ABSTRACT

While trying to isolate the enzymes, responsible for the biosynthesis of opium alkaloids, a distinct phenolase complex system has been recognised in *P.somniferum*. By the phenolase complex is meant that pair of enzymatic activities occurring together, associated with copper protein and responsible for the o-hydroxylation of phenols and dehydrogenation of o-diphenols.

The phenolase complex of *P.somniferum* differs from laccase in that it does not catalyse the oxidation of quinol, venillin and p-phenylenediamine. It differs from tyrosinase because it does not oxidise tyrosine either in presence or in absence of added catechol. However, it catalyses the oxidation of DOPAMINE, DOPA, catechol, p-cresol and tyramine. It differs from tyrosinases so far known because it does not oxidise tyrosine either in presence or in absence of added catechol. This distinct phenolase complex is distributed in all the tissues of *P.somniferum* studied which included roots, leaves, stems, buds and fruits.
The phenolase complex of *P. somniferum* was inhibited by sodium diethyldithiocarbamate, salicylaldoxime, potassium ethyl xanthate and thiourea which suggested that the enzyme is a copper containing protein. The enzyme was also inhibited by metallic ions which are known to compete with copper such as Ag⁺, Hg²⁺ and Au³⁺. These results together with other observed evidences suggested that phenolase complex of *P. somniferum* is a copper containing protein.

In order to gain more insight into the nature of the phenolase complex of *P. somniferum*, detailed kinetic studies were undertaken.

The formation of quinones during the oxidation of DOPAMINE, DOPA, catechol, p-cresol and tyramine, by phenolase complex of *P. somniferum* has been demonstrated. The anilino derivatives of quinones, i.e. dianilino-o-benzoquinone and dianilinohomoquinoneanil, produced by the action of phenolase complex on catechol and p-cresol, respectively, have been isolated and identified. The isolation of these derivatives as the products of catechol and p-cresol oxidation in presence of phenolase complex indicated that o-benzoquinones are formed under these conditions and first step
in the oxidation of monophenols is o-hydroxylation.

The oxidation of DOPAMINE, DOPA and tyramine, formation of o-benzoquinones and eventual deposition of black reaction products suggested that the pathway for melanin biosynthesis may be operative in *P. somniferum*.

The enzyme has been purified 47-fold from the acetone powder extracts of *P. somniferum* using ammonium sulphate precipitation method, CM-cellulose and DEAE-cellulose chromatography. The properties of partially purified preparations have been studied. With all the phenolic compounds mentioned above, except tyramine, straight lines have been obtained in Lineweaver and Burk reciprocal plot. With tyramine as substrate the reciprocal plot indicated substrate inhibition. The Km values obtained with these preparations show that phenolase complex of *P. somniferum* has highest affinity for DOPAMINE in comparison to other mono- and di-phenols tested.

The properties of phenolase complex in relation of melanin biosynthesis and the failure to demonstrate the presence of DOPA decarboxylase and amine oxidase in *P. somniferum* in relation to the biogenesis of opium alkaloids have been discussed.