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A SIMPLE AND EFFICIENT SYNTHESIS OF (7*E*, 9*E*, 11*Z*, 13*E*)-(5*S*, 6*R*, 15*S*)-TRIHYDROXYEICOSATETRAENOIC ACID (6*R*-LIPOXIN A)

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Summary: A practical seven-step synthetic route to 6*R*-lipoxin A from arachidonic acid is described which makes this biologically interesting eicosanoid easily available.

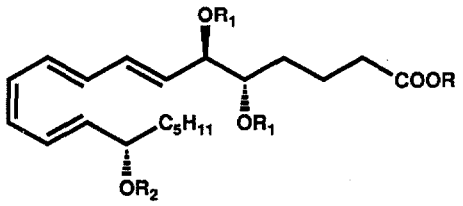
The versatility of arachidonic acid (and related C_{20} polyunsaturated fatty acids) as a precursor of biologically active short-range mammalian cell signalling agents has become increasingly evident in recent years. Initial 11-lipoxygenation leading to prostanoids and 5-lipoxygenation leading to leukotrienes are the most thoroughly studied routes to eicosanoids. However, 8-lipoxygenation, leading to marine prostanoids, 12-lipoxygenation leading to hepoxilins, and 15-lipoxygenation leading to the 14,15-epoxy analog of leukotriene A_4 and to lipoxins all appear to be of considerable significance, though much remains to be learned in these areas. This paper deals with the lipoxin pathway, an area afflicted with uncertainty and confusion because of the isolation of several position-isomeric and stereoisomeric lipoxins (in only low yield and tiny amount from human leukocytes) and the facile stereomutation of *Z* double bonds in the tetraene chromophore,[1] with specific reference to "lipoxin A." Lipoxin A is now used to describe 6*R* and 6*S* forms of (7*E*, 9*E*, 11*Z*, 13*E*)-(5*S*, 6,15*S*)-trihydroxyeicosatetraenoic acid. The 6*R* form seems to be more significant biologically at present since it is more active than the 6*S* diastereomer with regard to smooth muscle contraction,[1a] inhibition of neutrophile chemotaxis[2] and inhibition of the phosphatidyl inositol cycle.[2] We have previously described a short synthesis of the both 6*R*-lipoxin A (**1**) and 6*S*-lipoxin A.[3] We now describe a second generation synthesis which leads stereoselectively to the biologically more interesting 6*R*-lipoxin A by what we believe to be the simplest and most practical route known.[4]

A key observation in connection with the new process was the finding that the readily available enzyme soybean lipoxygenase could be used for the transformation of arachidonic acid (**2**) into 5*S*,15*S*-

diHETE (**3**). It had been reported previously that soybean lipoxygenase at pH 6.8 (0.2*M* phosphate buffer, 23°C) converts arachidonate via 15*S*-HPETE into 8*S*,15*S*-di-HPETE in good yield, leading after reduction to 8*S*,15*S*-diHETE.[5] We have discovered that at pH 11 (borate buffer, 0°C) under an atmosphere of O_2 soybean lipoxygenase catalyzes the transformation of arachidonate into a mixture of *two bis*-hydroperoxides (24 hour reaction time at 23°C) which upon reduction with sodium borohydride provides a corresponding mixture of 5*S*,15*S*-diHETE (**3**) and 8*S*,15*S*-diHETE (**4**) in a total yield of 80% and a ratio of 35 : 65.[6] This mixture could be used directly and conveniently for the synthesis of **1** in the following way. The mixture was treated with 1,1'-carbonyl diimidazole (1.5 equiv) in dry dichloromethane at 23°C for 5 hours. During this time, the 5*S*,15*S*-diHETE (**3**) underwent lactonization to give the 15*S*-hydroxy lactone **5**, whereas 8*S*,15*S*-diHETE (**4**) remained unchanged. Extractive isolation and silica gel filtration using hexane-ethyl acetate (4 : 1) as eluant gave pure lactone **5** in 91% of the theoretical yield. The 8*S*,15*S*-diHETE could be recovered from the column by eluting with a more polar solvent system, such as hexane-ethyl acetate-isopropanol (1 : 1 : 0.05).

The optically pure lactone **5** ($[\alpha]_D^{23}$ = +98.8°, *c* 1.3, chloroform) was transformed into the hydroxy ester **7** by the sequence: (1) silylation with *t*-butyldimethylsilyl chloride (2.0 equiv) and imidazole (3.0 equiv) in DMF at 23°C for 2 hours to produce the corresponding silyl ether **6** ($[\alpha]_D^{23}$ = +67.1°, *c* 2.45, chloroform) in 92% yield, and (2) lactone opening to provide the hydroxy methyl ester **7** ($[\alpha]_D^{23}$ = +17.8, *c* 2.95, chloroform) by treatment with triethylamine in dry methanol at 23°C for 2 hours (90% yield).

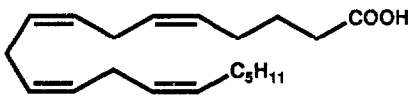
Attention was next turned to the stereoselective epoxidation at the 5,6-double bond of **7**. In our earlier work the epoxidation of **7** was utilized for the synthesis of both the 6*R*- and 6*S*-diastereomers (erythro and threo, respectively) but no attempt was made to optimize stereoselectivity. Therefore, the vanadyl acetoacetonate-catalyzed epoxidation of the allylic alcohol **7** had to be studied to improve the erythro/threo ratio. It was found that the best ratio could be obtained using dichloromethane as solvent (erythro/threo = 5/1 in dichloromethane compared to 2/1 in toluene or benzene[7]). Thus allylic alcohol **7** was treated in dichloromethane at 0°C with *t*-butylhydroperoxide (3.0 equiv), vanadyl acetoacetonate (1.0 equiv) and 2,6-lutidine (1.0 equiv) for 3 hours to yield the corresponding epoxy alcohols (72%, 5 : 1 ratio of erythro to threo). The use of 5A molecular sieves was also found to accelerate the reaction presumably by removing the co-product *t*-butyl alcohol which can inhibit reaction by coordination to the vanadium catalyst. The two isomeric epoxy alcohols were separated by use of silica gel chromatography using hexane-ethyl acetate (20 : 1) as eluent. The R_f values for the erythro epoxy alcohol **8** and its threo diastereomer are 0.39



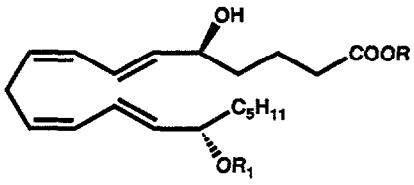
1 R, R_1, R_2 = H, 6*R*-Lipoxin A

9 R = Me, R_1 = TMS, R_2 = TBDMS

10 R = Me, R_1, R_2 = H

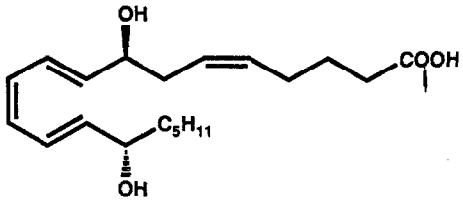


2 Arachidonic Acid

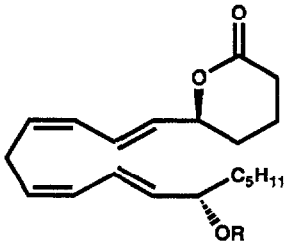


3 R, R_1 = H, 5*S*,15*S*-DiHETE

7 R = Me, R_1 = TBDMS

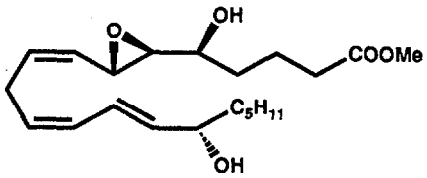


4 8*S*,15*S*-DiHETE



5 R = H

6 R = TBDMS



8 erythro

and 0.30 using silica gel plates with 9 : 1 methylene chloride-ether for development or 0.24 and 0.20 using 2 : 1 hexane-ethyl acetate. The pure erythro isomer **8** ($[\alpha]_D^{23}$ = +23.5°, *c* 1.0, chloroform) was converted to the tetraenoic trisilyl ether **9** by treatment with trimethylsilyl triflate (4.0 equiv) and 2,6-lutidine (6.0 equiv) in toluene at -78°C for 2 hours.[8] Desilylation of **9** with tetra-*n*-butylammonium fluoride (10 equiv) and acetic acid (10 equiv) in THF at 23°C for 1 hour gave rise to the lipoxin A methyl ester (**10**) in 82% overall yield after silica gel chromatography ($[\alpha]_D^{23}$ = +38.2°, c 0.86, methanol).[9]

The synthesis of 6*R*-lipoxin A recorded herein is recommended for use not only because it can provide this substance in ample quantities for experimental work with a minimum of time and effort, but also because it requires only easily available reagents and standard techniques. The synthesis is potentially useful for the large scale production which would be required if 6*R*-lipoxin A were to prove useful clinically, for example in inhalation therapy for respiratory illness.[10]

References and Notes

1. For recent discussions see (a) C. N. Serhan, K. C. Nicolaou, S. E. Webber, C. A. Veale, S.-E. Dahlén, T. J. Prustinen, and B. Samuelsson, *J. Biol. Chem.*, **1986**, *261*, 16340; (b) N. Ueda, S. Yamamoto, B. J. Fitzsimmons, and J. Rokach, *Biochem. Biophys. Res. Commun.*, **1987**, *144*, 996; (c) E. J. Corey and M. M. Mehrotra, *Tetrahedron Letters*, **1986**, *27*, 5171.
2. T. H. Lee and B. W. Spur, results in press and personal communications.
3. E. J. Corey and W. Su, *Tetrahedron Letters*, **1985**, *26*, 281.
4. For other syntheses see (a) J. Adams, B. J. Fitzsimmons, Y. Girard, Y. Leblanc, J. F. Evans, and J. Rokach, *J. Am. Chem. Soc.*, **1985**, *107*, 464; (b) J. Adams, B. J. Fitzsimmons and J. Rokach, *Tetrahedron Letters*, **1984**, *25*, 4713; (c) K. C. Nicolaou, C. V. Veale, S. E. Webber, and H. Katerinopoulos, *J. Am. Chem. Soc.*, **1985**, *107*, 7515.
5. (a) G. S. Bild, C. S. Ramadoss, S. Lim, and B. Axelrod, *Biochem. Biophys. Res. Commun.*, **1977**, *74*, 949; (b) S. Shak, H. D. Perez, and I. M. Goldstein, *J. Biol. Chem.*, **1983**, *258*, 14948.
6. The mixture of **3** and **4** was isolated by extraction and rapid silica gel chromatography using hexane-ethyl acetate-acetic acid (1 : 1 : 0.05) as eluent.
7. E. D. Mihelich, *Tetrahedron Letters*, **1979**, *20*, 4729.
8. The *E,E*-geometry of the newly formed double bonds (Δ^7, Δ^9) in **9** is dictated by the stereospecificity of the reaction. For its earlier precedents see: (a) S. Murata, M. Suzuki, and R. Noyori, *J. Am. Chem. Soc.*, **1979**, *101*, 2738; (b) E. J. Corey, A. Marfat, and J. O. Albright, *J. Am. Chem. Soc.*, **1980**, *102*, 1433.
9. Satisfactory spectroscopic data for all intermediates were obtained. For lipoxin A methyl ester (**10**): ^{1}H NMR (500 MHz, CD_3CN): 6.70 (m, 2H), 6.35 (m, 2H), 6.00 (m, 2H), 5.70 (m, 2H), 4.08 (m, 1H), 3.98 (m, 1H), 3.60 (s, 3H), 3.49 (m, 1H), 2.32 (t, 2H), 2.10 - 1.20 (m, 15H); FT-IR (neat): 3450, 1730 (cm^{-1}); UV (methanol): 284, 296, 309 (nm); RP-HPLC (C_{18} Zorbax reverse phase column, 4.6 x 250 mm, using methanol-water-acetic acid/75 : 25 : 0.001 as eluent): retention time 11.0 min at 1.5 ml/min.

10. This work was financially assisted by grants from the National Institutes of Health and the National Science Foundation.

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