- Janda, J. (1971) *Biochem. J.* **124**, 73P
 Janda, J. (1972) *Biochem. J.* **130**, 41P
 Janda, J. & Work, E. (1970) *J. Gen. Microbiol.* **61**, vii
 Osborn, M. J. (1963) *Proc. Nat. Acad. Sci. U.S.* **50**, 449

Spectral Evidence for the Presence of Cytochrome P-450 in Microsomal Fractions Obtained from some Higher Plants

By ANTHONY MARKHAM, GORDON C. HARTMAN and DENNIS V. PARKE (*Department of Biochemistry, University of Surrey, Guildford, Surrey, U.K.*)

Cytochrome P-450 has been detected in mitochondrial and microsomal fractions of a variety of mammalian tissues (Boyd *et al.*, 1971; Ernster & Orrenius, 1965). Studies on bacteria indicate the involvement of cytochrome P-450 in camphor hydroxylation by *Pseudomonas putida* (Gunsalus, 1971). In fungal metabolism a role for cytochrome P-450 has been demonstrated in *Claviceps purpurea* and *Candida tropicalis*. In the former it is involved in alkaloid biosynthesis, which may be stimulated by pretreatment with phenobarbital (Ambike & Baxter, 1970; Orrenius, 1971). In the yeast there is involvement in ω -hydroxylation of fatty acids (Lebean, 1971).

Studies on spermatophyta are limited. Murphy & West (1969) showed that the conversion of kaurene into kaurenol required NADPH as well as O₂, and was inhibited by CO. Similar results were obtained for the cinnamate 4-hydroxylase system in *Pisum sativum* (pea) by Russell (1971). The importance of cytochrome P-450 in higher-plant metabolism is still a matter for conjecture, other modes of hydroxylation having been described, e.g. phenolase (Butt & Vaughan, 1970) and peroxidase (Rathmell & Bendall, 1972).

Moore (1967) obtained evidence for the characteristic cytochrome P-450-CO binding spectra in microsomal fractions from *Pisum sativum* cotyledons (Chance *et al.*, 1968; D. S. Bendall, personal communication). We have obtained spectral evidence for cytochrome P-450 in a variety of etiolated plant tissues. The selected region of the plant was homogenized in 0.1 M-phosphate buffer, pH 7.4, containing 2M-glycerol and 0.02M-2-mercaptoethanol. The 220000g-min supernatant was recentrifuged for 1 h at 105000g and the resulting pellet was resuspended in the homogenizing medium.

Investigations of CO-binding spectra indicated the presence of material giving a 450nm peak within *Phaseolus vulgaris* (bean) cotyledons, *Pisum sativum* seedlings and *Zea mais* (maize) cotyledons. The concentration in bean and pea tissue was about 0.02nmol/mg of microsomal protein, whereas that in monocotyledons was about 0.007nmol/mg (ϵ taken to be 91mm⁻¹.cm⁻¹). These values are far lower

than those encountered in rat liver (0.8–1.2nmol/mg of microsomal protein). We have not obtained spectral evidence for cytochrome P-450 in *Phaseolus aureus* (mung bean), citrus fruit, microsomal preparations or in any of the 220000g-min pellets. The 450nm peak was transient, especially in the absence of the thiol, and was always accompanied by a peak at 420nm.

Preliminary studies *in vivo* and *in vitro* have shown no biphenyl hydroxylase activity in a variety of plant tissues. However, a trace of *p*-nitroanisole *O*-demethylase activity has been found in both the cotyledon and seedling of *Pisum sativum* (less than 0.012 μ mol of *p*-nitrophenol/h per mg of microsomal protein). So far pretreatment with phenobarbital or 2,4-dichlorophenoxyacetate has failed to influence cytochrome P-450 concentrations in *Pisum sativum* or *Phaseolus vulgaris*. Binding studies have produced no distinct spectra with biphenyl or aniline, but metyrapone [2-methyl-1,2-di-(3-pyridyl)propan-1-one] gave evidence of a type II complex in preparations from *Phaseolus vulgaris*.

Financial support for A. M. from the Science Research Council is gratefully acknowledged.

- Ambike, S. H. & Baxter, R. M. (1970) *Phytochemistry* **9**, 1959
 Boyd, G. S., Brownie, A. C., Jefcoate, C. R. & Simpson, E. R. (1971) *Biochem. J.* **125**, 1P
 Butt, V. S. & Vaughan, P. (1970) *Biochem. J.* **119**, 89
 Chance, B., Bonner, W. D. & Storey, B. T. (1968) *Annu. Rev. Plant. Physiol.* **19**, 295
 Ernster, L. & Orrenius, S. (1965) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **24**, 1190
 Gunsalus, I. C. (1971) *Biochem. J.* **125**, 5P
 Lebean, H. J. M. (1971) *Biochem. Biophys. Res. Commun.* **42**, 121
 Moore, C. W. (1967) Ph.D. Thesis, University of Cambridge
 Murphy, P. J. & West, C. A. (1969) *Arch. Biochem. Biophys.* **133**, 395
 Orrenius, S. (1971) *Chem.-Biol. Interact.* **3**, 313
 Rathmell, W. G. & Bendall, D. S. (1972) *Biochem. J.* **127**, 125
 Russell, D. (1971) *J. Biol. Chem.* **246**, 3870

Structure-Activity Relationships for the Induction and Inhibition of Hepatic Microsomal Enzymes in the Rat by Organohalogen Compounds

By CHARLES FRANKLIN and IDRIS B. EL-TAYEB (*Department of Pharmacology, Chelsea College, London SW3 6LX, U.K.*)

A number of organochlorine pesticides, such as DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane], cause the induction of several hepatic