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THE BERBERINE BRIDGE FORMING ENZYME IN TETRAHYDROPROTO-BERBERINE BIOSYNTHESIS

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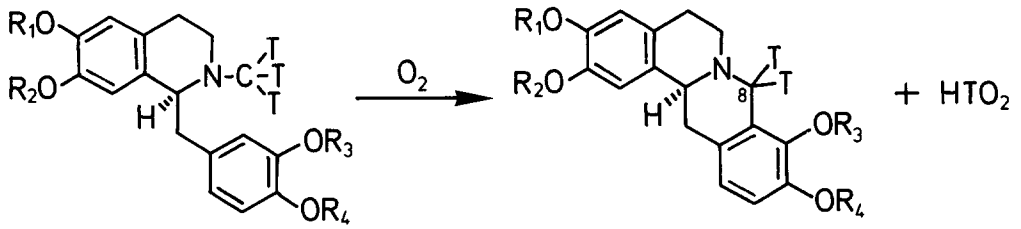
Abstract: The enzyme responsible for the berberine bridge formation was purified to homogeneity and shown to catalyze, in the presence of oxygen, the conversion of (S)-reticuline, (S)-protosinomenine and (S)-laudanosoline to the correspondingly substituted (S)-tetrahydroprotoberberines and stoichiometric amounts of H_2O_2.

Reticuline (I) has been recognized to be the precursor of the protoberberine carbon sceleton by precursor feeding and product degradation experiments[1]. During this reaction, the N-methyl group of I is converted to carbon atom 8 (the so-called berberine bridge) of the tetrahydroprotoberberine alkaloid scoulerine (II). A cell free system[2] of *Macleaya microcarpa* cell cultures was previously shown to catalyze the conversion of I to II, and the enzyme involved (purification factor 7-fold) was named the berberine bridge enzyme[2]. The mechanism of this conversion remained, however, unclear with regard to the discussed intermediate formation of reticuline-N-oxide[3], the participation of oxygen[2,4] and the steric course of the reaction[2].

Berberis beaniana Schneid. cell cultures are an excellent source for the synthesis of protoberberine alkaloids[5] and from these cells the berberine bridge enzyme was isolated and purified to homogeneity. The enzyme activity was conveniently monitored using N-CT_3-(S)-I as substrate and following the release of tritium in the aqueous phase of the incubation mixture; exactly 1/3 of the radioactivity contained in the N-methyl group was lost during the cyclisation of I to the tetrahydroprotoberberine molecule. The enzyme has a molecular weight of about 49.000 (gel filtration), pH optimum for maximal catalytic activity is at 8,9 and the isoelectric point at pH 4,9. The catalytic activity of the enzyme is inhibited by o-phenanthroline and by reducing agents such as dithioerythritol (50% inhibition at 6 µM and 4 mM, respectively). The enzyme does not need added pyridine nucleotides for catalytic activity. The product of the reaction using (R,S)-I as substrate was isolated from large scale incubation mixtures (1 ltr) and its identity was unequivocally established as (S)-scoulerine (II) (by HPLC, MS, NMR,

derivatisation). The enzyme is specific for the substrates with (S)-configuration and not for the substrates with (R)-configuration. Neither (S)- nor (R)-reticuline-N-oxide was transformed by the crude or by the homogenous enzyme. For each mol of (S)-I consumed one mol of O_2 is taken up and one mol each of (S)-tetrahydroprotoberberine and H_2O_2 is formed. The enzyme catalyzes the reaction sequence depicted in the scheme indicating also the assay principle. The K_M value for (S)-I is 0,14 µM. Of the three other possible isomers of I, only (S)-protosinomenine (I, R_1=R_3=H; R_2=R_4=Me) is accepted as substrate (145% of I); again, the (R)-stereoisomer is not a suitable substrate. Neither orientaline (I, R_1=R_3=Me; R_2=R_4=H) nor isoorientaline (I, R_1=R_4=H; R_2=R_3=Me) are affected, also zero activity of the enzyme is shown for (R,S)-laudanidine (I, R_1=R_2=R_4=Me; R_3=H) and (R,S)-laudanosine (I, R_1-R_4=Me). However, (R,S)-laudanosoline (I, R_1-R_4=H) is transformed (20% of I). This substrate specifity is at variance with the reported properties of the *Macleaya* enzyme[2]. The enzyme is exclusively contained in a particle which can be isolated from the cells using sucrose density gradient centrifugation (ρ = 1,14 g/ml).



N-CT_3-(S)-reticuline (I)
R_1=R_4=Me; R_2=R_3=H

C8-T_2-(S)-scoulerine (II)
R_1=R_4=Me; R_2=R_3=H

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