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# SIMPLE SYNTHESIS AND ASSIGNMENT OF STEREOCHEMISTRY OF LIPOXIN A

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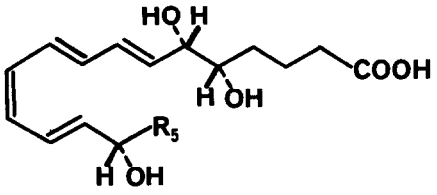
Summary: A synthesis of lipoxin A, a recently discovered biologically active eicosanoid, and the assignment of stereoformula 1 is reported herein.

Serhan, Hamberg and Samuelsson have recently described the isolation of a 5, 6, 15S-trihydroxy-7, 9, 11, 13-eicosatetraenoic acid from human leukocytes exposed to 15-HPETE (15S-hydroperoxy-5Z, 8Z, 11Z, 13E-eicosatetrenoic acid) in the presence of calcium ionophore A23187.[1] These workers have termed this substance lipoxin A (LX-A) and have shown it to have interesting biological activity (stimulation of elastase and superoxide ion release from neutrophils, an activity shared with leukotriene $B_4$).[2] We report herein an effective synthesis of this substance which makes it obtainable in quantity from arachidonic acid without the need for resolution. Native lipoxin A is available in submicrogram or microgram amounts only with difficulty (and biosynthesis requires access to a supply of human neutrophils). The availability of synthetic material (which has been identified by comparison with biosynthetic lipoxin A) should accelerate the study of the biological role of this new eicosanoid. The synthetic route reported herein allows the assignment of stereoformula 1 to lipoxin A.

The readily available 15S-HETE methyl ester ( 2 )[3] was transformed into the t-butyldimethylsilyl ether ( 3 ) by reaction with t-butyldimethylsilyl triflate (1.5 equiv) and lutidine (2.5 equiv) in methylene chloride at 0° for 20 min (95% yield).[4] The silyl ether 3 was converted into the 5-hydroxy-6E-silyl ether - methyl ester 4 in 74% overall yield by the standard iodolactonization method[5] consisting of the sequence: (1) methyl ester saponification ($H_2O$ - THF - methanolic lithium hydroxide for 4 hr at 23°); (2) neutralization to pH 7 and iodolactonization with iodine - potassium bicarbonate - potassium iodide for 10 hr at 0°; (3) treatment of the iodo δ-lactone with diazabicycloundecene (DBU) in benzene for 2 hr at 23°; and (4) methanolysis (methanol - triethylamine for 2 hr at 23°). Treatment of 4 with 1.5 equiv of anhydrous t-butylhydroperoxide and 0.2 equiv of vanadyl acetylacetonate in benzene for 1 hr at 23° afforded after chromatography on silica gel the threo epoxy alcohol 5 and the erythro isomer ( 6 ) in a ratio of 1:2 ($R_f$ values 0.30 and 0.39, respectively, using silica gel plates with 9 : 1 methylene chloride - ether for development).[6-9] Reaction of the threo epoxy alcohol ester 5[8] with 2.2 equiv of trimethylsilyl triflate and 4 equiv of 2, 6-lutidine in toluene at -78° for 1 hr afforded cleanly the tetraene triol triether 7, UV maximum 289.5, 302 and 316.5 nm (methanol), isolated in 94% yield after filtration through a silica gel column using 5 : 1 hexane - ether containing 1% triethylamine. The E, E-geometry of the newly formed

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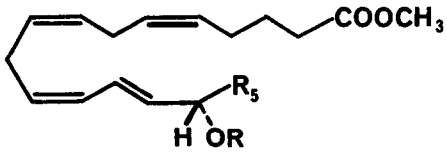
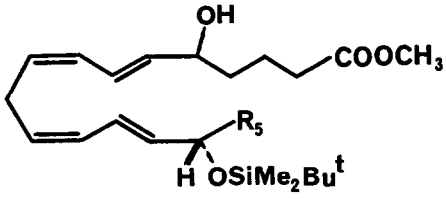



2 R=H

3 R=TBDMS

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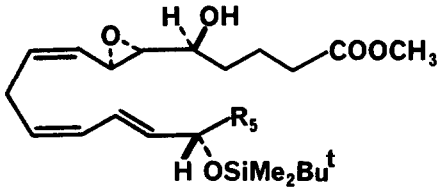


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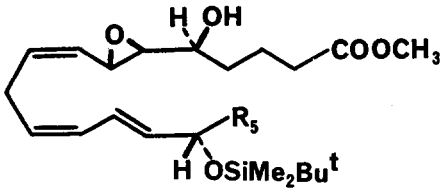


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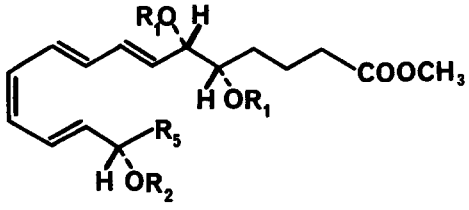
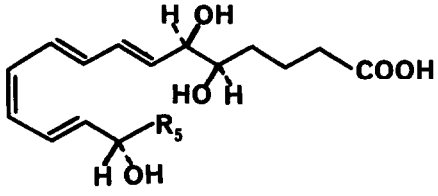



7 $R_1$=TMS $R_2$=TBDMS

8 $R_1=R_2$=H




9

$R_5$= $nC_5H_{11}$

double bonds ($\Delta^7$, $\Delta^9$) in 7 is indicated by the stereospecificity of the reaction and much earlier precedent.[10,11] Desilylation of 7 was effected cleanly using 30 equiv of tetra-n-butylammonium fluoride hydrate (Aldrich Chem. Co.) in concentrated solution in THF for 1 hr at 23° to give 90% of triol ester 8 after preparative thin layer chromatography on silica gel (7 : 3 ethyl acetate - hexane as eluent at 4°).[12]

Saponification of 8 with 3 equiv of lithium hydroxide in 4 : 1 : 1 THF - methanol - water at 0° for 2 hr afforded, after acidification to pH 5 and extraction with ether, lipoxin A ( 1 ) and the 5R, 6R, 15S-diastereomer ( 9 ) in a ratio of ca. 1 : 1 (100% yield) as determined by reversed phase HPLC analysis. Using 7 : 3 methanol - water as solvent with a Waters Assoc. 15 cm. NOVA PAC $C_{18}$ column (flow rate 1 ml/min) retention times were measured as 6.5 min for lipoxin A ( 1 ) and 5.6 min for diastereomer 9; the corresponding values for the methyl esters were 8.5 min for 1 methyl ester and 7.5 for 9 methyl ester. Authentic lipoxin A was prepared biosynthetically using human neutrophils, 15-HPETE and A23187 as described.[1,2] Both reversed phase HPLC mobilities and UV absorption (max 288, 301, 316 nm) were identical for native lipoxin A and synthetic 1 as the methyl esters. The assignment of the 5S, 6S stereochemistry to lipoxin A is dictated by the known stereochemistry of 5-lipoxygenation (5S) and the known S configuration of $LTB_4$ at C(5).

In a parallel sequence the erythro epoxy alcohol 6 was transformed into a pair of diastereomers of lipoxin A methyl ester. Although the UV absorption of these diastereomeric methyl esters was the same as for lipoxin methyl ester, the reversed phase HPLC retention times were clearly different. Under the standard HPLC conditions described above for lipoxin methyl ester and synthetic 1 methyl ester, the determined retention times were as follows: lipoxin A methyl ester, 8.5 min; lipoxin diastereomer # 1 from erythro epoxide 6, 5.5 min; and lipoxin diastereoisomer # 2 from erythro epoxide 6, 6.3 min. These results unambiguously confirm the assignment of 1 to lipoxin A.

Subsequent to the completion of this work a paper by Adams et al. appeared in which a synthesis of the 5S, 6R- and 5R, 6S-diastereomers of 1 is reported, without comparison of these synthetic products with biosynthetic lipoxin A.[13]

We are currently studying a modification of our synthesis utilizing a reagent for the selective synthesis of the threo epoxy alcohol 5 from 4. Synthetic lipoxin A has been supplied to our collaborators at the Harvard Medical School for detailed biological study.[14,15]

## References and Notes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5. (a) E. J. Corey and S. Hashimoto, Tetrahedron Letters, 22, 299 (1981); (b) E. J. Corey, J. O. Albright, A. E. Barton, and S. Hashimoto, J. Am. Chem. Soc., 102, 1435 (1980).

6. The threo (more polar) epoxy ketone 5 was distinguished from the erythro (less polar) isomer 6 and assigned that geometry on the basis of 270 MHz pmr spectra, and also the predominance of the erythro product (consistently observed in previous work with the vanadium-catalyzed epoxidation[7]). Observed values for $\delta H_5$ and $J(H_5, H_6$ for more polar (threo) and less polar (erythro) hydroxy epoxides were 3.30 ppm, 4.3 Hz and 3.55 ppm, 1.0 Hz, respectively. See (a) E. D. Mihelich, Tetrahedron Letters, 4729 (1979); (b) J. R. Falck, S. Manna, A. K. Siddhanta, and J. Capdevilla, Tetrahedron Letters, 24, 5715 (1983); (c) B. E. Rossiter, T. R. Verhoeven and K. B. Sharpless, Tetrahedron Letters, 4733 (1979); (d) E. J. Corey, M. M. Mehrotra, Tetrahedron Letters, 24, 4921 (1983).

7. This procedure generally provides the erythro isomer in larger amount than the threo form. See A. S. Narula, Tetrahedron Letters, 23, 5579 (1982); 22, 2017 (1981) and refs. 6a-6c.

8. Each product (threo or erythro) is a mixture of two diastereomers with respect to the stereo-relationship between C(15) (S) and C(5), both (S) and (R). For convenience only the 5S threo diastereomer 5 is shown.

9. Satisfactory spectroscopic data (270 MHz PMR, infrared, ultraviolet and mass spectral) were obtained for each reaction product using a chromatographically purified and homogeneous sample.

10. See, for example, (a) S. Murata, M. Suzuki and R. Noyori, J. Am. Chem. Soc., 101, 2738 (1979); and (b) E. J. Corey, A. Marfat, J. R. Falck, and J. O. Albright, J. Am. Chem. Soc., 102, 1433 (1980).

11. Analysis of this product by HPLC using a Waters Assoc. silica column (100 : 1 : 0.5 hexane - THF - triethylamine, detection at 302 nm) showed two equal peaks as expected for the C(15)/C(5) diastereomer mixture.[8]

12. The triol ester 8 showed two equal peaks by reversed phase HPLC using a $C_{18}$ column with 7 : 3 methanol-water as the mobile phase, as expected for the diastereomeric 5S, 6S/5R, 6R mixture.[8]

13. J. Adams, B. J. Fitzsimmons, and J. Rokach, Tetrahedron Letters, 25, 4713 (1984).



14. We are grateful to Drs. Robert A. Lewis, K. F. Austen, and R. A. Soberman of the Harvard Medical School for supplying a preparation of human neutrophils.

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