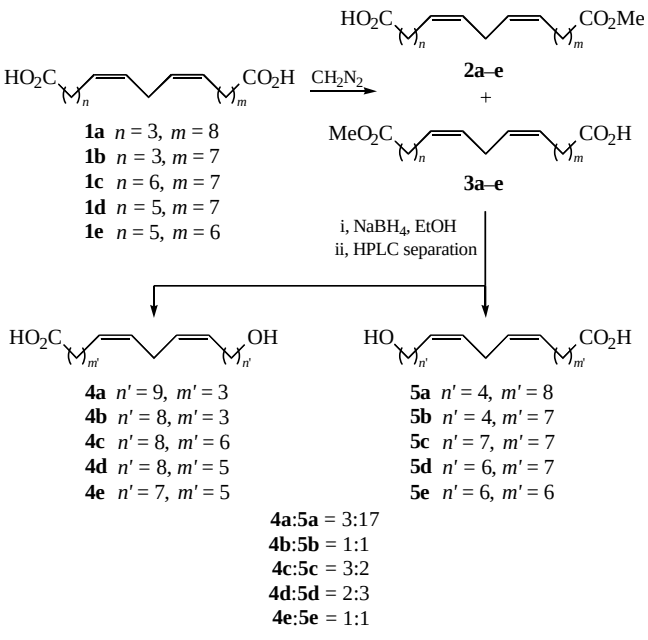
[a] *M. V. Lomonosov State Academy of Fine Chemical Technology, 117571 Moscow, Russian Federation. Fax: +7 095 434 8711; e-mail: myagkova@httos.mitht.msk.ru*

[b] *Institute of Biochemistry, University Clinics Charité, Humboldt University, D-10115 Berlin, Germany*

A set of new regioisomeric ω-hydroxydienoic fatty acids has been prepared *via* a three-step procedure involving the methylation of ω-carboxydienoic fatty acids followed by the $NaBH_4$ reduction of a mixture of the resulting monomethyl esters and HPLC separation.

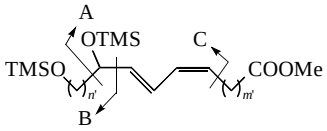
Lipoxygenases are lipid peroxidising enzymes which oxygenate polyenoic fatty acids containing a (1*Z*,4*Z*)-pentadienoic system to their corresponding 1-hydroperoxy-(2*E*,4*Z*)-derivatives.[1] Mammalian 15-lipoxygenase (LOX) is unique among other LOX due to its ability to oxidise polyunsaturated fatty acids incorporated in complex membrane lipids.[2,3] Although the first X-ray structure of mammalian 15-LOX has recently been solved, the structural features of the substrate binding cage are not completely understood.[4] Based on the theory[1,4–7] of 'hydrophobic binding cavity' we suggested that the incorporation of a polar (hydroxy) or evenly charged (carboxy) group at the ω-position of a fatty acid molecule could hinder the proper substrate alignment at the substrate binding site of 15-LOX. In order to prove this suggestion and test the substrate specificity of LOX we synthesised a set of isomeric ω-carboxydienoic fatty acids.[8,9] To avoid total synthesis in the preparation of ω-hydroxydienoic acids we propose an original three-step procedure which provides access to both possible regioisomeric ω-hydroxy acids (**4a**–**e** and **5a**–**e**) from dicarboxylic acid **1a**–**e** (Scheme 1).

The methylation of dicarboxylic acid **1a**–**e** with a small molar excess of diazomethane in diethyl ether leads to a mixture of two monomethyl esters, **2a**–**e** and **3a**–**e**. The sequential reduction of this mixture with an ethanol solution of sodium borohydride produced two ω-hydroxy acids **4a**–**e** and **5a**–**e**.† Both isomeric products were eluted in reverse phase HPLC as a single peak when the chromatogram was developed with the solvent system methanol–water–acetic acid (85:15:0.1, v/v) at a flow rate of 1 ml min^{-1}. However, when the mixture of ω-hydroxydienoic acids was analysed by normal phase HPLC the isomeric compounds, **4a**–**e** and **5a**–**e**, were resolved (Figure 1). In carrying out the isomer separation we prepared both product isomers with a degree of purity exceeding 99%. The yield of the entire preparation procedure calculated for the mixture of **4** and **5** varied between 61–65%. The analysis of the ratio of isomeric products **4a**–**e** and **5a**–**e** (Scheme 1) shows that the methylation of dienedicarboxylic acids **1b**–**e** with diazomethane occurs without any significant preference for either one or the other carboxylic group. Although the data observed were reproducible and the ratio of isomeric products **4a**–**e** and **5a**–**e**, and hence, that of monomethyl esters **3a**–**e** and **4a**–**e** in the starting mixture varied slightly, compound **1a** failed to follow the trend. Whether methylation of one of the carboxylic groups has any preference or the predominant formation of one of the methyl esters in the case of **1a** was accidental, should be studied further.



Scheme 1

Table 1 Analytical data for the oxygenated ω-hydroxydienoic fatty acid isomers.[a,b]



ω-Hydroxyacid	Oxygenation product	
	Retention time/min	Key ion fragmentation, *m/z*
4a (*n′* = 9, *m′* = 3)	5.84	470 (M^+), 255 (A), 317 (B)
4b (*n′* = 8, *m′* = 3)	6.57	456 (M^+), 255 (A), 303 (B)
4c (*n′* = 8, *m′* = 6)	6.72	498 (M^+), 355 (C), 297 (A), 303 (B)
4d (*n′* = 8, *m′* = 5)	7.98	484 (M^+), 355 (C), 283 (A), 303 (B)
4e (*n′* = 7, *m′* = 5)	8.63	470 (M^+), 341 (C), 283 (A), 289 (B)
5a (*n′* = 4, *m′* = 8)	13.78	470 (M^+), 325 (A), 247 (B)
5b (*n′* = 4, *m′* = 7)	12.63	456 (M^+), 311 (A), 247 (B)
5c (*n′* = 7, *m′* = 7)	7.58	498 (M^+), 341 (C), 311 (A), 289 (B)
5d (*n′* = 6, *m′* = 7)	9.28	484 (M^+), 327 (C), 311 (A), 275 (B)
5e (*n′* = 6, *m′* = 6)	9.00	470 (M^+), 327 (C), 297 (A), 275 (B)

[a]Only the early eluting compound in SP-HPLC (Figure 2) was analysed. A Nucleosil 100-7 column (250×5 mm, 7 μm particle size; Machery and Nagel, Germany) with the solvent system hexane–isopropanol–acetic acid (100:10:0.1, v/v) was used, flow rate 1 ml min^{-1}. The oxygenated products were analysed by GC-MS as the corresponding trimethylsilylmethyl ester derivatives. [b](C) is the minor intensity fragmentation.

† In a typical experiment, the dicarboxylic acid **1a**–**e** (0.10 mmol) dissolved in 1 ml diethyl ether was methylated with a small molar excess of diazomethane (0.12 mmol). The ether was removed by argon flow and 7 ml of a 0.5 M ethanol solution of sodium borohydride (the solution was prepared as described by Brown *et al.*,[10] and the concentration of borohydride was determined by the hydrolysis method) was added to the residue at room temperature under argon. The reaction mixture was stirred for 6–16 h at 24 °C. After the organic solvent was evaporated under vacuum, the reaction mixture was quenched with 2 ml of water and then acidified to pH 3. The fatty acid derivatives were extracted twice with 5 ml of ethyl acetate, the combined organic extracts were concentrated under vacuum and the resulting products **4** and **5**, preliminary purified by RP-HPLC, were separated by normal phase HPLC.

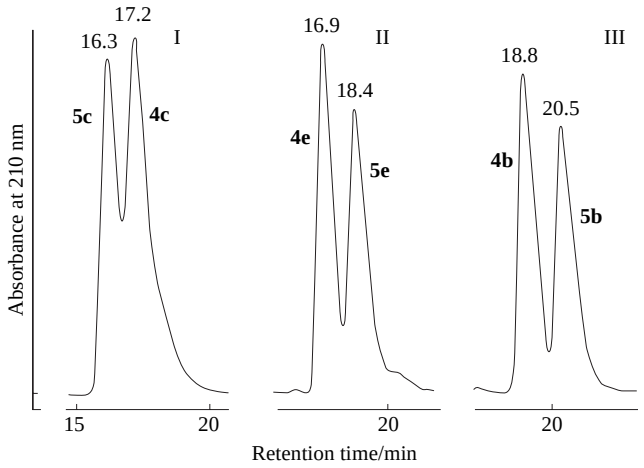


Figure 1 SP-HPLC separation of the products formed by $NaBH_4$ reduction of a mixture of monomethyl esters of **1c** (I), **1e** (II) and **1b** (III). [The compounds were separated on an analytical Nucleosil 100-7 column (250×5 mm, 7 μm particle size; Machery and Nagel, Germany) with the solvent system hexane–isopropanol–acetic acid (100:2:0.1, v/v), flow rate 1 ml min^{-1}.]

Unfortunately, neither ^{1}H NMR spectra nor GC-MS data of the trimethylsilylmethyl ester provided sufficient information to localise the position of the double bond system in products **4** and **5**, especially when the difference between *n′* and *m′* is less then 2 carbon atoms.‡ To solve this problem the ω-hydroxylated fatty acids **4a**–**e** and **5a**–**e** were oxygenated to their corresponding hydroperoxy derivatives in the presence of 2,2′-azobis-(amindiopropane)hydrochloride which induces peroxy radical mediated lipid peroxydation.§,[11] This procedure involves a double bond conjunction associated with a *Z*–*E* isomerisation of the double bond shifted. HPLC analysis of the reaction mixture indicated the formation of four oxygenation products (Figure 2) containing conjugated diene chromophores with absorbance maxima between 230 and 235 nm (inset). The UV spectra of the fraction eluting early from the oxygenated products, with absorbance maxima at 235 nm, indicated the geometry,[12] and the GC-MS data in Table 1 indicated the position of the double bonds. The data observed confirmed the position of the double bond system in compounds **4a**–**e** and **5a**–**e**.

This work was supported in part by a DAAD fellowship awarded to I. V. Ivanov (A/96/28729), a grant from the Russian Foundation for Basic Research (grant no. 96-03-327-68a) awarded to G. I. Myagkova and I. V. Ivanov and a grant awarded by the Deutsche Forschungsgemeinschaft [Ku 961(2-2)] to H. Kühn.

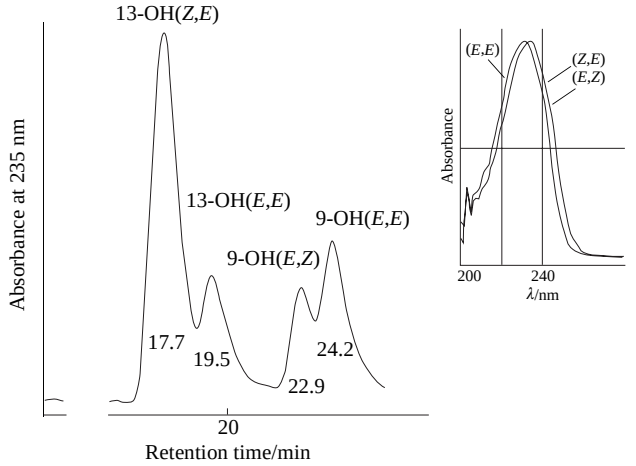


Figure 2 Analytical SP-HPLC of non-enzymatically oxidised 17-hydroxy-(9*Z*,12*Z*)-heptadecadienoic acid **5b** [this HPLC run was performed with the solvent system hexane–isopropanol–acetic acid (100:7.5:0.1, v/v)].

References

1 W. D. Lehman, *Free Radical Biol. Med.*, 1994, **16**. 241.
2 H. Kühn and A. Brash, *J. Biol. Chem.*, 1990, **265**, 1454.
3 T. Schewe, W. Halangk, C. Hiebsch and S. M. Rapoport, *FEBS Lett.*, 1975, **60**, 149.
4 S. A. Gillmor, A. Villasenor, R. Fletterick, E. Sigal and M. F. Browner, *Natur. Struct. Biol.*, 1997, **4**, 1003.
5 Q.-F. Gan, M. F. Browner, D. L. Sloane and E. Sigal, *J. Biol. Chem.*, 1996, **271**, 25412.

‡ All the compounds **4** and **5** gave satisfactory analytical data and were characterised by ^{1}H NMR spectroscopy and GC-MS analysis.

4a (*n′* = 9, *m′* = 3): ^{1}H NMR ($CDCl_3$) *δ*: 1.25–1.35 (m, 12H, CH_2), 1.56–1.70 (m, 4H, 3-CH_2 and 17-CH_2), 2.04 (m, 2H, 10-CH_2), 2.09 (m, 2H, 4-CH_2), 2.35 (t, 2H, CH_2COO, *J* 7 Hz), 2.78 (m, 2H, 7-CH_2), 3.67 (t, 2H, 18-CH_2, *J* 2.5 Hz), 5.30–5.38 (m, 4H, CH=CH). GC-MS, *m/z*: 382 (M^+), 367 (M^+ – Me), 351 (M^+ – MeO).

4b (*n′* = 8, *m′* = 3): early eluting product in normal phase HPLC (see Figure 1); ^{1}H NMR ($CDCl_3$) *δ*: 1.30–1.40 (m, 10H, CH_2), 1.55–1.65 (m, 4H, 3-CH_2 and 16-CH_2), 2.04 (m, 2H, 10-CH_2), 2.10 (m, 2H, 4-CH_2), 2.37 (t, 2H, CH_2COO, *J* 7 Hz), 2.77 (m, 2H, 7-CH_2), 3.67 (t, 2H, 17-CH_2, *J* 2.5 Hz), 5.35–5.42 (m, 4H, CH=CH). GC-MS, *m/z*: 368 (M^+), 353 (M^+ – Me), 337 (M^+ – MeO).

4c (*n′* = 8, *m′* = 6): ^{1}H NMR ($CDCl_3$) *δ*: 1.30–1.40 (m, 16H, CH_2), 1.60 (m, 2H, 3-CH_2), 1.65 (m, 2H, 19-CH_2), 2.07 (m, 4H, 7-CH_2 and 13-CH_2), 2.37 (t, 2H, CH_2COO, *J* 7 Hz), 2.79 (m, 2H, 10-CH_2), 3.67 (t, 2H, 18-CH_2, *J* 2.5 Hz), 5.35–5.45 (m, 4H, CH=CH). GC-MS, *m/z*: 410 (M^+), 395 (M^+ – Me), 379 (M^+ – MeO).

4d (*n′* = 8, *m′* = 5): ^{1}H NMR ($CDCl_3$) *δ*: 1.30–1.40 (m, 14H, CH_2), 1.60 (m, 2H, 3-CH_2), 1.66 (m, 2H, 18-CH_2), 2.06 (m, 2H, 12-CH_2), 2.10 (m, 2H, 6-CH_2), 2.35 (t, 2H, CH_2COO, *J* 7 Hz), 2.78 (m, 2H, 9-CH_2), 3.64 (t, 2H, 19-CH_2, *J* 2.5 Hz), 5.30–5.40 (m, 4H, CH=CH). GC-MS, *m/z*: 396 (M^+), 381 (M^+ – Me), 365 (M^+ – MeO).

4e (*n′* = 7, *m′* = 5): early eluting product in normal phase HPLC (see Figure 1); ^{1}H NMR ($CDCl_3$) *δ*: 1.30–1.40 (m, 12H, CH_2), 1.60 (m, 2H, 3-CH_2), 1.65 (m, 2H, 17-CH_2), 2.07 (m, 4H, 6-CH_2 and 12-CH_2), 2.35 (t, 2H, CH_2COO, *J* 7 Hz), 2.78 (m, 2H, 9-CH_2), 3.65 (t, 2H, 18-CH_2, *J* 2.5 Hz), 5.30–5.40 (m, 4H, CH=CH). GC-MS, *m/z*: 382 (M^+), 367 (M^+ – Me), 351 (M^+ – MeO).

5a (*n′* = 4, *m′* = 8): early eluting product in normal phase HPLC (see Figure 1); ^{1}H NMR ($CDCl_3$) *δ*: 1.25–1.35 (m, 12H, CH_2), 1.59 (m, 2H, 3-CH_2), 1.72 (m, 2H, 17-CH_2), 2.05 (m, 2H, 9-CH_2), 2.14 (m, 2H, 15-CH_2), 2.35 (t, 2H, CH_2COO, *J* 7 Hz), 2.78 (m, 2H, 12-CH_2), 3.67 (t, 2H, 18-CH_2, *J* 2.5 Hz), 5.30–5.38 (m, 4H, CH=CH). GC-MS, *m/z*: 382 (M^+), 367 (M^+ – Me), 351 (M^+ – MeO).

5b (*n′* = 4, *m′* = 7): ^{1}H NMR ($CDCl_3$) *δ*: 1.30–1.40 (m, 10H, CH_2), 1.57 (m, 2H, 3-CH_2), 1.73 (m, 2H, 16-CH_2), 2.05 (m, 2H, 8-CH_2), 2.15 (m, 2H, 14-CH_2), 2.37 (t, 2H, CH_2COO, *J* 7 Hz), 2.78 (m, 2H, 11-CH_2), 3.68 (t, 2H, 17-CH_2, *J* 2.5 Hz), 5.30–5.38 (m, 4H, CH=CH). GC-MS, *m/z*: 368 (M^+), 353 (M^+ – Me), 337 (M^+ – MeO).

5c (*n′* = 7, *m′* = 7): early eluting product in normal phase HPLC (see Figure 1); ^{1}H NMR ($CDCl_3$) *δ*: 1.30–1.40 (m, 16H, CH_2), 1.57 (m, 2H, 3-CH_2), 1.64 (m, 2H, 19-CH_2), 2.05 (m, 4H, 8-CH_2 and 14-CH_2), 2.37 (t, 2H, CH_2COO, *J* 7 Hz), 2.79 (m, 2H, 11-CH_2), 3.64 (t, 2H, 20-CH_2, *J* 2.5 Hz), 5.30–5.40 (m, 4H, CH=CH). GC-MS, *m/z*: 410 (M^+), 395 (M^+ – Me), 379 (M^+ – MeO).

5d (*n′* = 6, *m′* = 7): early eluting product in normal phase HPLC (see Figure 1); ^{1}H NMR ($CDCl_3$) *δ*: 1.30–1.40 (m, 14H, CH_2), 1.57 (m, 2H, 3-CH_2), 1.65 (m, 2H, 18-CH_2), 2.05 (m, 4H, 8-CH_2 and 14-CH_2), 2.35 (t, 2H, CH_2COO, *J* 7 Hz), 2.79 (m, 2H, 11-CH_2), 3.64 (t, 2H, 19-CH_2, *J* 2.5 Hz), 5.30–5.40 (m, 4H, CH=CH). GC-MS, *m/z*: 396 (M^+), 381 (M^+ – Me), 365 (M^+ – MeO).

5e (*n′* = 6, *m′* = 6): ^{1}H NMR ($CDCl_3$) *δ*: 1.30–1.40 (m, 12H, CH_2), 1.59 (m, 2H, 3-CH_2), 1.66 (m, 2H, 17-CH_2), 2.05 (m, 4H, 7-CH_2 and 13-CH_2), 2.35 (t, 2H, CH_2COO, *J* 7 Hz), 2.78 (m, 2H, 10-CH_2), 3.65 (t, 2H, 18-CH_2, *J* 2.5 Hz), 5.30–5.40 (m, 4H, CH=CH). GC-MS, *m/z*: 382 (M^+), 367 (M^+ – Me), 351 (M^+ – MeO).

§ The ω-hydroxylated acids **4a**–**e** or **5a**–**e** (0.01 mmol) were incubated at 40 °C for 2 h with 100 mmol of 2,2′-azo-bis(2-amindiopropane)-hydrochloride in 2 ml of 0.1 M borate buffer (pH 9.0) containing 10% (v/v) of methanol. Then the reaction was stopped and hydroperoxides were reduced to the corresponding hydroxides by addition of an equimolar amount of sodium borohydride. The mixture was acidified to pH 3.5, the fatty acids were extracted with 2 ml of ethyl acetate, concentrated under vacuum and analysed by normal phase HPLC and GC-MS as their thrimethylsilylmethyl ester derivatives [the carboxylic group was methylated with diazomethane, the hydroxylic groups were silylated with bis(trimethylsilyl)trifluoroacetamide in pyridine].

6 S. Bongräber, R. J. Kuban, M. Anton and H. Kühn, *J. Mol. Biol.*, 1996, **264**, 1145.
7 H. Kühn, H. Sprecher and A. R. Brash, *J. Biol. Chem.*, 1990, **265**, 16300.
8 I. V. Ivanov, N. V. Groza, G. M. Malchenko, G. I. Myagkova and T. Schewe, *Bioorg. Khim.*, 1997, **23**, 519 (*Russ. J. Bioorg. Chem.*, 1997, **23**, 481).
9 I. V. Ivanov, N. V. Groza, H. Kühn and G. I. Myagkova, *Bioorg. Khim.*, 1998, **24**, 454 (*Russ. J. Bioorg. Chem.*, 1998, **24**, 398).
10 N. C. Brown, S. Narasimhan and Y. M. Choi, *J. Org. Chem.*, 1988, **47**, 4702.
11 C. D. Ingram and A. R. Brash, *Lipids*, 1998, **23**, 340.
12 V. W. Bowry and R. Stocker, *J. Am. Chem. Soc.*, 1993, **115**, 6029.

Received: Moscow, 3rd August 1998
Cambridge, 29th September 1998; Com. 8/06230G