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EICOSANOID BIOSYNTHESIS: DIFFERENTIAL INHIBITION OF CYTOCHROME P450 EPOXYGENASE AND ω-HYDROXYLASE

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Abstract: Biphenyl **4** and vinyldibromide **8** were prepared on a multigram scale and shown to be comparatively specific arachidonic acid epoxygenase and ω-hydroxylase inhibitors, respectively, in rat kidney microsomal fractions. © 1997 Elsevier Science Ltd.

Cytochromes P450 (Cyt P450) are now recognized[1] as major contributors to eicosanoid biosynthesis and comprise one of the three primary branches of the arachidonic acid (AA) cascade together with the canonical cyclooxygenases and lipoxidases.[2] The predominate Cyt P450 arachidonate metabolites in vivo originate in the epoxygenase and ω-hydroxylase pathways (Figure 1) which give rise to four regioisomeric epoxyeicosatrienoic acids (EETs) and 20-hydroxyarachidonic acid (20-OH AA), respectively.[1] The ubiquitous EETs have been implicated in gene expression,[3] ion and water transport,[4] steroidogenesis,[5] hypertension,[6] and vasomodulation.[7] In coronary arteries, they have been proposed as endothelium-derived hyperpolarizing factors (EDHFs) where their effects are mediated via G-proteins.[8] In some instances, EET specific receptors have been identified.[9] Interest in 20-OH AA resides largely in its vasoactivity in the microcirculation,[10] stimulation of erythropoiesis,[11] and regulation of kidney function.[12]

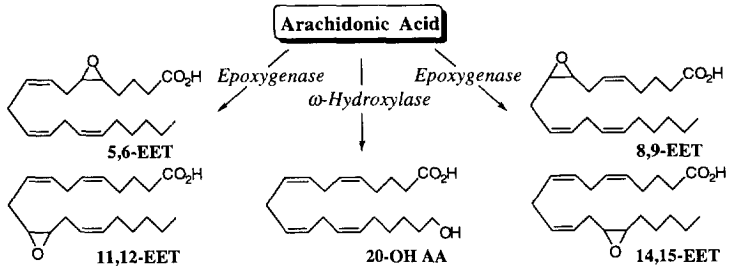


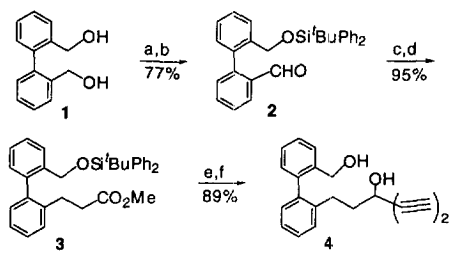
Figure 1 Cytochrome P450 Arachidonate Metabolism.

Efforts to intervene pharmacologically with Cyt P450 arachidonate oxidases (i.e., to specifically inhibit either epoxygenase or ω-hydroxylase activity) have been limited.[13] Despite the widely held view[14] that azoles are broadly applicable inhibitors of all P450s, Capdevila[15] observed microsomal ω-hydroxylation of arachidonic acid was not attenuated with any of a variety of imidazoles, even at effector concentrations >100 μM. The

acetylenic fatty acid 17-octadecynoic acid (17-ODYA), introduced by Ortiz de Montellano as a suicide-substrate inhibitor of ω-hydroxylase, is now known[16] to simultaneously block renal epoxygenase at comparable concentrations. Likewise, the series of isonitrile-containing arachidonate and heteroatom analogs prepared by Falck et al.[17] potently suppress both pathways. To help expedite current investigations into the physiologic role(s) of the third branch of the AA cascade, we report herein the outcome of a screen for differential inhibitors of AA metabolism by renal Cyt P450 epoxygenase and ω-hydroxylase. Our strategy took into account the sterically restricted binding cavity of most fatty acid ω-hydroxylases relative to other classes of Cyt P450.[14]

A multigram synthesis (Scheme 1) the the epoxygenase inhibitor commenced with the selective monosilylation of commercial 2,2′-biphenyldimethanol (**1**) followed by MnO_2 oxidation of the remaining alcohol. The resultant aldehyde **2** was elaborated to ester **3** by condensation with methyl (triphenylphosphoranylidene)acetate at room temperature and mild reduction over P-2 Ni; while somewhat slow, these conditions avoided hydrogenolysis of the benzylic silyloxy ether. Exhaustive addition of acetylide to ester **3** and removal of all silyl protecting groups secured diol **4**.[18]

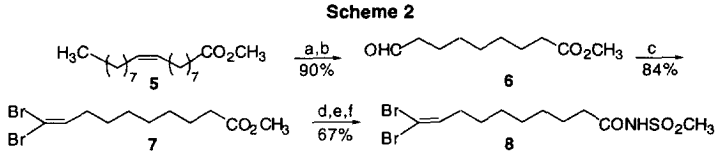
Scheme 1



Reagents and conditions: (a) $PH_2{}^t$BuSiCl, $AgNO_2$, THF/C_5H_5N (15:1), 23 °C, 14 h; (b) MnO_2, CH_2Cl_2, 23 °C, 16 h; (c) Ph_3P = $CHCO_2Me$, CH_2Cl_2, 23°C, 12 h; (d) H_2, P-2 Ni, EtOH, 23 °C, 40 h; (e) Li (trimethylsilyl)acetylide, THF, 0–23 °C, 1 h; (f) Bu_4NF, THF, 23 °C, 3 h.

The ω-hydroxylase inhibitor was accessed by oxidative degradation of methyl oleate (**5**) using OsO_4 and subsequent $Pb(OAc)_4$ cleavage (Scheme 2). Olefination of the resultant aldehyde **6** under standard conditions smoothly afforded dibromide 7,[19] which was transformed to the metabolically more stable carboxylate mimic **8**,[20] mp 40–43 °C, by sequential saponification, N-hydroxysuccinimide (NHS) activation, and methanesulfonamide displacement in HMPA.

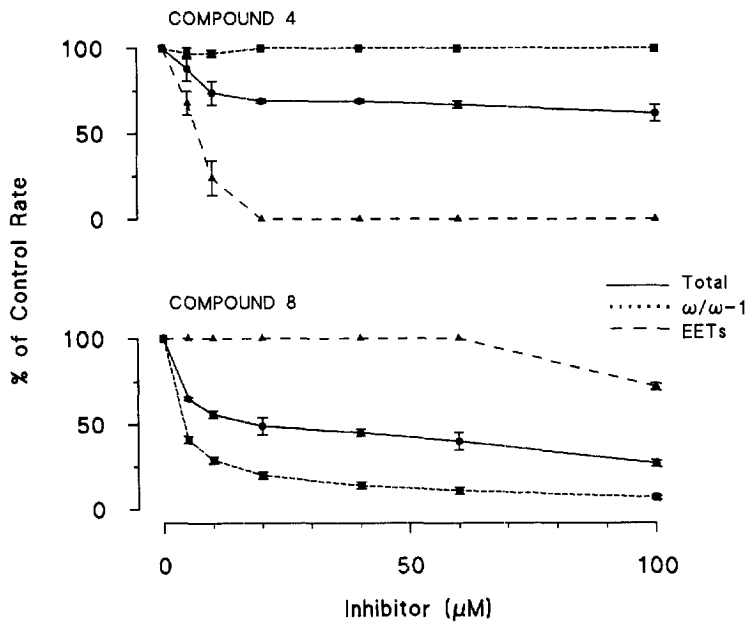
Scheme 2



Reagents and conditions: (a) OsO_4/MNO, acetone/H_2 (9:1), 23 °C, 8 h; (b) $Pb(OAc)_4$, CH_2Cl_2, –40 °C, 0.5 h; (c) CBr_4/Ph_3P, CH_2Cl_2, 0 °C, 1 h; (d) LiOH, THF/H_2O (5:1), 23 °C, 10 h; (e) NHS/DCC, THF, 23 °C, 24 h; (f) $H_2NSO_2CH_3$, DMAP (0.1 equiv), HMPA, 90 °C, 4 h.

To assess specificity, increasing amounts of inhibitor were added to kidney microsomal fractions (1 mg protein/mL) isolated from male Sprague–Dawley rats as previously described.[15] After 2–3 min at room temperature, sodium [1-^{14}C]arachidonate was added (100 mM, 0.5–1.5 mCi/mmol) and the oxidation was initiated with NADPH (1 mM final concentration). The incubation was quenched after 10 min at 30 °C and the reaction products were extracted with acidified Et_2O, resolved via HPLC, and quantified. Diol **4** (Fig. 2, upper panel) potently (IC_{50}~5 μM) and completely abolished EET formation. In the low μM range, there is a slight, yet reproducible, decline in 20-OH AA production which then levels off. This may represent the contribution to overall metabolism from a "dual function" Cyt P450 as described by Schwartzman et al.[21] and would suggest that its active site more closely resembles that of an epoxygenase. Evaluations of **7** and **8** (Fig. 2, lower panel; shown for **8** only) revealed they have comparable activities (IC_{50}~6 μM) and preferentially constrain ω-hydroxylation versus epoxidation.

Figure 2. Rat Kidney Microsomal Metabolism of AA



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18. ^{1}H NMR (250 MHz, $CDCl_3$) of **4**: δ 2.03 (dd, J = 7.6, 8.6 Hz, 2H), 2.40 (s, 1H), 2.43 (d, J = 2.9 Hz, 2H), 2.57–2.83 (m, 2H), 4.36 (s, 2H), 7.15–7.56 (m, 8H); inhibitor **8**: δ 1.20–1.49 (m, 8H), 1.55–1.74 (m, 2H), 2.09 (q, J = 7.2 Hz, 2H), 2.33 (t, J = 7.6 Hz, 2H), 3.31 (s, 3H), 6.38 (t, J = 7.2 Hz, 1H), 8.59 (br s, 1H).
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