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ANGIOGENIC FACTORS: SYNTHESES OF 12(R)- AND 12(S)-HYDROXYEICOSA-5(Z),8(Z),14(Z)-TRIENOIC ACID AND THEIR 14,15-DEHYDRO ANALOGS

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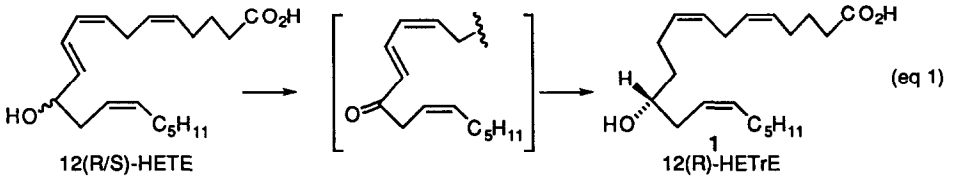
Abstract: The title autacoids and acetylenic analogs were conveniently prepared from 2-deoxy-D-ribose via Barton deoxygenation, cuprate coupling, and Wittig homologation. 12(R)-HETrE, but not the 12(S)-isomer, significantly stimulated microvessel endothelial cell proliferation and proto-oncogene mRNA levels at sub-nano to picomolar concentrations. A high affinity binding site for 12(R)-HETrE was detected on microvessel endothelial cells.

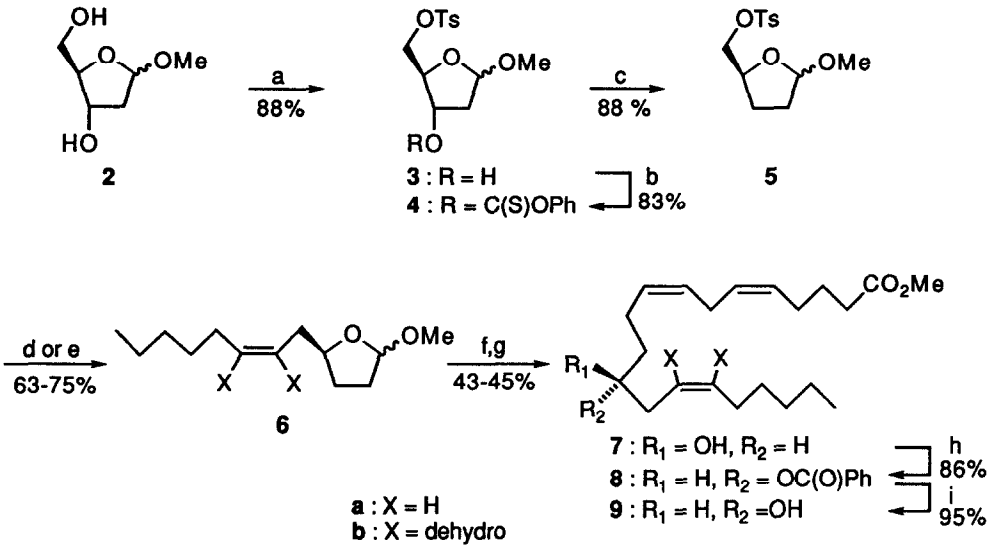
In mammals, 12(R)- and 12(S)-hydroxyeicosatetraenoic acids (HETE) are biosynthesized by unrelated enzymatic pathways, viz., cytochrome P450 and 12-lipoxidase, respectively, and also exhibit substantially different biological activities.[1] Both, however, are rapidly and stereoselectively transformed[2] to the same 10,11-dihydro-12(R)-metabolite **1** [12(R)-HETrE] via a keto intermediate (eq 1) by a recently recognized oxido-reductase pathway[3] that is most prominent in neutrophils, corneal epithelium, and skin. More importantly, the pharmacologic profile of **1** differs markedly from its progenitors and has consequently evoked unusually intense interest in this new class of autacoids. For example, **1** enhances delayed-type skin hypersensitivity,[4] capillary permeability,[5] neutrophil chemotaxis,[6] and is a potent vasodilator.[5] Its 12(S)-antipode, in contrast, is comparatively inactive, but has been useful as a competitive antagonist of **1**.[4] To meet the urgent need for increasing supplies of these eicosanoids and labeled standards,[7] we report herein a concise, multi-millimole scale synthesis of **1**, 12(S)-HETrE, and their 14,15-acetylenic analogs from a readily available carbohydrate precursor. We also describe initial indications of **1** as a potent angiogenic factor with high affinity binding sites on microvessel endothelial cells.



12(R/S)-HETE — **1** — 12(R)-HETrE (eq 1)

Methyl furanoside **2**, obtained[8] in nearly quantitative yield as an anomeric mixture from commercial 2-deoxy-D-ribose, was sequentially derivatized to **4**[9,10] by way of primary tosylate **3** (Scheme 1). Tributyltin hydride mediated cleavage of the phenylthionocarbonate as described by Barton[11] furnished 2,3-dideoxy-D-riboside **5** that was elaborated to **6a** by coupling with the higher order cuprate generated from (Z)-1-iodo-1-heptene.[12] Mild acidic hydrolysis of the methyl lactol followed by Wittig *cis*-olefination using 7-carbomethoxy-3(Z)-en-1-ylidenetriphenylphosphorane[13] (**10**) afforded methyl 12(S)-HETrE (**7a**) after chromatographic purification[14] to remove a minor amount (5-10%) of 8(E)-isomer. Methyl 12(R)-HETrE (**9a**) was secured from **7a** by conventional Mitsunobu inversion[15] of the C(12)-alcohol and methanolysis of the resultant benzoate **8a**.

Scheme 1



[a]TsCl, C_5H_5N/CH_2Cl_2 (4:5), 0°C, 12 h.[b]ClC(S)OPh, C_5H_5N/CH_2Cl_2 (1:1), 0° to 23°C, 5 h. [c]Bu_3SnH (1.2 equiv), AIBN (cat.), C_6H_6, 80°C, 2 h.[d](Z)-1-iodo-1-heptene, BuLi, Et_2O, -40°C, 20 min; CuCN (0.5 equiv), -40°C, 2 h; **5**, -40° to -5°C, 14 h.[e]1-Heptyne, EtMgBr, Et_2O, 0°C, 0.5h; CuI (0.5 equiv), -40° to 0°C, 1h; **5**,-40°to 0°C, 4h.[f]THF/H_2O/HOAc (1:1:2), 60°C, 6 h. [g]**10** (3 equiv), THF/HMPA (10:1), -20°C, 1h.[h]$PhCO_2H/Ph_3P$/DEAD (1 equiv each), C_6H_6, 23°C , 2 h.[i]25% NaOMe/MeOH, THF, 23°C, 0.5 h.

Alkylation of **5** with the Cu(I) salt of 1-heptyne and repetition of the remaining synthetic sequence from **6b** provided the 14,15-acetylenes **7b** and **9b**. These analogs were useful for the regiospecific introduction of ^2H- and ^3H-isotope labels for binding, quantitation, and metabolism studies (*vide infra*). Esters **7a,b** and **9a,b** were

converted to their free acids by saponification (NaOH, MeOH, 23°C, 4h), adjustment to pH 4.5, and extractive isolation.

Biological Evaluations

12(R)-HETrE (**1**) maximally stimulated rabbit limbal and coronary microvessel endothelial cell proliferation at 0.1 nM within 48 h [$3.2x10^5 \pm 2x10^4$ vs. $4.87x10^5 \pm 3.2x10^4$ cells for quiescent and 12(R)-HETrE-stimulated cells, respectively; $p<0.01$, n=6] supporting its potential role as a direct acting mitogen. 12(S)-HETrE did not significantly increase cell numbers. Notably, the mitogenic activity of **1** is comparable to that of bFGF (10 ng/ml), a potent endothelial cell mitogen. At the molecular level, **1** raises the mRNA levels of the proto-oncogenes *c-jun* and *c-myc* up to 8-fold in quiescent microvessel endothelial cells at concentrations as low as 1 pM. Additionally, nuclear extracts prepared from these cells treated for 15 min with 0.1 nM **1** showed a sharp increase in NFκB binding activity as demonstrated by electrophoretic mobility shift assay. Initial studies using [14,15-^{3}H]-**1** have also revealed a high affinity binding site for **1** on microvessel endothelial cells with a K_d of 0.043 nM and a B_{max} of 24,700 sites/cell. These data help qualify **1** as an intrinsic angiogenic factor and warrant further investigation.

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9. Satisfactory spectral data ($^1H/^{13}C$ NMR, MS) were obtained for all new compounds using chromatographically homogeneous samples.
10. Spectral data for **4** (mixture of anomers): ^1H NMR (250 MHz, $CDCl_3$) δ 2.30-2.55 (m, 2H), 2.50 (s, 3H), 3.25 (s, 3H), 4.10-4.55 (m, 3H), 5.19-5.28 (m, 1H), 5.53-5.62 (m, 1H), 7.10-7.53 (m, 9H); ^{13}C NMR: δ 21.6, 38.9, 55.3, 69.7, 80.8, 82.9, 83.7, 105.2, 105.7, 121.7, 126.7, 127.9, 128.0, 128.4, 129.5, 132.6, 145.0, 153.2, 194.5. **5** (mixture of anomers): ^1H NMR ($CDCl_3$) δ 1.60-2.23 (m, 4H), 2.51 (s, 3H), 3.22 and 3.25 (s, 3H), 3.89-4.13 (m, 2H), 4.21-4.33 (m, 1H), 4.87-4.93 (m, 1H), 7.26-7.38 (m, 2H), 7.73-7.82 (m, 2H); ^{13}C NMR: δ 21.61, 25.46, 25.86, 31.73, 32.50, 54.37, 54.71, 71.32, 72.93, 75.08, 76.49, 105.41, 105.53, 127.95, 129.80, 133.05, 133.09, 144.76, 144.80. **7a**:^1H NMR ($CDCl_3$) δ 0.84 (t, J~6.7 Hz, 3H), 1.18-1.40 (m, 6H), 1.47 (dt, J~1.8, 7.6 Hz, 1H), 1.52 (t, J~7.5 Hz, 1H), 1.59-1.75 (m, 4H), 1.95-2.22 (m, 8H), 2.30 (t, J~7.5 Hz, 2H), 2.77 (br t, J~5.6 Hz, 2H), 3.51-3.69 (complex m overlapping a singlet at 3.63,4H), 5.22-5.63 (m, 6H); ^{13}C NMR: δ 14.05, 22.55, 23.66, 24.78, 25.60, 26.55, 27.39, 29.34, 31.51, 33.44, 35.44, 36.56, 51.50, 71.01, 124.96, 128.34, 128.72, 129.04, 129.63, 133.57, 174.32. **7b**: ^1H NMR ($CDCl_3$) δ 0.85 (t, J~6.3 Hz, 3H), 1.20-1.41 (m, 4H), 1.47-1.79 (m, 6H), 2.01-2.25 (m, 6H), 2.28-2.48 (complex m overlapping a triplet at 2.31, J~7.4Hz, 4H), 2.73 (br t, J~5.7 Hz, 2H), 3.61 (s, 3H), 3.69 (br s, 1H), 5.22-5.48 (m, 4H); ^{13}C NMR δ 13.97, 18.70, 22.19, 23.51, 24.78, 25.59, 26.54, 27.81, 28.68, 31.08, 33.43, 36.00, 51.48, 69.72, 76.00, 83.43, 128.48, 128.80, 129.01, 129.39, 174.01.**9a,b** were spectrally identical with their enantiomers **7a,b**.
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