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Synthesis of 11,12-leukotriene A_4, 11*S*,12*S*-oxido-5*Z*,7*E*,9*E*,14*Z*-eicosatetraenoic acid, a novel leukotriene of the 12-lipoxygenase pathway

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A simple and efficient method for preparing 11,12-leukotriene A_4 has been established by the stereospecific biomimetic route from arachidonic acid. 12*S*-Hydroperoxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid was synthesized using a partially purified 12-lipoxygenase of porcine leukocytes. The methyl ester of the compound was then chemically converted to two labile epoxides with a conjugated triene structure. These compounds were identified by proton NMR and mass spectrometry to be 11*S*,12*S*-oxido-5*Z*,7*E*,9*E*,14*Z*-eicosatetraenoic acid (11,12-leukotriene A_4) and its geometric isomer.

12-Lipoxygenase; Leukotriene; 11,12-Leukotriene A_4; 12-Hydroperoxyicosatetraenoic acid

1. INTRODUCTION

Leukotrienes (LTs) are a family of biologically active compounds involved in inflammation and in allergic responses [1,2]. These LTs are produced by way of a labile allylic epoxide, LTA_4 (5*S*,6*S*-oxido-7*E*,9*E*,11*Z*,14*Z*-eicosatetraenoic acid), a 5-lipoxygenase product [3–8]. The biological significance of 12-lipoxygenase, another lipoxygenase abundant in the blood platelets [9,10], remains unclear. Samuelsson [1,11] proposed the analogous transformation of arachidonic acid into 12-series LTs via 11,12-LTA_4. However, the presence, synthesis and properties of 11,12-LTA_4 have not yet been determined.

We designed a simple, appropriate method for preparing novel LTs, 11,12-LTA_4 and its isomer. Acquisition of these compounds paves the way for determination of the significance of the 12-lipoxygenase pathway.

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Abbreviations: LT, leukotriene; 12-H(P)ETE, 12*S*-hydro(pero)xy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid; 5-H(P)ETE, 5*S*-hydro(pero)xy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid; 15-H(P)ETE, 15*S*-hydro(pero)xy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid; GC-MS, gas chromatography-mass spectrometry; PMP, 1,2,2,6,6-pentamethylpiperidine; SP, straight-phase; RP, reversed-phase

2. EXPERIMENTAL

2.1. *Materials*

Arachidonic acid was obtained from Nu Chek Prep (Ellysian). PMP was purchased from Fluka (Buchs) and trifluoromethanesulfonic anhydride (triflic anhydride) was from Aldrich (Milwaukee). Iatrobeads 6RS-8060 was from Iatron (Tokyo). All other reagents were of analytical grade and the solvents were distilled just before use.

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2.2. *Preparation of 12-HPETE*

12-HPETE was prepared using a partially purified porcine leukocyte 12-lipoxygenase [12]. Details of the procedures will be published elsewhere. Briefly, 10 mg arachidonic acid was incubated with 12-lipoxygenase in 0.1 M Tris-HCl buffer (pH 7.4) at 4°C for 20 min. After extraction with diethyl ether [4], the extract was purified by silica gel (Iatrobeads) column chromatography and RP HPLC (TSK ODS-120T, 7.8 × 300 mm, Toyosoda, Japan). About 4 mg (36% yield) 12-HPETE was obtained. The methyl ester of 12-HPETE was prepared with ethereal diazomethane. For structural determination by GC-MS, 12-HPETE methyl ester was reduced to 12-HETE methyl ester using triphenylphosphine, followed by derivatization to the trimethylsilyl ether derivative. GC-MS was performed using a Shimadzu QP-1000 (Kyoto) equipped with 2% OV-1 coated column (3 mm × 2 m) at 230°C. The derivatized compound appeared with a *C* value of 21.3. Characteristic ions were observed at *m/z* 406 (M), 391, 375, 316, 295 (base peak), 229, 205 and 173, such being in good agreement with those for 12*S*-HETE [9] prepared from human blood platelets.

2.3. *Preparation of 11,12-LTA$_4$*

11,12-LTA$_4$ was prepared from 12-HPETE with a slight modification of Corey and Barton [13] for the synthesis of LTA$_4$ methyl ester from 5-HPETE. Azeotropically dried 12-HPETE methyl ester (1 mg, 2.8 μmol) was dissolved in 30 μl dry dichloromethane and 30 μl freshly distilled tetrahydrofuran under argon. PMP (2.4 μl, 5.0 equiv.) was added, and the solution cooled to −78°C. Triflic anhydride (1.4 μl, 2.9 equiv.) was added to the resulting solution, and the reaction mixture stirred rapidly for 2 h at −78°C. The reaction was quenched with triethylamine (5 μl), and the solution allowed to warm up to −10°C. Hexane-diethyl ether-triethylamine (50:50:1, v/v) was added (3 ml), followed by washing three times with saturated brine (0.3 ml). The organic layer was dried with anhydrous sodium sulfate and evaporated in vacuo. The resulting yellowish oily residue, dissolved in a minimum volume of diethyl ether, was applied on a silica gel column (Iatrobeads, 0.8 × 2 cm) and eluted with hexane-diethyl ether-triethylamine (97:3:1, v/v). Fractions containing the desired products were combined, and the solvent was removed in vacuo to give crude 11,12-LTA$_4$ methyl ester (0.7 mg, 76% yield) determined by UV spectrum analysis. Further purification was performed at 4°C by preparative SP HPLC (Nucleosil 50-5, 10 × 300 mm) using hexane-diethyl ether-triethylamine (100:0.4:0.4, v/v), as a running solvent.

2.4. *NMR and mass spectrum analyses of 11,12-LTA$_4$ and its isomer*

The structures of the compounds (0.1 mg each) were assigned by ^{1}H on a JEOL JNM-GX400 FT NMR spectrometer (400 MHz) in $CDCl_3$ containing 1% deuterated pyridine at 0°C, and the chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane used as the internal standard. Mass spectra were obtained using 10 μg of each sample with a Hitachi M-80A (EI).

3. RESULTS

3.1. *Preparation of 11,12-LTA$_4$*

A typical HPLC profile of the 12-lipoxygenase reaction is shown in fig.1. The major product was identified by GC-MS to be 12-HPETE (see section 2), and a small amount of 12-HETE was also observed. Under these conditions, the yields of 12-HPETE and 12-HETE were 68.1 and 4.4%, respectively. 12-HPETE methyl ester was converted to epoxide LT as described in section 2, and the products were analyzed by SP-HPLC (TSK gel silica-60, 4.6 × 250 mm, Toyosoda) using hexane-diethyl ether-triethylamine (100:0.4:0.4, v/v) at 4°C. When detected at 280 nm, two compounds appeared, named P-1 (major peak) and P-2 (fig.2). The ratio of P-1 to P-2 was about 7:3 (w/w). The characteristic UV spectra of these compounds (λ_{max} in methanol 268, 278, 289 nm) as shown in fig.3 indicated conjugated triene structures. Both triplet spectra shifted immediately by 10 nm to the shorter wavelength after addition of one drop of 1 N HCl, thereby suggesting dihydroxy acid formation from the epoxide LTs.

3.2. *Identification of 11,12-LTA$_4$ and its isomer*

The geometrical structures of these compounds were determined by ^{1}H NMR and mass spectrometry. The ^{1}H NMR spectral data were as

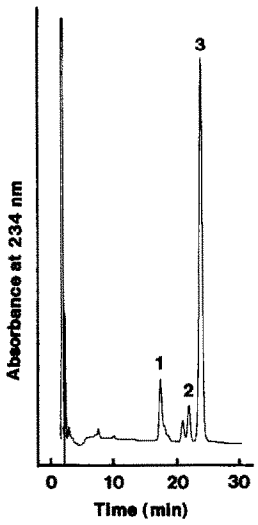

Fig.1. HPLC of porcine 12-lipoxygenase products. An aliquot of the incubation mixture obtained at 20 min, quenched by 2 vols acetonitrile-methanol-acetic acid (350:150:1, v/v) containing internal standard (13-hydroxylinoleic acid), was injected onto the RP HPLC column (TSK ODS-80TM, 4.6 × 150 mm) eluted with acetonitrile-methanol-water-acetic acid (350:150:250:1, v/v, 1 ml/min): peak 1, internal standard; peak 2, 12-HETE; peak 3, 12-HPETE.

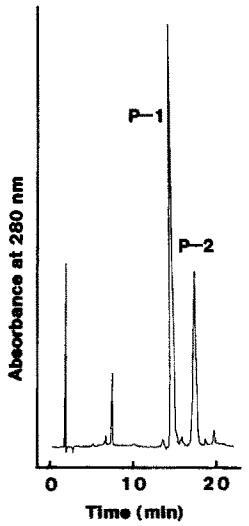

Fig.2. HPLC of 11,12-LTA$_4$ methyl ester and its isomer. The crude sample (25 μg) was injected onto the SP-HPLC column (TSK gel silica-60, 4.6 × 250 mm) eluted at 4°C with hexane-ethyl acetate-triethylamine (100:0.4:0.4, v/v, 1 ml/min).

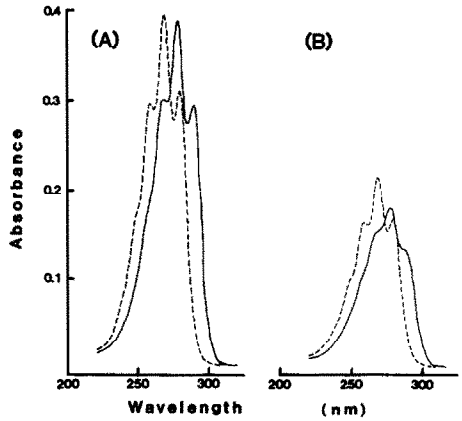

Fig.3. UV spectra of samples (P-1, P-2) purified by HPLC in fig.2. The HPLC fractions were evaporated and dissolved in the same volume of methanol. When HCl was added to the cuvette, the spectra immediately changed (dashed lines). (A) P-1, (B) P-2.

follows. Chemical shifts (δ) are expressed as ppm, and s, d, t, and m denote singlet, doublet, triplet, and multiplet, respectively.

(P-1) δ 6.52 (1H,dd,J=16,11 Hz; H_7 or H_8), 6.37 (1H,dd,J=15,11 Hz; H_8 or H_7), 6.18 (1H,dd,J=14,11 Hz; H_9), 6.07 (1H,t,J=10 Hz; H_6), 5.4–5.6 (4H,m; H_5,H_{10},H_{14},H_{15}), 3.67 (3H,s; $O\underline{CH_3}$), 3.10 (1H,dd,J=8,2 Hz; H_{11}), 2.92 (1H,dt,J=8,2 Hz; H_{12}), 2.43 (1H,m; H_{13}), 2.34 (2H,t; $\underline{CH_2}CO_2Me$), 2.25 (1H,m; H_{13}), 2.03 (4H,m; H_4,H_{16}), 1.74 (2H,m; H_3), 1.2–1.4 (6H,m; H_{17},H_{18},H_{19}), 0.87 (3H,t; H_{20}).

(P-2) δ 6.47 (1H,t,J=11 Hz; H_7 or H_8), 6.3 (1H,t,J=11 Hz; H_8 or H_7), 6.25 (1H,t,J=11 Hz; H_9), 6.01 (1H,t,J=11 Hz; H_6), 5.4–5.6 (4H,m; H_5,H_{10},H_{14},H_{15}), 3.67 (3H,s;$O\underline{CH_3}$), 3.23 (1H,dd, J=8,2 Hz; H_{11}), 2.92 (1H,dt,J=5,2 Hz; H_{12}), 2.44 (1H,m; H_{13}), 2.33 (2H,t; $\underline{CH_2}CO_2Me$), 2.25 (1H,m; H_{13}), 2.03 (4H,m; H_4,H_{16}), 1.74 (2H,m; H_3), 1.2–1.4 (6H,m; H_{17},H_{18},H_{19}), 0.89, 0.87 (3H,t,t; H_{20}).

The mass spectra of P-1 and P-2 were practically identical. Characteristic ions were observed at m/z 332 (M), 301 (M − OCH_3), 221 (M − 111), 111 (CH_2-CH = CH-$(CH_2)_4$-CH_3), and 69 (base peak). These results plus the UV spectra suggest that P-1 is 11S,12S-oxido-5Z,7E,9E,14Z-eicosatetraenoic acid methyl ester (11,12-LTA$_4$ methyl ester). P-2 was identified as one of the isomers of 11S,12S-oxido-5Z,7,9,14Z-eicosatetraenoic acid. The ab-

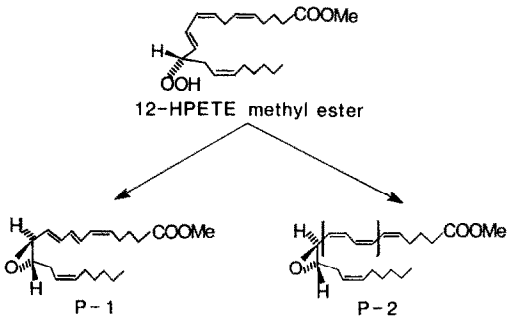

Fig.4. Proposed structures of 11,12-LTA_4 and its isomer. The absolute configuration of the double bonds at C-7 and C-9 of P-2 remains to be clarified.

solute configuration of the double bonds at C-7 and C-9 of P-2 was not clearly determined because this compound was gradually decomposed during the ^1H NMR analysis. The proposed structures of these compounds are shown in fig.4.

4. DISCUSSION

The racemic mixture of 11,12-LTA_4 methyl ester was chemically synthesized from undeca-2,5-diyn-1-ol by Corey et al. [14], and 11*S*,12*S*-LTA_4 was prepared from 2-deoxy-D-ribose by Zamboni et al. [15]. Since these methods require expertise for chemical synthesis and a large quantity of the starting materials, 11,12-LTA_4 has not been readily available. We designed a simple, appropriate method for the stereospecific preparation of purified 11,12-LTA_4 methyl ester from arachidonic acid, with a good yield (about 34%, starting from 12-HPETE). 12-HPETE was biosynthesized from arachidonic acid using porcine leukocyte 12-lipoxygenase. Use of the enzyme is efficient, providing that the optimal ratio of the substrate and enzyme amount is arranged. Detailed procedures of the synthesis of 12-HPETE will be published elsewhere (Kitamura et al., in preparation).

For the preparation of 11,12-LTA_4 from 12-HPETE, we used the method of Corey and Barton [13] which was originally described for the synthesis of LTA_4 methyl ester from 5-HPETE. Using this method, we obtained two methyl esters of epoxy conjugated triene, P-1 and P-2, and these esters were separated by HPLC and proved to be 11*S*,12*S*-oxido-5*Z*,7*E*,9*E*,14*Z*-eicosatetraenoic acid (11,12-LTA_4) and its geometric isomer, respectively (fig.4). Whether or not 12-HPETE is converted to 11,12-LTA_4 in vivo and which isomer is produced are the subjects of ongoing investigation. From what is known of the enzymatic transformations of 5-HPETE and 15-HPETE, 11*S*,12*S*-oxido-5*Z*,7*E*,9*E*,14*Z*-eicosatetraenoic acid (P-1) may be the correct isomer of 11,12-LTA_4. In this case the L (pro-*R*) hydrogen at C-7 is assumed to be stereoselectively eliminated.

12-Lipoxygenase activity has been detected in blood platelets [9,10], porcine leukocytes [11], rabbit aortic endothelial cell [16] and the brain of rodents [17,18]. However, the biological significance and enzymatic transformations of 12-HPETE were not determined. Although the formation of 11,12-LTA_4 and its further transformation into 12-series LTs are considered [1,11], no confirmative data have so far been available. Using a substantial amount of 11,12-LTA_4 and its isomer, the elucidation of the significance of the 12-lipoxygenase pathway is under extensive investigation in our laboratory.

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REFERENCES

[1] Samuelsson, B. (1983) Science 220, 568–575.
[2] Lewis, R.A. and Austen, K.F. (1984) J. Clin. Invest. 73, 889–897.
[3] Borgeat, P. and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. USA 76, 3213–3217.
[4] Borgeat, P. and Samuelsson, B. (1979) J. Biol. Chem. 254, 7865–7869.

[5] Shimizu, T., Rådmark, O. and Samuelsson, B. (1984) Proc. Natl. Acad. Sci. USA 81, 689–693.
[6] Rouzer, C.A., Matsumoto, T. and Samuelsson, B. (1986) Proc. Natl. Acad. Sci. USA 83, 857–861.
[7] Shimizu, T., Izumi, T., Seyama, Y., Tadokoro, K., Rådmark, O. and Samuelsson, B. (1986) Proc. Natl. Acad. Sci. USA 83, 4175–4179.
[8] Ueda, N., Kaneko, S., Yoshimoto, T. and Yamamoto, S. (1986) J. Biol. Chem. 261, 7982–7985.
[9] Hamberg, M. and Samuelsson, B. (1974) Proc. Natl. Acad. Sci. USA 71, 3400–3404.
[10] Nugteren, D.H. (1975) Biochim. Biophys. Acta 431, 651–654.
[11] Samuelsson, B. (1983) Adv. Prostaglandin, Thromboxane, Leukotriene Res. 11, 1–13.
[12] Yoshimoto, T., Miyamoto, Y., Ochi, K. and Yamamoto, S. (1982) Biochim. Biophys. Acta 712, 638–646.
[13] Corey, E.J. and Barton, A.E. (1982) Tetrahedron Lett. 23, 2351–2354.
[14] Corey, E.J., Marfat, A. and Goto, G. (1980) J. Am. Chem. Soc. 102, 6607–6608.
[15] Zamboni, R., Milette, S. and Rokach, J. (1984) Tetrahedron Lett. 25, 5835–5838.
[16] Greenwald, J.E., Bianchine, J.R. and Wong, L.K. (1979) Nature 281, 588–589.
[17] Yoshimoto, T., Kusaka, M., Shinjo, F., Yamamoto, S. and Dray, F. (1984) Prostaglandins 28, 279–285.
[18] Shimizu, T., Takusagawa, Y., Izumi, T., Ohishi, N. and Seyama, Y. (1987) J. Neurochem. 48, in press.