Tetrahedron Letters,Vol.27,No.43,pp 5173-5176,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

A STEREOSELECTIVE AND PRACTICAL SYNTHESIS OF 5,6(*S,S*)-EPOXY-15(*S*)-HYDROXY-7(*E*),9(*E*),11(*Z*),13(*E*)-EICOSATETRAENOIC ACID (4), POSSIBLE PRECURSOR OF THE LIPOXINS

E. J. Corey and Mukund M. Mehrotra
Department of Chemistry, Harvard University, Cambridge, Massachusetts, 02138

Summary: A stereoselective synthesis of **4**, a possible biosynthetic precursor of lipoxins, is reported. A pathway is suggested by which **4** can give rise to the biologically active lipoxins **1** and **2**.

In 1984 Serhan, Hamberg and Samuelsson reported that human leukocytes convert 15*(S)*-hydroperoxy-5,8,11*(Z)*, 13*(E)*-eicosatetraenoic acid (15-HPETE) to two different trihydroxyeicosatetraenoic acids and assigned to these the trivial names lipoxin A and lipoxin B.[1] Lipoxin A was proposed to be a 5,6,15*(S)*-trihydroxy-7,9,11,13-eicosatetraenoic acid and lipoxin B was proposed as a 5*(S)*, 14,15*(S)*-trihydroxy-6,8,10,12-eicosatetraenoic acid. Because these compounds were obtained in low yield and in only microgram amount, it was obvious that chemical synthesis was needed to allow assignment of exact structure and to facilitate the study of biological properties and significance. The work of our group[2] and of others[3,4] resulted in the synthesis of **1** and its correlation with the material which had been designated as lipoxin A. Two synthetic 5, 14, 15-trihydroxy acids, diastereomers **2** and **3** which we synthesized were found to correspond to two other peaks in the biosynthetic lipoxin mixture,[5] but unambiguous identification with lipoxin B was not possible since requests for comparison with the material obtained by the original investigators remained unanswered. The minor component of our biosynthetic mixture corresponded to **2** and the major component to **3**. As a consequence of the unavailability of authentic "lipoxin B" and the facile isomerization of *Z*-double bonds in the tetraene chromophore at pH *ca.* 3, the literature now presents conflicting views as to the detailed structure of lipoxin B. A group at Merck Frosst Canada has reported that the geometry of the tetraene unit of lipoxin B is all *trans*.[6] On the other hand workers at the Upjohn Company have concluded that "natural" lipoxin B is a mixture of **2** and the 14*(R)*-8-*trans* and 14 *(S)*-8-*trans* isomers.[7] It has also been found in tests of stimulation of protein kinase C that lipoxin A (**1**) is the most active of the naturally derived lipoxin isomers, but that **2** and the 14*(R)*-8-*trans* and 14*(S)*-8-*trans* isomers also show significant activity.[8] Recently, in a publication by the originators of the trivial name "lipoxin," the name lipoxin B (LXB) was given to compound **2**,[9] and it was also reported that the oxygen at C (14) of native **2** is derived biosynthetically from water rather than dioxygen,[9] an indication that **2** may be formed from a precursor such as **4** (formally a 15-lipoxygenated (15-LO) derivative of leukotriene A_4 (LTA_4)).

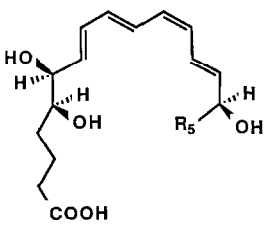
Although the physiological role of the various trihydroxy acids of the lipoxin class is still unclear, the question of the mechanism of formation of **1**, **2**, and the other isomers obtained from 15-HPETE (or 15-HETE)[9] by incubation with neutrophils is both interesting and relevant. It has been generally accepted that the oxygens at C (5) and C (15) are both introduced by lipoxygenation of arachidonic acid. The third oxygen at C (6) or C (14)

could in principle be derived either by a LO process or by hydration of a suitable reactive intermediate, although the former pathway now seems excluded for **2**.[9]

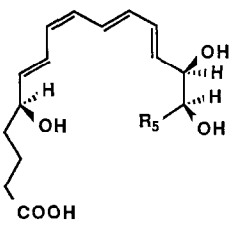
For some time we have been interested in the question of whether 5, 6, 15-oxygenated and 5, 14, 15-oxygenated lipoxins such as **1** and **2** might arise from **4** as a common precursor. In this note we describe a highly practical, stereoselective synthesis of **4** and a simple explanation for the formation of **1** and **2** biosynthetically from **4** under enzymic control.

The readily available dienal **5**, a key intermediate in the original stereocontrolled synthesis of leukotriene A,[10] was an obvious starting point for the synthesis of **4**. The other requisite Wittig coupling component, phosphonium salt **6**, was synthesized by the route shown in Scheme 1 and is thus readily available. The *Z*-stereoselective coupling of **5** and **6** was expected to pose a problem since our experience and that of others has shown that free alkoxy groups in the Wittig ylide component tend to favor *E*-olefination.[11] *Z*-Selective Wittig condensation of **5** and **6** was achieved by the use of a new *in situ* alkoxide protection method. The alkoxide of phosphonium salt **6** was generated (-78°C) using potassium hexamethyldisilazide in THF and stannylated by reaction with tri-*n*-butyltin triflate[12] at -78°C. The resulting phosphonium salt was converted to the corresponding ylide by further reaction with 1.1 equivalent of potassium hexamethyldisilazide at -78°C and then allowed to react with epoxy ester **5**. Aqueous workup at pH 9.5 resulted in destannylation to give the desired coupling product, **4** methyl ester, stereoselectively (*Z/E* ratio 6:1) in 59% yield after rapid chromatography on silica gel deactivated with triethylamine (for **4**, R_f = 0.27 using 65:35 hexane-ethyl acetate containing 5% triethylamine, UVmax (CH_3OH) 292, 305, 320 nm).[13,14] Saponification of the methyl ester using 1*N* lithium hydroxide in 3:1 THF-methanol gave solutions of the salt of **4** having the expected UV absorption at 292, 305 and 320 nm.[15]

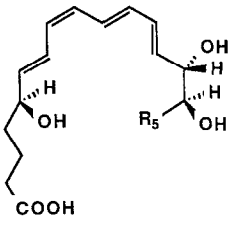
When aqueous solutions of **4** (produced by saponification) were adjusted to pH 6.7 - 7 at 23° hydrolysis ensued to give mixture of products which was examined by reversed phase HPLC analysis on a Waters μ-Bondapak column (30 cm x 3.9 mm) using methanol-water-acetic acid (65: 35: 0.05). We found no appreciable amount of the 8-*Z*-lipoxin B isomers **2** and **3**. The major products, in agreement with an earlier report,[16] were the all *trans* tetraene isomers corresponding to LXB's **2** and **3**, all *trans* tetraenes corresponding to **1** and 6-*epi*-**1**, and finally 6-*epi*-**1** and **1** itself. Thus, it seems likely that **1** and **2** produced by human leukocytes result from enzymic rather than non-enzymic hydrolysis of **4**. A simple explanation of the origin of **1** and **2** is that the lipoxin synthetase binds to the bottom pi face of **4** which is then converted by proton transfer to the enzyme-bound delocalized cation **7**. Attack by water on **7** subsequently occurs from the opposite (top) pi face either at C(6) to form **1** or at C(14) to form **2**. If **1** and **2** are both formed from **4** by the same enzyme, it may be that the relative amounts are regulated by the conformational state of the enzyme. The incubation of 15-HPETE with human neutrophils *in vitro* produces a more complex mixture than originally described, apparently as function of conditions used, isomerization during isolation, and perhaps also concurrent non-enzymatic hydrolysis of **4**.[17]



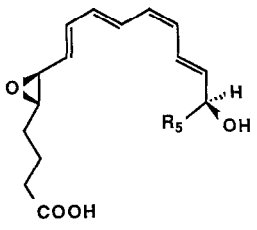
1



2

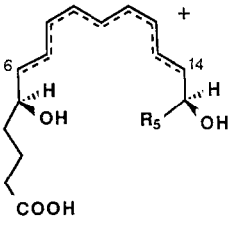


3



4

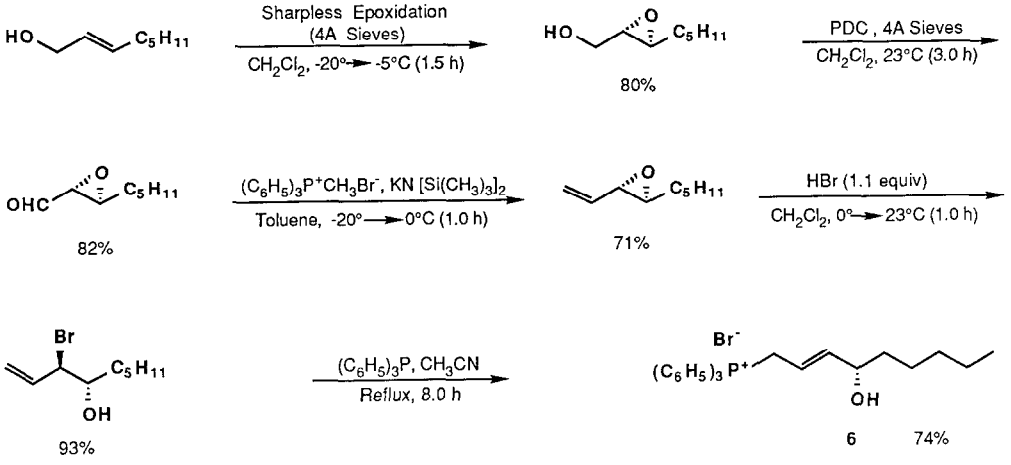
5



7

$R_5 = C_5H_{11}$

SCHEME 1



References and Notes

1. C. N. Serhan, M. Hamberg and B. Samuelsson, *Biochem. Biophys. Res. Commun.*, **118**, 943 (1984); *Proc. Natl. Acad. Sci. USA*, **81**, 5335 (1984).

2. E. J. Corey and W. Su, *Tetrahedron Letters*, **26**, 281 (1985).

3. J. Adams, B. J. Fitzsimmons, Y. Gerard, Y. Leblanc, J. F. Evans and J. Rokach, *J. Am. Chem. Soc.*, **107**, 464 (1985).

4. K. C. Nicolaou, C.A. Veale, S. E. Webber, and H. Katerinopoulos, *J. Am. Chem. Soc.*, **107**, 7515 (1985).

5. E. J. Corey, M. M. Mehrotra, and W. Su, *Tetrahedron Letters*, **26**, 1919 (1985).

6. Y. Leblanc, B. Fitzsimmons, J. Adams, and J. Rokach, *Tetrahedron Letters*, **26**, 1399 (1985).

7. J. Morris and D. G. Wishka, *Tetrahedron Letters*, **27**, 803 (1986).

8. A. Hansson, C. N. Serhan, J. Haeggström, M. Ingelman-Sundberg, B. Samuelsson, and J. Morris, *Biochem. Biophys. Res. Commun.*, **134**, 1215 (1986).

9. C. N. Serhan, M. Hamberg, B. Samuelsson, J. Morris, and D. G. Wishka, *Proc. Natl. Acad. Sci. USA* , **83**, 1983 (1986).

10. (a) E. J. Corey, D. A. Clark, G. Goto, A. Marfat, C. Mioskowski, B. Samuelsson, and S. Hammarstrom, *J. Am. Chem. Soc.*, **102**, 1436 (1980). (b) E. J. Corey and J. O. Albright, *J. Org. Chem.*, **48**, 2114 (1983).

11. (a) W. G. Salmond, M. A. Barta, and J. L. Havens, *J. Org. Chem.*, **43**, 790 (1978). (b) E. J. Corey and J. Kang, *J. Am. Chem. Soc.*, **104**, 4724 (1982); A. Marfat, unpublished observations in this laboratory.

12. E. J. Corey and T. E. Eckrich, *Tetrahedron Letters*, **25**, 2419 (1984).

13. When coupling of **5** and the ylide from **6** was carried out with *in situ* protection of alkoxide by trimethylsilyl (TMS) the yield of **4** methyl ester was only 37% but *Z*/*E* selectivity was still 6:1.

14. Product ratio was determined by HPLC analysis of the 15- TMS, methyl ester derivative of **4** with a DuPont Zorbax silica column using 2% triethylamine-hexane for elution; retention times were 7 min. for the major (11,12*Z*) isomer and 8.4 min. for the minor (11,12*E*) isomer of the 15-TMS ether, methyl ester of **4**. The stereochemistry about the 11, 12 double bond in **4** methyl ester was determined from 500 MHz decoupled PMR spectra which showed $J_{11,12}$ of 8.5 Hz.

15. Compare a previously outlined non-stereoselective synthesis of **4** reported by J. Adams, B. J. Fitzsimmons, and J. Rokach, *Tetrahedron Letters* **25**, 4713 (1984).

16. B. J. Fitzsimmons, J. Adams, J. F. Evans, Y. Leblanc, and J. Rokach, *J. Biol. Chem.*, **260**, 13008 (1985); see also ref. 6.

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

(Received in USA 14 July 1986)