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NEW SYNTHETIC ROUTES TO LEUKOTRIENES AND OTHER ARACHIDONATE DERIVED EPOXY EICOSATETRAENOIC ACIDS (EPETE'S). EXCLUSION OF THE HYDROXY EPOXIDE PATHWAY FOR LEUKOTRIENE BIOSYNTHESIS

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Summary: Synthetic hydroxy epoxides 3 and 5 have been utilized for the synthesis of the methyl esters of leukotriene A (4) and 14,15-EPETE (6) and for the demonstration that the acid corresponding to 3 is not an intermediate in leukotriene biosynthesis.

The foregoing paper[1] describes the total synthesis and identification of two major metabolites of arachidonate in mammalian blood platelets, the C(10) diastereomeric 12-(S)-10-hydroxy-trans-11,12-epoxyeicosa-5,9,14-(Z)-trienoic acids. The biosynthesis of these naturally occurring eicosanoids can be understood in terms of the known[2] lipoxygenase (LO) promoted rearrangement of LO-produced allylic hydroperoxides to hydroxy epoxides, that is:

It occurred to us that this pathway represents a possible biosynthetic pathway from 5-(S)-HPETE to leukotriene A (LTA) and, indeed, might also provide a synthetic route to LTA and the analogous epoxy tetraenes (EPETE's)[3] which in principle can originate from other HPETE's (for example, 8- , 12- , and 15-HPETE's). This note reports studies along these lines which demonstrate new syntheses of LTA and 14,15-EPETE and also exclude the involvement of the allylic hydroperoxide ⟶ hydroxy epoxide rearrangement in the biosynthesis of leukotrienes.

The synthesis of LTA methyl ester via a hydroxy epoxide was accomplished in two steps from known compounds. We have previously employed the iodo triene 1 as an intermediate in the synthesis of 5,6-dehydroarachidonic acid (an inhibitor of LT biosynthesis)[4] and the epoxy aldehyde 2 in the synthesis of leukotriene A itself.[5] Treatment of the iodide 1 at -110° in 1 : 1 ether-tetrahydrofuran (THF) with 2 equiv. of t-butyllithium generated the corresponding vinyllithium reagent which upon treatment with 2 (-78° to -20°, 3 hr.), extractive isolation and rapid chromatography on silica gel gave two C(7)-

diastereomeric 7-hydroxy-5, 6-epoxides 3 in a ratio of 60 : 40.[6] The ester epoxides 3 could be saponified using 0.07 N sodium hydroxide in 2 : 1 water-methanol at 20° for 4 hr. and recovered by esterification of the resulting carboxylic acid epoxides with diazomethane in ether. Mesylation of the diastereomeric mixture 3 (2.5 equiv. of mesyl chloride, 4 equiv. of triethylamine in methylene chloride at -78° for 30 min.) followed by stereospecific elimination (diazabicycloundecene, DBU, -78 to 0°, 3 hr.) and flash chromatography on triethylamine treated silica gel with 1:3 hexane-ether containing 2% triethylamine for elution provided in 90% yield the methyl ester of LTA (4), identification of which was made by chromatographic (HPLC), pmr and ultraviolet comparison with an authentic sample.[5] This new route to LTA is direct, efficient, and experimentally straightforward.

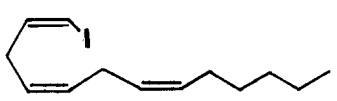
The hydroxy epoxide 3 was synthesized with a tritium label for biosynthetic studies as follows. Epoxy aldehyde 2 in 1 : 1 dimethoxyethane-ethanol was treated with ^{3}H-containing sodium borohydride (340 mCi/mmole) at 0° for 40 min. to afford after acidification with glacial acetic acid and extractive isolation the corresponding primary alcohol-epoxide-ester (8.67 mCi/mmole). Oxidation of this alcohol with $CrO_3 \cdot$ 2 pyridine in methylene chloride at 20° for 20 min. followed by extractive isolation and chromatography on silica gel gave tritiated epoxy aldehyde 2 (5.1 mCi/mmole). The labeled 2 (2.6 mg.) was then diluted with 8 mg. of unlabeled 2 and transformed by the above described route to the mixture of 7-tritiated C(7)-diastereomeric 7-hydroxy-5, 6-epoxides 3.

The tritiated methyl ester 3 was saponified at 20° with aqueous sodium hydroxide to the corresponding carboxylate which was tested as a substrate for LTB biosynthesis using the rat basophilic leukemic cell (RBL-1) enzyme preparation[8] which effects conversion of arachidonate to LTB via LTA. Under standard conditions which resulted in 11% conversion of tritiated arachidonate to LTB, less than 0.15% of the tritiated carboxylate of 3 was converted to product(s) within the tlc band corresponding to LTB (duplicate experiments).[9] From these results in a homogeneous soluble enzyme system it is apparent that the carboxylic acid corresponding to 3 is not an intermediate in the biosynthesis of leukotrienes from arachidonic acid.

The successful conversion of the 7-diastereomeric 7-hydroxy epoxides 3 to LTA methyl ester suggested the practicality of a new and simple route to 14, 15-EPETE[3] from 15-(S)-HPETE. The original synthesis of 14, 15-EPETE and the proposal that this substance might be a naturally occurring metabolite of arachidonate[3] actually preceded the recent discovery of this substance in biological systems by several groups.[10] 15-(S)-HPETE[11] (22 mg.) in ca. 2 ml of 0.5 M pH 7 phosphate buffer under argon was treated with 25 mg. of type I soybean lipoxygenase (Sigma Chem., 125,000 u. $mg.^{-1}$) at 20° for 40 min. Subsequently three 10-mg. portions of lipoxygenase were added at 40 min. intervals. Extractive isolation (after a total reaction time of 3 hr.) followed by esterification with ethereal diazomethane and chromatography on silica gel (3 : 1 hexane - ether for elution) afforded 11.3 mg. of methyl 13-hydroxy-15-(S)-trans-14, 15-epoxyeicosa-5, 8, 11-(Z)-trienoate (48% overall yield from arachidonic acid). Dehydration as described above for the conversion of 3 to 4 gave pure 14, 15-EPETE methyl ester 6 (82% yield) after rapid chromatography on silica gel (3 : 1 hexane-ether containing 1%

triethylamine for elution). The product so obtained was identical in all respects with an authentic sample of 6.[3]

The new syntheses of LTA_4 and 14, 15-EPETE described herein can be recommended as simple and efficient.[12]



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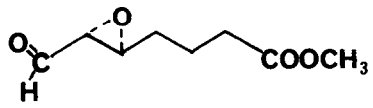

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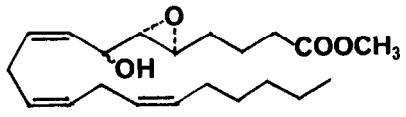

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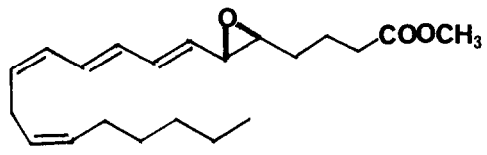

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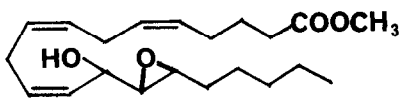

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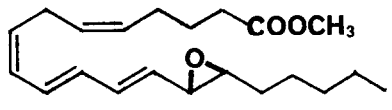

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References and Notes

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2. (a) M. Hamberg and B. Gotthammar, Lipids, 8, 737 (1973); (b) G. J. Garssen, G. A. Veldink, J. F. G. Vliegenthart and J. Boldingh, Eur. J. Biochem., 62, 33 (1976); (c) M. R. Egmond and R. J. P. Williams, Biochim. Biophys. Acta, 531, 141 (1978).
3. E. J. Corey, A. Marfat and G. Goto, J. Am. Chem. Soc., 102, 6607 (1980).
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5. (a) E. J. Corey, D. A. Clark, G. Goto, A. Marfat, A. Mioskowski, B. Samuelsson and S. Hammarström, J. Am. Chem. Soc., 102, 1436,3663 (1980); (b) E. J. Corey, S. Hashimoto and A. E. Barton, ibid., 103, 721 (1981).

6. The two diastereomers of 3 are readily separated by thin layer chromatography (silica gel, 60:40 ether-hexane); the major isomer has R_f 0.33 and pmr peak due to CHOH at 4.33 δ and the minor has R_f 0.26 and pmr peak due to CHOH at 4.68 δ.

7. Satisfactory infrared, proton magnetic resonance and mass spectral data were obtained for each synthetic substance.

8. E. J. Corey, S. S. Kantner and P. T. Lansbury, Jr., Tetrahedron Letters, 24, 265 (1983).

9. Most of the tritiated epoxy carboxylate corresponding to 3 was recovered unchanged, but ca. 3% was transformed into a product which appeared from chromatographic and mass spectral analysis to be the triol acid derived from hydration of the epoxide function.

10. J. Turk, R. L. Maas, L. J. Roberts II, A. Brash and J. A. Oates, Adv. Prostaglandin Thromboxane Res., 11, 123 (1983) and refs. cited therein. Unfortunately, the misleading term 14,15-leukotriene A_4 appears in the biochemical literature rather than the original designation,[3] 14,15-EPETE.

11. M. Hamberg and B. Samuelsson, Proc. Natl. Acad. Sci. USA, 71, 3400 (1974).

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