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Biochemistry

A second pathway of leukotriene biosynthesis in porcine leukocytes

[arachidonic acid/(14*S*,15*S*)-*trans*-oxido-5,8-*cis*-10,12-*trans*-icosatetraenoic acid/lipoxygenase/reactive oxygen metabolites]

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ABSTRACT **Incubation of suspensions containing polymorphonuclear and eosinophilic leukocytes with arachidonic acid led to the formation of two pairs of diastereomeric 8,(15*S*)-dihydroxy-5,9,11,13-icosatetraenoic acids and two *erythro*-14,15-dihydroxy-5,8,10,12-icosatetraenoic acids. The structures were elucidated by ultraviolet spectroscopy and gas chromatography–mass spectrometric analysis of several derivatives of each compound, catalytic hydrogenation, oxidative ozonolysis with steric analysis of alcohols, and comparison to reference compounds prepared by chemical synthesis. Experiments with $^{18}O_2$ and $H_2{}^{18}O$ indicated that in all six compounds the hydroxyl group at C-15 was derived from molecular oxygen. Two of the diastereomeric 8,15-dihydroxy acids incorporated $H_2{}^{18}O$ at C-8, while the other two 8,15-dihydroxy products (also diastereomers) and the 14,15-dihydroxy compounds (geometric isomers) incorporated $^{18}O_2$ at C-8 and C-14, respectively, in addition to C-15. Two of the 8,15-dihydroxy acids are formed by reaction of water with an unstable allylic epoxide intermediate, (14*S*,15*S*)-oxido-5,8,10,12-icosatetraenoic acid; the two 14,15-dihydroxy acids are proposed to be formed by reaction of activated molecular oxygen with the same epoxide, which in turn originates via 15*S* oxygenation of arachidonic acid.**

Leukotrienes (abbreviated LT) are a family of biologically active lipids and lipid conjugates that are formed by leukocytes and that contain as a common structural feature a conjugated triene chromophore (1). These compounds can be formed from arachidonic acid and other polyunsaturated fatty acids by C-5 lipoxygenation to give (5*S*)-hydroperoxyicosatetraenoic acid [(5*S*)-HPETE], followed by enzyme-catalyzed dehydration to an unstable epoxide intermediate, LTA_4 (2). Other families of leukotrienes, arising from 12*S*- and 15*S*-lipoxygenation, however, are also mechanistically possible. Previously, compounds tentatively identified as 14,15- and 8,15-dihydroxy trienes were isolated after incubation of dihomo-γ-linolenic acid with human platelets (3); more recently, the 12- and 15-lipoxygenase-derived analogues of LTA_4 and LTC_4 have been chemically synthesized (4).

This report describes a pathway of leukotriene biosynthesis in porcine leukocytes that proceeds from 15*S*-lipoxygenation of arachidonic acid via an analogue of LTA_4, (14*S*,15*S*)-*trans*-oxido-5,8-*cis*-10,12-*trans*-icosatetraenoic acid, and which leads to the formation of 8,15- and 14,15-dihydroxy acids.

MATERIALS AND METHODS

Preparation of Porcine Leukocytes and Incubation Conditions. Porcine leukocytes were isolated from peripheral blood collected with one-sixth volume 6% dextran in isotonic saline with heparin, 6 units/ml of blood, and purified by centrifugation over lymphocyte separation medium (Bionetics, Kensington, MD), lysis with ammonium chloride, washing, and resuspension to 30×10^6 cells per ml in Earle's balanced salt solution (GIBCO). After preincubation at 37°C with shaking under a normal atmosphere for 3 min, the incubation was made 100 μM in arachidonic acid (NuChek Prep) and in some experiments 10 μM in ionophore A23187 (Calbiochem). After 5 min, incubations were terminated with 1.5 vol of methanol, and the supernatant was diluted and extracted with 2 vol of diethyl ether at pH 3.8, washed, and evaporated to dryness under reduced pressure. $^{18}O_2$ (99.7%) and $H_2{}^{18}O$ (97.3%, normalized) were from Miles Laboratories.

High-Pressure Liquid Chromatography (HPLC), Derivatizations, Steric Analysis, and Gas Chromatography–Mass Spectrometry (GC–MS). Analytical methods were as described in the legends to Figs. 1–3 and Table 1. C-values refer to retention time on GC relative to saturated straight-chain fatty acid methyl esters.

Synthesis of Reference Compounds. *(15S)-HPETE methyl ester* (1) *and methyl (8S,15S)-dihydroxy-5,11*-cis-*9,13*-trans-*icosatetraenoate* (2): Compounds 1 and 2 were prepared from the sodium salt of arachidonic acid essentially as described (5), using soybean lipoxygenase, type IV (Sigma), at enzyme concentrations of 1.2 and 60.0 μg of protein per ml, respectively. Satisfactory UV, HPLC, GC–MS (reduced product), and ^1H and ^{13}C NMR were obtained for 1. UV, HPLC, GC–MS, and stereochemical data for 2 were indistinguishable from those obtained for leukocyte compound II (see *Results*). ^1H NMR for 2 was in agreement with the published spectral data (5).

Methyl (14S,15S)-trans-oxido-5,8-cis-*10,12*-trans-*icosatetraenoate* (3): Compound 3 was prepared by the Corey procedure (4, 6) in *ca.* 10% yield from 1 by reaction with trifluoromethanesulfonic anhydride (Alfa) and dry pentamethylpiperidine at 30 mg/ml in Et_2O/CH_2Cl_2 (1:1, vol/vol) at −110°C and rapidly isolated as described. *UV*: λ_{max} in hexane = 278 nm, shoulders at 268 and 289 nm. *^1H NMR* (in completely deuterated benzene) (chemical shifts of resonances in ppm relative to tetramethylsilane; t, triplet; br, broad; m, multiplet; s, singlet): 0.88 (t, 3H, CH_3); 1.07–1.49 (br, CH_2 at C-16, 17, 18, and 19); 1.49–1.78 (m, CH_2 at C-3); 1.96 (m, CH_2 at C-4); 2.13 (t, coupling constant J = 7, CH_2-$COOCH_3$); 2.6–3.1 (m, 4H, CH_2 at C-7, H-14 and 15); 3.39 (s, 3H, OCH_3); 5.3–5.6 (m, 4H, H-5, 6, 8, and 13); 5.9–7.0 (m, 4H, H-9, 10, 11, and 12). *MS (probe)* (*m/z*, mass-to-charge ratio; M, parent ion): *m/z* 332 (M), 314

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Abbreviations: RP- and SP-HPLC, reverse-phase and straight-phase high-pressure liquid chromatography; LTA_4, (5*S*,6*S*)-*trans*-oxido-7,9-*trans*-11,14-*cis*-icosatetraenoic acid; LTB_4, (5*S*,12*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid; LTC_4, (5*S*)-hydroxy-(6*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid; HETE and HPETE, hydroxy- and hydroperoxy-icosatetraenoic acid; GC–MS, gas chromatography–mass spectrometry.

(M − 18), 301 (M − 31), 283 [M −(18 + 31)], 261 (M − 71), 218, 131, 129, 117, 105, and 91 (base). $[\alpha]_D^{27} \approx -8°$ (0.3 g/100 ml of cyclohexane + 0.2% Et_3N).

Methyl (8R,15S)- and (8S,15S)-dihydroxy-5-cis-9,11,13-trans-icosatetraenoates (**4, 5**): Compound **3** was converted to **4, 5, 6,** and **7** by exposure to dimethoxyethane/0.1 mM HCl (1:1, vol/vol) at 1 mg/ml for 5 min, followed by CH_2Cl_2 extraction and reverse-phase (RP)- and straight-phase (SP)-HPLC. RP-HPLC analysis showed **4, 5, 6,** and **7** as the only conjugated triene-containing products, in a ratio of *ca.* 3:3:1:1. HPLC, UV, GC–MS, and stereochemical data for **4** and **5** are as described for leukocyte products **I** and **IV**, respectively (*Results*). *^{1}H NMR of 4 and 5, in $(C^2H_3)_2C{=}O$*: 0.88 (t, 3H, CH_3); 1.29 (br, 6H, CH_2 at C-17, 18, and 19); 1.56–1.78 (m, H-3); 2.31 (m, 4H, CH_2COOCH_3 and H-7); 3.62 (s, 3H, OCH_3); 3.96–4.17 (m, 2H, CH-OH at C-8 and 15); 5.46 (m, 2H, H-4 and 6); 5.61 (m, 2H, probably H-9 and 14); 6.20 (br s, 4H, probably H-10–13; see ref. 7 for the vinyl region of β-eleostearic acid).

Threo- *and* erythro-*methyl 14,(15S)-dihydroxy-5-cis-8,-10,12-icosatetraenoates* (**6** *and* **7**) *and icosanoates* (**6a** *and* **7a**): Compounds **6** and **7** showed λ_{max} in MeOH = 272.5 nm, shoulders at 263 and 282 nm. Thermal breakdown on GC analysis [1% or 3% SP2100 (Supelco)] precluded satisfactory analysis of the unsaturated compounds by GC–MS; after hydrogenation, satisfactory GC–MS data were obtained (see below). The (±)-*threo*-icosanoate (**6a**) was prepared from **8**, (±)-*cis*-14,15-oxido-5,8,11-*cis*-icosatrienoic acid (ref. 8) by the sequence: (*i*) methylation with CH_2N_2, (*ii*) hydrogenation with Pd/C at 1 atmosphere in tetrahydrofuran, and (*iii*) perchlorate-catalyzed ring opening in dimethoxyethane/water (1:1, vol/vol). The (±)-*erythro*-icosanoate (**6b**) was prepared from **6a** via the *trans*-epoxide by the sequence (9): (*i*) reaction of **6a** with 15% (wt/vol) HBr in acetic acid, (*ii*) reflux with 0.5 M methanolic KOH, (*iii*) treatment with CH_2N_2, and (*iv*) ring opening as above. For **8**, GC–MS (Me ester, C-20.9, SP2100) and ^{1}H and ^{13}C NMR (which showed C-15 and 14 at 56.41, 57.28 ppm, in C^2HCl_3 at 77.00) were in accord. The saturated *cis*- and *trans*-epoxides showed (as Me esters) C-21.6 and 21.4, respectively [3% OV-1 (Applied Science)], with *m/z* 340 (M), 322 (M − 18), 309 (M − 31), 291 (M − 18 + 31), 269 (M − 71). Compounds **6a** and **6b** showed (as the Me, Me_3Si derivatives) C-23.6 and 23.8 (3% OV-1), with *m/z* 502 (M), 487 (M − 15), 471 (M − 31), 431 (M − 71), 402, 387, 329, 173 (base), and 129, and also showed the expected relative mobilities on thin-layer plates treated with boric acid (10) [R_F (2% MeOH in $CHCl_3$) = 0.76 and 0.59, respectively].

RESULTS

Analysis of the ether extracts obtained after incubation of porcine leukocytes with arachidonic acid by RP-HPLC revealed six major peaks corresponding in retention time to dihydroxy acids and absorbing at 280 nm (Fig. 1). Peak 5 consisted of a mixture of LTB_4 and (5S,12S)-6,8,10,14-icosatetraenoic acid (**11**) (formed by incorporation of two atoms of ^{18}O from molecular oxygen) in a typical ratio of 1 to 2.5. RP-HPLC peak 3 also consisted mainly of a 5,12-dihydroxy acid. In contrast, RP-HPLC peaks 1 and 2, when analyzed by SP-HPLC, were each found to consist mainly of an 8,15-dihydroxy-icosatetraenoic acid, termed compounds **I** and **II**, respectively. RP-HPLC peak 4 contained two such 8,15-dihydroxy acids, defined here as compounds **III** and **IV**, in addition to larger quantities of a 5,12-dihydroxy acid. Two 14,15-dihydroxy acids, termed compounds **V** and **VI**, eluted in RP-HPLC peak 6. Although small amounts of several other 5,12-dihydroxy acids were identified in some of the RP-HPLC peaks, the present report concerns the structures and possible mode of formation of the 8,15- and 14,15-dihydroxy acids, compounds **I–VI**. UV, GC, and HPLC data are tabulated for these compounds in Table 1.

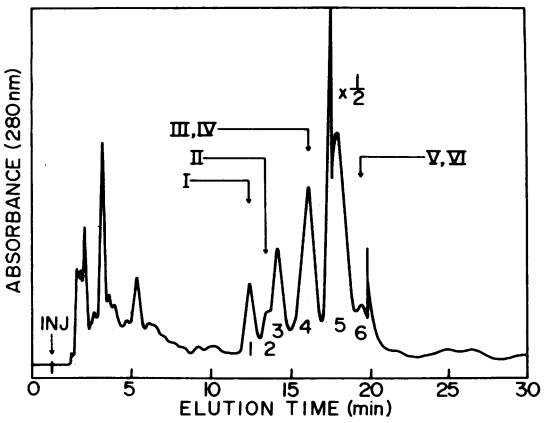

FIG. 1. RP-HPLC analysis of the ether extract obtained after incubation of porcine leukocytes with arachidonic acid (100 μM), using an analytical C_{18} column (Waters), 3.9 mm × 30 cm, with mobile phase 65% methanol/35% water/0.01% acetic acid (vol/vol), at 2 ml/min and 2000 pounds/inch^2 (14 MPa). The proportions of the six main RP-HPLC peaks were fairly constant in over 20 different experiments. The eluate was monitored at 280 nm with a model 450 variable-wavelength detector (Waters). INJ, injection. In larger scale incubations, the dihydroxy acid fraction obtained from a semipreparative C_{18} column (7.8 mm × 30 cm) was collected and rechromatographed on the analytical C_{18} column. Compounds I–IV above were each 8,(15S)-dihydroxy acids, while compounds V and VI were 14,15-dihydroxy acids. RP-HPLC peaks 1–6 were further analyzed as Me esters by SP-HPLC (Table 1). After incubations using [^{14}C]arachidonic acid, compounds I–VI were each found to be ^{14}C-labeled.

Structures of Compounds I and IV. The mass spectrum of the Me, Me_3Si derivative of **I** showed informative ions at *m/z* 494 (M), 479 (M − 15; loss of CH_3·), 463 (M − 31; loss of $^{\cdot}OCH_3$), 423 (M − 71; loss of $^{\cdot}[CH_2]_4CH_3$), 404 (M − 90; loss of Me_3SiOH), 353 (M − 141; loss of $^{\cdot}CH_2$—CH=CH—$[CH_2]_3COOCH_3$), 263 (353 − 90), 243 (Me_3SiO^+=$CHCH_2CH$=CH—$[CH_2]_3COOCH_3$), and 173 (Me_3SiO^+=CH—$[CH_2]_4CH_3$), establishing the positions of the hydroxyl groups at C-8 and C-15. Other ions in the spectrum included *m/z* 217 (Me_3SiO^+=CH—CH=CH—$OSiMe_3$), 199 ($^+$CH=CH—CH($OSiMe_3$)—$[CH_2]_4CH_3$), 191 (Me_3SiO^+=CH—$OSiMe_3$), and 129 (base) and 73. These assignments were supported by spectra recorded on the Et ester and Me_3Si ester Me_3Si ether derivatives, the Me ester [$^2H_9$]Me_3Si ether derivative, and on the Me ester Me_3Si ether derivative of the PtO_2 hydrogenated compound, which showed major fragment ions at *m/z* 173, 245, and 359. Mass spectrometric analysis of compound **I** isolated after incubation of cells under an atmosphere of $^{18}O_2$ and N_2 showed that 70% of the molecules had incorporated an atom of ^{18}O at C-15 (Fig. 2 *Upper*). Similar ^{18}O incorporation of 62% was found for the fragment ions containing both hydroxyl groups, *m/z* 355 and 353. In accord, the ion at *m/z* 243 corresponding to C-1 to C-8 was retained as such. A similar experiment conducted in $H_2{}^{18}O$ indicated 85% incorporation of ^{18}O from $H_2{}^{18}O$ at C-8, but none at C-15. Oxidative ozonolysis (Fig. 3) showed that compound **I** had the *R* configuration at C-8 and the *S* configuration at C-15 and, moreover, that the Δ^5 *cis* double bond of arachidonic acid was retained in its original position. Comparison of compound **I**-Me with methyl (8*R*,15*S*)-dihydroxy-5-*cis*-9,11,13-*trans*-icosatetraenoate showed that the two compounds cochromatographed on both RP- and SP-HPLC and on GC (3% SP2100); the two compounds also had identical UV and mass spectra. On the basis of UV and GC data [reported for 5,12-dihydroxy-6,8,10-*trans*-14-*cis*-icosatetraenoate, λ_{max} in MeOH = 268 nm, C-24.8 (1%

Table 1. Analytical data for compounds I–VI

Compounds	UV spectrometric data*: λ_{max}; hypso- and bathochromic shoulders, nm	GC data (3% SP2100 or OV-1), C-value		SP-HPLC retention times† of Me esters, min
		Me, Me_3Si	Me, Me_3Si/catalytic $-H_2$	
I, IV	269; 260, 280‡	24.9	24.1 (I–IV)	11.5, 15.0
II, III	268.5; 259, 279‡	23.6		9.5, 12.3
V	272.5; 263, 283	23.9	23.8 (V, VI)§	10.8
VI	270: 261, 281	24.9		11.4

* UV spectra recorded in methanol on a Beckman 25 spectrophotometer, calibrated with LTB_4.

† Waters μPorasil, silicic acid, 10-μm particles, hexane/isopropyl alcohol, 100:3 (vol/vol), 2 ml/min, 100 pounds/inch^2 (0.4 MPa). V-Me and VI-Me could be better resolved by using hexane/isopropyl alcohol, 100:2 (vol/vol), 0.5 ml/min (separation factor, VI/V = 1.10).

‡ UV spectra for I and IV were identical; similarly for II and III. I and IV characteristically showed a more deeply defined bathochromic shoulder than did II and III.

§ Synthetic *threo*-14,15-dihydroxy-icosanoate Me, Me_3Si derivative had a C-value of 23.6 under these conditons (3% OV-1, 215°C); the *erythro* derivative gave C-23.8.

SE-30) (14)], synthetic comparisons, and proposed mechanism of formation, it would seem likely that the triene system in compound I is all-*trans*. Compound I is thus assigned as (8*R*,15*S*)-dihydroxy-5-*cis*-9,11,13-*trans*-icosatetraenoic acid.

UV,GC-MS, and ^{18}O-labeling data for compound IV were identical to those for I. Steric analysis of compound IV (Fig. 3) revealed that IV was an epimer of I at C-8, with the *S* configuration at both C-8 and C-15. Compound IV-Me was compared by RP- and SP-HPLC, GC–MS, and UV spectroscopy to synthetic methyl (8*S*,15*S*)-dihydroxy-5-*cis*-9,11,13-*trans*-icosatetraenoate and found to be identical. Compound IV is thus assigned as (8*S*,15*S*)-dihydroxy-5-*cis*-9,11,13-*trans*-icosatetraenoic acid.

Structures of Compounds II and III. The mass spectrum of compound II showed fragmentation very similar to that described for compound I, indicating that compound II was also an 8,15-dihydroxy-5,9,11,13-icosatetraenoate. Differences in ion intensities and in UV spectra and GC retention times made it likely that compounds I and II were geometric isomers within the triene. Analysis of compound II after incubation of cells with $^{18}O_2$ (Fig. 2 *Lower*) showed that II incorporated ^{18}O at both C-8 and C-15. Ions were found at *m/z* 175, 245, and 357 (*m/z* 357:355:353,47:41:12), with additional high-mass ions at *m/z* 498 (M), 483 (M − 15), and 467 (M − 31). This agreed with the data obtained on compound II after incubation with $H_2{}^{18}O$, which showed no evidence for incorporation of ^{18}O into either hydroxyl group. Steric analysis of compound II indicated that it had the *S* configuration at both C-8 and C-15, and that Δ^5 was retained. Compound II-Me and methyl (8*S*,15*S*)-dihydroxy-5-*cis*-9-*trans*-11-*cis*-13-*trans*-icosatetraenoate, prepared by double dioxygenation of arachidonic acid by soybean lipoxygenase, were indistinguishable by UV spectroscopy, RP- and SP-HPLC, and GC–MS. However, the ability of these methods to distinguish between different *cis–trans* arrangements in the conjugated triene is uncertain. Thus, while it is clear that compound II is an (8*S*,15*S*)-dihydroxy-5-*cis*-9,11,13-icosatetraenoic acid, the exact geometry of the conjugated triene is not specified. The all-*trans* geometry, however, is excluded on the basis of chromatographic separation from the synthetic (8*S*,15*S*)-all-*trans* isomer.

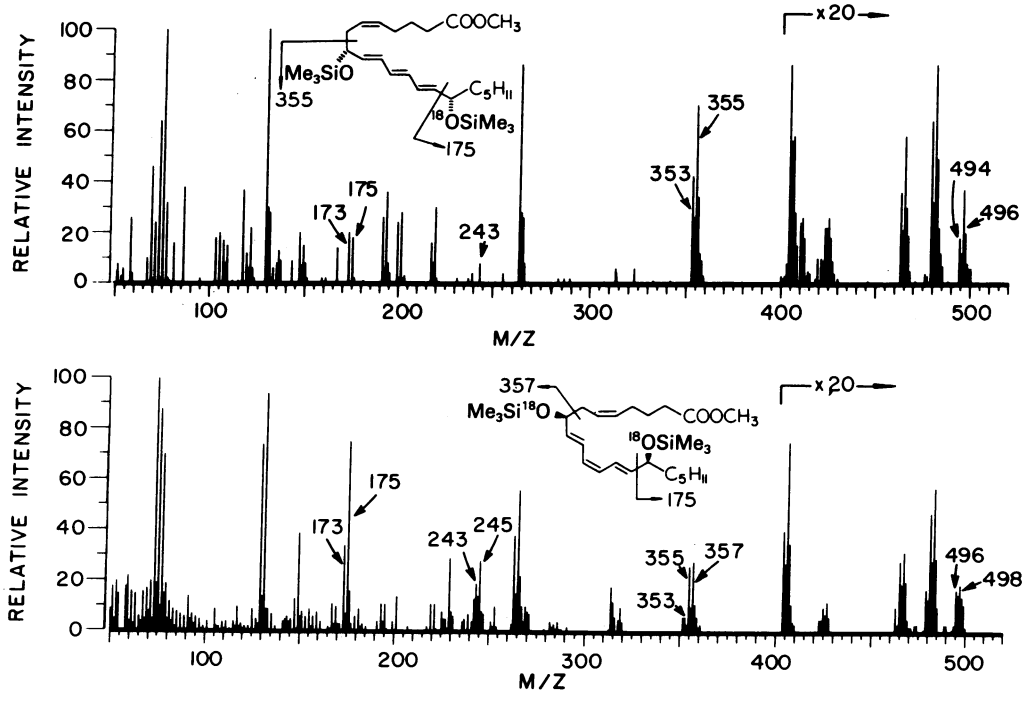

FIG. 2. Mass spectrum of molecular oxygen ^{18}O-labeled Me, Me_3Si derivatives of compound I (*Upper*) and compound II (*Lower*). GC-MS was performed on HP5980, Riber 10-10, or LKB 9000 mass spectrometers, using 1–3% SP2100 on Supelcoport at 220–235°C. Electron energy was 70 eV. Diazomethane or diazoethane was used for esterification, and pyridine and *N,O*-bis(Me_3Si) trifluoroacetamide or [$^2H_9$]bis(Me_3Si)acetamide at room temperature for 15 min for silylation.

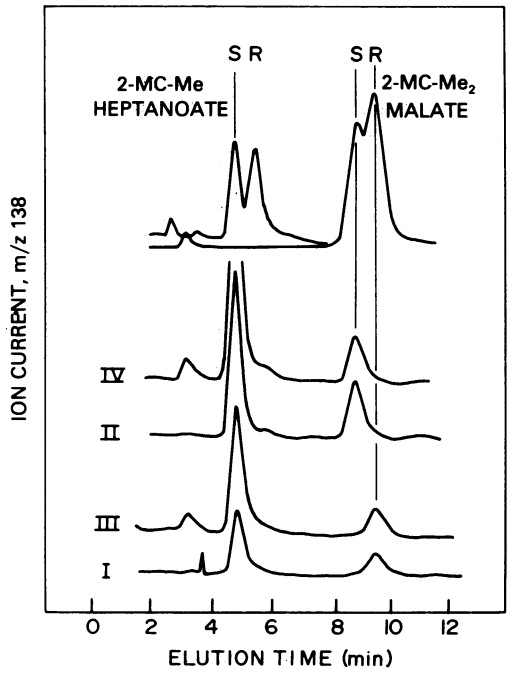

FIG. 3. Steric analysis of the four 8,15-dihydroxy acids, compounds I–IV. The bis(menthoxycarbonyl) (MC) derivatives were prepared as described (12) and repurified by RP-HPLC [C_{18} column, methanol/water, 970:30 (vol/vol), retention vol = 13 ml] with UV detection at 280 nm. Oxidative ozonolysis was performed as described (13), with analysis by GC–MS on a 6-foot (1.8-m) 3% QF-1 (Supelco) column at 165°C. Standards were prepared from *RS*-malic acid, (15*RS*)-HETE, and (15*S*)-HETE. HETE, hydroxyicosatetraenoic acid.

Compound III-Me showed identical UV, GC–MS, and ^{18}O-labeling data to compound II, but had the *R* configuration at C-8. Compound III is thus described as (8*R*,15*S*)-5-*cis*-9,11,13-icosatetraenoic acid, an epimer of compound II at C-8; as with II, the all-*trans* geometry is excluded for the conjugated triene.

Structures of Compounds V and VI. The mass spectra of the Me, Me_3Si derivatives of compounds V and VI were very similar, with ions at *m/z* 494 (M), 479 (M − 15), 463 (M − 31), 423 (M − 71), and 404 (M − 90) and prominent fragmentations at *m/z* 394 (M − 100; by rearrangement), 321 (M − 173; loss of $Me_3SiO^+ {=}CH{-}[CH_2]_4CH_3$), and 173 (base). These assignments were supported by spectra recorded on the Et and Me_3Si ester Me_3Si ether derivatives. After catalytic hydrogenation, V and VI Me, Me_3Si derivatives were compared to synthetic *threo* and *erythro* icosanoates (see *Materials and Methods*) on 3% OV-1; both V and VI clearly chromatographed with the latter standard. Assuming that the Δ^5-*cis* double bond remains unchanged, the mass spectral and UV data indicate that V and VI are isomeric *erythro*-14,15-dihydroxy-5-*cis*-8,10,12-icosatetraenoic acids. Despite similar UV maxima, the UV spectrum of V differed from that of the synthetic 14,(15S)-dihydroxy-5-*cis*-8,10-12-icosatetraenoates, prepared from acid/water treatment of (14*S*,15*S*)-oxido-5,8-*cis*-10,12-*trans*-icosatetraenoate, in showing more deeply defined shoulders. RP-HPLC comparisons revealed that compounds V-Me and VI-Me eluted between the synthetic compounds. Finally, compounds V and VI were readily analyzable by GC employing SP2100, whereas the synthetic diastereomers were not. Additional evidence for a difference between compounds V and VI and the synthetic standards was obtained from cell incubations performed under an atmosphere of $^{18}O_2$. Measurement of ion ratios in the Me, Me_3Si derivatives at *m/z* 175 and 173 showed 78% and 75% ^{18}O incorporation at C-15 in compounds V and VI, respectively. The ^{18}O incorporation in the fragment ions containing C-14, *m/z* 323 and 321, was measured as 69% and *ca.* 55% (weak signal) for compounds V and VI. Additional experiments with selected ion monitoring of the M − 15 ion confirmed the incorporation of two atoms of ^{18}O from different molecules of oxygen into V and VI. Experiments with $H_2{}^{18}O$ were compatible with these findings.

DISCUSSION

Porcine leukocytes converted arachidonic acid to a complex mixture of leukotrienes. Products derived from LTA_4 were present and also several dihydroxy leukotrienes dervied via C-15 lipoxygenation of arachidonic acid (compounds I–VI). MS analysis of cell incubations performed under an atmosphere of $^{18}O_2$ or in $H_2{}^{18}O$ indicated that the oxygen in the C-15 hydroxyl group of compounds I–VI was derived from molecular oxygen. The four 8,15-diols each had the *S* configuration at C-15, compatible with their formation by C-15 lipoxygenation. The absolute configuration of the 14,15-diols (compounds V and VI) was not determined, although the relative configuration was shown to be *erythro*, a significant finding in relation to their possible mechanism of formation. Compounds V and VI differed from the 14,15-*threo* and *erythro*-dihydroxy diastereomers formed by hydrolysis of the *trans*-14,15-oxido-5,8,10,12-icosatetraenoate. The latter compounds were prepared by chemical synthesis (see *Materials and Methods*) and found to be thermally unstable on GC. It is very likely that they were formed in small amounts in these incubations but simply were not detected by GC–MS.

Compounds I and IV were diastereomeric (8*R*)- and (8*S*,15*S*)-dihydroxy acids that incorporated oxygen from water at C-8. They were identical to the major products of hydrolysis of synthetic (14*S*,15*S*)-*trans*-oxido-5,8-*cis*-10,12-*trans*-icosatetraenoic acid, the 14,15-oxido analog of LTA_4. They are analogous to the 5,12-dihydroxy acids formed nonenzymatically from LTA_4 via acid-catalyzed formation of a carbonium ion (2). It is of interest that no 8,15-dihydroxy acid strictly analogous to LTB_4 was detected in these incubations.

Compounds II, III, V, and VI were found to incorporate ^{18}O from molecular oxygen at both hydroxyl groups. The overall resemblance of these compounds to compounds I and IV, and to 14,(15S)-oxido-5,8,10,12-icosatetraenoate suggests that they may also originate from the epoxide. At the outset of this study, compound II was presumed to be formed by double lipoxygenation of arachidonic acid, because it incorporated ^{18}O from molecular oxygen into, and had the *S* configuration at, each hydroxyl group, and because double lipoxygenation is known to occur in arachidonate-stimulated cell preparations, leading to the formation of 5,12- and 5,15-dihydroxy acids (11,15). In addition, incubation of soybean lipoxygenase with arachidonic acid leads to the formation of an 8,15-dihydroxy-icosatetraenoic acid by double lipoxygenation (5), and leukocyte compound II could not be distinguished from this latter product as the methyl ester by any of the chromatographic or spectroscopic methods at our disposal. However, other findings obtained upon further study would mitigate against formation of compound II by double lipoxygenation. These include the identification of compound III, which is an epimer of compound II at C-8, whereas all known lipoxygenase transosformations are stereospecific, the absence of significant amounts of 8-HETE in these incubations (12-HETE/5-HETE, *ca.* 50% each, with 8-,9-,11-, and 15-HETEs each ≤0.5%) and finally, the finding of *erythro*-14,15-dihydroxy-icostatetraenoates that also show incorporation of two atoms of ^{18}O from molecular oxygen. Although double lipoxygenation is not excluded by the present study as a mechanism for the formation of compound II, it would seem distinctly unlikely.

The possibility that reactive oxygen metabolites such as su-

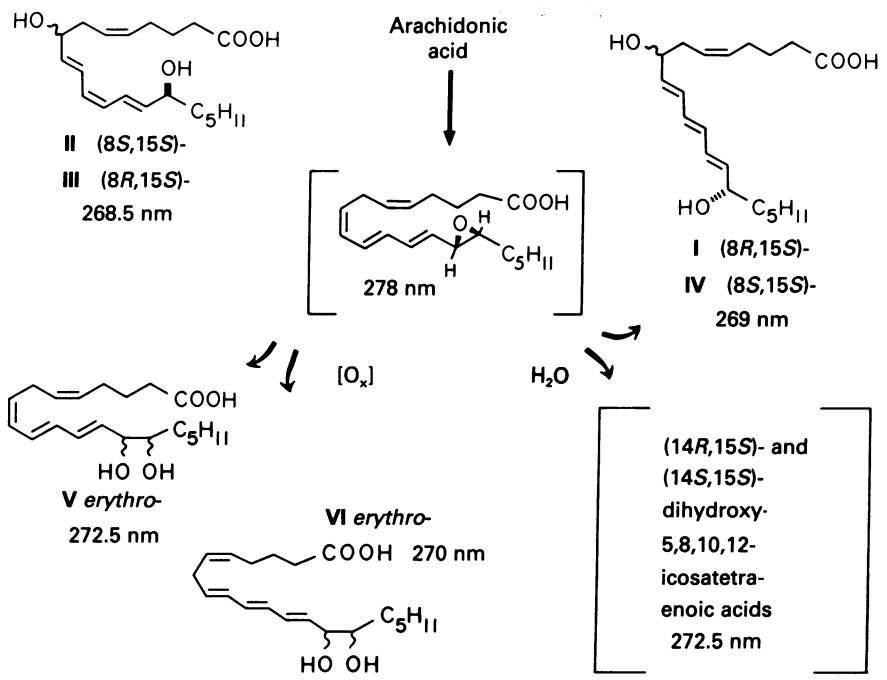

FIG. 4. Hypothetical scheme to account for the formation of compounds **I**, **IV**, **V**, and **VI** via an epoxide intermediate. λ_{max} values are given. The mechanism of formation of compounds **II** and **III** is not known. The geometry of the conjugated triene is unassigned in compounds **II**, **III**, **V**, and **VI**; the absolute configuration is not specified for the two *erythro* diols, compounds **V** and **VI**. [O_x] denotes reaction with an unspecified oxygen metabolite. Compounds in brackets were not isolated from the cells.

peroxide anion or its derivatives, hydroxyl radical or singlet oxygen, might react with the epoxide intermediate to form compounds **II**, **III**, **V**, and **VI** appears attractive for several reasons. Reactive oxygen metabolites are known to be generated in appreciable quantities in the polymorphonuclear leukocytes of several species as part of the "respiratory burst" after exposure to a variety of stimuli, and they are an essential part of the microbicidal mechanisms of this cell (16). Attack on the epoxide intermediate by either hydroxyl radical or superoxide anion by direct nucleophilic attack or by electron transfer (17) could theoretically account for the existence of compounds **II**, **III**, **V**, and **VI** and also for the different triene geometry of **II** and **III** as compared to the all-*trans* geometry of the carbonium ion-derived products **I** and **IV**. Neutralization of the carbonium ion obtained from protonation of the epoxide would result in racemization at C-14 and lead to the formation of diastereomeric 14,15-diols. Compounds **V** and **VI** were not diastereomers. Both had the *erythro* configuration about the 14,15-diol unit. The absence of a diastereomeric relationship between compounds **V** and **VI** and the fact that they are both *erythro* would be compatible with a reactive oxygen-mediated opening of the *trans* epoxide ring, with inversion of configuration at C-14. The reason for, and exact nature of, the geometric isomerism of compounds **V** and **VI** remains to be determined. The stereochemical data do not exclude the possibility that the formation of one or both compounds could be enzymatic, although they are formed in only small amounts. However, these possibilities will require further detailed investigation.

A hypothetical scheme for the formation of compounds **I–VI** is given as Fig. 4. Our structural analysis of compounds **I** and **IV** establishes the existence of the epoxide. The available evidence for the formation of **II** and **III** from the epoxide is less compelling than that for **V** and **VI**. Recently, some 8,15- and 14,15-dihydroxy compounds were identified in preparations of human leukocytes, although the stereochemistry and origins of the hydroxyl oxygens were not determined (18, 19), thus making direct comparison with the compounds identified in this study difficult. Additonal studies in our laboratory have revealed that the formation of compounds **I–VI** also occurs in mouse peritoneal cells and human leukocytes isolated from a patient with mastocytosis. Further studies are necessary to clarify the biological activities and the mechanism of biosynthesis of these compounds, and to investigate the possibility of other products formed through this pathway.

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