Communications to the Editor

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SYNTHESIS OF 5-FLUOROARACHIDONIC ACID AND ITS BIOTRANSFORMATION TO 5-FLUORO-12-HYDROXYEICOSATETRAENOIC ACID

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The synthesis of 5-fluoroarachidonic acid (1) was achieved from the intermediate (E)-5,6-dihydroxy-2-fluorohex-2-enoate 5,6-acetonide (5). Incubation of 1 with human platelets yielded 5-fluoro-12-hydroxyeicosatetraenoic acid (5F-12-HETE, 2).

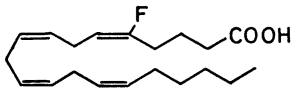
KEYWORDS ——— arachidonic acid; fluoroarachidonic acid; hydroxyeicosatetraenoic acid; fluorohydroxyeicosatetraenoic acid; lipoxygenase; platelet

Incorporation of fluorine into biologically active molecules often yields compounds with enhanced, prolonged or selective activities, or with new physiological properties, probably due to the characteristics of fluorinated compounds.[1] Yet, fluorine, as the second smallest substituent, closely mimics hydrogen with respect to steric requirement at binding sites of enzymes or target tissues. Fluorinated analogs are useful tools for the pharmaco-physiological and pathophysiological studies of the natural compounds.

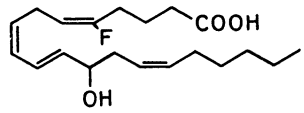
Eicosanoids display a wide diversity of pharmacological activities[2] and therefore study of these lipid mediators is one of the most active areas of clinical research. Although early studies of eicosanoids have been focused on cyclooxygenase products, lipoxygenase products are now emerging as topics of clinical interest. A significant number of fluorinated prostaglandins has been synthesized and many of them show increased metabolic stability or higher selectivity of action.[3] In contrast, synthesis of fluorinated lipoxygenase products has not been reported.

Biotransformation involves relatively simple techniques and short procedures, and generally, enzymic reaction proceeds in stereoselective manner. Therefore, we planned to prepare fluorinated lipoxygenase products by biotransformation of chemically synthesized fluorinated arachidonic acid. In addition to the products, this work will provide information on the effects of fluorine incorporation on enzymic reactions.

In this paper, we report the synthesis of 5-fluoro-5(E),8(Z),11(Z), 14(Z)-eicosatetraenoic acid (5F-AA, 1) and its biotransformation to 5-fluoro-12-hydroxyeicosatetraenoic acid (5F-12-HETE, 2).



1, 5F-AA



2, 5F-12-HETE

Chart 1

To synthesize fluorinated AAs including 1, ethyl (E)-5,6-dihydroxy-2-fluorohex-2-enoate 5,6-acetonide (5) obtained in high stereoselectivity was chosen as a key intermediate.

Treatment of the fluorophosphonoacetate (4)[4] with LDA in ether followed by the reaction with 3,4-dihydroxybutanal 3,4-acetonide (3)[5] (-78°C, 2 h) gave after chromatography the (E)-fluoroenoate [5, 80%; ^{1}H-nmr($CDCl_3$)δ 6.00 (dt, J=21 and 7.5 Hz, CH=CF), ^{19}F-nmr($CDCl_3$) δ -55.1 (d, J=21 Hz)][6] and the Z-isomer of 5[5%, ^{1}H-nmr ($CDCl_3$)δ 6.18 (dt, J=33 and 7.5 Hz, CH=CF), ^{19}F-nmr($CDCl_3$)δ -63 (d, J=33 Hz)]. Reduction of 5 with DIBAL-H (0.7 M hexane solution, Et_2O, -78°C, 30 min) gave the alcohol (6, 90%), which was converted to the bromide (7) by treating with butyllithium (THF, -78°C, 5 min), followed by the reaction with methanesulfonyl chloride in the presence of LiBr (THF, -78°C, 2 h) to give 7 [95%, ^{1}H-nmr($CDCl_3$)δ 5.30 (dt, J=18 and 7.5 Hz, CH=CF), ^{19}F-nmr($CDCl_3$) -36.5 (dt, J=18 and 22.5 Hz)].

The carbon chain extension reaction of the phosphate of 6 or the acetate of 6 with Grignard reagent [$BrMg(CH_2)_2CH\langle^{O}_{O}\rangle$, $CuBr\cdot Me_2S$][7] or lithium enolate of ethyl acetate with the bromide (7) in the presence of copper(I)[8] gave the undesired allylic rearranged products. Reaction of 7 with lithium salt derived from deprotonation of ethyl phenylthioacetate [LDA, Et_2O-HMPA (10:3), -78°C, 20 min] at room temperature for 1.5 h afforded the ester (8, 82%). Desulfurization of 8 with Raney-Ni (W-2) in acetone (9, 69%) and the subsequent reduction of 9 with DIBAL-H (Et_2O-hexane, -78°C, 1 h) afforded the alcohol (10, 93%). Conversion of the hydroxyl group to the ester group was achieved by mesylation (MsCl-Et_3N, CH_3Cl_2, -23°C), cyanation (KCN-18-crown-6, DMF, 50°C, 5 h), hydrolysis (NaOH, H_2O-MeOH, refl., 24 h then 10% HCl) followed by esterification (CH_2N_2, Et_2O) to give the methyl ester (11, 64%).

Deprotection of 11 (0.75 M HCl in 15:1 MeOH-H_2O, 1 h) gave the diol (12, 68%), which was cleaved to the corresponding β,γ-unsaturated aldehyde [1.4 equiv. $Pb(OAc)_4$, CH_2Cl_2, 10 min, -78°C].[9] This was allowed to react with the ylide derived from deprotonation of the phosphonium salt (14) [BuLi, THF-HMPA (8:1), -78°C][10] to give after chromatography methyl 5-fluoroarachidonate (15) in 24% yield. Hydrolysis of 15 [KOH, H_2O-MeOH-THF (1:4:4), r.t., 1 h] afforded 5F-AA (1) in 87% yield.[11]

Human platelets were incubated with 5F-AA (1) or AA in phosphate buffer in the presence of ionophore A23187 at 37°C for 20 min[12] followed by the reversed-phase HPLC of the extract. The reaction products from AA (Fig. 1,A) were compared with those from 5F-AA (Fig. 1,B). Compounds in (B) corresponding to those in (A) are probably non-fluorinated metabolites produced from endogenous AA released from cell membrane phospholipids[2] while peaks which appeared only in (B) are probably fluorinate compounds. The most abundant product derived from 5F-AA (Fig. 1,B, cross hatched peak) was collected, esterified (CH_2N_2) and chromatographed on a straight phase HPLC (Zorbax Sil column, Dupont; 1% 2-propanol in hexane).

UV absorption of the product showed an absorpsion maximum of 235 nm, character-

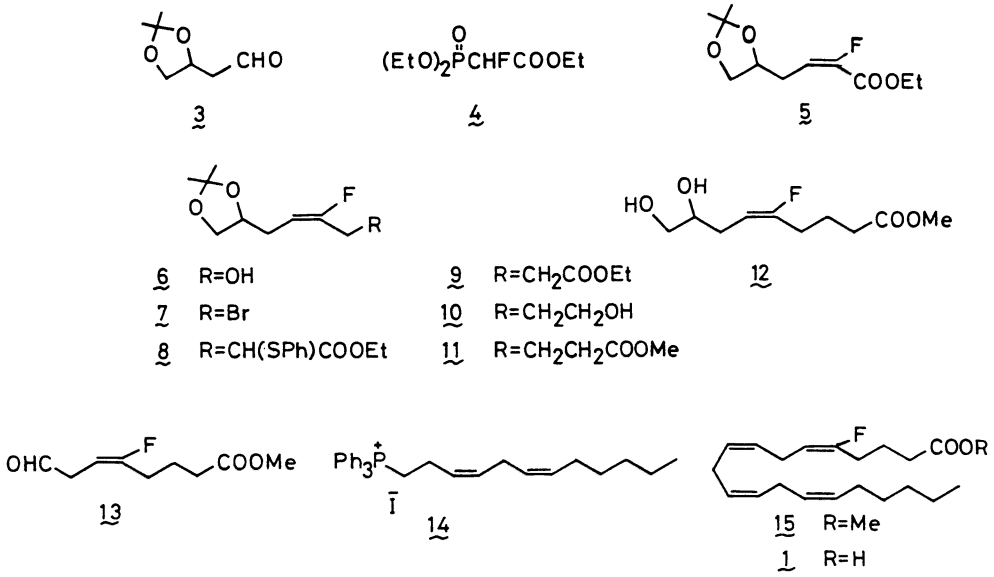
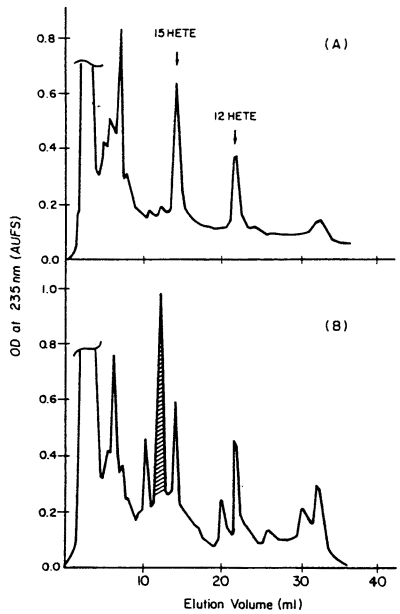


Chart 2



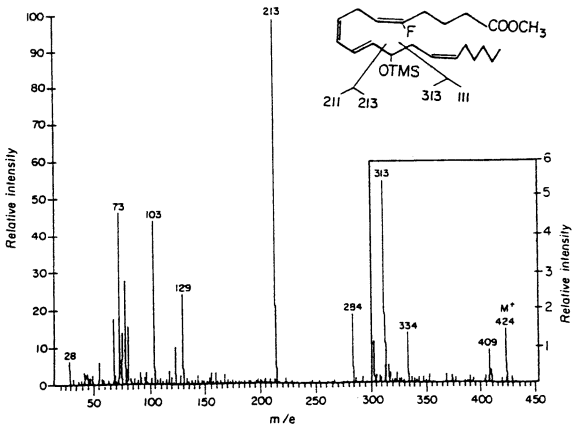


Figure 2. Mass Spectrum of the Product

Figure 1. RP-HPLC profiles of extracts of incubation media [(A) AA, (B) 5F-AA] chromatographed on a Zorbax ODS column (6.2 mm x 8 cm, Dupont) with MeOH/H_2O/acetic acid (75/25/0.01, v/v/v) at a flow rate of 2 ml/min produced by 2000 psi pressure. Arrows indicate elution position of authentic 15-HETE and 12-HETE (both purchased from Cayman Chemical, Ann Arbor, MI).

istic for HETE compounds.[13] A mass spectrum of the methylester trimethylsilylated with N,O-bis(trimethylsilyl)trifluoroacetamide showed ions at m/z 424 (M^+), 409 (M^+-15), 334 (M^+-90; loss of H+OTMS), 313 (M^+-111) and 213 (M^+-211; base peak)(Fig. 2). In comparison with that of natural 12-HETE, m/z 406 (M^+), 391 (M^+-15) and 295 (M^+-111),[13] this compound was identified as 5F-12-HETE (2). In this incubation about 11 μg of 2 was produced from 100 μg of 1. These data suggest that a significant amount of analog sufficient to perform preliminary biological assay can be produced by biotransformation.

Despite the fact that 12-HETE is the major product of AA by platelets,[13,14] its physiological significance has not been precisely defined. If a specific position of AA is substituted with fluorine(s) and incubated with platelets, 12-HETE fluorinated at the specific position can be produced. Such analogs represent new tools for investigating structure-activity relationships, and the metabolic and functional importance of natural 12-HETE.

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REFERENCES AND NOTES

1) R. Filler and S. M. Naqvi, "Biomedicinal Aspects of Fluorine Chemistry", ed. by R. Filler and Y. Kobayashi, Elsevier Biomedical Press, 1982, pp. 1-32.
2) A. J. Marcus, Am. J. Med., 78, 805(1985).
3) a) P. A. Grieco, W. Owens, C. Wang, E. Williams, and W. J. Schillinger, J. Med. Chem., 23, 1072(1980); b) J. Fried, D. K. Mitra, M. Nagarajan, and M. M. Mehrotra, ibid., 23, 234(1980); c) K. Bannai and S. Kurozumi, J. Synth. Org. Chem., Japan, 42, 794(1984).
4) a) H. Machleidt and G. Strehlke, Justus Liebigs Ann. Chem., 681, 21(1965); b) G. Etemad-Moghadam and J. Seyden-Penne, Bull. Soc. Chim. Fr., 448(1985).
5) K. Mori, T. Takigawa,and T. Matsuo, Tetrahedron, 35, 933(1979).
6) In ^{19}F-nmr, benzotrifluoride was used as an external standard.
7) J. A. Marshall and K. E. Flynm, J. Am. Chem. Soc., 105, 3360(1983).
8) I. Kuwajima and Y. Doi, Tetrahedron Lett., 1163(1973).
9) E. J. Corey and P. T. Lansbury, Jr., J. Am. Chem. Soc., 105, 4093(1983).
10) J. R. Pfister and D. V. Krishna Murthy, J. Med. Chem., 26, 1099(1983).
11) 1: ^{1}H-nmr (400 MHz; $CDCl_3$)δ 0.891 (3H, t, J=6.8 Hz, 20-H), 1.25-1.40 (6H, m, 17, 18 and 19-H), 1.868 (2H, quintet, J=7.3 Hz, 3-H), 2.055 (2H, q, J=7 Hz, 16-H), 2.328 (2H, dt, J=22.7 and 7.2 Hz, 4-H), 2.409 (2H, t, J=7.4 Hz, 2-H), 2.691 (2H, t, J=7.5 Hz), 2.807 (2H, t, J=6.4 Hz), 2.822 (2H, t, J=6.5 Hz), 5.065 (1H, dt, J=21.4 and 8 Hz, 6-H), 5.28-5.45 (6H, m, 8, 9, 11, 12 and 15-H).
12) W. F. Stenson, S. M. Precatt, and H. Spiecher, J. Biol. Chem., 259, 11784(1984).
13) M. Hamberg and B. Samuelsson, Proc. Natl. Acad. Sci. U.S.A., 71, 3400(1974).
14) P. Y. K. Wong, P. Westlund, M. Hamberg, E. Granström, P. H. W. Chao, and B. Samuelsson, J. Biol. Chem., 260, 9162(1985).

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