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Synthesis and Structure Confirmation Of Compound D, A Proinflammatory Arachidonate Metabolite

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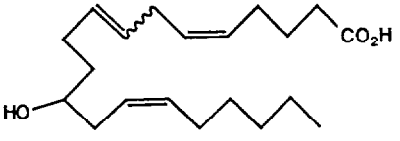
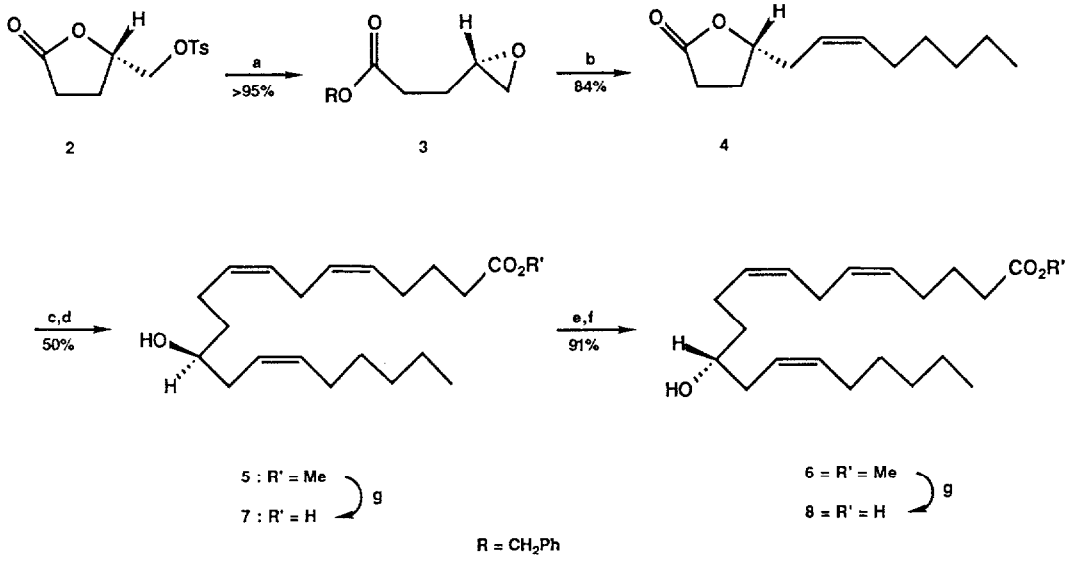
Summary: 12(S)- and 12(R)-Hydroxyeicosa-5(Z),8(Z),14(Z)-trienoic acids (7 and 8, respectively) and their 8(E)-analogues were prepared from L-glutamic acid. Only 8 was comparable by bioassay to natural compound D isolated from corneal epithelium.

Recently, a novel cytochrome P-450 derived arachidonate metabolite[1], compound D, was isolated from corneal epithelium by Murphy et al.[2] and assigned structure 1. Compound D elicits profound vasodilatory and angiogenic responses and stimulates protein influx into the aqueous humor of the eye[2]. At present, there remains uncertainty concerning the stereochemistry of the C(12)-alcohol and $\Delta^{8,9}$-olefin due to mechanistic ambiguities in the proposed biosynthesis and to the limited availability of natural material. To clarify the structure and to expedite the biological evaluation of compound D, we report herein the enantiospecific total synthesis of the four stereoisomers implicit in structure 1 and their comparisons by bioassay with natural compound D.



1

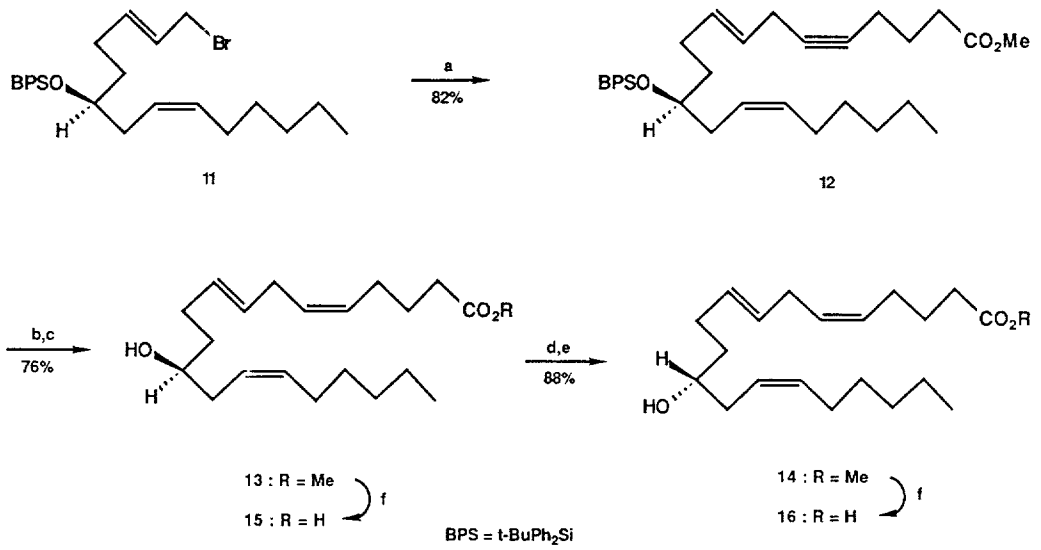
SCHEME I



[a]$PhCH_2OH$, n-BuLi, THF, -78°C to 0°C, 2h. [b]**9** (1.1 equiv), n-BuLi, Et_2O, -60°C, 20 min; CuCN, -78°C to -45°C, 30 min; **3**, -50°C, 30 min. [c]DIBAL-H, $PhCH_3$, -78°C, 3h. [d]**10** (2.1 equiv), THF/HMPA (3:1), -78°C to -24°C, 2h. [e]$PhCO_2H/Ph_3P$/DEAD (1.5 equiv each), THF, 0°C, 2h. [f]NaOMe, MeOH, 23°C, 6h. [g]LiOH, $MeOH/H_2O$ (3:1), 23°C, 6h.

γ-Butyrolactone **2**[3] readily accessible from L-glutamic acid, was converted almost quantitatively into **3**[4] by the lithium salt of benzyl alcohol (Scheme I). Addition of the higher order cuprate[5] generated from (Z)-1-iodo-1-heptene[3] (**9**) to a 0.1 M solution of **3** with concomitant lactonization furnished **4**, $[\alpha]_D^{23}$ + 17.7° (c 3.7, MeOH); lit.[6] $[\alpha]_D^{24}$ + 16.5° (c 2.6, MeOH). The lactol obtained from **4** by diisobutylaluminum hydride (DIBAL-H) reduction was condensed with 7-carbomethoxyhepta-3(Z)-en-1-ylidenetriphenylphosphorane[7] (**10**) to give methyl 12(S)-hydroxyeicosa-5(Z),8(Z),14(Z)-trienoate[8] (**5**), $[\alpha]_D^{23}$ + 2.8° (c 1.7, acetone), after chromatographic purification [TLC: SiO_2, hexanes/Et_2O (7:3), $R_f \approx 0.31$]. Mitsunobu inversion[9] of **5** gave rise to the corresponding epimeric C(12)-benzoate, $[\alpha]_D^{23}$ + 20.6° (c 1.0, acetone), which was solvolyzed to methyl 12(R)-hydroxyeicosa-5(Z),8(Z),14(Z)-trienoate[8] (**6**), $[\alpha]_D^{23}$ - 3.0° (c 0.8. acetone).

SCHEME II



[a]17, EtMgBr, CuBr•Me_2S, THF, 0° to 45°C, 20h; CH_2N_2. [b]$Ni(OAc)_2$, $NaBH_4$, $(CH_2NH_2)_2$, H_2, MeOH, 24°C, 12h. [c]n-Bu_4NF, THF, 45°C, 10h. [d]$PhCO_2H/Ph_3P$/DEAD (1.5 equiv each), THF, 0°C, 2h. [e]NaOMe, MeOH, 24°C, 6h. [f]LiOH, MeOH/H_2O (3:1), 23°C, 6h.

Synthesis of the 8(E)-analogues (Scheme II) commenced with copper catalyzed coupling[10] of allylic bromide 11, previously prepared[11] from L-glutamic acid, with the bis-magnesium bromide salt of 5-hexynoic acid (17) followed by diazomethane esterification. Chromatographic purification [TLC: SiO_2, hexane/EtOAc (9:1), R_f≈0.47] afforded acetylenic ester 12 which was transformed into methyl 12(S)-hydroxyeicosa-5(Z),8(E),14(Z)-trienoate[12] (13), $[\alpha]_D^{23}$ - 3.0° (c 1.5, acetone), by selective hydrogenation over P-2 Ni[13] in MeOH and fluoride promoted desilylation. Epimerization of 13 as described for the conversion of 5 to 6 generated methyl 12(R)-hydroxyeicosa-5(Z),8(E),14(Z)-trienoate[12] (14) after chromatographic purification [TLC: SiO_2, hexane/Et_2O (3:2), R_f≈0.38].

Free acids 7, 8, 15, and 16 were obtained from their respective methyl esters by saponification, adjustment to pH 4.5, and extractive isolation. Only 8 was comparable and equipotent to natural compound D in eliciting vasodilation of rabbit conjuctival blood vessels and increasing aqueous humor protein concentration. Topical application of 5-60 ng of 16 evoked vasodilation which was about 10% of that observed with a similar amount of compound D, but did not change aqueous humor protein concentration. The 12(S)-isomers 7 and 15 were inactive under similar circumstances.

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References and Notes:

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8. Identical data were obtained for **5** and **6**. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.88(t, J≈6.8 Hz, 3H), 1.23-1.40 (m,6H), 1.49-1.76 (m,6H), 1.99-2.30 (m,6H), 2.32 (t, J≈7.4 Hz, 3H), 2.79(t, J≈5.5 Hz, 2H), 3.57-3.67 (m, 1H), 3.67(s, 3H), 5.28-5.47 (m, 5H), 5.51-5.63 (m, 1H); MS (PICI, CH_4) of TMS ether: m/e 175, 207, 297, 319 (base), 393, 409 (M^+ + 1), 437 (M^+ + 29); HPLC: μPorasil (7.8 x 300 mm), 0.5% isopropanol/hexane, 3 ml/min flow rate, R_t≈16 min.
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11. P. Yadagiri, Sun Lumin, J.R. Falck, A. Karara, and J. Capdevila, *Tetrahedron Lett.* **30**: 429-432 (1989).
12. Identical data were obtained for **13** and **14**. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.88(t, J≈6.8 Hz, 3H), 1.23-1.40 (m, 6H), 1.51-1.78 (m, 6H), 1.98-2.25 (m, 6H), 2.35 (t, J≈7.4 Hz, 3H), 2.64-2.76 (m, 2H), 3.57-3.67 (m, 1H), 3.65 (s, 3H), 5.26-5.45 (m, 5H), 5.48-5.62 (m, 1H); MS (PICI, CH_4) of TMS ether: m/e 175, 207, 297, 319 (base), 393, 409 (M^++1), 437 (M^++29); HPLC: μPorasil (7.8 x 300mm), 0.5% isopropanol/hexane, 3ml/min flow rate, R_t≈20 min.
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