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ARACHIDONATE EPOXYGENASE: TOTAL SYNTHESIS OF BOTH ENANTIOMERS OF 8,9- AND 11,12-EPOXYEICOSATRIENOIC ACID

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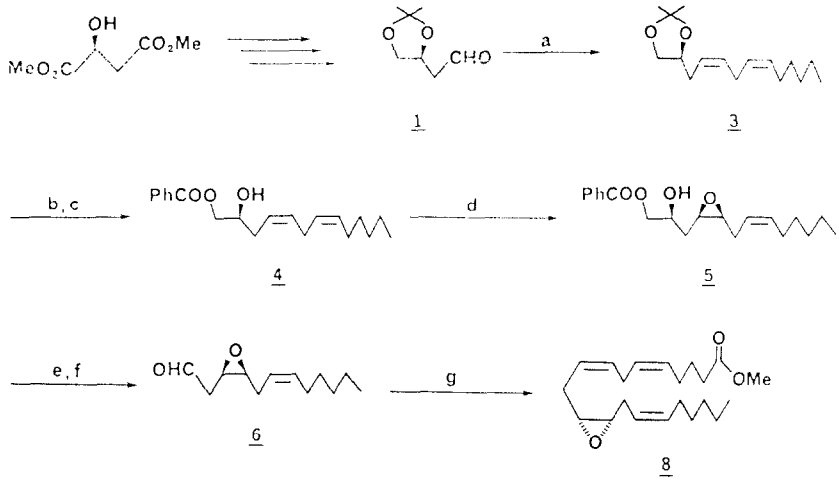
Summary: Both enantiomers of the epoxygenase metabolites 8,9- and 11,12-epoxyeicosatrienoic acid (EET) were synthesized by a convergent strategy utilizing dimethyl D- or L-malate and erythrospecific epoxidation.

Cytochrome P-450, depending on the particular isozyme(s) present, catalyzes the metabolism of arachidonic acid by any of three pathways: ω/ω-1 hydroxylation, lipoxygenase-like oxidation to Z,E-dienols, or epoxidation[1]. The latter epoxygenase activity generates four regioisomeric cis-epoxyeicosatrienoic acids (EETs) whose absolute configurations have been determined using the major phenobarbital inducible isozyme of rat liver microsomal cytochrome P-450[2]. While the EETs are present in mammalian tissue[3] and human urine[4], the in vivo significance of the epoxygenase pathway and the physiological role of its metabolites have not been defined. Recent studies, however, suggest that the EETs may participate in kidney function[5] and peptide hormone secretion[1,6]. As part of our efforts to evaluate the pharmacology and metabolic fate[7] of monooxygenase fatty acid metabolites, a versatile and convergent enantiospecific approach to cytochrome P-450 derived eicosanoids was developed. The synthetic potential[8] of this route is illustrated herein by the syntheses of both enantiomers of 8,9- and 11,12-EET.

Aldehyde 1, prepared[9] (47%) from dimethyl L-malate, was elaborated to diene 3[10,11] (87-91%) under cis-olefination conditions using 3-(Z)-nonenylidenetriphenylphosphorane (2) (Scheme I). Acetonide hydrolysis and selective protection of the primary alcohol afforded monobenzoate 4 (84-87%) which was converted by vanadium catalyzed epoxidation[12] to 5 (81-86%) obtained as an ca. 94:6 diastereomeric mixture (^{13}C NMR, 300 MHz). Epoxide 5 was transformed to aldehyde 6 by exposure to methanolic $KHCO_3$, quenching with 0°C pH 7 buffer, extractive isolation, and flash column purification (SiO_2:2% MeOH/Et_2O, R_f~0.35) followed by buffered periodate cleavage of the resultant diol. Crude 6 was dried azeotropically with benzene under reduced pressure and added dropwise in a minimum volume of THF to a 36 mM solution of 7-carbomethoxyhepta-3-(Z)-en-1-ylidenetriphenylphosphorane (7) [2.4 equiv; generated at -78°C, THF, 45 min, $NaN(SiMe_3)_2$] in THF/toluene (1:4.6) at -100°C. Gradual warming to -15°C over 4h, quenching with ice-cold 25% NH_4OAc, extractive isolation and chromatography furnished methyl 11(R),12(S)-EET (8) in 50-60% overall yield from 5 accompanied by a small amount of methyl 12(S)-HETE[8] arising from isomerization of 6 prior to Wittig coupling.

Methyl 8(S),9(R)-EET(12) was assembled in comparable yields utilizing the same reagents by simply reversing the order of Wittig homologations (Scheme II). Thus, treatment of 1 with 7,

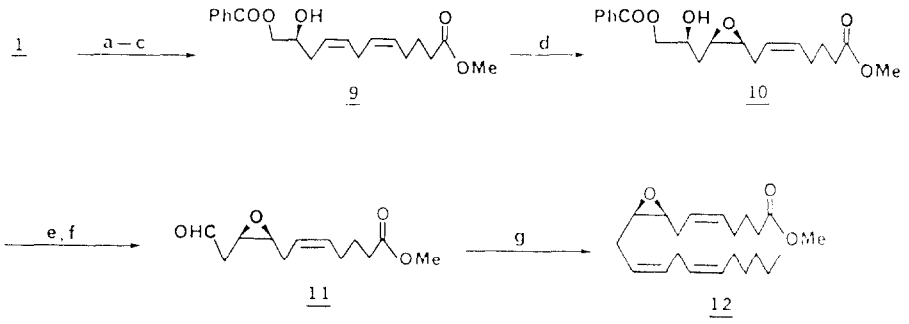
Scheme I



[a] 2, THF/HMPA (4:1), -78 to -20°C, 3h. [b] 1N HCl/MeOH (1:5), 4°C, 12h; $NaHCO_3$. [c] PhCOCN, 10 mol % ET_3N, CH_2Cl_2, 0°C, 0.5h. [d] t-BuOOH, $VO(acac)_2$, CH_2Cl_2, 0°C, 10h. [e] $KHCO_3$, MeOH, 25°C, 10h. [f] $NaIO_4$, $MeOH/H_2O$ (2:1), Na_2HPO_4. [g] 7, THF/ $PhCH_3$ (1:4.6), -100 to -15°C, 4h.

diol liberation, and benzoylation gave 9. Consecutive epoxidation, deprotection, and glycol cleavage as described above secured 11 via ester 10. Union of 11 and 2 completed the sequence.

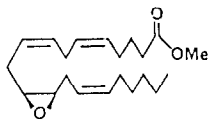
Scheme II



[a] 7, THF/HMPA (4:1), -78 to -20°C, 3h. [b] 1N HCl/MeOH (1:5), 4°C, 12h; $NaHCO_3$. [c] PhCOCN, 10 mol % ET_3N, CH_2Cl_2, 0°C, 0.5h. [d] t-BuOOH, $VO(acac)_2$, CH_2Cl_2, 0°C, 10h. [e] $KHCO_3$, MeOH, 25°C, 10h. [f] $NaIO_4$, $MeOH/H_2O$ (2:1), Na_2HPO_4. [g] 2, THF/$PhCH_3$ (1:4.6), -100 to -15°C, 4h.

Repetition of the syntheses in Schemes I and II using dimethyl D-malate afforded methyl 11(S),12(R)-EET(13) and methyl 8(R),9(S)-EET(14), respectively. Except for inverse optical rotations, both enantiomeric series had identical physical and spectral characteristics.

Esters 8 and 12-14 were converted to the corresponding acids with NaOH in MeOH/H_2O (3:1), acidification to pH 4, and extractive isolation.



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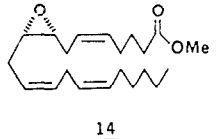

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The foregoing syntheses, in conjunction with previous synthetic efforts[13], secure ready access to sufficient quantities of the EETs in enantiomerically pure form for biological testing. It is anticipated[14] that these studies will help to answer the many urgent questions concerning the physiological function of arachidonate epoxygenase.

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References and Notes

1. J. Capdevila, G. Snyder, and J.R. Falck in "Microsomes and Drug Oxidations"; Eds., A.R. Boobis, J. Caldwell, F. DeMatteis, and C.R. Elcombe; Taylor and Francis, Ltd.: Philadelphia, 1985, pp. 84-94.
2. J.R. Falck, S. Manna, H.R. Jacobson, R.W. Estabrook, N. Chacos, and J. Capdevila, J. Am. Chem. Soc. 106: 3334-3336, 1984.
3. J. Capdevila, B. Pramanik, J.L. Napoli, S. Manna, and J.R. Falck, Arch. Biochem. Biophys. 231: 511-517, 1984.
4. R. Toto, A. Siddhanta, J.R. Falck, and J. Capdevila, submitted for publication.
5. H.R. Jacobson, S. Corona, J. Capdevila, N. Chacos, S. Manna, A. Womack, and J.R. Falck in "Prostaglandins and Membrane Ion Transport"; Eds., P. Braquet, J.C. Frolich, S. Nicosia and R. Garay; Raven Press: New York, 1984, pp. 311-318. M. Schwartzman, N.R. Ferreri, M.A. Carroll, E. Songu-Mize, and J.C. McGiff, Nature 314: 620-622, 1985. M. Schwartzman, M.A. Carroll, N.G. Ibraham, N.R. Ferreri, E. Songu-Mize, and J.C. McGiff, Hypertension 7 (Suppl I), I136-I144, 1985.
6. A. Negro-Vilar, G.D. Snyder, J.R. Falck, S. Manna, N. Chacos, and J. Capdevila, Endocrinology 116: 2663-2668, 1985. S.R. Ojeda, J. Capdevila, G. Snyder, S.M. McCann, A. Negro-Vilar, and J.R. Falck, Adv. Prost. Throm. Leuk. Res. 15: 559-560, 1985.
7. M.E. Spearman, R.A. Prough, R.W. Estabrook, J.R. Falck, S. Manna, K.C. Leibman, R.C. Murphy, and J. Capdevila, Arch. Biochem. Biophys. 242: 225-230, 1985. N. Chacos, J. Capdevila, J.R. Falck, S. Manna, C. Martin-Wixtrom, S.S. Gill, B.D. Hammock, and R.W. Estabrook, ibid. 223: 639-648, 1983.
8. For the synthesis of HETEs by this strategy see, P. Yadagiri, S. Lumin, P. Mosset, J. Capdevila, and J.R. Falck, following communication.

9. For large scale preparations, the method of S. Saito, T. Hasegawa, M. Inaba, R. Nishida, T. Fujii, S. Nomizu, and T. Moriwake was modified by LAH reduction (Et_2O, 1h) of the intermediate methyl 3,4-O-isopropylidenebutanoate and oxidation (PCC, CH_2Cl_2, 3Å molecular sieves, 45 min) to 1.

10. Except as noted, satisfactory nmr, uv and mass spectral data were obtained for all new compounds using chromatographically homogeneous samples.

11. Physical data for 3: NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, J~6.8 Hz, 3H), 1.23-1.45 (m, 6H), 1.36 (d, J~0.5 Hz, 3H), 1.43 (d, J~0.5 Hz, 3H), 2.05 (br q, J~6.7 Hz, 2H), 2.31 (dddd, J~1.5, 6.5, 7.2, 14.7 Hz, 1H), 2.45 (dddd, J~1.5, 6.2, 7.0, 14.7 Hz, 1H), 2.80 (br t, J~7.0 Hz, 2H), 3.56 (dd, J~7.0, 7.9 Hz, 1H), 4.03 (dd, J~6.0, 7.9 Hz, 1H), 4.13 (dddd, J~6.0, 6.2, 6.5, 7.0 Hz, 1H), 5.32 (dtt, J~10.8, 7.0, 1.3 Hz, 1H), 5.38 (dtt, J~10.7, 7.1, 1.3 Hz, 1H), 5.40 (dtt, J~10.7, 7.0, 1.2 Hz, 1H), 5.50 (dtt, J~10.7, 7.2, 1.5 Hz, 1H); TLC, SiO_2, hexane/Et_2O (3:1) R_f~0.55. 4: NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, J~6.8 Hz, 3H), 1.19-1.45 (m, 6H), 2.04 (br q, J~6.8 Hz, 2H), 2.21 (br s, 1H), 2.41 (t, J~6.7 Hz, 2H), 2.83 (t, J~7.0 Hz, 2H), 4.04 (dddd, J~3.5, 6.3, 6.6, 6.7 Hz, 1H), 4.28 (dd, J~6.7, 11.5 Hz, 1H), 4.42 (dd, J~3.5, 11.5 Hz, 1H), 5.32 (dtt, J~10.7, 6.8, 1.3 Hz, 1H), 5.41 (dtt, J~10.7, 6.9, 1.4 Hz, 1H), 5.48 (dtt, J~10.8, 7.3, 1.4 Hz, 1H), 5.59 (dtt, J~10.8, 7.2, 1.3 Hz, 1H), 7.41-7.49 (m, 2H), 7.58 (ddt, J~6.6, 8.0, 1.4 Hz, 1H), 8.03-8.10 (m, 2H); TLC, SiO_2, Et_2O/hexane (1:1) R_f~0.36. 5: NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, J~6.8 Hz, 3H), 1.19-1.42 (m, 6H), 1.75 (ddd, J~8.0, 8.0, 14.5 Hz, 1H), 1.98 (ddd, J~4.2, 4.2, 14.5 Hz, 1H), 1.98-2.08 (m, 2H), 2.22 (dddd, J~1.4, 6.5, 7.1, 15.0 Hz, 1H), 2.42 (dddd, J~1.4, 6.5, 7.1, 15.0 Hz, 1H), 2.79 (br s, 1H), 2.99 (ddd, J~4.2, 6.5, 6.5 Hz, 1H), 3.21 (ddd, J~4.2, 4.2, 8.0 Hz, 1H), 4.29 (dddd, J~4.2, 4.5, 6.0, 8.0 Hz, 1H), 4.37 (dd, J~6.0, 11.5 Hz, 1H), 4.43 (dd, J~4.5, 11.5 Hz, 1H), 5.41 (ddddd, J~7.1, 7.1, 10.8, 1.4, 1.4 Hz, 1H), 5.54 (ddddd, J~7.2, 7.2, 10.8, 1.4, 1.4 Hz, 1H), 7.45 (tt, J~1.3, 7.5 Hz, 2H), 7.58 (tt, J~1.3, 7.4 Hz, 1H), 8.03-8.09 (m, 2H); TLC, SiO_2, 5% MeOH/CH_2Cl_2 R_f~0.44. 8: NMR ($CDCl_3$, 90 MHz) δ 0.88 (t, J~7 Hz, 3H), 1.13-1.46 (m, 6H), 1.54-1.81 (m, 2H), 2.00-2.37 (m, 10H), 2.68-3.00 (m, 4H), 3.61 (s, 3H), 5.20-5.54 (m, 6H); TLC, SiO_2, hexane/Et_2O (4:1) R_f~0.37; $[\alpha]_D^{23}$+4.94° (c 1.64, acetone). 12: $[\alpha]_D^{23}$+2.33° (c 1.37, acetone).

12. E.D. Mihelich, K. Daniels, and D.J. Eickhoff, J. Am. Chem. Soc. 103: 7690-7692, 1981.

13. C.A. Moustakis, J. Viala, J. Capdevila, and J.R. Falck, J. Am. Chem. Soc. 107: 5283-5285, 1985. J.R. Falck, S. Manna, and J. Capdevila, Tetrahedron Letters 25: 2443-2446, 1984. Also see, M.D. Ennis and M.E. Baze, accompanying report.

14. For example, 14(R),15(S)-EET is a potent in vitro inhibitor of cyclooxygenase, whereas the 14(S),15(R)-isomer is essentially inactive. Dr. F.A. Fitzpatrick (Upjohn Co.), personal communication

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