catalysing the formation of 3,4,3',4'-tetrahydroxybiphenyl, was demonstrated.

The enzyme system oxidizing catechol to biphenylene dioxide 2,3-quinone was purified 120-fold by protamine sulphate precipitation, tricalcium phosphate-gel treatment and molecular-sieve chromatography on DEAE-Sephadex A-50. The approximate molecular weight was found to be 200000 by gel filtration on Sephadex G-150. The enzyme moved as a single band when subjected to electrophoresis on polyacrylamide gels. It had optimum pH 7.1. The K_m was 4×10^{-4} M. The enzyme was not susceptible to inhibition by copper-chelating agents. Dialysis of the enzyme against copper-chelating agents did not have any inhibitory effect on the enzyme. The enzyme was not inhibited by CO. The enzymic reaction could not proceed anaerobically. Reducing agents such as cysteine, GSH and cysteamine inhibited the oxidation of catechol by the purified enzyme. We did not observe the production of any H_2O_2 during the catalytic activity. Another observation was that the enzyme did not display any hydroxylase activity against *p*-cresol and other monohydric phenols. The most significant feature of this enzyme was its specificity: it was highly specific for catechol, the dihydric phenols and flavonoids not being acted upon by the enzyme. The stoichiometric conversion of catechol into biphenylene dioxide 2,3-quinone was also studied.

The enzyme system that converted catechol into 3,4,3',4'-tetrahydroxybiphenyl was purified 70-fold from the leaves of *Tecoma stans* by ammonium sulphate precipitation, negative adsorption on tricalcium phosphate gel and chromatography on DEAE-Sephadex. The partially purified enzyme was maximally active at pH 7.2. The K_m was 2×10^{-4} M. The enzyme activity was partially stimulated by Mg^{2+} . The enzyme was resistant to the action of copper-chelating agents. The formation of C-C- and C-O-linked oxidation products observed in this study can be explained on the basis of the formation of aryloxy radicals (Thomson, 1964).

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Flavoprotein Semiquinone

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On anaerobic irradiation in the presence of EDTA, flavoproteins produce either a red or a blue semiquinone (Massey & Palmer, 1966). An exception to this is glucose oxidase, which gives a red semiquinone in alkaline media and a blue semiquinone in acidic media, and it was considered (Massey & Palmer, 1966) that the former corresponds to the anion radical and the latter to the neutral radical. D-Amino acid oxidase generates the red semiquinone even when the pH of the solution is lowered. However, this red semiquinone can easily be converted into the blue semiquinone by the addition of an excess of benzoate, a competitive inhibitor for the substrate (Yagi *et al.*, 1968b). The conversion of the red semiquinone into the blue semiquinone by benzoate was achieved at pH 8.3, 7.0 and 6.0. The conversion of the red radical into the blue radical of the flavin moiety could therefore be ascribed to the modification of the environment around the buried flavin but not to the exposure of the flavin to the medium, water. A Benesi-Hildebrand plot for the formation of the blue radical on the addition of benzoate gave a straight line, indicating complex-formation. The dissociation constant was calculated to be 2×10^{-2} M. Our earlier work (Kotaki *et al.*, 1966; Yagi *et al.*, 1968a) indicated that the complex-formation between benzoate and the oxidized form of D-amino acid oxidase induces a modification in the environment of the flavin moiety from hydrophobic to hydrophilic, as judged by the change in 370 nm absorption band. If this alteration occurs around the semiquinone, facilitating the protonation of the radical, it results in the conversion from the red semiquinone into the blue semiquinone. Müller *et al.* (1970) found that the highest spin density is located at N-5 of the flavin nucleus. Thus their prediction that in the blue semiquinone N-5 interacts with the proton-donating residue of the protein molecule (Müller *et al.*, 1970) is probably applicable to the blue complex of D-amino acid oxidase.

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