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Supplementary Material:

Epoxyalcohols 4 and 5 - These were identified as 11-hydroxy-14,15-epoxyeicosa-trienoic acids on the basis of the EI mass spectra of their methyl ester TMS ether and TMS ester TMS ether derivatives. For example, the spectrum of the TMS ester TMS ether of product 4 showed a molecular ion at *m/z* 480 and structurally significant fragment ions at *m/z* values of 465 (M-15), 409 (M-71), 390 (M-90), 375 (M-[90+15]), 341 (M-[C_{12}-C_{20}]), 312, and 241 (base peak, C_{11}-C_{20}). Catalytic hydrogenation gave three products in the ratio of ≈1:1:4. The first was identified as a saturated 11-hydroxy-14,15-epoxyeicosanoic acid on the basis of the M-15 ion at *m/z* 413 (2% relative abundance), (the molecular ion was absent), and structurally diagnostic ions at *m/z* 287 (C_1 - C_{11}, 40% relative abundance) and *m/z* 243 (C_{11} - C_{20}, 70% relative abundance); the base peak was observed at *m/z* 129 ($C_2H_3CO_2{}^+Si(CH_3)_2$) (Tulloch, 1985). The second hydrogenated product also gave no molecular ion, although it appeared to have the same molecular weight based on a weak ion at *m/z* 413 (M-15) and a more prominent *m/z* 357 (M-71, loss of C_{16} - C_{20}). The presence of a silylated 15-hydroxyl was strongly suggested by the *m/z* 357 ion and the base peak at *m/z* 173 (C_{15} - C_{20}), although a structure could not be assigned from this information. The largest peak was identified as an 11,15-dihydroxyeicosanoic acid based on the structurally diagnostic ions at *m/z* 431(M-71, ≤ 1% relative abundance), 402 (M-100, 2%), 317 (C_{11} - C_{20}, 25%), 287 (C_1 - C_{11}, 50%), 269, 227, and 173 (C_{15} - C_{20}, base peak). This type of dihydroxy derivative is precedented as a main rearrangement product of hydrogenation of allylic epoxyalcohols (Hamberg et al., 1986).

The stereochemistry of the 11-hydroxyl of the two 11-hydroxy-14,15*trans*-epoxyeicosatrienoic acids was established using a method developed originally by Hamberg (Hamberg, 1971). In this procedure the configuration of the hydroxyl is established by derivatization of the diastereomers with menthylchloroformate, cleavage at the double bonds by oxidative ozonolysis and finally comparison of the GC retention times of the fragments containing the original hydroxyl (as the methyl ester, menthyl carbonate) with authentic standards prepared from R and S malic acid (Figure 1). Thus, products 4 and 5 are assigned respectively as 11S-hydroxy- and 11R-hydroxy-14S,15S-*trans*-epoxyeicosa-5*Z*,8*Z*,12*E*-trienoic acids. The elution of the two diastereomers on straight phase chromatography (silica HPLC or TLC) is in the order of the less polar 11S-hydroxy-14S,15S-*trans*-epoxide (product 4) followed by the more polar 11R diastereomer (product 5), identical to the relative mobilities observed for other closely related diastereomeric epoxyalcohols (Corey & Su, 1990; Lumin & Falck, 1992).

The structures of epoxyalcohols 4 and 5 were confirmed by ^{1}H-NMR. Chemical shifts and key coupling constants are given in Table 1. Their spectra are virtually indistinguishable. Only when the spectra are overlaid are some minor differences in chemical shift discernible. The NMR spectra confirm the epoxyalcohol structures including the *trans* configuration of the 14,15-epoxide ($J_{14,15}$ = 2.0 Hz for products 4 and 5, cf. for *cis* epoxides, J = 4-5 Hz (Mercier & Agoh, 1974). The coupling constant $J_{12,13}$ =

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15.9 Hz indicates the *trans* configuration of the 12,13 double bond. Decoupling of H4 clarified the signal for H5 and allowed designation of the *cis* configuration of the 5,6 double bond ($J_{5,6}$ = 10.8). The H8 and H9 protons gave overlaying signals that were not deciphered; this double bond is not involved in formation of the epoxyalcohol products and is assumed to retain the original *cis* configuration.

Epoxyalcohols 7 and 8 - The EI mass spectra of the methyl ester TMS ether derivatives were very similar to a published spectrum of 13-hydroxy-14,15-epoxyeicosatrienoic acids (Narumiya et al., 1981). There are very few structurally diagnostic ions. Apart from very weak ions corresponding to M^+ (*m/z* 422, ≤1% relative abundance) and M-15 (*m/z* 407) the main diagnostic feature of the spectrum is an ion at *m/z* 309, arising by α-cleavage at the C13 hydroxyl and corresponding to C_1-C_{13}. Epoxyalcohols 7 and 8 were hydrogenated without any significant degradation using palladium on alumina. The spectra of the saturated products (MeTMS derivative) had the heaviest ion at *m/z* 413 (M-15), with prominent ions at *m/z* 315 (C_1 - C_{13}), 299, 281, 267, 215 (C_{13} - C_{20}), 199, 187, 159, 143, and 129 (base peak); this is very similar to a published spectrum (Narumiya et al., 1981) except that we observed a weaker ion at *m/z* 413 ion (≈2% relative abundance).

The structures and main stereochemical features of the products were established conclusively by ^{1}H-NMR (Tables 2 and 3). Like the main allylic epoxides 4 and 5, products 7 and 8 are *trans* 14,15 epoxides ($J_{13,14}$ = 2.3 Hz in product 7 and 2.1

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Hz in product 8) (Mercier & Agoh, 1974). The *erythro* and *threo* diastereomers can be distinguished based on the value of the coupling constant between the α-hydroxyl and the *trans* epoxide (Mercier & Agoh, 1974), and by two additional properties that consistently segregate with *erythro* and *threo* (Bernart & Gerwick, 1994). Thus, the relative configuration of the 13-hydroxyl can be assigned from the values of the coupling constant $J_{13,14}$, which is 2.7 Hz in product 7 (*erythro*), and 5.9 Hz in product 8 (*threo*) (Mercier & Agoh, 1974). Secondly, the mobility of the two diastereomers on silica (SP-HPLC or TLC) is in the order of the more mobile *erythro* (product 7) followed by *threo* (product 8), again matching the properties of other closely related diastereomers (Gardner & Crawford, 1981; Dix & Marnett, 1985; Corey et al., 1983; Corey & Mehrotra, 1983; Vasiljeva et al., 1993). Third, the chemical shift of the hydroxyl proton, H13, is significantly further downfield in the *erythro* isomer; the signal is located at 4.55 ppm in product 7 (*erythro*) and 4.3 ppm in product 8 (*threo*). The overlaying signals from the olefinic protons confounded measurement of most of the coupling constants. As the three remaining double bonds are not involved in formation of products 7 and 8, they are assumed to retain their original *cis* configuration. The complete structures of hematin products 7 and 8 are respectively 13S-hydroxy- and 13R-hydroxy-14S,15S-*trans*-epoxyeicosa-5Z,8Z,11Z-trienoic acids.

Epoxyalcohols 1, 2, 3, and 6 - These are minor hematin products and the evidence points to their being *cis* epoxides. Mass spectra were recorded on derivatives of each (± hydrogenation), indicating that 1 and 3 are 11-hydroxy-14,15-epoxyeicosatrienoic

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acids and 2 and 6 are 13-hydroxy-14,15-epoxyeicosatrienoic acids. ^{1}H-NMR spectra were obtained on product 1 from hematin reactions with 15S-HPETE and on product 6 (obtained from an incubation of 15R-HPETE) (Tables 4 and 5). The NMR data established the *cis* configuration of the epoxide in 1 and 6 ($J_{14,15}$ = 4.3 Hz). Products 2 and 3 are assumed also to be *cis* epoxides that are the hydroxy diastereomers of the other two compounds. The absolute configurations of the individual diastereomers were not defined.

Metabolism of 15R-HPETE - The mass spectrum of the methyl ester TMS ether derivative of the main 15R metabolite of control microsome incubations was essentially indistinguishable from the spectrum of the 11-hydroxy-14,15-epoxyeicosatrienoic acid standard. Catalytic hydrogenation gave three products as described earlier for other 11-hydroxy-14,15-epoxyeicosatrienoic acids. Based on these analyses, and the ^{1}H-NMR analysis of hematin product 1 (Table 4), (and assuming that the 5,6 and 8,9 double bonds retain their original *cis* configuration), the main 15R-HPETE metabolite in control microsomes was identified as 11-hydroxy-14S,15R-*cis*-epoxyeicosa-5*Z*,8*Z*,12*E*-trienoic acid.

In the incubation of phenobarbital-induced microsomes, the main 15R-HPETE product (product 6) corresponds to a minor peak in the hematin reactions. The mass spectrum of the methyl ester TMS ether derivative of product 6 was consistent with a 13-hydroxy-14,15-epoxyeicosatrienoic acid (not shown). As product 6 could not be

produced in large amounts in the hematin reaction, we prepared sufficient product for NMR analysis in a 30 ml microsomal incubation with 1mg 15R-HPETE (Table 5). On the basis of the GC-MS and ^{1}H-NMR data, product 6 was identified as 13-hydroxy-14S,15R-*cis*-epoxyeicosa-5Z,8Z,11Z-trienoic acid. The configuration of the 13-hydroxyl remains unassigned. From the literature on the proton NMR of α-hydroxy epoxides it is known that on the basis of the coupling constants it is not possible to distinguish reliably the relative *erythro* or *threo* configuration of a hydroxyl α to a *cis* epoxide (e.g. ref. Song et al., 1993; Pierre et al., 1968; Lellouche et al., 1988; Roush et al., 1991).

Technical considerations in epoxyalcohol analysis - The allylic epoxyalcohols are unstable and certain precautions are required for extraction, HPLC and NMR analysis. Under neutral conditions the epoxyalcohols are stable, and during the short time course of the microsomal incubations apparently the epoxides are not subject to significant enzymatic hydrolysis. During isolation of the products, hydrolysis can be avoided simply by ignoring the usual convention of acidification prior to extraction. For HETEs and epoxyalcohols, acidification is not required for efficient extraction into solvents of the polarity of chloroform, methylene chloride, diethyl ether or ethyl acetate. Also, if the sample is acidified briefly to pH 3-4 for extraction, hydrolysis of the epoxyalcohols is mimimal if the extraction is carried out promptly, the sample is kept cold, and the organic phase is washed to neutral reaction (≥ pH 5) with water prior to evaporation to dryness. During HPLC analysis, we noticed that the relatively unstable allylic epoxyalcohols (products 1, 3, 4 and 5) tend to degrade markedly on "well-used"

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reversed-phase HPLC columns. More stable molecules (HETEs, non-allylic epoxyalcohols) chromatographed perfectly well on the older columns, but relatively new reversed-phase columns were need to achieve the chromatographic performance shown in Figures 1 and 2 of the main section of the paper.

Our first attempts to characterize the epoxyalcohol products by ^{1}H-NMR were complicated by their tendency to hydrolyze to trihydroxy derivatives on exposure to the $CDCl_3$ solvent (that contains DCl impurity); this occurred with both the allylic and non-allylic epoxides. The use of a trace of deuterated pyridine in the $CDCl_3$ is reported for analysis of the labile epoxide leukotriene A_4 methyl ester (Baker et al., 1980; Atrache et al., 1981). We added 2 μl of deuterated pyridine per 0.5 ml of $CDCl_3$, but the four main epoxyalcohol samples were 20-100% degraded after several hours in this solvent; (spectra were recorded at room temperature). Fresh samples were prepared, purified as the methyl ester derivatives and the spectra were recorded successfully in deuterated benzene. In this solvent there was no problem with sample degradation.

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K-471-MG

Table 1 **^{1}H-NMR (300 MHz) of the methyl esters of hematin products 4 and 5 recorded in C_6D_6 [a]**

Chemical shift Product 4 methyl ester	Product 5 methyl ester	# H		Assignment, coupling constant (Hz)	
5.825	5.82	1H	dd	H12	$J_{11,12}$ = 5.4, $J_{12,13}$ = 15.5
5.51 (5.55 - 5.45)	5.495 (5.54-5.44)	2H	dd + m	H13, H8	$J_{12,13}$ = 15.5, $J_{13,14}$ = 7.6
5.39 (5.45 - 5.33)	5.39 (5.45-5.33)	2H	m	H6, H9	
5.28 (5.33 - 5.22)	5.28	1H	m	H5	$J_{5,6}$ = 10.8
3.96	3.95	1H	m *	H11	
3.34	3.345	3H	s	OCH_3	
2.97	2.96	1H	dd	H14	$J_{13,14}$ = 7.6, $J_{14,15}$ = 2.0
2.745	2.74	2H	t	H7	
2.65	2.645	1H	dt	H15	$J_{14,15}$ = 2.0, $J_{15,16}$ = 5.3
2.22 (2.32 - 2.13)	2.225 (2,325-2.135)	2H	m	H10	
2.085	2.085	2H	t	H2	
1.96	1.96	2H	q	H4	
1.58	1.58	2H	p	H3	
1.38 (1.43 - 1.34)	1.38 (1.43-1.34)	2H	m	H16	
1.30 (1.34 - 1.26)	1.30 (1.34-1.26)	2H	m	H17	
1.19 (1.26 - 1.13)	1.19 (1.26-1.14)	4H	m	H18, H19	
0.85	0.85	3H	t	H20	

[a] The two diastereomers have very similar spectra. The only readily apparent difference is the pattern of overlay of H13 and H8. Other differences are discernable only when the two spectra are superimposed. For example, in product 4 the signals from H12,

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H14, H7, and H15 are approximately 0.07 - 0.1 ppm further downfield, while the signal from H10 is ≈0.07 ppm upfield.

[b] H11 appears as a multiplet (actually a poorly resolved dt) in deuterated benzene because of the additional coupling to the hydroxyl proton (O<u>H</u>)

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Table 2 **^{1}H-NMR (300 MHz) of the methyl ester of hematin product 7 recorded in C_6D_6**

Chemical shift Product 7 methyl ester	**# H**		**Assignment, coupling constant (Hz)**
5.48 (5.55-5.44)	2H	m	H11, H12
5.39 (5.44 - 5.34)	3H	m	H6, H8, H9
5.30 (5.34 - 5.23)	1H	m	H5 $J_{5,6}$ = 10.7
4.56	1H	ddd	H13 $J_{13,-OH}$ = 2.7, $J_{12,13}$= 7.2 $J_{13,14}$ = 3.4
3.35	3H	s	OCH_3
2.92	1H	m	H15 $J_{15,16}$ = 4.9 and 6.0
2.90-2.80	2H	m	H10
2.77	2H	t	H7
2.70	1H	dd	H14 $J_{13,14}$ = 3.3, $J_{14,15}$ = 2.3
2.10	2H	t	H2
1.98	2H	q	H4
1.78	1H	d	CH-OH $J_{13,-OH}$ = 2.7
1.58	2H	p	H3
1.45 - 1.22	4H	m	H16, H17
1.22 - 1.12	4H	m	H18, H19
0.84	3H	t	H20

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Table 3 **^{1}H-NMR (300 MHz) of the methyl ester of hematin product 8 recorded in C_6D_6**

Chemical shift			**Assignment, coupling constant (Hz)**	
Product 8 methyl ester	**# H**			
5.50 (5.57-5.44)	2H	m	H11, H12	
5.44 - 5.35	3H	m	H6, H8, H9	
5.35 - 5.23	1H	m	H5	
4.29	1H	dt	H13	$J_{13,\text{-OH}} = J_{13,14} = 5.2$, $J_{12,13} = 7.6$
3.35	3H	s	$OC\underline{H_3}$	
2.92 - 2.80	2H	m	H10	
2.82	1H	m	H15	
2.77	2H	t	H7	
2.71	1H	dd	H14	$J_{13,14} = 5.2$, $J_{14,15} = 2.1$
2.10	2H	t	H2	
1.98	2H	q	H4	
1.83	1H	d	CH-O<u>H</u>	$J_{13,\text{-OH}} = 5.2$
1.58	2H	p	H3	
1.45 - 1.22	4H	m	H16, H17	
1.22 - 1.12	4H	m	H18, H19	
0.84	3H	t	H20	

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Table 4 **^{1}H-NMR spectrum (300 MHz) of product 1 methyl ester in C_6D_6**

Very little sample was available and decoupling was not carried out. Probable assignments are :

Chemical shift			**Assignment, coupling constant (Hz)**
Product 1 methyl ester	**# H**		
5.84	1H	dd	H12 $J_{11,12}$ = 5.5, $J_{12,13}$ = 15.7
5.65	1H	ddd	H13 $J_{12,13}$ = 15.7, $J_{13,14}$ = 7.1, J = 1.3
5.55-5.22	4H	m	H5, H6, H8, H9
3.98	1H	m	H11
3.35	3H	s	OCH_3
3.24	1H	dd	H14 $J_{13,14}$ = 7.2, $J_{14,15}$ = 4.3
2.85	1H	ddd (m)	H15
2.74	2H	t	H7
2.32-2.13	2H	m	H10
2.08	2H	t	H2
1.96	2H	q	H4
1.58	2H	p	H3
1.5-1.15	6H	m	H16,17,18,19
0.85	3H	t	H20 (partly obscured by impurity)

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Table 5 **^{1}H-NMR spectrum (400 MHz) of product 6 methyl ester in C_6D_6**

Chemical shift			**Assignment, coupling constant (Hz)**	
Product 6 methyl ester	**# H**			
5.48-5.43	2H	m	H11, H12	
5.43-5.33	3H	m	H6, H8, H9	
5.33-5.25	1H	m	H5	
4.39	1H	br. dt	H13	$J_{12,13}$ = 3.2, $J_{13,14}$ = 7.6 Hz
3.35	3H	s	OCH_3	
2.95-2.87	1H	m	H10a	
2.91	1H	dd	H14	$J_{13,14}$ = 7.7, $J_{14,15}$ = 4.3
2.83-2.79	1H	m	H10b	
2.77	2H	t	H7	
2.72	1H	dt	H15	$J_{14,15}$ =4.5, $J_{15,16}$ =6.9
2.09	2H	t	H2	
1.99	1H	s, br.d?	CH-OH	
1.98	2H	q	H4	
1.58	2H	p	H3	
1.42-1.31	4H	m	H16, H17	
1.31-1.17	4H	m	H18, H19	
0.86	3H	t	H20	

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Figure Legends

Figure 1. **GC-MS analysis of the stereochemistry of malic acid obtained by oxidative ozonolysis of hematin product 4.**

The configuration of the 11-hydroxyl of product 4 was determined by GC-MS analysis of the methyl ester menthyl carbonate (Me-MC) derivative of malic acid obtained by oxidative ozonolysis of the Me-MC derivative of product 4. The peaks from the GC-MS analysis are shown. A: authentic standards. B: product 4. The analyses were performed using a SPB-2250 fused silica column (30 m x 0.25 mm inner diameter). The oven temperature was programmed from 150° to 250°C at 10°C/min with a 20 min hold at the final temperature. The Me-MC derivatives eluted at 14.5 min and were detected in the EI mode by selected ion monitoring at *m/z* 138.

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FIGURE 1