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Biochemistry

On the stereochemistry and biosynthesis of lipoxin B

(arachidonic acid/conjugated tetraenes/lipoxygenase interaction products)

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ABSTRACT **Lipoxin B (LXB) was prepared by incubation of (15*S*)-15-hydroperoxy-5,8,11-*cis*-13-*trans*-icosatetraenoic acid (15-HPETE) with human leukocytes. Comparison with a number of trihydroxyicosatetraenes prepared by total synthesis showed that biologically derived LXB is (5*S*,14*R*,15*S*)-5,14,15-trihydroxy-6,10,12-*trans*-8-*cis*-icosatetraenoic acid. Two isomers of LXB were identified by using an improved isolation procedure. These compounds were shown to be (5*S*,14*R*,15*S*)-5,14,15-trihydroxy-6,8,10,12-*trans*-icosatetraenoic acid (8-*trans*-LXB) and (5*S*,14*S*,15*S*)-5,14,15-trihydroxy-6,8,10,12-*trans*-icosatetraenoic acid [(14*S*)-8-*trans*-LXB]. Experiments with $^{18}O_2$ showed that formation of LXB and its two isomers occurred with incorporation of molecular oxygen at C-5 but not at C-14. These results together with the finding that (15*S*)-hydroxy-5,8,11-*cis*-13-*trans*-icosatetraenoic acid (15-HETE) is a precursor of LXB compounds in activated leukocytes suggest that 15-hydroxy-5,6-epoxy-7,9,13-*trans*-11-*cis*-icosatetraenoic acid or its equivalent is a common intermediate in the biosynthesis of LXB and its two isomers.**

The formation of oxygenated derivatives of arachidonic acid is associated with the activation of a variety of cell types and with various aspects of inflammatory responses (for review, see ref. 1). Therefore, events leading to the release of arachidonic acid, its oxygenation, and regulation of the products formed are of interest. Of the major lipoxygenases observed in mammalian tissues (i.e., 5-, 12-, and 15-lipoxygenase), the 5- and 15-lipoxygenases are highly active routes in human leukocytes (2). The major products of these enzymes are (5*S*)-5-hydroperoxy-6-*trans*-8,11,14-*cis*-icosatetraenoic acid (5-HPETE) and (15*S*)-15-hydroperoxy-5,8,11-*cis*-13-*trans*-icosatetraenoic acid (15-HPETE), respectively. 5-HPETE and 15-HPETE are both subject to further transformations, which lead to the production of mono-, di-, and trihydroxy acids, including leukotrienes (1). In addition to either reduction or epoxidation, other metabolic fates of HPETEs exist. For example, they may serve as substrates for double dioxygenation. Thus, each mono-HPETE may be subject to several metabolic fates. The regulation of each of these metabolic events as well as the identity of the products formed remain to be fully elucidated.

In view of the multiple metabolic fates of lipoxygenase products we have studied the consequences of initial lipoxygenation at the C-15 position of arachidonic acid as well as interactions between the 5- and 15-lipoxygenase pathways. Recently, we described the isolation of a series of oxygenated derivatives of arachidonic acid and proposed the name lipoxins for this series (3, 4). When added to human neutrophils, lipoxin A, 5,6-(15*S*)-trihydroxy-7,9,11,13-icosatetraenoic acid (LXA), stimulates superoxide anion generation without provoking aggregation (4). Human natural killer cells exposed to either LXA or lipoxin B (LXB) are unable to provoke target cell lysis (5). In addition, LXA also provokes contraction of parenchymal strips and stimulates microvascular changes (6). In this paper, we report studies on the biosynthesis of LXB, the isolation of two of its natural isomers, and the assignment of the stereochemistry of these compounds.

MATERIALS AND METHODS

Materials and procedures employed were essentially the same as described (3, 4) unless indicated. (5*S*,15*S*)-5,15-Dihydroxy-6,13-*trans*-8,11-*cis*-icosatetraenoic acid (5,15-DiHETE) and (5*S*,15*S*)-5,15-dihydroperoxy-6,13-*trans*-8,11-*cis*-icosatetraenoic acid (5,15-DiHPETE) were prepared as in ref. 7. Human leukocytes were prepared from peripheral blood of healthy donors by means of dextran sedimentation followed by hypotonic lysis. The preparations consisted of mixed populations of leukocytes (neutrophils, eosinophils, etc.) in which the neutrophil contribution represents >90%. Cells were washed twice and suspended in Dulbecco's phosphate-buffered saline at pH 7.45 (4). Human platelets were prepared as in ref. 8.

Purification of LXB (Compound III), Compound I, and Compound II and ^{18}O Labeling. Leukocytes (50–100 ml of 30 $\times 10^6$ cells per ml) were warmed to 37°C. 15-HPETE (50 μM) and A23187 (2.5 μM) were added simultaneously in EtOH (1%, vol/vol) and incubations were continued for 15 min. In other incubations, (15*S*)-15-hydroxy-5,8,11-*cis*-13-*trans*-icosatetraenoic acid (15-HETE) (50 μM) and A23187 (2.5 μM) were added simultaneously to the cells and incubations were continued for 15 min at 37°C. All incubations were stopped with addition of MeOH (2 vol). Procedures for extraction, silicic acid chromatography, and TLC were as described (4). Briefly, ethyl acetate fractions from silicic acid chromatography were evaporated, dissolved in MeOH, treated with CH_2N_2, and subjected to TLC with added methyl 11,12,15-trihydroxy-5,8,13-[1-^{14}C]icosatrienoate and methyl 11,14,15-trihydroxy-5,8,12-[1-^{14}C]icosatrienoate. Following TLC the zone containing methyl esters exhibiting tetraene UV was eluted with MeOH (cf. ref. 9). Samples were treated again with CH_2N_2 and injected onto a reversed-phase (RP)-

Abbreviations: 15-HPETE, (15*S*)-15-hydroperoxy-5,8,11-*cis*-13-*trans*-icosatetraenoic acid; 15-HETE, (15*S*)-15-hydroxy-5,8,11-*cis*-13-*trans*-icosatetraenoic acid; 5,15-DiHETE, (5*S*,15*S*)-5,15-dihydroxy-6,13-*trans*-8,11-*cis*-icosatetraenoic acid; 5,15-DiHPETE, (5*S*,15*S*)-5,15-dihydroperoxy-6,13-*trans*-8,11-*cis*-icosatetraenoic acid; LXA, lipoxin A, 5,6-(15*S*)-trihydroxy-7,9,11,13-icosatetraenoic acid; synthetic I, (5*S*,14*R*,15*S*)-5,14,15-trihydroxy-6,10,12-*trans*-8-*cis*-icosatetraenoic acid; synthetic II, (5*S*,14*R*,15*S*)-5,14,15-trihydroxy-6,8,10,12-*trans*-icosatetraenoic acid; synthetic III, (5*S*,14*S*,15*S*)-5,14,15-trihydroxy-6,8,10,12-*trans*-icosatetraenoic acid; synthetic IV, (5*S*,14*S*,15*S*)-5,14,15-trihydroxy-6,10,12-*trans*-8-*cis*-icosatetraenoic acid; Me_3Si, trimethylsilyl; THETE, trihydroxyicosatetraenoic acid; RP-HPLC, reversed-phase HPLC; LXB, lipoxin B (compound III); (14*S*)-LXB, synthetic IV.

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HPLC column (Altex Ultrasphere ODS, particle diameter = 5 μm, 10 mm inner diameter × 25 cm) eluted with MeOH/H_2O, 65:35 (vol/vol), at 3 ml/min (UV detector set at 301 nm). Materials (Fig. 1) were collected separately and rechromatographed on RP-HPLC columns before further analysis. To achieve further separation between compounds II and III, materials obtained from the preceding HPLC were injected onto a Nucleosil C_{18} column (4.6 mm × 25 cm, 5 μm) with MeOH/H_2O, 60:40 (vol/vol), at 1.0 ml/min.

The incorporation of ^{18}O from molecular oxygen into trihydroxytetraene compounds was essentially as described (10). In these incubations, either 15-HPETE (50 μM) with A23187 (2.5 μM) or 15-HETE (50 μM) with A23187 (2.5 μM) was added to leukocytes maintained in an atmosphere enriched with ^{18}O, and the compounds were purified (*vide supra*).

In other experiments, human platelets (1×10^9 cells per ml) suspended in 25 ml of phosphate-buffered saline at pH 7.45 were incubated with various icosanoids for 20 min at 37°C. Incubations were stopped with MeOH and extractions and purifications were performed as above.

GLC/mass spectrometry was performed with a Dani 3800 gas chromatograph HR PRV-2CH equipped with a fused silica capillary column (20 m × 0.32, Orion) SE-30 and 7070E VG analytical mass spectrometer. The current was set at 22.5 eV, with an oven temperature of 230°C. Mass spectra were taken by utilizing selection ion monitoring at either *m/e* 203, 205, or 173 for various derivatives. Synthetic materials were prepared as in ref. 11.

RESULTS

Human leukocytes transform 15-HPETE to a series of C20:4-derived compounds that contain four conjugated double bonds. The formation of these compounds was increased when the ionophore A23187 (5 μM) was added to the cells (3, 4).

To further examine the formation of LXB, 15-HPETE and A23187 were added simultaneously to human leukocytes and the 5,14,15-trihydroxytetraene compounds were isolated and characterized. Following ether extraction, silicic acid chromatography, and TLC (cf. ref. 9), samples were injected onto RP-HPLC columns. In addition to the 5,14,15-trihydroxyicosatetraenoic acid (5,14,15-THETE) described previously (4), two other components showing absorption at 301 nm were obtained (n = 16) (Fig. 1*A*). Material eluting in peak I was collected and rechromatographed in the same HPLC system. To achieve further resolution, material eluting under peaks labeled II and III was pooled and injected onto a second RP-HPLC system (Fig. 1 *A* and *B*). Virtually identical HPLC profiles were obtained when leukocytes were incubated with 15-HETE (50 μM) and A23187 (2.5 μM) (n = 15). In contrast, leukocytes incubated with 15-HETE (50 μM), in the absence of A23187, did not generate these materials (n = 4).

The UV spectrum of material eluting in peak I (compound I) showed a triplet of adsorption bands at 288, 301, and 316 λ_{max}^{MeOH} (Fig. 1 *A* and *C*). GC/MS analysis of the trimethylsilyl (Me_3Si) derivative of this material showed a compound with a C value of 28.0, which is greater than the value observed for LXB (cf. ref. 4). The prominent ions in its mass spectrum were at *m/e* 173 [base peak, Me_3SiO^+=CH—$(CH_2)_4$—CH_3], 203 [Me_3SiO^+=CH—$(CH_2)_3$—$COOCH_3$], 289 (379 − 90), and 379 (*M*-203). Ions of lower intensities were at *m/e* 582 (*M*), 492 (*M*-90; loss of Me_3SiOH), 482 [*M*-100; rearrangement followed by loss of O=HC—$(CH_2)_4$—CH_3], 409 (*M*-173), 402 [*M*-(2 × 90)], 329, 319 [*M*-(173 + 90)], and 307 (*M*-275). The presence of these ions suggests that compound I was a methyl icosatetraenoate with three hydroxyl groups located at C-5, C-14, and C-15. The location of the vicinal hydroxyl groups was supported by the high intensity of the ion at *m/e* 173 (base peak) (also see Fig. 2).

The material eluting in peak II (compound II) (Fig. 1*B*) also showed three main bands of intense absorption with λ_{max}^{MeOH} = 288, 301, 315 (Fig. 1*C*, inset b), indicating the presence of a conjugated tetraene. GC/MS analysis of its Me_3Si derivative (C value = 28.3) showed ions of high intensity at *m/e* 173 [base peak; Me_3SiO^+=CH—$(CH_2)_4$—CH_3], 203 [Me_3SiO^+=CH—$(CH_2)_3$—$COOCH_3$], 289 (379 − 90), and 379 (*M*-203). Ions of lower intensities were at *m/e* 582 (*M*), 492 (*M*-90; loss of Me_3SiOH), 482 [*M*-100; rearrangement followed by loss of O=HC$(CH_2)_4$—CH_3], 409 (*M*-173), 402 [*M*-(2 × 90); 2Me_3SiOH], 329, 319 [*M*-(173 + 90)], and 307 (*M*-275). The presence of these ions suggests that compound II was also a methyl icosatetraenoate with three hydroxyl groups located at C-5, C-14, and C-15.

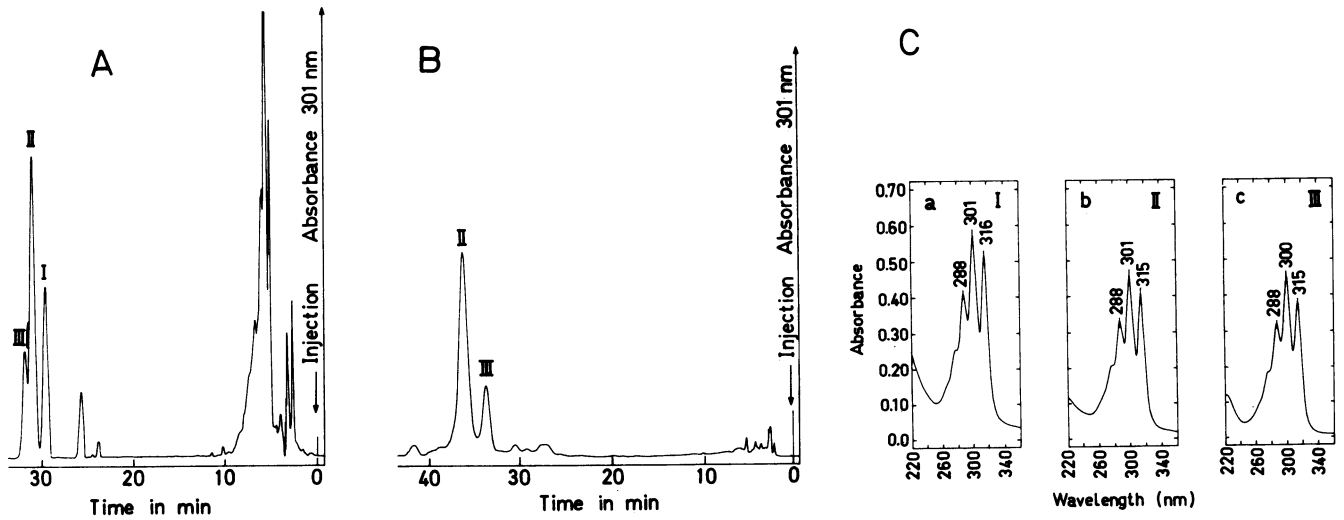

FIG. 1. RP-HPLC chromatograms and UV spectra of products obtained from incubation of human leukocytes with 15-HPETE and ionophore A23187. The incubation (15 min, 37°C) was stopped and the materials were obtained as described in the text. (*A*) RP-HPLC chromatogram. The column (Altex Ultrasphere ODS, particle diameter = 5 μm, 10 mm × 25 cm) was eluted with MeOH/H_2O, 65:35 (vol/vol), at 3.0 ml/min. (*B*) RP-HPLC chromatogram of compounds II and III. The column (Nucleosil C_{18}, 4.6 mm × 25 cm, 5 μm) was eluted with MeOH/H_2O, 60:40 (vol/vol), at 1.0 ml/min. (*C*) UV spectra of compounds I–III in MeOH. Inset a: compound I in MeOH. Inset b: compound II in MeOH. Inset c: compound III.

Similar analyses were performed with material eluting in peak III (compound III) (Fig. 1*B*). Its UV spectrum also displayed three main bands of absorption at 288, 300, and 315 λ_{max}^{MeOH} (Fig. 1*C*, inset c). The UV spectrum was similar to those obtained for compounds I and II, with the exception of a λ_{max}^{MeOH} at 300 nm rather than 301 nm. This shift in λ_{max} was a highly consistent finding. This material was converted to the Me_3Si derivative and analyzed by GC/MS. The C value was 24.0, as reported for LXB (cf. ref. 4). The prominent ions were at *m/e* 173 (base peak), 203, and 289. Ions of lower intensities were observed at 582 (*M*), 492, 482, 402, 329, 319, and 307. These ions and the C value are consistent with those reported for LXB. It follows then that compounds I–III are isomers since their mass spectra are virtually identical. It is noteworthy that compounds I and II have C values of 28.0 and 28.3, respectively, whereas compound III gave a C value of 24.0 (Table 1). Further evidence in support of the notion that these compounds are isomers comes from examining the products formed upon catalytic hydrogenation. Hydrogenation of compound II gave a major peak on GC (C-25.3), which was due to the saturated Me_3Si derivative. Ions of high intensity were at *m/e* 173, 203, 297 [*M*-(203 + 90)], 399 [*M*-(101 + 90)], and 417 (*M*-173). Ions of lower intensity were at *m/e* 575 (*M*-15) and 490 (*M*-100). The product formed upon hydrogenation of compound III also gave a major peak on GC at C-25.3, which was due to the saturated derivative of compound III. The prominent ions here were identical to those obtained with compound II (*vide supra*) (cf. ref. 4). Thus, upon reduction of their double bonds, compound II (C-28.3) and compound III (C-24.0) gave a single peak on GC at C-25.3.

Leukocytes, heated (50°C, 30 min) followed by incubation with either 15-HPETE (50 μM) or 15-HETE (50 μM) plus A23187 (5 μM) at 37°C for 30 min, did not form compounds I–III ($n = 3$). Also, elimination of residual hydroperoxy acids (by $SnCl_2$ reduction), which may have been present following extraction of the incubation mixtures, did not increase the yield of trihydroxytetraenes nor did it change the profile of compounds I–III ($n = 8$).

Labeling Studies with $^{18}O_2$. To examine the origins of oxygen in compounds I–III, leukocytes were incubated with either 15-HPETE (50 μM) and A23187 (2.5 μM) or 15-HETE (50 μM) and A23187 (2.5 μM) under an atmosphere enriched in $^{18}O_2$ and compounds I–III and 5,15-DiHETE were isolated ($n = 4$). GC/MS analysis of these compounds clearly demonstrated the incorporation of ^{18}O into each of the compounds at the C-5 position. The percentage of incorporation of ^{18}O into 5,15-DiHETE was 62.7%, and it was 63.0%, 63.0%, and 62.1% for compounds I–III, respectively. The ion *m/e* 203, which contains carbons originating from C-1 through C-5 positions of compounds I–III (cf. ref. 10), and 5,15-DiHETE were in each case shifted to *m/e* 205 (Fig. 2). The mass spectrum of 5,15-DiHETE, obtained under ^{18}O atmosphere, was essentially identical to that reported by Maas *et al.* (7).

In addition, GC/MS analysis clearly showed that the oxygen atoms at C-14 of compounds I–III were not derived from $^{18}O_2$, since the prominent ions showed shifts of $M + 2$ rather than $M + 4$ (Fig. 2). The ^{16}O atom located at the C-15 positions of compounds I–III was in all cases retained. In the case of either 15-HPETE or 15-HETE, virtually identical results were obtained.

Comparison of Synthetic Compounds with Biologically Derived Materials. To substantiate the structures of compounds I–III, samples of the leukocyte-derived compounds were compared with materials prepared by total synthesis. In view of biogenic considerations, four compounds were synthesized (see Table 1). Each of the synthetic samples were compared with biologically derived materials by several criteria (Table 1), including UV, HPLC (several systems), GC, GC/MS, and cochromatography on GC and HPLC. Utilizing these criteria, compound I gave identical properties as synthetic III, compound II as synthetic II, and compound III (LXB) as synthetic I (Table 1). Thus, on the basis of these findings the appropriate stereochemistries of the biologically derived compounds I–III were assigned.

Incubations with Human Platelets. Since it is possible to obtain 14,15-dihydroxy-5,8,10,12-icosatetraenoic acid (14,15-DiHETE) from platelets by way of the 12-lipoxygenase (12), we examined whether platelets could generate 5,14,15-THETEs from appropriate substrates. When incubated with either 5,15-DiHETE (65 μM), 5,15-DiHPETE (100 μM), 15-HETE (100 μM), or 15-HPETE (100 μM) human platelets did not generate compounds I–III ($n = 4$). Moreover, when synthetic IV [(14*S*)-LXB], a possible candidate of a 12-lipoxygenase mechanism, was compared to products obtained from *vide supra* platelet incubations or incubations with human leukocytes, it could not be matched with the biologically derived materials.

Recovery and Stability of LXB. When synthetic LXB was carried through the protocol used to obtain compounds I–III, 34.9% of the material was recovered following HPLC ($n = 4$). In addition, of the recovered material, 44.8% of the compound was recovered as compound II ($n = 4$), as assessed by criteria presented in Table 1. Next, acid stability studies were performed. The compound was added to MeOH/H_2O (65:35), acidified pH 3.5 (HCl), followed by direct injection onto HPLC columns at various intervals. Here the amounts of native compound recovered diminished with the time of exposure to acid. At 1 min of exposure, 88% of the native compound was recovered, at 30 min 20% was recovered, and by 1 hr, the compound was not detected ($n = 2$). Together these findings indicate that considerable losses may be incurred upon isolation of LXB.

Table 1. Comparison of leukocyte-derived materials with synthetic materials

Parent compound	Altex,* R_f in min	Nucleosil,† R_f in min	UV λ_{max}^{MeOH}‡	C value§ SE-30
Compound I	29.5	36.2	288, 301, 316	28.0
Compound II	31.6	36.2	288, 301, 315	28.3
Compound III (LXB)	32.2	34.0	288, 300, 315	24.0
Synthetic I	32.2	34.0	288, 300, 315	24.0
Synthetic II	31.6	36.2	288, 301, 315	28.3
Synthetic III	29.5	36.2	288, 301, 316	28.0
Synthetic IV	48.0	40.1	288, 300, 315	23.4

Synthetic I, (5*S*,14*R*,15*S*)-5,14,15-trihydroxy-6,10,12-*trans*-8-*cis*-icosatetraenoic acid; synthetic II, (5*S*,14*R*,15*S*)-5,14,15-trihydroxy-6,8,10,12-*trans*-icosatetraenoic acid; synthetic III, (5*S*,14*S*,15*S*)-5,14,15-trihydroxy-6,8,10,12-*trans*-icosatetraenoic acid; synthetic IV, (5*S*,14*S*,15*S*)-5,14,15-trihydroxy-6,10,12-*trans*-8-*cis*-icosatetraenoic acid.

*The column was eluted with MeOH/H_2O, 65:35 (vol/vol), at 3.0 ml/min, 3500 psi (1 psi = 6.89 kPa), 25°C. Coinjections were also performed in this system (biological plus synthetic).

†The column was eluted with MeOH/H_2O, 60:40 (vol/vol), at 1 ml/min, 3000 psi. Cochromatographies were also performed with this system.

‡UV spectra were recorded in MeOH for the free acids and esterified materials. In each case the λ_{max} values for methyl esters and free acids were identical.

§Materials were treated with CH_2N_2 and then converted to Me_3Si derivatives before injection onto the column.

DISCUSSION

In previous papers we have reported the isolation, basic structures, and some of the biological activities of two products of a series of compounds obtained from human leukocytes (i.e., LXA and LXB). Here we describe the

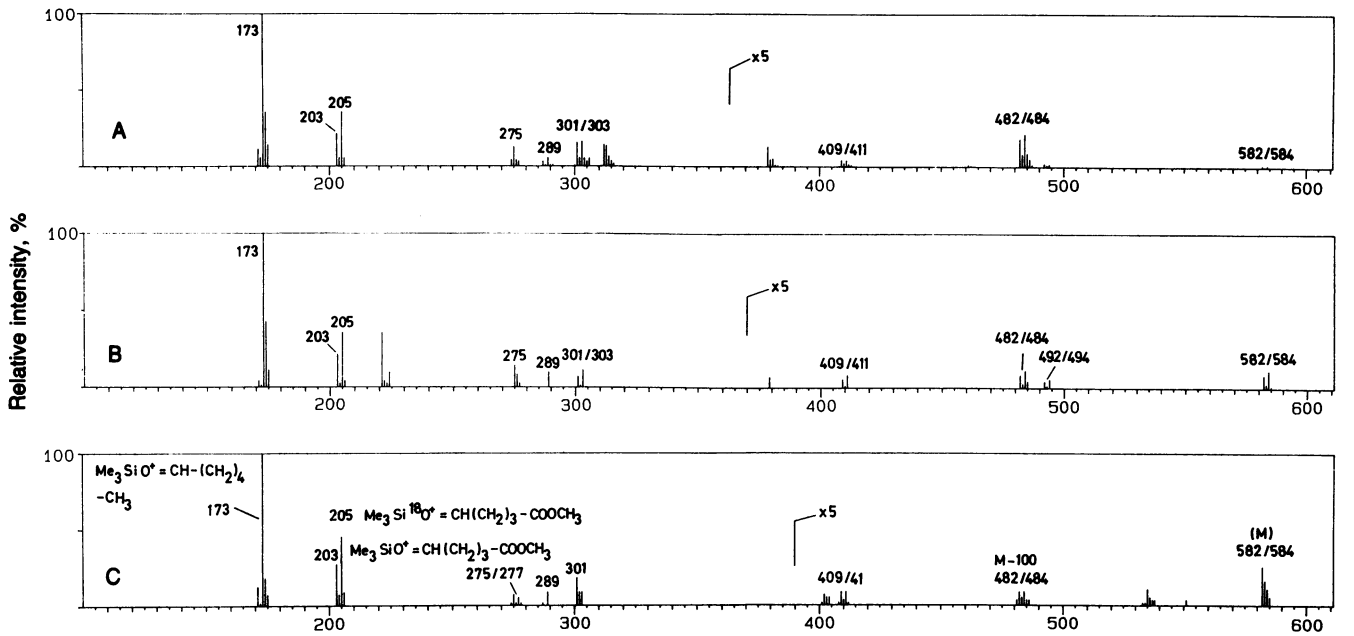

FIG. 2. Mass spectra of the Me_3Si derivatives of compounds I–III obtained from leukocytes exposed to 15-HETE (50 μM) and A23187 (2.5 μM) incubated in an atmosphere rich in $^{18}O_2$. (*A*) ^{18}O-labeled Me_3Si derivative of compound I. (*B*) ^{18}O-labeled Me_3Si derivative of compound II. (*C*) ^{18}O-labeled Me_3Si derivative of compound III.

isolation, biosynthesis, and stereochemistry of LXB as well as two of its naturally occurring isomers.

Compounds I–III were isolated from extracts of human leukocytes exposed to either 15-HPETE and A23187 as in refs. 3 and 4 or 15-HETE (50 μM) and A23187 (2.5 μM). Purification was achieved by column chromatography of the free acids, TLC, and RP-HPLC of esterified materials (Fig. 1). The presence and positions of the three hydroxyl groups in compounds I–III were established by GC/MS of several derivatives of each of the compounds (see *Results*). Together with UV data and previously reported criteria (4, 9), these findings indicate that compounds I–III are 5,14,15-trihydroxy-tetraene isomers.

$^{18}O_2$-labeling studies with either 15-HETE or 15-HPETE added to leukocytes exposed to A23187 clearly showed that the oxygen atom located at the C-5 position in compounds I–III and 5,15-DiHETE was derived from molecular oxygen (Fig. 2 and *Results*). Also from these studies it is possible to conclude that the oxygen atoms at the C-14 position of compounds I–III are not derived from molecular oxygen. Together with our previous findings (3, 4, 13), results of these studies present strong evidence that compounds I–III arise by way of interactions with a leukocyte 5-lipoxygenase. Thus, following lipoxygenation of arachidonic acid at the C-15 position, either 15-HPETE or 15-HETE may be subject to enzymatic attack by a 5-lipoxygenase, giving rise to 5,15-DiHPETE or its partially reduced form, which may then serve as an intermediate in the formation of compounds I–III. It is noteworthy that 5,15-DiHETE has been shown to enhance neutrophil degranulation (14).

Several distinct biosynthetic routes may be operative in the formation of trihydroxytetraenes. For example, once formed, 5,15-DiHPETE could be expected to undergo enzymatic dehydration to form either a 5,6-epoxide tetraene or a 14,15-epoxide tetraene, which, by analogy to the hydrolysis of LTA_4, could be transformed to trihydroxytetraenes. Alternatively, 5,15-DiHPETE may be subject to another lipoxygenation involving a 12-lipoxygenase to yield 5,14,15-THETEs. A similar mechanism has been proposed in the formation of 14,15-DiHETE from 15-HETE (12). Since 15-HETE is not expected to form an epoxide at the C-14,15 position, our findings indicate that a 14,15-epoxide tetraene intermediate is not formed during this particular route of biosynthesis. Moreover, since compounds I–III isolated from cells exposed to 15-HETE and A23187 under an atmosphere enriched in $^{18}O_2$ carried only one ^{18}O atom it is unlikely that a lipoxygenase is involved in the insertions of oxygen at the C-14 positions (Fig. 2). Together these findings strongly suggest a (15*S*)-hydroxy-5,6-epoxy-7,9,13-*trans*-11-*cis*-icosatetraenoic acid intermediate in the formation of compounds I–III (by way of this route of biosynthesis).

The stereochemistries of compounds I–III were established by comparison with materials prepared by total synthesis. By criteria presented in Table 1 the parent acids of compounds I–III were assigned. The total synthesis of lipoxins has been reported (15–19) and structures for LXB have been assigned based on HPLC retention times (16, 19). LXB was tentatively assigned the structure (5*S*,14*S*,15*S*)-5,14,15-trihydroxy-6,10,12-*trans*-8-*cis*-icosatetraenoic acid (16). However, when this compound was synthesized in the present study and its properties were compared to either leukocyte- or platelet-derived materials it did not match the properties of biologically derived materials (Table 1 and *Results*). In other studies, LXB was reported to be two isomers [(5*S*,14*R*,15*S*)-all-*trans*-THETE and (5*S*,14*S*,15*S*)-all-*trans*-THETE] based on HPLC comparisons (19). As observed in the present study, biologically derived compounds I–III can be resolved effectively by using two HPLC systems and the identities of LXB and its isomers can be established by GC together with the criteria presented in Table 1. Moreover, when human natural killer cells were exposed to either synthetic LXB or biologically derived LXB the cytotoxicity towards K562 target cells was inhibited (20).

A hypothetical scheme for the formation of LXB and its isomers is given in Fig. 3. In this particular route, (5*S*)-hydroperoxy-(15*S*)-hydroxy-6,13-*trans*-8,11-*cis*-icosatetraenoic acid may be transformed to a (15*S*)-hydroxy-5,6-epoxy-7,9,13-*trans*-11-*cis*-icosatetraenoic acid intermediate. This 5,6-epoxide tetraene may then be opened enzymatically to compound III (LXB) by hydration at the C-14 position, generating an 8-*cis* double bond. In addition, hydrolysis of the 5,6-epoxide tetraene would give rise to a pair of epimers at C-14. This scheme is supported by three lines of evidence: (*i*) 15-HETE serving as a precursor for the formation of compounds I–III in activated

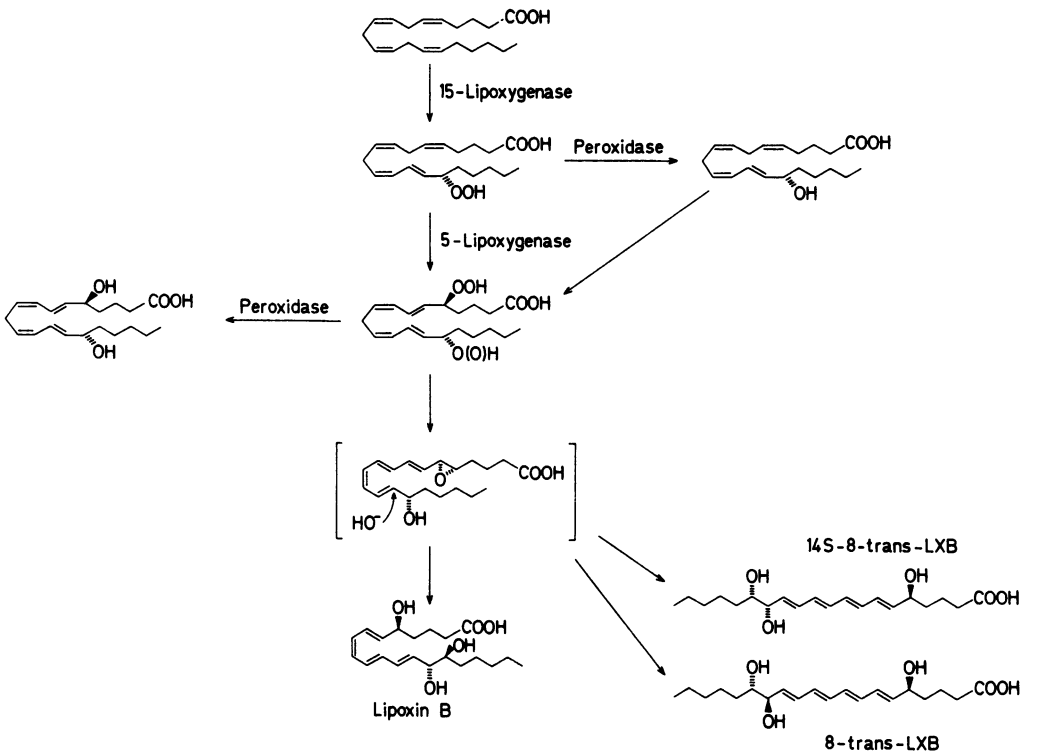

FIG. 3. Scheme of formation of LXB and its isomers. The stereochemistry of LXB and its isomers has been established. The 5,6-epoxide tetraene has not been isolated in the present study.

leukocytes, (*ii*) the pattern of ^{18}O incorporation in 5,15-DiHETE and compounds I–III, and (*iii*) the absolute stereochemistry of compounds I–III.

In theory, nonenzymatic hydrolysis of the 5,6-epoxide tetraene would lead to equal amounts of compounds I and II. The fact that larger amounts of compound II were recovered from leukocytes (Fig. 1) may, in part, be explained by the observation that LXB (compound III) can, upon standard workup, undergo isomerization to compound II (>40%) (see *Results*).

As in the case of leukotrienes, subtle changes in geometric configurations of lipoxins result in different biological activities. For example, LXA activates protein kinase C *in vitro*. LXB also activates the kinase but at higher concentrations, whereas (14*S*)-LXB and 8-*trans*-LXB are without effect (21).

In summary, our findings establish one of several possible biosynthetic routes in the formation of LXB and its natural isomers.

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