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Synthesis, Properties, and Identification of Epimeric Hepoxilins (-)-(10R)-B_3 and (+)-(10S)-B_3[1]

Ludmila L.Vasiljeva, Tatjana A.Manukina, Peter M.Demin, Margarita A.Lapitskaja, and Kasimir K.Pivnitsky*

Institute of Experimental Endocrinology, National Endocrinology Scientific Center RAMS, 1 Moskvorechje St., Moscow 115522, RUSSIA

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Abstract: *To characterize the individual epimers of hepoxilin B_3, a new total synthesis procedure was developed consisting of subsequent condensations of enantiomerically pure (2R,3S)-epoxy-5-undecynal with $LiC{\equiv}CCH_2Cl$ and $HC{\equiv}C(CH_2)_3COOMe$, followed by Lindlar hydrogenation, and finally epimer separation. Derivatives of hepoxilin <u>syn</u>-(10R,11R,12S)-B_3 (free acid, methyl ester and acetate of methyl ester) are more polar on silica gel, have negative optical rotations $[\alpha]^{25}_D$ -60.6°, -62.6°, -25.2°, respectively, and in ^1H NMR spectra have the larger splitting of carbinolic H-10 signal ($J_{10,11}$ 5.0-6.1 Hz). Corresponding values for less polar hepoxilin <u>anti</u>-(10S,11R,12S)-B_3 derivatives are: $[\alpha]^{25}_D$ +73.5°, +61.9°, and -2.0°; $J_{10\text{-}11}$ 2.95-3.3 Hz. These data suggest that an epimer of hepoxilin B_3 recently isolated by W.H.Gerwick et al. from red algae is <u>syn</u>-(10R,11R,12S)-B_3.*

INTRODUCTION

Hepoxilin B_3 (HxB_3) epimers, metabolites from the arachidonic acid cascade, are synthesized in numerous mammalian tissues as mixtures of two epimers at the 10-hydroxyl group.[2] The most important physiological role of hepoxilin is the stimulation of basal and glucose-induced insulin secretion in rat pancreatic islets.[3] This bioactivity has created a steady increase in the interest of hepoxilin in biochemical and medical research. Recently, hepoxilin has been found in other natural systems, most notably HxB_3 was isolated from several tropical red marine algae[4,5] and identified in the *Aplysia* mollusk tissue.[6]

Although the chemical synthesis of hepoxilin B_3 was first reported in 1983,[7] and the stereochemistry later studied,[8] the physical properties of the non-racemic epimers of HxB_3 have not been yet described. This restricts the identification of HxB_3 epimers to only "more" or "less" polar.[7,9,10]

The aim of this study was to provide the means to differentiate and identify the HxB_3 epimers. To accomplish this, a new synthesis process of individual HxB_3 epimers (**1a,b**) was developed, and their properties relevant to epimer differentiation and identification are described.

RESULTS AND DISCUSSION

Chemical Synthesis of Hepoxilin B₃ Epimers

A synthesis of non-racemic epimers of HxB₃ **1a,b** was performed by a new method (Scheme 1). Enantiodirected epoxidation of 1-hydroxyundec-2(E)-en-5-yne **2**[8] by the catalytical Sharpless method[11] using L-(+)-diethyl tartrate produced (2S,3S)-epoxyalcohol **3** with enantiomeric excess (e.e.) 88%. Enantiomeric purity of the product was easily enhanced with high yield up to e.e. >98% by low-temperature recrystallization. E.e. values were determined by ^{1}H NMR analysis of corresponding acetates in the presence of the chiral shift reagent europium(III) tris[3-(heptafluoropropylhydroxymethylene)-D-camphorate] [Eu(hfc)₃] which induced different shifts of the OAc-signal in the acetate enantiomers.

Pyridinium dichromate oxidation of the epoxyalcohol **3**, entirely analogous to those described[8] for the racemic compound *rac*-**3**, afforded (2R,3S)-epoxyaldehyde **4**.[12] In this case, as distinguished from the oxidation of the racemic substrate, the dimeric ester **5** formed as a by-product was a diastereomerically homogenous product, in accordance with the expectations. This fact constitutes an additional evidence of the enantiomeric purity of epoxyalcohol **3**.

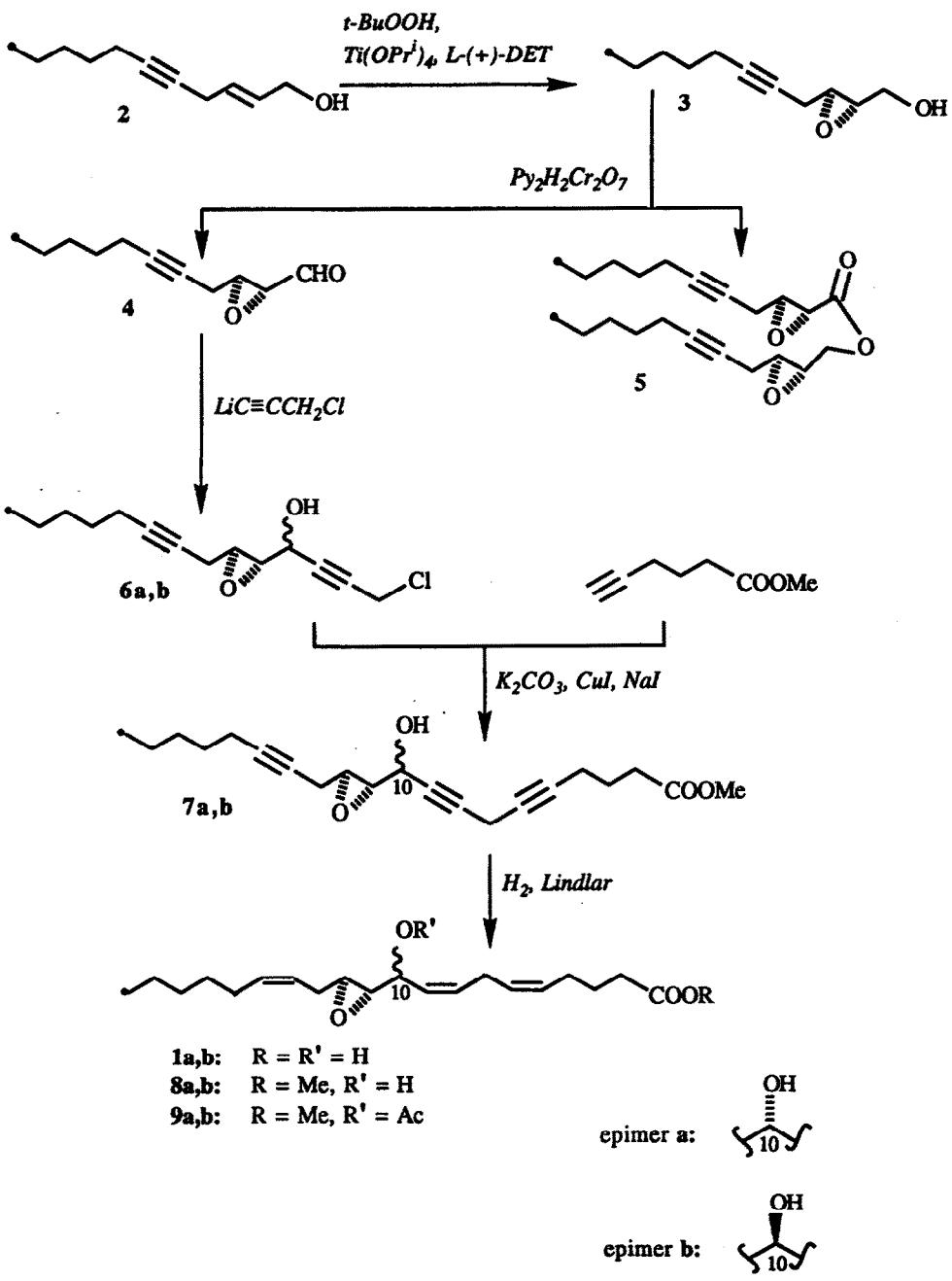
Previously,[8] the further processing of the racemic aldehyde *rac*-**4** to construct the hepoxilin 20-carbon chain involved a condensation with 5,8-nonadiynoic acid. As this condensation could be performed at best with modest yields (≤32%), we developed a new method for carbon chain elongation. A condensation of aldehyde **4** with lithiated propargyl chloride, $ClCH_2C{\equiv}CLi$[13] proceeded in satisfactory yield (71%) giving rise to chloroalcohols **6a,b** as a mixture of epimers in a ratio of 71:29. The epimer configurations at the newly formed C^{10} asymmetric center were deduced from the NMR chemical shifts of carbinolic 10-H-signals. By analogy with HxB₃ derivatives[8] the more shielded signal (δ 4.48 ppm) was attributed to the major *syn* epimer **6a**. This assignment was subsequently verified by the conversion of the epimeric mixture **6a,b** into the mixture of HxB₃ epimers **1a,b** with the same *syn:anti* ratio (*vide infra*).

The final chain elongation to reach 20-carbon chain was performed by condensation of chloroalcohol mixture **6a,b** with methyl 5-hexynoate in the presence of CuI, NaI, and K_2CO_3 in DMF, which produced a high yield of hexadehydro-HxB₃ methyl ester mixture **7a,b**. This new method of terminal acetylene alkylation with propargylic halogenides in mild conditions will be published in a separate communication.[14] The developed two-step method of hepoxilin carbon chain construction from aldehyde **4** doubles the total yield in comparison with the direct condensation with 5,8-nonadiynoic acid and does not require the preparation of the latter. This constitutes an uncommon example of a non-convergent synthetic scheme which is more effective than a convergent one.

The final step in the synthesis is the simultaneous Lindlar hydrogenation of 3 triple bonds of hexahydro-HxB₃ ester **7a,b** into (Z)-double bonds of HxB₃ ester **8a,b**. Similar multiple hydrogenations of the arrays of different triple bonds are believed to be low-yielding and difficult to control.[7] Previously we have succeeded in Lindlar hydrogenation of the ester *rac*-**7a,b** with selectivity up to 80%.[8] At present we selected the conditions for this and similar hydrogenations which secure the selectivity up to 95%. The detailed conditions for this hydrogenation yielding 97% of the HxB₃ ester mixture **8a,b** (epimer ratio a:b = 74:26) are presented in the Experimental section.

The individual epimers of HxB₃ methyl esters **8a,b** were isolated from the mixture by high performance flash chromatography (HPFC). Corresponding free acids **1a,b** and acetates **9a,b** were prepared by standard methods.

Scheme 1


t-BuOOH,
Ti(OPr^i)_4, L-(+)-DET
2
OH
3
OH
Py_2H_2Cr_2O_7
CHO
4
5
LiC≡CCH_2Cl
OH
6a,b
Cl
COOMe
K_2CO_3, CuI, NaI
OH
7a,b
10
COOMe
H_2, Lindlar
OR'
10
COOR
1a,b: R = R' = H
8a,b: R = Me, R' = H
9a,b: R = Me, R' = Ac
epimer a:
OH
10
epimer b:
OH
10

Properies of Hepoxilin B₃ Epimers

Selected properties of these hepoxilin HXB₃ epimer derivatives most significant for epimer differentiation are presented in the Table 1. (10R)- or 10,11-*syn*-epimers (indexed "a") of all investigated hepoxilin B₃ derivatives were more polar on silica gel chromatography and possessed negative optical rotations. As the values of optical rotation are not easily reproducible for small quantities of unstable oily substances, such as hepoxilins, and therefore could not always be used for identification (for example see below), we recommend the application of the difference in optical rotations at two wave lengths, e.g. 366 and 578 nm, for the purpose of hepoxilin epimer identification. These increments of optical rotation are usually of significantly larger magnitude (see Table 1) and are less sensitive to impurities than single $[\alpha]_D$ values. A very similar criterion has already been used as a simple indicator of chirality of weakly rotating eicosanoids.[15] An even more valuable means of epimer identification is provided by ^{1}H NMR data. Already published regularities in NMR data of HXB₃ epimers[8] are found to hold for acetates **9a,b** as well.

Table 1. The Properties of C-10 Epimers of Hepoxilin B₃

Compound	Derivative	C-10-epimer	R_f, silica gel, TLC	$[\alpha]^{25}_D$ (c in $CHCl_3$)	$[\alpha]_{366}-[\alpha]_{578}$	^{1}H NMR data: solvent	H-C^{10} signal: δ, ppm	J, Hz: 9–10	J, Hz: 10–11
1a	free acid	R (*syn*)	0.25[a]	−60.6° (c 1.17)	−138°	$CDCl_3$	4.33	7.4	5.1
1b		S (*anti*)	0.29	+73.5° (c 0.67)	+175°		4.70	7.9	2.95
8a	methyl ester	R	0.13[b]	−62.6° (c 0.89)	−130°	$CDCl_3$	4.33	7.7	5.0
8b		S	0.16	+61.9° (c 1.09)	+173°		4.67	8.5[c]	3.3
9a	acetate methyl ester	R	0.41[d]	−25.2° (c 1.68)	−54°	C_6D_6	5.61	8.8	6.1
9b		S	0.42	−2.0° (c 0.82)	+4°		5.89	8.4	3.3

a) system EtOAc–hexane (2:3); b) EtOAc–hexane (1:4); c) note the correction of published data[8]; d) C_6H_6–Et_2O (85:15).

Configuration Assignment of an Unknown Hepoxilin B3 Epimer

The data in the Table 1 make it possible to identify hepoxilin B3 epimers even if the data for a single epimer only are available. This may be illustrated by the following example. W.H.Gerwick et al.[4,5] isolated from tropical red algae *Platysiphonia miniata*, *Cottoniella filamentosa*, and *Murrayella periclados* an epimer of HxB3 in the form of the acetate methyl ester for which the following data were published: $[\alpha]^{23}_D$ -10.9° (c 0.11, $CHCl_3$), $\delta_{10\text{-}H}$ 5.67 ppm, $J_{9\text{-}10}$ 9.2 Hz, $J_{10\text{-}11}$ 6.4 Hz (in C_6D_6). From the sign of optical rotation the absolute (12S) configuration of algae HXB3 can be deduced, but the relative (10,11)-configuration remains unknown. However, the later can be estimated with ease by comparing cited NMR data with those in Table 1. An agreement is observed with the data for epimer "a" only. Therefore HxB3 isolated from red algae[4] is (10R,11R,12S)-HXB3 1a.

We hope that the information presented herein will help in differentiation and identification of hepoxilin B3 epimers in other instances as well.

EXPERIMENTAL

General. Optical rotation were measured on polarimeter Polamat A at the wavelengths of mercury lamp lines. Rotation at the sodium D-line (λ 589 nm) were calculated using the equation: $[\alpha]_D = 1.34 \times [\alpha]_{578} - 0.34 \times [\alpha]_{546}$. Melting points were determined with a Boetius micro hot stage. Infrared spectra were obtained on a Specord 75 IR-spectrophotometer with liquid films or CCl_4 solutions. ^1H NMR spectra were obtained on either Bruker MSL-200 (200 MHz) or a Tesla BS-587A (80 MHz) spectrometers, with Me_4Si as an internal standard (δ=0). Gas-liquid chromatography was performed on a chromato-mass-spectrometer LKB-2091 using an open tubular fused silica capillary column (0.3 mm x 25 m) with SE-30 stationary phase, other conditions see ref.8. Electron-impact mass-spectra (22.5 eV) were obtained on the same apparatus using a direct inlet of the sample into the ion source at stated temperature.

Thin layer chromatography (TLC) was with Silufol UV-254 aluminium-backed TLC sheets with silica gel layer (0.2 mm). Thick layer preparative chromatography (TLPC) was run using Whatman glass TLC plates with silica gel layer of 2 mm thickness. High performance flash chromatography[16] was performed using columns 2 x 16 cm or 3.5 x 18 cm with Kieselgel H (Fluka). Column effectiveness was determined to be 2000 theoretical plates. Vacuum distillation was done using Kugelrohr apparatus (Aldrich) at stated temperatures of an oven.

"Usual workup" refers to extraction by the stated solvent, drying of the extract by anhydrous $MgSO_4$, concentration *in vacuo* on rotoevaporator at <40°C, and drying of the residue until constant weight at 20°C in 1.3 GPa vacuum. All reactions and sample storage were performed in a static atmosphere of purified argon.

Pyridinium dichromate, Lindlar catalyst, n-BuLi solution (Fluka), L-(+)-diethyl tartrate and $Ti(OPr\text{-}i)_4$ (Aldrich) were used as received. A solution of anhydrous t-BuOOH in CH_2Cl_2 was prepared according to K.B.Sharpless et al.[11]. 1-Hydroxyundec-2(E)-en-5-yne 2 and 5-hexynoic acid were synthesized by known methods. Methyl ester of the latter was prepared by etherial diazomethane methylation. All other reagents and solvents were purified by standard methods. Solvents were distilled in glass before use.

(2S,3S)-1-Hydroxy-2,3-epoxyundec-5-yne (3). A suspension of powdered, activated 4A molecular sieves (230 mg) in 40 ml of CH_2Cl_2 was cooled to -5°C. L-(+)-Diethyl tartrate (80 mg, 0.39 mmol) and $Ti(OPr\text{-}i)_4$ (88 mg, 0.31 mmol) were added sequentially. After the mixture was cooled to -20°C, t-BuOOH (1.72 ml, 6.5 mmol, 3.8 M in CH_2Cl_2) was added and the resulting mixture was stirred 10 min, whereupon a solution of alcohol 2 (515 mg, 3.1 mmol) in CH_2Cl_2

(3 ml) was added. Stirring was maintained at -20°C for 2.5 h before addition of water (2 ml). The mixture was allowed to warm up to 25°C for 45 min resulting in a stable white emulsion. An aqueous NaOH solution (0.4 ml, 30%) saturated with NaCl, and after 30 min methanol (5 ml), were added for layer separation. After the usual workup (CH_2Cl_2, 4 x 2 ml) and distillation of residue, epoxyalcohol 3 was obtained as a colorless crystalline mass (514 mg, 91%), b.p. 100–110°C/0.03 GPa, m.p. 32.5–33.1°C, $[\alpha]^{25}_D$ -11.1° (c 1.83, $CHCl_3$), e.e. 88% according ^1H NMR analysis of corresponding acetate with addition of 20 molar % of Eu(hfc)$_3$ using the ratio of singlet OAc-group sygnals at ca. 3.58 (major) and 3.60 (minor). Three recrystallizations from hexane (10 ml) at -78°C afforded an optically pure substance: yield 65%, e.e. >98%, m.p. 34°C, $[\alpha]^{25}_D$ -11.8° (c 1.14, $CHCl_3$) (lit.[17]: $[\alpha]^{20}_D$ +10.7° for enantiomer). Spectral data of epoxide 3 are identical to those published[8] for the racemic compound *rac*-3.

(2R,3S)-2,3-Epoxyundec-5-yn-1-al (4) and (9S,10R,14S,15S)-9,10;14,15-Bisepoxy-12-oxatricosa-6,17-diyn-11-one (5). These were obtained concurrently by oxidation of the alcohol 3 in an analogous manner to that described for corrésponding racemate.[8] The aldehyde 4: a colorless liquid, $[\alpha]^{25}_D$ +40.4° (c 1.25, $CHCl_3$). The dimeric ester 5: a colorless oil, $[\alpha]^{25}_D$ -21.8° (c 2.27, $CHCl_3$); unlike the product from the racemic starting material, the ester 5 is an individual diastereomer. ^1H NMR (80 MHz, $CDCl_3$): δ 0.90 (t, J 6.1 Hz, 2 x Me), 1.37 (m, 6 x CH_2), 2.14 (m, 5-H_2 + 19-H_2), 2.60 (m, 8-H_2 + 16-H_2), 2.94–3.42 (m, 9-H + 14-H + 15-H), 3.48 (d, J 1.6 Hz, 10-H), 4.04 (dd, J 6.3 and 12.2 Hz, 13-H), 4.54 (dd, J 3.0 and 12.2 Hz, 13-H). Other properties of these optically active substances are identical to those described for corresponding racemic samples.[8]

(4RS,5RS,6SR)- and (4SR,5RS,6SR)-1-Chloro-4-hydroxy-5,6-epoxytetradeca-2,8-diynes (rac-6a,b). A solution of propargyl chloride (909 mg, 12.2 mmol) in diethyl ether (10 ml) was cooled to -78°C. n-BuLi (7.3 ml, 1.5 M in hexane, 11.0 mmol) was added over the period of 5 min followed after 3 min by a solution of the racemic aldehyde *rac*-4 (500 mg, 2.78 mmol) in diethyl ether (10 ml). After 10 min the reaction was quenched by addition of saturated NH_4Cl solution (20 ml). The usual workup (EtOAc, 100 ml) resulted in an orange oil which in benzene solution was adsorbed on silica gel column (5–40 nm, 10 g). Gradient elution by EtOAc solutions in hexane (from 5 to 20%) and solvent removal gave epimer mixture of racemic alcohols *rac*-**6a,b** as a colorless oil (505 mg, 71%, epimer ratio 71:29 by ^1H NMR analysis); R_f 0.17 (EtOAc–hexane, 1:4); IR (film, cm^{-1}): 701 (C-Cl), 1259 and 3423 (OH); ^1H NMR (80 MHz, $CDCl_3$): δ 0.89 (t, J 6.0 Hz, Me), 1.38 (m, 11-H_2 + 12-H_2 + 13-H_2), 2.14 (m, 10-H_2), 2.58 (m, 7-H_2 + OH), 3.21 (m, 5-H + 6-H), 4.18 (d, J 1.8 Hz, 1-H_2), 4.48 (m, intensity 0.71H, 4-H of "a" epimer), 4.69 (m, intensity 0.29H, 4-H of "b" epimer); mass spectrum (100°C, m/z, % of rel.intensity): 237 ($[M-H_2O]^+$, 0.4), 219 ($[M-Cl]^+$, 0.7), 211 (0.8), 201 ($[M-Cl-H_2O]^+$, 0.7), 183 ($[M-C_5H_{11}]^+$, 1.4), 151 ($[C^5-C^{14}]^+$, 9), 95 ($[C^8-C^{14}]^+$, 100), 81 (76), 67 (88), 55 (55), 53 (49), 43 (33), 41 (56).

(4R,5R,6S)- and (4S,5R,6S)-1-Chloro-4-hydroxy-5,6-epoxytetradeca-2,8-diynes (6a,b). The condensation of the enantiomerically pure aldehyde 4 was performed as described above for the racemic sample to give a colorless oil which crystallized in the refrigerator: m.p. 17.5°C, b.p. 190°C/0.06 GPa, $[\alpha]^{25}_D$ -6.3° (c 1.53, $CHCl_3$); other properties were identical to those described above for the racemic sample.

Methyl (10RS,11RS,12SR)- and (10SR,11RS,12SR)-10-Hydroxy-11,12-epoxyeicosa-5,8,14-triynoates (rac-7a,b). A mixture of each anhydrous, pulverized CuI (64.1 mg, 0.34 mmol), NaI (169 mg, 1.13 mmol), K_2CO_3 (123 mg, 0.89 mmol), and DMF (0.5 ml) was prepared. The solutions of methyl 5-hexynoate (98 mg, 0.78 mmol, in 0.4 ml of DMF) and chloroalcohol *rac*-**6a,b** (85 mg, 0.33 mmol, in 0.4 ml of DMF) were successively added. The mixture was stirred for 5 h at 21°C

before quenching with saturated aqueous NH_4Cl solution (40 ml). Usual workup (benzene, 3 x 40 ml) followed by filtration of the resulting yellow oil in EtOAc–hexane (1:4) solution through silica gel (3 g) resulted in isolation of triyne *rac*-**7a,b** as a yellow oil (104 mg, 91.5%, epimer ratio a:b = 74:26 by GLC analysis of Bu^tMe_2Si ether, see ref.7). This sample was identical in all respects except the epimer ratio to those obtained by the other way.[8]

Methyl (10R,11R,12S)- and (10S,11R,12S)-10-Hydroxy-11,12-epoxyeicosa-5,8,14-triynoates (7a,b). The mixture of optically active epimers was obtained from chloroalcohol mixture **6a,b** entirely analogous to the racemic sample as a pale yellow oil, $[\alpha]^{25}_D$ −7.7° (c 1.25, $CHCl_3$); all other properties were identical to those of the racemic sample.

Methyl (10R,11R,12S)- and (10S,11R,12S)-10-Hydroxy-11,12-epoxyeicosa-5(Z),8(Z),14(Z)-trienoates (Hepoxilin B_3 Methyl Esters) (8a,b). A mixture of Lindlar catalyst (350 mg), quinoline (770 µl), and benzene (15 ml) was saturated at 15°C with hydrogen (35 ml, 1 atm). A solution of triyne **7a,b** (350 mg) in benzene (17 ml) was added and the mixture was stirred in a hydrogen atmosphere at 15°C until hydrogen absorption had ceased (60 min, 38 ml of hydrogen was absorbed). The suspension was filtered, a hydrogen-presaturated Lindlar catalyst (350 mg) was added to the filtrate, and hydrogenation repeated for 50 min (total hydrogen absorption 123 ml, 180% of theoretical amount). The mixture was filtered, the filtrate poured on a aluminium oxide column (19 g, II activity according Brockman, pH 7.0), and the quinoline was eluted by EtOAc–hexane (5:95, 120 ml). The column was eluted by EtOAc (120 ml) to give a yellow oil (346 mg) containing (by GLC analysis of Bu^tMe_2Si ether) 95% of trienes **8a,b**. Individual epimers were isolated using HPFC (EtOAc–hexane, 15:85) followed by TLPC (Et_2O–benzene, 15:85, 4 developments) of mixed fractions. Epimer **8a**: a colorless oil, yield 211 mg (59%), $[\alpha]^{25}$ (λ, nm): −195° (366), −147° (406), −121.4° (436), −71.8° (546), −64.9° (578) (c 0.89, $CHCl_3$). Epimer **8b**: a colorless oil, yield 74 mg (21%), $[\alpha]^{25}$ (λ, nm): +238° (366), +172° (406), +141.1 (436), +75.8° (546), +65.4° (578) (c 1.09, $CHCl_3$). For other data, see Table 1. Chromatographic as well as spectral properties of optically active samples were identical to those of the corresponding racemic ones.[8]

Hepoxilin (10R)-B_3 (1a). A solution of methyl ester **8a** (13 mg) and LiOH (47.6 mg) in a mixture of methanol (7.3 ml) and water (4 ml) was stirred at 23°C for 90 min. The mixture was diluted with water (30 ml) and diethyl ether (30 ml) and carefully acidified to pH 3 by 3% hydrochloric acid. The usual workup (Et_2O, 3 x 30 ml) followed by TLPC (EtOAc–hexane, 2:3) afforded hepoxilin **1a** (11.4 mg, 91%) as a colorless oil, $[\alpha]^{25}$ (λ, nm): −201° (366), −150° (406), −123.9° (436), −70.9° (546), −63.2° (578) (c 1.17, $CHCl_3$); IR (CCl_4, cm^{-1}): 1715 (C=O), 3400 (OH); ^1H NMR (80 MHz, $CDCl_3$): δ 0.88 (t, J 6.0 Hz, 20-H_3), 1.28 (m, 17-H_2 + 18-H_2 + 19-H_2), 1.75 (quintet, J 7.0 Hz, 3-H_2), 2.04 (m, 4-H_2 + 16-H_2), 2.36 (m, 2-H_2 + 13-H_2), 2.92 (m, 7-H_2 + 11-H + 12-H), 4.33 (dd, J 5.1 and 7.4 Hz, 10-H), 5.16 (br.s, 2 x OH), 5.45 (m, olefinic 6H). See also Table 1.

Hepoxilin (10S)-B_3 (1b). This was obtained in an analogous fashion to epimer "a" starting from ester **8b**: a colorless oil, yield 78%, $[\alpha]^{25}$ (λ, nm): +252° (366), +186° (406), +156.2° (436), +85.6° (546), +76.6° (578) (c 0.67, $CHCl_3$); ^1H NMR (80 MHz, $CDCl_3$): δ 0.89 (t, J 6.0 Hz, 20-H_3), 1.27 (m, 17-H_2 + 18-H_2 + 19-H_2), 1.74 (quintet, J 7.1 Hz, 3-H_2), 2.05 (m, 4-H_2 + 16-H_2), 2.36 (m, 2-H_2 + 13-H_2), 2.87 (m, 7-H_2 + 11-H), 3.06 (dt, J 2.56 and 5.51 Hz, 12-H), 4.15 (br.s, 2 x OH), 4.70 (dd, J 2.95 and 7.87 Hz, 10-H), 5.41 (m, olefinic 6H). See also Table 1.

Hepoxilin (10R)-B_3 Acetate Methyl Ester (9a). A mixture of ester **8a** (17 mg), acetic anhydride (157 mg), and pyridine (430 µl) was maintained at 20°C for 18 h. After dilution with benzene (2 ml) the mixture was evaporated at 20°C *in vacuo* at 1.3 GPa to dryness. The residue was

purified by TLPC (Et_2O-benzene, 15:85) to give the acetate **9a** (17.7 mg, 93%) as a colorless oil, $[\alpha]^{25}$ (λ, nm): -79.8° (366), -66.7° (406), -50.0° (436), -29.2° (546), -26.2° (578) (c 1.68, $CHCl_3$); IR (CCl_4, cm^{-1}): 1745 (C=O); ^1H NMR (200 MHz, C_6D_6): δ 0.90 (t, J 7.0 Hz, 20-H_3), 1.24 (m, 17-H_2 + 18-H_2 + 19-H_2), 1.64 (quintet, J 7.0 Hz, 3-H_2), 1.70 (s, OAc), 1.97 (m, 4-H_2 + 16-H_2), 2.14 (t, J 7.4 Hz, 2-H_2), 2.30 (m, 13-H_2), 2.92 (m, 7-H_2 + 11-H + 12-H), 3.40 (s, COOMe), 5.45 (m, olefinic 6H), 5.61 (dd, J 6.1 and 8.8 Hz, 10-H); mass spectrum (70°C, m/z, % of rel.intensity): 392 ($[M]^+$, 0.2), 361 ($[M-OMe]^+$, 0.5), 350 ($[M-CH_2CO]^+$, 0.5), 332 ($[M-AcOH]^+$, 8), 301 ($[M-OMe-AcOH]^+$, 5), 281 ($[C^1-C^{12}]^+$, 4), 221 ($[281-AcOH]^+$, 48), 210 (51), 160 (44), 81 (100). See also Table 1.

Hepoxilin (10S)-B₃ Acetate Methyl Ester (9b). This epimer was obtained from ester **8b** similarly to epimer "a" as a colorless oil, yield 91%, $[\alpha]^{25}$ (λ, nm): +1.2° (366), +3.6° (406), -1.2° (436), -3.6° (546), -2.4° (578) (c 0.82, $CHCl_3$); IR (CCl_4, cm^{-1}): 1745 (C=O); ^1H NMR (200 MHz, C_6D_6): δ 0.92 (t, J 7.0 Hz, 20-H_3), 1.28 (m, 17-H_2 + 18-H_2 + 19-H_2), 1.63 (m, 3-H_2), 1.72 (s, OAc), 1.98 (m, 4-H_2 + 16-H_2), 2.16 (t, J 7.4 Hz, 2-H_2), 2.27 (m, 13-H_2), 2.92 (m, 7-H_2 + 11-H + 12-H), 3.40 (s, COOMe), 5.48 (m, olefinic 6H), 5.89 (dd, J 3.3 and 8.4 Hz, 10-H). See also Table 1.

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REFERENCES AND NOTES

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