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SYNTHESIS OF ARACHIDONIC ACID METABOLITES PRODUCED BY PURIFIED KIDNEY CORTEX MICROSOMAL CYTOCHROME P-450

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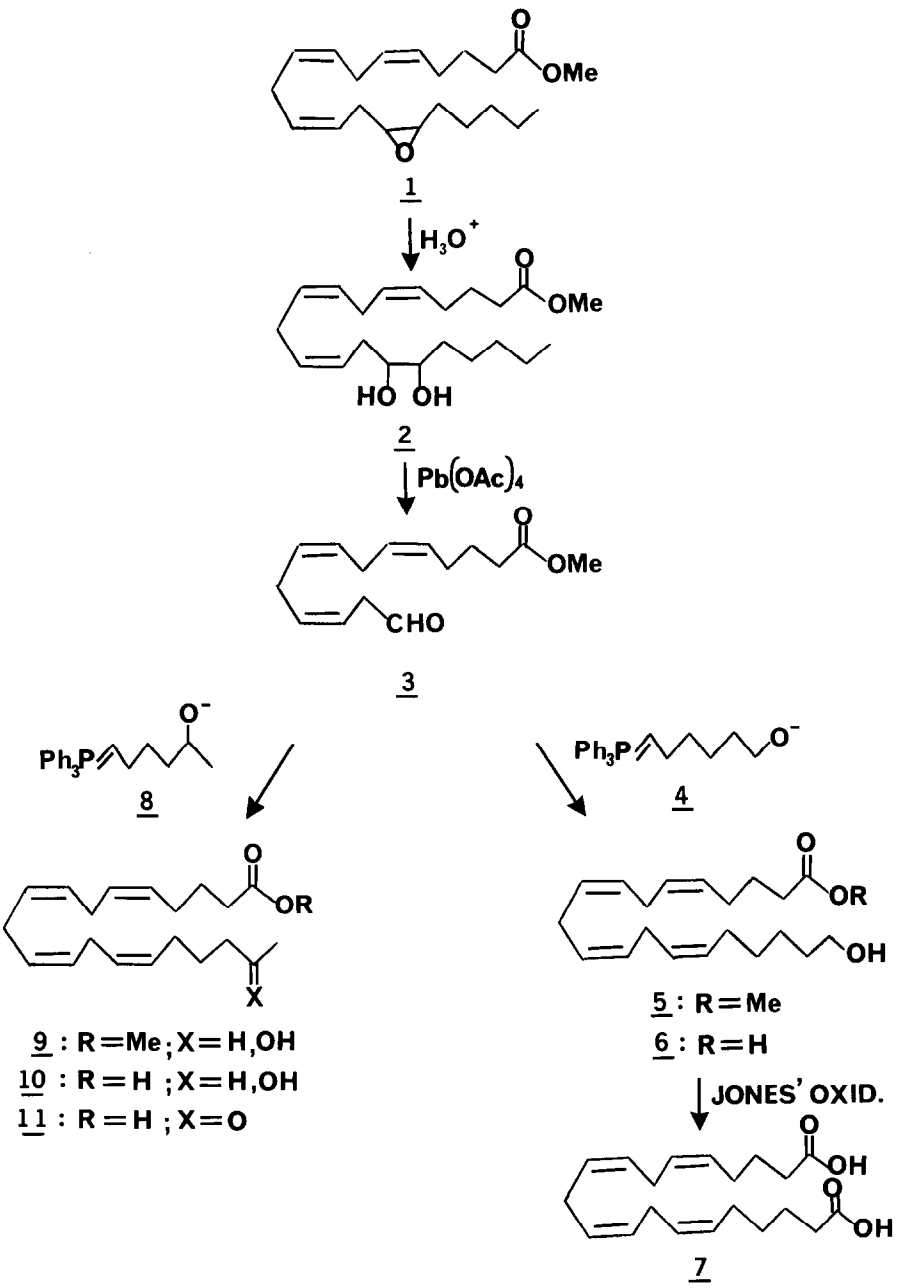
Summary: Reported are the synthesis and structure confirmation of the major metabolites produced during the NADPH dependent oxidation of arachidonic acid by a reconstituted enzyme system containing purified kidney cortex microsomal cytochrome P-450.

Recently, Capdevila[1] and Others[2] have demonstrated a NADPH-dependent cytochrome P-450 mediated pathway for the rapid and efficient conversion of arachidonic acid to a rich variety of oxygenated metabolites. In addition to various lipoxygenase-type products, several novel metabolites unique to the cytochrome pathway have been isolated[3], some of which have potent and meaningful biological activity[4]. Significantly, both the identity and ratio of metabolites are associated closely with the tissue source from which the cytochrome P-450 is isolated[1c]. In the case of cytochrome P-450 purified from pig kidney cortex microsomes, the major products[5] of incubation with arachidonic acid are 19- and 20-hydroxyeicosatetraenoic acid, 19-oxoeicosatetraenoic acid, and eicosatetraen-1,20-dioic acid, (10), (6), (11), and (7), respectively. These metabolites have been reported previously[2] from incubations with rabbit renal cortical supernatant or microsomal suspensions based on mass spectroscopic data. We report herein their structure confirmation and synthesis in sufficient quantities for biological testing and biochemical study.


OMe
1
H_3O^+
OMe
HO OH
2
$Pb(OAc)_4$
OMe
CHO
3
Ph_3P
8
OR
X
9 : R=Me ; X=H,OH
10 : R=H ; X=H,OH
11 : R=H ; X=O
Ph_3P
4
OR
OH
5 : R=Me
6 : R=H
JONES' OXID.
OH
OH
7

Methyl 14,15-epoxyarachidonate[6] (1) was hydrolyzed in 10% perchloric acid-tetrahydrofuran (3:8) at 0° for 24 h to diol 2[7] (SiO_2:5% MeOH/CH_2Cl_2, $R_f \sim 0.43$). Lead tetraacetate (1.05 equiv) cleavage in dry methylene chloride at -20°C for 15 min and warming to 0°C over 15 min afforded unstable aldehyde 3 (SiO_2:5% MeOH/CH_2Cl_2, $R_f \sim 0.59$). The reaction mixture was filtered rapidly through a silica gel-Celite bed, evaporated with heptane and dried in vacuo. Wittig cis-olefination[8] with phosphonium ylide 4[9] (1.5 equiv) in tetrahydrofuran-hexamethylphosphoric triamide (9:1) at -78°C for 30 min and warming to 0°C over 90 min afforded methyl ester 5 in 34% yield over-all from 1 [nmr ($CDCl_3$, δ):1.15-2.24 (m,13H), 2.36 (t,J ~7Hz,2H), 2.72-3.00 (m,6H), 3.70 (s,3H), 3.68 (t,J ~7Hz,2H), 5.20-5.56 (m,8H); tlc (SiO_2):ether/hexane 1:1, $R_f \sim 0.26$]. Saponification of 5 produced 6 (SiO_2:5% MeOH/CH_2Cl_2, $R_f \sim 0.25$) identical in all respects with the enzymatic product[10]. Jones oxidation of 5 at 0°C and saponification furnished 7 indistinguishable from natural material[10].

Similarly, Wittig cis-olefination[8] of 3 using ylide 8[11] gave methyl ester 9 in 30% yield over-all from 1 [nmr ($CDCl_3$, δ):1.23 (d, J ~7 Hz,3H), 1.38-2.24 (m,10H), 2.36 (t, J ~7 Hz,2H), 2.68-3.02 (m,6H), 3.68-3.74 (m,1H), 3.70 (s,3H), 5.20-5.56 (m,8H); tlc (SiO_2):ether/hexane 1:1, $R_f \sim 0.22$]. Saponification yielded 10 (SiO_2:5% MeOH/CH_2Cl_2, $R_f \sim 0.14$). Jones oxidation and hydrolysis of 9 afforded keto-acid 11 [nmr($CDCl_3$, δ): 1.24-2.56 (m,12H), 2.18 (s,3H), 2.68-3.00 (m,6H), 5.20-5.56 (m,8H); tlc (SiO_2):ether/hexane 1:1, $R_f \sim 0.17$]. Both 10 and 11 were identical with enzymatically derived material[10]. We are currently investigating the biological activity of the above described metabolites and the unprecedented[12] conversion by cytochrome P-450 of an unactivated methyl to carboxyl using synthetic material prepared from radiolabeled arachidonic acid.

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References and Notes

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5. J. Capdevila, N. Chacos, R.W. Estabrook, unpublished results.

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7. Satisfactory infrared, proton magnetic resonance, and mass spectral data were obtained for all new compounds using chromatographically homogeneous samples, except for aldehyde 3 which was used without purification.

8. The olefin stereochemistry in 5 and 9 was confirmed by the sequence: tosylation, lithium aluminum hydride reduction, and Jones oxidation affording in both cases a single product (IR: 680 cm^{-1}) identical in all respects with arachidonic acid and distinguishable from eicosa-cis-5,8,11-trans-14-tetraenoic acid (IR:970 cm^{-1}) by TLC ($AgNO_3/SiO_2$). Trans isomer prepared from the bromo-mesylate of 1 using Zn/HOAc, E.J. Corey, A. Marfat, J.R. Falck, J.O. Albright, J. Amer. Chem. Soc., 102, 1433-1435 (1980).

9. Prepared from 6-chlorohexanol by displacement with sodium iodide in refluxing acetone for 8 h, then triphenylphosphine/potassium carbonate in refluxing acetonitrile for 10 h, salt mp 136-137°C (C_6H_6). Ylide generation using two equivalents n-BuLi in THF at -78°C and warming to -30°C over 30 min.

10. Comparisons of enzymatic and synthetic products based on normal and reverse phase high pressure liquid chromatography (HPLC) and positive chemical ionization mass spectroscopy with methane as carrier gas. Consult ref. 2.

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