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(S)-TETRAHYDROPROTOBERBERINE OXIDASE THE FINAL ENZYME IN PROTOBERBERINE BIOSYNTHESIS

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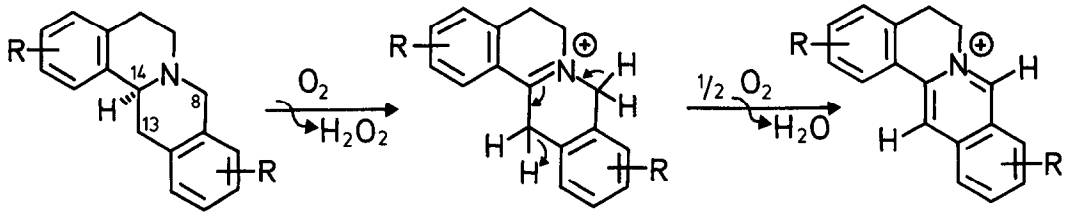
Abstract: A new flavin enzyme has been discovered which in the presence of O_2 catalyzes the oxidation of (S)-tetrahydroprotoberberines to protoberberines via the intermediate 7,14-dehydroberberinium.

As a result of previous studies, it has been proposed that the final step in the biosynthesis of the berberine alkaloids consists in the specific oxidation of ring C of tetrahydroprotoberberines[1]. We report here on a new enzyme which catalyzes the dehydrogenation of some 20 different (S)-tetrahydroprotoberberines to the corresponding protoberberine alkaloids. The enzyme was assayed for its catalytic activity either by following the increase in absorption at 345 nm (log ∈: 4,40) during the oxidation of canadine to berberine or by measuring the release of tritium into the aqueous medium during the dehydrogenation of [8,13,14-$^3H_3$]-(R,S)-canadine. The enzyme was found to occur in a number of plant cell cultures, especially those straines of *Berberis* which produce considerable amounts of protoberberines[2] but also in whole plants of *Papaver somniferum*. The enzyme was first isolated from *Berberis wilsonae* var. *subcaulialata* Schneid. cell cultures, which were grown as previously described and purified to homogeneity (purification factor 150; yield: 25%). The enzyme contains flavin, has a molecular weight of 100.000, a pH-optimum for maximal catalytic activity at 8,9 and shows a yellow-green fluorescence (excitation at 450 nm, max. emission spectrum 520 nm) which is quenched by the addition of $Na_2S_2O_4$. The catalytic activity is inhibited by morine (50% inhibition at 0,18 µM). The enzyme is specific for tetrahydroprotoberberines of the (S)-configuration, and none of the (R)-enantiomers tested acted as substrates. The K_M value for (S)-tetrahydrojatrorrhizine is 1,3 µM and for (S)-canadine is 26,7 µM. The aromatic substitution pattern of rings A and D of the tetrahydroprotoberberine nucleus only quantitatively influences the catalytic activity. 1,5 Mole of oxygen is consumed and one mole of H_2O_2 and H_2O produced by each mole of substrate which is oxidized. Addition of catalase reduces the oxygen consumption rate to 67% of the

original value. In the presence of $NaBD_4$ the enzyme does not oxidize canadine to berberine but reisolated canadine shows incorporation of 1 atom of deuterium which is present at the C-14 carbon (by mass and NMR spectra). 13,14-Dehydrocanadine and canadine methosalt are not oxidized by the enzyme. Methyl substitution at C-8 or C-13 renders these tetrahydroprotoberberines unsuitable for enzymatic oxidation. It is therefore concluded that the enzyme catalyzes the dehydrogenation of the tetrahydroprotoberberine molecule at C-14 and N-7 and that the 7,14-dehydroberbinium intermediate aromatizes further in ring C to yield the protoberberine molecule as shown in scheme 1. The enzyme is exclusively found in a type of organelle which can be isolated from the cells using sucrose density gradient centrifugation (ρ = 1,14 g/cm^3). These organelles do not correspond in density to known particles such as mitochondria, microbodies, proplastids etc.



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