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7-Ethoxycoumarin: substrate for a rapid, sensitive, fluorometric assay of microsomal monoxygenase activity

The mixed-function oxidase system in liver microsomes plays an important part in the metabolism of drugs. For this reason, sensitive and reliable tests are necessary for determining enzyme activity and consequently the drug-metabolizing capacity of the system. 7-Ethoxycoumarin has been found to be an excellent substrate for the direct fluorometric determination of microsomal monoxygenase activity. The assay is based on the O-dealkylation of 7-ethoxycoumarin to produce the highly fluorescent 7-hydroxycoumarin (umbelliferone, catalog number H2400-3).

This system involves cytochrome P-450 containing monooxygenases and is dependent on NADPH and molecular oxygen.

CH₃CH₂O
\[ \text{7-ethoxycoumarin} \] \[ \text{NADPH, O₂} \]
\[ \text{cytochrome-P-450} \]
\[ \text{7-ethoxycoumarin} \]
\[ \text{O-deethylase} \]

Since it is well known that microsomal monooxygenases are induced typically by phenobarbital and polycyclic aromatic hydrocarbons, e.g., 3-methylcholanthrene (MC), the effect of these on O-deethylation of 7-ethoxycoumarin was studied. Phenobarbital and MC induced O-deethylation of 7-ethoxycoumarin in hepatic tissues and in isolated rat liver cells, whereas only phenobarbital induced the O-deethylation in extrahepatic tissues.

It has also been demonstrated that MC inducibility of 7-ethoxycoumarin O-deethylase and aryl hydrocarbon hydroxylase is determined genetically in the Ah locus. Poland et al. have shown that O-deethylation is also inducible by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

An improved fluorometric assay for microsomal monoxygenase determination via 7-ethoxycoumarin O-deethylation has been developed recently. This in vitro fluorometric assay is at least ten times as sensitive as present methods, and has facilitated the kinetic studies of O-deethylase activity as well as a reevaluation of the use of 7-ethoxycoumarin O-deethylation as an indicator of phenobarbital-induced monooxygenase activity in mice.

This assay enables nearly quantitative recovery of the major product, 7-hydroxycoumarin, by extraction and the product is essentially free of fluorescent contaminants. Maximal fluorescence of 7-hydroxycoumarin in aqueous solution is obtained at pH 9.5 or higher. O-Deethylase activity induced by phenobarbital, MC and TCDD was studied by this method.

The advantages of using 7-ethoxycoumarin as a substrate are that the compound is not known to be carcinogenic, it is not particularly light-sensitive, and it is dealkylated to a single, highly fluorescent product. 7-Ethoxycoumarin has been rigorously purified to eliminate as much background fluorescence as possible.

7-Ethoxycoumarin should prove to be a useful indicator of a wide range of monooxygenase inducers, particularly in cancer research.

References:

19,564-2 7-Ethoxycoumarin, 99.9 +%+, GOLD LABEL 100mg $11.00; 1g $44.00

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