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Proceedings of the Third European Symposium on Vitamin B₁₂ and Intrinsic Factor. University of Zurich, March 5-8, 1979, Zurich, Switzerland. Editors: B. Zagalak · W. Friedrich.

1979. 17 cm x 24 cm. XVI, 1239 pages. Numerous illustrations.
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These proceedings of the Vitamin B₁₂ Symposium present the current state of knowledge and research as well as future trends in the chemistry, biochemistry and physiological role of this vitamin. Many distinguished and authoritative scientists from throughout the world have contributed their most recent research results to this volume.

This book is dedicated to the memory of Robert Burns Woodward, winner of the Nobel Prize for chemistry in 1965, who died in July 1979. Professor Woodward's contribution to this volume represents his last research work in the field of synthetic vitamin B₁₂.

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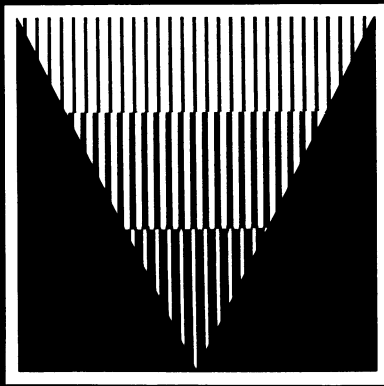
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SYMPOSIA SERIES No. 44

Biochemistry of Genetic Engineering

Edited by **P. B. Garland** and **R. Williamson**

pp. 145 (ISBN 0 904498 08 5) £12.50 (US\$27.50)

A Biochemical Society Symposium held in London in July 1978

The Biochemical Society's Forty-Fourth Symposium held at University College London in July 1978 reviewed in a two day meeting the exciting and rapidly expanding area of Genetic Engineering. Leaders in the field gave general introductions to the biochemical basis, practice and aims of many aspects of the subject, illustrated with accounts of current research. Subjects included ranged from the enzymology of restriction nucleases, ligases and polymerases, proceeded through vectors and hosts for recombinant DNA, considered in depth selected plant and animal systems, and concluded with industrial prospects and social perspectives. These excellent and well-received presentations form the basis of this publication, which will serve not only as a readable introduction to the biochemistry of genetic engineering but also as a valuable account of the activities of a number of leading laboratories as of summer 1978.

List of contents and authors:

Preface.

Restriction Nucleases, Ligases and Polymerases in Genetic Manipulation by **A. D. B. Malcolm.**
Safe and Useful Vector Systems by **W. J. Brammar.**

Plasmid Vectors for Genetic Manipulation in vitro by **D. J. Sherratt.**

Analysis of Restriction-Fragment Patterns from Complex Deoxyribonucleic Acid Species by **E. M. Southern.**

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Genetic Engineering: Do We Need It? How Would We Do It? by **A. J. Hale.**

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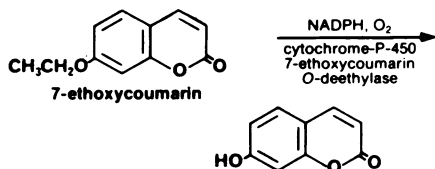
7-Ethoxycoumarin

Substrate for a Rapid, Sensitive, Fluorometric Assay of Microsomal Monooxygenase 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 found¹ to be an excellent substrate for the direct fluorometric determination of microsomal monooxygenase 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.



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 tissues² and in isolated rat liver cells,³ whereas only phenobarbital induced the O-deethylation in extrahepatic tissues.⁴

It has also been demonstrated that MC inducibility of 7-ethoxycoumarin O-deethylase and aryl hydrocarbon hydroxylase is determined genetically in the *Ah* locus.⁵ Poland *et al.*⁶ have shown that O-deethylase is also inducible by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

An improved fluorometric assay for microsomal

monooxygenase determination via 7-ethoxycoumarin O-deethylation has been developed recently.⁷ 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.⁷

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.⁷

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 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.

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