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5- AND 8-HETE PRODUCTION BY RAT HEPATIC MICROSOMAL CYTOCHROME P-450

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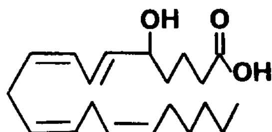
Summary: The NADPH dependent, cytochrome P-450 mediated oxidation of arachidonic acid by rat hepatic microsomes produces 5- and 8-HETE in addition to the previously reported products.

Many mammalian tissues metabolize arachidonic acid to one or more regioisomeric hydroxyeicosatetraenoic acids (HETEs) by way of the corresponding hydroperoxides (HPETEs)[1]. The HETEs have attracted considerable attention because of their biological activities and possible involvement in disease states[2]. In addition, they can undergo further metabolism[3], e.g., 5-HETE to 5,12- and 5,15-DiHETE. Progress in these areas would benefit from an increased understanding of HETE production and of the enzymatic systems involved. Recently, Capdevila[4] and others[5] have elucidated an alternative pathway leading to eicosanoids mediated by cytochrome P-450 and consuming NADPH and O_2 in a 1:1 stoichiometric ratio. In continuation of these studies, we report the isolation of 5-HETE (1) and 8-HETE (2) from the incubation of arachidonic acid with rat hepatic microsomal cytochrome P-450.



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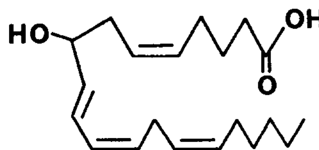

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Arachidonic acid was incubated at 25°C for 15 min in the presence of 0.5 mM NADPH with hepatic microsomes isolated from phenobarbital treated rats and the ethyl acetate soluble products purified by high pressure liquid chromatography (HPLC) on a Waters μ-Bondapak C_{18} column as described[4]. The metabolites absorbing at 235 nm were pooled and resolved on a Waters μ-Porasil column using a 30 min linear solvent gradient of 0.5-1.5% isopropanol in hexane /0.1% acetic acid

at a flow rate of 3 ml/min[4]. In addition to the previously reported products[4], two metabolites with the same retention times (30.1 and 20.6 min) as authentic 5-HETE and 8-HETE, respectively, were isolated. Their identities were confirmed by uv spectroscopy (235 nm, cis-trans diene) and mass spectral comparisons of the methyl ester trimethylsilyl ether derivatives with synthetic standards[6]. Variable amounts of the δ-lactone of 1, retention time 11 min, were also isolated and characterized by HPLC and spectral comparisons with a known sample.

Using radiolabeled arachidonic acid, it was found that the diene-containing metabolites accounted for ca. 30% of the total metabolites formed by rat hepatic microsomes. Dienes 1 and 2 each comprise 8% of the total diene fraction and are produced at a rate of 0.1 $nmol.min^{-1}.mg^{-1}$ microsomal protein. Although no HPETEs were detected, this does not exclude them as transient intermediates. Cytochrome P-450 exhibits a peroxidase activity which would make it unlikely that hydroperoxides could be isolated under the in vitro conditions described here[7].

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