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LEUKOTRIENES PRODUCED BY INCUBATION OF DIHOMO-γ-LINOLENIC ACID WITH HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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SUMMARY: Two new leukotrienes, 8,15-dihydroxy-9,11,13-icosatrienoic acid (8,15-LTB_3) and 14,15-dihydroxy-8,10,12-icosatrienoic acid (14,15-LTB_3) were identified in incubations of human polymorphonuclear leukocytes with dihomo-γ-linolenic acid (8,11,14-icosatrienoic acid). The yield of these compounds was very low (0.5% of the total radioactivity) and did not increase with indomethacin alone or in combination with ionophore A-23187. Stereochemistry and biologic activity of the two leukotrienes remain to be established.

Incubations of arachidonic acid with human (1) or porcine (2) polymorphonuclear leukocytes (PMNL) and of 15-hydroperoxy-5,9,11,13-icosatetraenoic acid (15-HPETE) with human PMNL (3,4) or hemoglobin (5) have resulted in the synthesis of 8,15-dihydroxy-5,9,11,13-icosatetraenoic acid (8,15-LTB_4) and 14,15-dihydroxy-5,8,10,12-icosatetraenoic acid (14,15-LTB_4). It was expected that incubations of PMNL with other free fatty acid precursors would yield the corresponding dihydroxy acids. In fact, we wish to report that incubations of human PMNL with dihomo-γ-linolenic acid (8,11,14-icosatrienoic acid) lead to the production of 8,15-dihydroxy-9,11,13-icosatrienoic acid (8,15-LTB_3) and 14,15-dihydroxy-8,10,12-icosatrienoic acid (14,15-LTB_3).

MATERIALS AND METHODS

The procedures for isolation of human PMNL, extraction of the products and silicic acid chromatography have been reported from this laboratory (6). Cell suspensions (50-100 ml containing 100 x 10^6 cells/ml) in Hank's buffer pH 7.4 with Hepes (6 g/L) were preincubated at 37°C for 15 minutes and then incubated at the same temperature for 5 minutes with 150 μM dihomo-γ-linolenic acid (Nu Chek Prep, Inc., Elysian, MN) and 10 μCi of

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^{14}C-dihomo-γ-linolenic acid, specific activity 40-60 mCi/mmol (New England Nuclear, Boston, MA). Following ether extraction and separation by silicic acid chromatography, the ether/methanol 95/5 v/v eluate was evaporated to dryness, dissolved in methanol and treated with diazomethane as previously reported (1). Samples were injected into a Waters high performance liquid chromatography (HPLC) equipped with a model U6K injector, a model M-45 solvent delivery system, a model 450 variable wavelength detector and an Omni Scribe B-500 recorder (Houston Instruments, Austin, TX). Compounds were eluted from a straight phase preparatory column (30 cm x 7.8 mm ID, packed with Porasil), using hexane/isopropanol 96/4 v/v as the solvent system at 4 ml/min. Peaks absorbing at 270 nm were collected and the solvent was evaporated in vacuo. The residue was dissolved in 1 ml of methanol and subjected to UV spectroscopy and gas chromatography - mass spectrometry (GC-MS). A small portion (5%) of the samples was injected into a straight phase analytical column (30 cm x 3.9 mm ID, packed with Porasil) and eluted at 1 ml/min with the same system. Other samples obtained from similar experiments were injected as free acids into a reversed phase Radial-PAK cartridge (C_{18}, 8mm ID x 10 cm, 5 µ) in a Z-module system and eluted with methanol/water/acetic acid 70/30/0.01 v/v/v at 4 ml/min. The UV detector was set at 270 nm for the first 10 minutes and at 235 nm for 20 additional minutes. Fractions were collected every minute for counting of radioactivity. All radioactive peaks were subjected to UV spectroscopy and GC-MS.

The effect of 10^{-5} M indomethacin (Sigma, St. Louis, MO) and 5 µM ionophore A23187 (Calbiochem-Behring Corp., La Jolla, CA) on the products of dihomo-γ-linolenic acid by human PMNL was investigated with incubations at 37°C for 15 minutes (indomethacin) or 5 minutes (ionophore). Samples were injected into the Z-module system as described above.

Procedures for UV spectroscopy and GS-MS (6), including catalytic hydrogenation (1) have been previously reported.

RESULTS

A straight phase HPLC chromatogram of a methylated extract obtained by incubating human PMNL with dihomo-γ-linolenic acid is shown in Figure 1. Six peaks, absorbing at 270 nm can be appreciated. All compounds exhibited a typical UV leukotriene spectrum with absorption bands at 258, 268 and 278 for peaks I, IV, V and VI, at 262, 272 and 282 for peak II and at 260, 270 and 280 for peak III. The equivalent chain lengths (C values) were as follows: I = 23.8, II = 24.2, III = 24.9, IV = 24.9, V = 24.2 and VI = 24.9. The mass spectra of compounds I, IV, V and VI were the same. Analysis of the mass spectra of the methyl ester trimethylsilyl ether derivatives of the unsaturated compounds (Figure 2) and saturated compounds (Figure 3) allowed the identifiation of I, IV, V and VI as isomers of 8,15-dihydroxy-9,11,13-icosatrienoic acid. Informative ions for the unsaturated derivative (Figure 2) were: 481 (M-15; loss of CH_3), 465

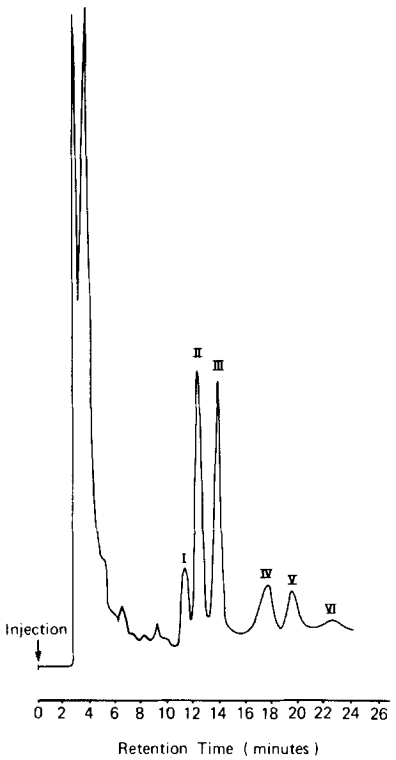

FIGURE 1 Straight phase HPLC chromatogram of methylated products obtained by incubating human PMNL with dihomo-γ-linolenic acid. Column: μ Porasil, 7.8 mm ID x 30 cm. Solvent system: hexane/isopropanol 96/4 v/v. Flow rate; 4 ml/min. The UV absorption was monitored at 270 nm.

(M-31; loss of $^{\bullet}OCH_3$), 406 (M-90; loss of Me_3SiOH), 353 (M-143; loss of $^{\bullet}[CH_2]_6\ COOCH_3$), 335 (425-90), 323 (M-173; loss of $Me_3SiO^+ = CH\text{-}[CH_2]_4CH_3$), 263 (353-90), 251 (M-245; loss of $Me_3SiO^+ = CH\text{-}[CH_2]_6COOCH_3$), 245 (M-251) and 173 ($Me_3SiO^+ = CH\text{-}[CH_2]_4CH_3$). The saturated derivative (Figure 3) had the following informative ions: 487 (M-15; loss of CH_3), 471 (M-31; loss of $^{\bullet}OCH_3$), 412 (M-90; loss of Me_3SiOH), 341 (M-[71+90]), 269 (M-[143+90], 257 (M-245; loss of $Me_3SiO^+{=}CH\text{-}[CH_2]_6COOCH_3$), 245 (M-257) and 173 ($Me_3SiO^+{=}CH\text{-}[CH_2]_4CH_3$).

Compounds II and III had identical unsaturated (Figure 4) and saturated (Figure 5) mass spectra of the methyl ester trimethylsilyl derivatives. Based on analysis of these mass spectra compounds II and III were identified as 14,15-dihydroxy-8,10,12-icosatrienoic acid. Informative

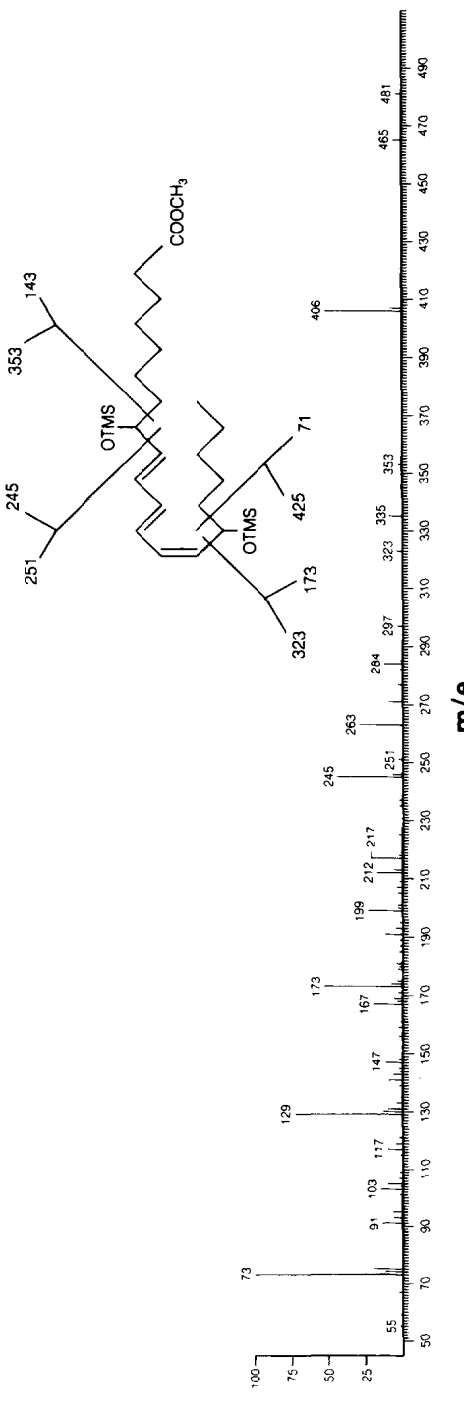

FIGURE 2 Mass spectrum of the methyl ester trimethylsilyl ether derivative of compounds I, IV, V and VI from Figure 1.

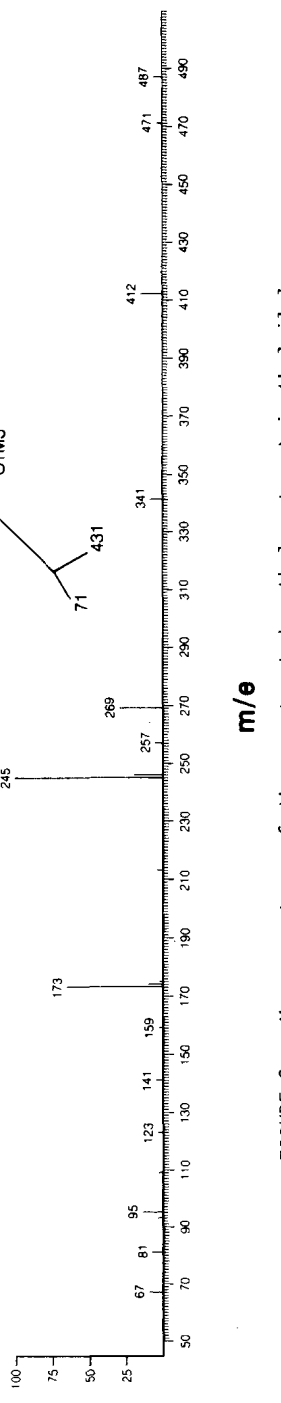

FIGURE 3 Mass spectrum of the unsaturated methyl ester trimethylsilyl ether derivative of compounds I, IV, V and VI from Figure 1.

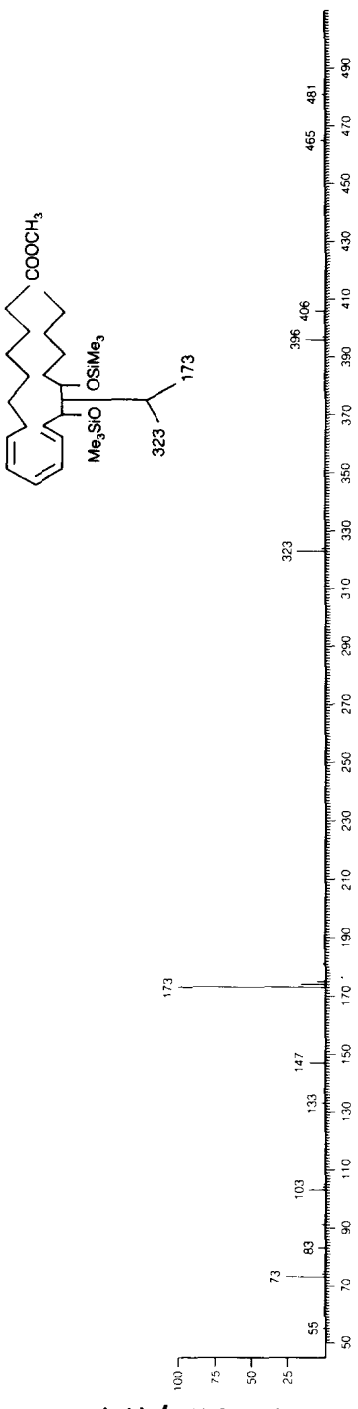

FIGURE 4 Mass spectrum of the methyl ester trimethylsilyl ether derivative of compounds II and III from Figure 1.

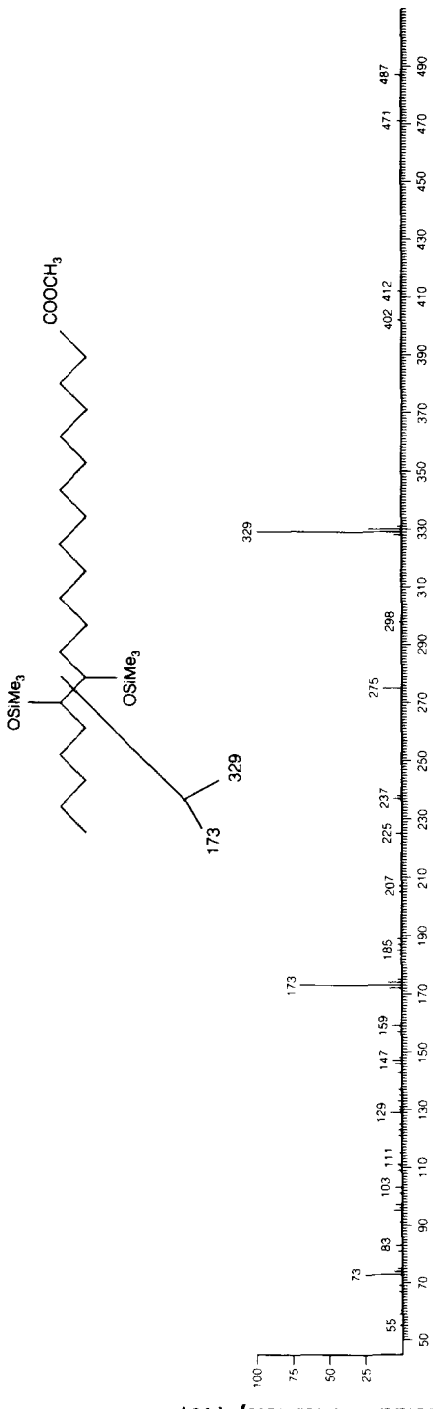

FIGURE 5 Mass spectrum of the unsaturated methyl ester trimethylsilyl ether derivative of compounds II and III from Figure 1.

TABLE 1. Distribution of the radioactivity during ether extraction and silicic acid column chromatography.

Step	Percent of Initial Counts	
Cell homogenate	16.3	Extraction
H_2O Wash	2.2	
Ether	81.2	
Hexane/ether 85/15	69.1	Silicic Acid Chromatography of Ether Extract
Ether/methanol 95/5	6.2	

ions for the unsaturated derivative were: 481 (M-15), 465 (M-31), 406 (M-90), 396 (M-100, by rearrangement), 323 (M-173) and 173 (Me_3SiO^+ = $CH\text{-}[CH_2]_4CH_3$) and for the saturated derivative: 487 (M-15), 471 (M-31), 412 (M-90), 402 (M-100, by rearrangement), 329 (M-173) and 173 (Me_3SiO^+ = $CH - [CH_2]_4CH_3$). The distribution of the Carbon-14 counts during ether extraction and silicic acid chromatography is shown in Table 1. The majority of the counts present in the ether extract eluted with hexane/ether (unpolar). This fraction contains phospholipids, neutral lipids, unreactive dihomo-γ-linolenic acid and other unidentified compounds. Only 6.2% of the initial radioactivity was recovered in the ether/methanol fraction (polar). As shown in Table 2, the radioactivity in

TABLE 2. Distribution of the radioactivity in the peaks from the reversed phase HPLC in methanol/water/acetic acid 70/30/0.01 v/v/v. Radioactivity present in the ether/methanol 95/5 v/v extract (6.2% of the initial counts) was injected into the HPLC.

Compound	Percent of Initial Counts	Percent of Counts in Ether/ Methanol 95/5	Retention Time (min)
I) PGE_1, $PGF_{1\alpha}$, Thromboxane B_1	3.8	16.5	3.0
II) 8,15-LTB_3 and 14,15-LTB_3	0.5	3.3	6.8
III) 12-hydroxy-8,10-heptadecadienoic acid	0.3	14.0	10.8
IV) 12-hydroxy-8,10-14-icosatrienoic acid	1.7	56.0	25.0

this polar fraction was distributed in four major areas on the reversed phase HPLC. I contained PGE_1, $PGF_{1\alpha}$ and thromboxane B_1, II contained 8,15-LTB_3 and 14,15-LTB_3 and III and IV contained respectively 12-hydroxy-8,10-heptadecadienoic acid and 12-hydroxy-8,10,14-icosatrienoic acid. With the exception of the leukotrienes, all of these compounds have been identified previously (7). In contrast to a previous report (8), despite multiple attempts, we could not isolate 8-hydroxy-9,11,14-icosatrienoic acid.

Indomethacin effectively blocked the production of the prostaglandins, thromboxane B_1 and 12-hydroxy-8,10-heptadecadienoic acid, all known products of the cyclo-oxygenase system. Synthesis of the lipoxygenase product 12-hydroxy-8,10,14-icosatrienoic acid was unaffected. Indomethacin alone or in combination with ionophore did not increase the yield of 8,15-LTB_3 and 14,15-LTB_3.

DISCUSSION

Dihomo-γ-linolenic acid is metabolized by human PMNL into a series of compounds. The bulk of the radioactivity is recovered among unpolar compounds which include phospholipids, neutral lipids, unreactive dihomo-γ-linolenic acid and other uncharacterized metabolites. Only 6.2% of the initial radioactivity was present in the fraction that contained polar compounds. The predominant compounds in this fraction were PGE_1, $PGF_{1\alpha}$, thromboxane B_1, 12-hydroxy-8,10-heptaodecadienoic acid and 12-hydroxy-8,10,14-icosatrienoic acid. A very small portion (0.5% of the initial radioactivity and 3.3% of the radioactivity in the polar region) was present in 8,15-LTB_3 and 14,15-LTB_3. The yield of these two compounds was not increased by indomethacin alone or in combination with ionophore. Thus, although human PMNL are capable of synthesizing leukotrienes from dihomo-γ-linolenic acid, they are quantitatively minor products. The stereochemistry of 8,15-LTB_3 and 14,15-LTB_3 could not be investigated because of the small quantities that were produced. The biologic activity of these compounds remains to be established.

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