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TOTAL SYNTHESIS OF 5S,12S-DIHYDROXY-6,10-E,8,14-Z-EICOSATETRAENOIC ACID (5S,12S-di-HETE) (2), A NEW HUMAN METABOLITE OF ARACHIDONIC ACID

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SUMMARY: A synthesis of 5S,12S-di-HETE (2) is reported which confirms the proposed structure and stereochemistry for this biologically active, natural eicosanoid.

Leukotriene B[1] (LTB) is an important human metabolite of arachidonic acid which is chemotactic for neutrophils and macrophages at concentrations as low as 1 ng/ml. The detailed structure of this substance was assigned recently as 1 on the basis of total syntheses[2,3] which also make this rare compound readily available. In the course of these studies various stereoisomers of 1 were also synthesized to ascertain whether any of these might be naturally occurring, and it was discovered that a (±)-6-E,8-Z isomer of 1 possessed considerable chemotactic activity, although ca. two orders of magnitude less potent than 1.[4] Because the method of synthesis employed racemic intermediates, it was not possible to determine whether the biologically active substance was 2 or the 5S,12R-diastereomer and for this reason we undertook an unambiguous synthesis of 2. Concurrently, a substance isomeric with LTB was isolated by a Canadian group from the incubation of arachidonic acid with mixed peripheral blood leukocytes,[5] and the same substance was obtained either from incubation of 5S-HETE with blood platelets or 12S-HETE with leukocytes.[5] The ultraviolet absorption spectra (UV_{max} in CH_3OH 258, 268, 278 nm) of the metabolite and previously synthesized[4] racemic 5,12-di HETE were identical as were proton magnetic resonance (pmr) spectra and reversed phase high pressure liquid chromatographic (RP-HPLC) behavior.[6,7]

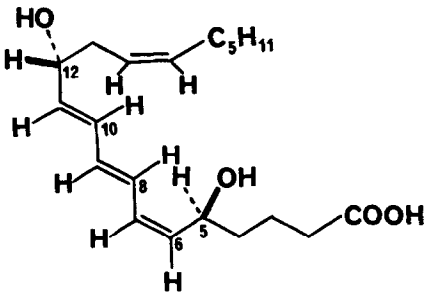
Methyl 5S-benzoyloxy-5-formylvalerate (3)[2], $[\alpha]_D^{25}$ -33° (c = 2.5 in $CHCl_3$) was heated in benzene at reflux with 1.1 equiv of formylmethylenetriphenylphosphorane[8] for 2 hr to afford in 90% yield after chromatography on silica gel the oily trans-α,β-unsaturated aldehyde 4, $[\alpha]_D^{25}$ +61.8° (c = 3.35 in $CHCl_3$), IR (neat) 1725, 1695, 1600, 1585 cm^{-1}, pmr (δ) 9.60 (d, J = 7.3 Hz, 1H, CHO); 8.1-7.5 (m, 5H aromatic); 6.85 (dd, J = 15.7, 4.6 Hz, 1H, β-olefinic) 6.27 (ddd, J = 15.7, 7.3, 1.4, 1H, α-olefinic).[9] 2S,3S-epoxy-5Z-undecen-1-ol (5) ($[\alpha]_D^{25}$ -17° (c = 2.2 in $CHCl_3$))

was prepared as described previously for the dextro enantiomer[3,4] except that dextrorotatory dimethyl tartrate was used as the chiral director. Oxidation of 5 with *in situ* generated $CrO_3 \cdot 2$ pyridine (6 equiv) in methylene chloride (2 hr at 23°) afforded epoxy aldehyde 6 which upon treatment with methylenetriphenylphosphorane (1.1 equiv) in tetrahydrofuran (THF) at 23° for 2 hr provided after chromatography on silica gel (6:1 pentane-ether containing 1% triethylamine) the epoxy diene 7 in 85% yield. Exposure of 7 to 1 equiv of dry hydrogen bromide in methylene chloride at 23° for 10 min produced the allylic bromide 8 which was treated without isolation with excess triphenylphosphine at reflux in methylene chloride for 40 hr to give the hydroxy phosphonium salt 9 as a colorless amorphous solid, $[\alpha]_D^{25}$ +7.27° (c = 2.4 in $CHCl_3$).

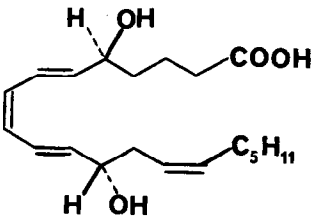
Treatment of 9 in THF with 2 equiv of n-butyllithium at -78° for 1 hr and -40° for 0.5 hr gave a deep red solution of oxido ylide to which was added at -78° 20 equiv of hexamethylphosphorictriamide and 1.0 equiv of the trans-α,β-unsaturated aldehyde 4. After 1 hr at -78° and 2.5 hr at -78° to 0° the product was separated by extractive isolation and thin layer chromatography (tlc) on silica gel. The desired coupling product, tetraene ester 10, $[\alpha]_D^{25}$ +112.67° (c = 1.46 in $CHCl_3$), UV_{max} in CH_3OH 261, 269.5, 280.5 ± 0.5 nm, R_f 0.30 (silica gel tlc, 1:1 hexane-ether) was obtained in ca. 25% yield.[10] Hydrolysis of 10 using 20 equiv of potassium carbonate in methanol at 23° for 12 hr followed by excess lithium hydroxide in methanol water at 23° for 1 hr afforded quantitatively 5S,12S-dihydroxy-6,10-E,8,14-Z-eicosanoate (2), UV_{max} in CH_3OH 258, 268.5, 279.0 ± 0.5 nm of > 95% purity by RP-HPLC analysis using a Waters Associates C_{18} μ-Bondapak column with 3:1 methanol-water containing 0.01% acetic acid for elution (retention vol 3.7).

Synthetic 2 produced in this way was identical with native 5,12-di-HETE[5,6] by RP-HPLC, UV and bioassay[11] of chemotactic effect on neutrophils. In addition it was converted to the methyl ester diacetate which was identical with the corresponding derivative prepared from native 5,12-di-HETE by pmr spectroscopy at 270 MHz.[12]

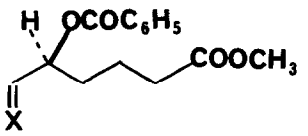
The synthesis of 2 reported herein provides unambiguous support for this formulation of the new metabolite of arachidonic acid recently isolated in low yield (ca. 2%) from leukocytes.[5] The availability of synthetic material by a short, convergent synthesis from intermediates which are common to the syntheses of other leukotrienes should facilitate the development of a radioimmuno assay for 2 which is essential for the understanding of its role in cell function. In addition the supply of 2 no longer limits the direct study of the full range of its biological effects.[13]



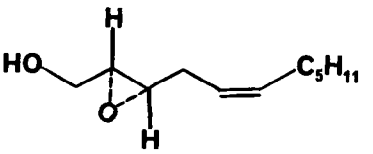
1



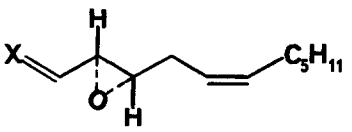
2



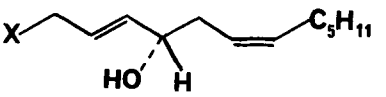
3, X = O
4, X = CHCHO



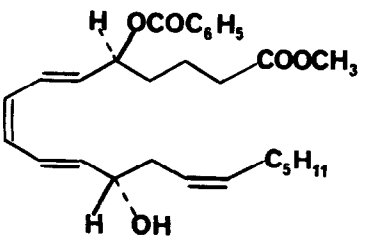
5



6, X = O
7, X = CH_2



8, X = Br
9, X = $(C_6H_5)_3P^+Br^-$



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References and Notes

1. P. Borgeat and B. Samuelsson, J. Biol. Chem., 254, 2643 (1979).

2. E. J. Corey, A. Marfat, G. Goto, and F. Brion, J. Am. Chem. Soc., 102, 7984 (1980).

3. E. J. Corey, A. Marfat, K. S. Kim, P. B. Hopkins, and F. Brion, Tetrahedron Letters, 22, 1077 (1981).

4. E. J. Corey, P. B. Hopkins, J. E. Munroe, A. Marfat, and S. Hashimoto, J. Am. Chem. Soc. 102, 7986 (1980).

5. P. Borgeat, S. Picard, P. Vallerand, and P. Sirois, Biochem. Biophys. Res. Commun., in press.

6. A 25 μg sample of native 2 was kindly supplied to us by Dr. P. Borgeat.

7. In comparison the UV_{max} in CH_3OH for LTB appear at 260, 269.5 and 280.5 nm.

8. S. Tripett and D. M. Walker, J. Chem. Soc., 1266 (1961).

9. Satisfactory pmr, IR, UV and mass spectral data were obtained for each synthetic intermediate using chromatographically homogeneous samples. All reactions were conducted under an atmosphere of argon.

10. The coupling step produced in addition to 10 the isomeric trans olefination product, UV_{max} in CH_3OH 261, 270, 281.5 nm, R_f 0.33 (silica gel tlc, 1:1 hexane-ether) in comparable amount. Saponification of this 8-trans isomer of 10 produced 6-trans-12-epi-LTB identical with a previously synthesized reference sample; see E. J. Corey, A. Marfat, and D. J. Hoover, Tetrahedron Letters, 22, 1587 (1981).

11. Bioassay courtesy of Prof. Robert Lewis, Harvard Medical School.

12. Details of the pmr analysis including complete assignment of chemical shifts and coupling constants were carried out by P. B. Hopkins in these laboratories and will be published separately.

13. Financial support of this research by the National Institutes of Health is gratefully acknowledged. We are grateful to Dr. Pierre Borgeat for helpful discussions of his biochemical studies.

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