Tetrahedron Letters,Vol.23,No.17,pp 1755-1756,1982 0040-4039/82/171755-02$03.00/0
Printed in Great Britain

8,9-EPOXYARACHIDONIC ACID: A CYTOCHROME P-450 METABOLITE

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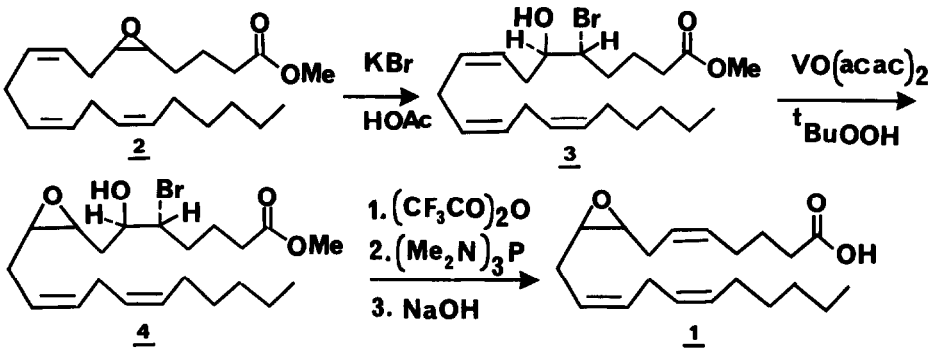
Summary: The title compound was prepared from methyl 5,6-epoxyarachidonate and confirmed as a cytochrome P-450 metabolite of arachidonic acid.

Our knowledge of eicosanoids[1], a family of C_{20} polyunsaturated fatty acid metabolites, and their crucial physiological role has progressed considerably in recent decades. This achievement is especially noteworthy when one considers that these metabolic products are present normally in only minute amounts. Accordingly, structure determinations and biological evaluation have in many instances relied on the availability of authentic synthetic material[1]. We report herein the synthesis and confirmation of structure for a new eicosanoid isolated from incubation of arachidonic acid with cytochrome P-450.

Estabrook[2] and others[3] have observed that both microsomal and purified cytochrome P-450 rapidly and efficiently convert arachidonic acid to a wide variety of oxygenated products. In addition to various lipoxygenase-type products, several novel metabolites unique to the cytochrome system have been isolated[4]. We provisionally assigned one of these as the previously unknown[5] 8,9-epoxide of arachidonic acid 1 on mass spectroscopic data alone. To confirm the structure and provide sufficient quantities for biological testing, isomerically homogeneous 1 was synthesized as described below.

Methyl 5,6-epoxyarachidonate [6a] 2 was transformed quantitatively in acetic acid-saturated aqueous potassium bromide-tetrahydrofuran (20·3·4) for 10 h to an ca. 1.5·1 mixture of bromohydrin 3[7] and its regioisomer [tlc (SiO_2):ether/hexane 1·1, $R_f \sim 0.23$ and 0.26 for 3 and regioisomer, respectively]. The bromohydrin mixture was catalytically epoxidized[8] using vanadyl acetylacetonate (0.25 equiv) and 90% t-butyl hydroperoxide (1.6 equiv) in dry benzene (0.15 mmole bromohydrin /10 ml) for 2.5 h. After quenching with excess dimethyl sulfide (30 min), extractive isolation, and chromatography (SiO_2, ether/hexane 2:1, $R_f \sim 0.21$) 4 was obtained as a colorless oil in 42% over-all yield from 2. The regioisomer of 3 was essentially unchanged and could be recycled to 2 by base. In the final step, Corey's remarkably effective elimination procedure [6b] was followed exactly. Treatment of 4 in methylene chloride at 0° with pyridine (2 equiv) and trifluoromethanesulfonic anhydride (1.1 equiv) followed after 40 min with hexamethylphosphorous triamide (10 equiv) and stirring for an additional 30 min afforded the methyl ester of 1[9] in nearly quantitative yield after extractive isolation [tlc (SiO_2): ether/hexane 1.1, $R_f \sim 0.54$). Saponification gave 1 which was identical [10] with enzymatically produced material [tlc (SiO_2):ether/hexane 1:1, $R_f \sim 0.19$; nmr ($CDCl_3$, δ): 0.88 (t,3H), 1.04-2.62 (complex m,18H), 2.65-3.06 (m, 4H), 5.08-5.70 (m,6H)].



We are currently investigating the physiological significance of cytochrome P-450 dependent fatty acid oxidations and its involvement in eicosanoid production. The biological function of epoxyarachidonic acids, some of which have weak contractile activity[11] on rat stomach strips, is unknown at present.

Acknowledgement· This work was supported generously by the Robert A. Welch Foundation. R.W. Estabrook, J. Capdevila and N. Chacos are thanked for chromatographic comparisons of synthetic and enzymatic products.

References and Notes

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7. For all new compounds satisfactory infrared, proton magnetic resonance, and mass spectral data were obtained on chromatographically homogeneous samples.

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9. The methyl ester was degraded by hydrogenation over Pd/C in ethyl acetate and cleavage with periodic acid to lauraldehyde identified by nmr, ir, and mass spectroscopy.

10. Compared by HPLC analysis using a μ-porosil column and an isocratic mixture of 99.4% hexane, 0.5% isopropanol, and 0.1% acetic acid. Mass spectra of enzyme-derived and synthetic acid, methyl ester, and hydrogenated methyl ester were identical in detail.

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(Received in USA 5 February 1982)