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# A PREVIOUSLY UNIDENTIFIED LEUKOTRIENE PRODUCED BY HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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SUMMARY: A previously unidentified leukotriene was isolated from incubations of human polymorphonuclear leukocytes with ionophore A23187. The compound eluted between $LTB_4$ and 20-carboxy $LTB_4$ on SP-HPLC and between the two 6-trans isomers of $LTB_4$ on RP-HPLC. Ultraviolet spectroscopy revealed three absorption bands at 258 nm, 268 nm and 278 nm. $^{18}O_2$ was incorporated by the OH at both $C_5$ and $C_{12}$. Oxidative ozonolysis indicated the presence of 5S, 12S configuration. The structure of the newly identified leukotriene is 5S, 12S-dihydroxy-icosatetraenoic acid. Stereochemistry of the double bonds and biologic activity were not investigated.

Arachidonic acid is the precursor of a variety of compounds via three enzymatic pathways catalyzed by cyclooxygenase, lipoxygenase and an NADPH-dependent oxidizing system (1-3). The products of the lipoxygenase system include a series of monohydroxy, dihydroxy and trihydroxy acids exhibiting diverse biologic activity (1). A group of these compounds, the leukotrienes, are characterized by the presence of three conjugated double bonds or triene (4). Initial oxidation of arachidonic acid at carbons 5, 8, 12 and 15 leads to the formation of hydroperoxi-icosatetraenoic acids which are metabolized into leukotrienes through several intermediates. While working with leukotriene $B_4$ ($LTB_4$) we have observed a small but consistent peak eluting between $LTB_4$ and 20-carboxy $LTB_4$ on straight-phase high performance liquid chromatography (SP-HPLC). Ultraviolet spectroscopy revealed a typical leukotriene spectrum with absorption bands at 258 nm, 268 nm and 278 nm and the mass spectrum is characteristic of a dioxygenated 5, 12-dihydroxy acid. Oxidative ozonolysis revealed that both hydroxy groups had an S configuration. Thus, the structure of this new leukotriene is 5S, 12S-dihydroxy-icosatetraenoic acid.

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## MATERIALS AND METHODS

Procedures for isolation and stimulation of polymorphonuclear leukocytes (PMNL) and for extraction of the leukotrienes released into the medium have been described previously (5). In short, PMNL obtained from human peripheral blood were sedimented with dextran, purified by Ficoll-Hypaque gradient centrifugation, suspended in Hank's buffer pH 7.4, incubated at 37° C and stimulated with 5 uM ionophore A23187 for five minutes. Reaction was stopped with 1.5 volumes of methanol and the leukotrienes were extracted with ether at pH 3.0. Following treatment with diazomethane, samples were injected into a Waters HPLC system equipped with a 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, Texas 78753). Leukotrienes were eluted from a straight-phase Radial-PAK cartridge (SI, 8 mm x 10 cm, 5u) in a Z-module with hexane/isopropanol/acetic acid 97/3/0.01 V/V/V at 4 ml/min. Peaks at 270 nm were collected for scanning ultraviolet (UV) spectroscopy and gas chromatography-mass spectrometry (GC-MS). In addition, the peaks obtained from the SP-HPLC were injected into a reverse-phase Radial-PAK cartridge ($C_{18}$, 8 mm ID x 10 cm, 5 u) and eluted with methanol/water/acetic acid 75/25/0.01 V/V/V at 2 ml/min. This procedure allowed to establish a relationship between compound A and the other 5,12-dihydroxy compounds. Incubations were carried out in the presence or absence of 18 $O_2$. Oxidative ozonolysis and preparation of the products for ultraviolet spectroscopy and GC-MS were performed by published methods (5-7).

## RESULTS

A SP-HPLC chromatogram of the products (methyl esters) obtained by incubating PMNL with ionophore A23187 is shown in Figure 1. Compound A, eluting at 18.4 minutes and between $LTB_4$ and its 20-carboxy metabolite exhibited a characteristic UV pattern with absorption bands at 258 nm, 268 nm and 278 nm. The quantities of compound A are extremely small, accounting for less than 2% of those of $LTB_4$. Gas chromatography of the methyl ester trimethylsilylether derivative of compound A revealed the presence of two isomers with a chain length equivalent (c-value) of 23.6 and 24.9. Mass spectra of both compounds were identical (Figure 2A) and it corresponded to 5,12 dihydroxy-icosatetraenoic acid. Shifting of ion 203 to 205, ion 383 to 387 and an increase in the molecular ion by four mass units 494 to 498 indicates incorporation of $O_{18}$ at $C_5$ and $C_{12}$ (Figure 2B). Products of oxidative ozonolysis of compound A cochromatographed with the (-)-menthoxycarbonyl derivatives of dimethyl-L-malate and dimethyl-2-L-hydroxyadipate. As it happens, GC-MS of 5S, 12S-dihydroxy-6, 8, 10, 14 (trans, cis, trans, cis)-icosatetraenoic acid (5,12-diHETE), the first peak in the SP-HPLC chromatogram shown in Figure 1 revealed the

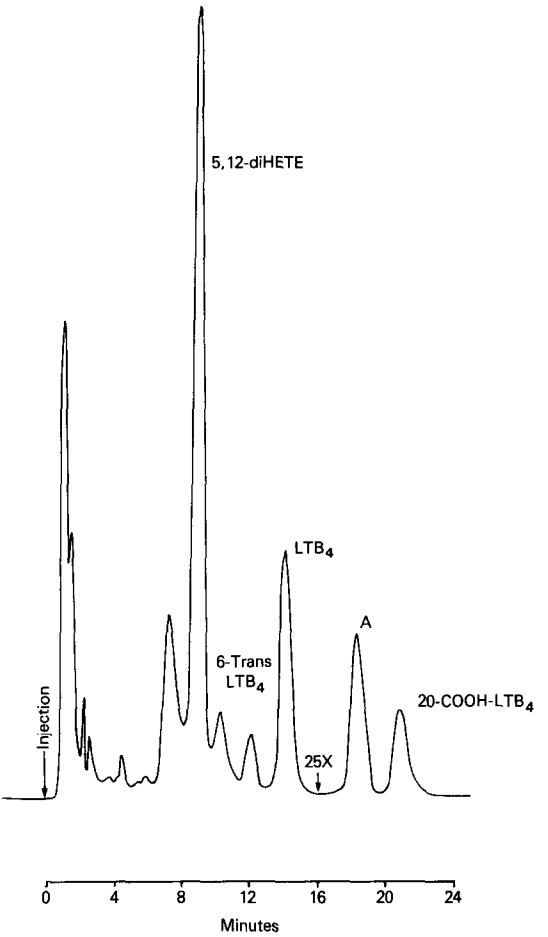


FIGURE 1. SP-HPLC chromatogram of an ether extract from an incubation of human PMNL (60 ml of 100 x $10^6$ cells/ml) with 5 uM ionophore A-23187 for 5 min at 37° C. Z-module system, Radial-PAK cartridge SI, 8 mm ID x 10 cm, 5 u. System: hexane/isopropanol/acetic acid 97/3/0.01 V/V/V. Pump rate - 4 ml/min. Ultraviolet absorption was monitored at 270 nm. Sample was treated with diazomethane prior to injection. 25X indicates where the detector sensitivity was increased 25 times.

presence of an isomer, not previously identified, with a c-value of 24.9 and incorporation of $O_{18}$ at $C_5$ and $C_{12}$. On RP-HPLC compound A eluted between the two 6-trans isomers of $LTB_4$.

## DISCUSSION

A previous unidentified leukotriene has been isolated from incubations of human PMNL with ionophore A-23187. The compound migrates between $LTB_4$ and 20-carboxy $LTB_4$ on SP-HPLC and between the 6-trans isomers of

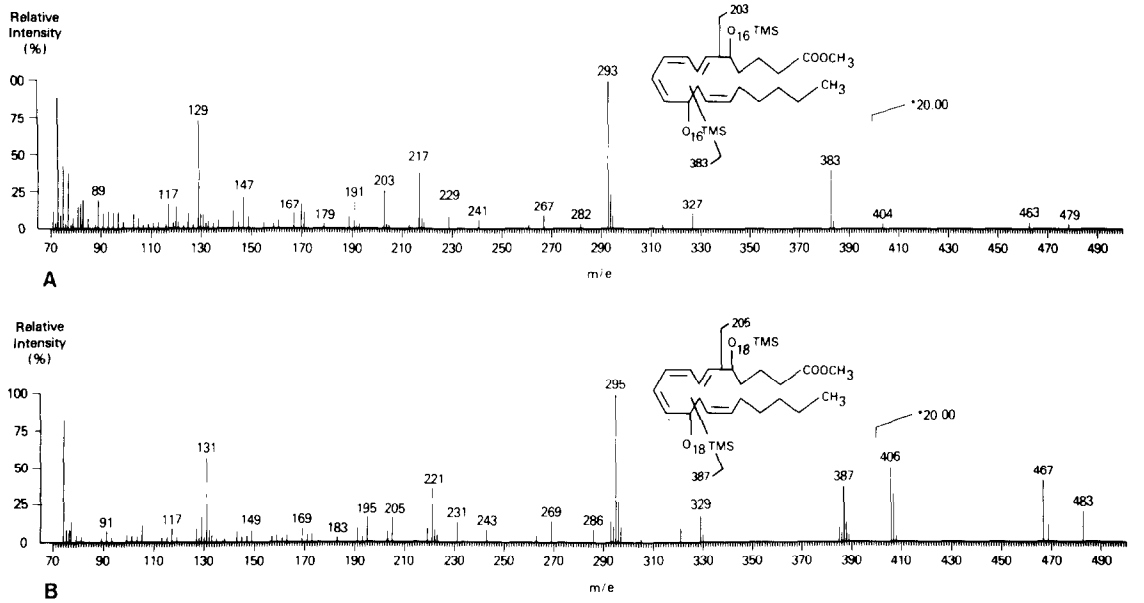


FIGURE 2. Mass spectra of compound A, C value = 23.6. A = $O_{16}$, B = $O_{18}$. Shift on ion 203 to 205, ion 383 to 387 and increase in the molecular ion by four mass units indicate incorporation of $O_{18}$ at $C_5$ and $C_{12}$. The structure corresponds to 5S, 12S-dihydroxy-icosatetraenoic acid.

$LTB_4$ on RP-HPLC. Ultraviolet absorption at 258 nm, 268 nm and 278 nm was observed. The mass-spectrum is that of a dioxygenated 5,12-dihydroxy compound and oxidative ozonolysis revealed an S configuration for both hydroxyl groups. The small quantities of material obtained did not allow investigating the stereochemistry of the double bonds. It is possible that the isomer with C=23.6 may have the cis, trans, trans, cis configuration like $LTB_4$ and that the C=24.9 isomer may have the trans, trans, trans, cis configuration. However, other possibilities cannot be excluded. In addition, we have identified an isomer of the dioxygenated 5,12-dihydroxy-trans-cis-trans-cis compound (5,12-diHETE). The C value of 24.9 suggests a trans-trans-trans-cis configuration for this new compound. The biologic effects of this elusive 5S, 12S-dihydroxy-icosatetraenoic acid compound remains to be determined.

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