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S-ADENOSYL-L-METHIONINE: (S)-7,8,13,14-TETRAHYDROBERBERINE-CIS-N-METHYLTRANSFERASE, A BRANCH POINT ENZYME IN THE BIOSYNTHESIS OF BENZOPHENANTHRIDINE AND PROTOPINE ALKALOIDS.

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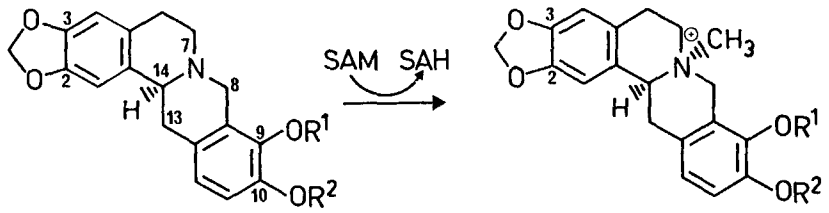
Abstract: The enzyme which transfers the CH_3-group of S-adenosylmethionine to the nitrogen atom of (S)-tetrahydroberberine and (S)-stylopine is found to occur in a number of plant cell cultures originating from species containing alkaloids; it is located at an important branch point in isoquinoline alkaloid biosynthesis.

Protopine and benzophenanthridine alkaloids are prominent members of the large group of isoquinoline alkaloids in higher plants. Previous work has verified the incorporation of the N-methyl derivatives of canadine and stylopine into both classes of alkaloids [1,2]. Takao et al. [3] further demonstrated that only the cis-N-methyl derivatives of tetrahydroprotoberberines can be stereospecifically metabolized into the benzophenanthridine type and protopine type skeleton. As part of our investigations involving the elucidation of reaction sequences in the isoquinoline biosynthesis, we searched for that enzyme which catalyzes the N-methylation of tetrahydroprotoberberines. The enzyme was assayed for its catalytic activity by using ($^{14}CH_3$)-S-adenosyl-L-methionine (SAM; 2 nMol) and (R,S)-canadine (10 nMol) as a substrate at pH 8 (glycine-NaOH buffer) in a total volume of 300 µl containing 50-300 µg protein. The enzyme reaction was followed by monitoring the transfer of radioactivity from the aqueous into the isoamyl phase [4] after an incubation period of 30 min at 40°C. Enzymatically catalyzed methylation of canadine occurred in a number of cell-free extracts from cell suspension cultures of for instance Berberis stenophylla (0.22 pkat/mg protein), Corydalis vaginans (0.74), Dicentra spectabilis (0.94), Fumaria officinalis (0.43), Papaver somniferum (0.41). The product of the reaction was unequivocally identified as N-methyltetrahydroprotoberberine by large scale (500-fold) incubation (10 h) of the above incubation mixture containing 80 mg protein from C. vaginans. The incubation mixture was extracted with ethylacetate, which was chromatographed (TLC) and the newly formed compound isolated. MS of the unknown product showed e/z M^+ at 353 (5.6%); 339 (19.4%); 176 (27.8%); 174 (30.6%); 164 (100%); 149 (83.5%). The MS was in every respect identical to N-methylcanadine [5]. The enzyme was purified 40-fold, it has a molecular weight of 72 000 ± 10%, a pH-optimum at 8.0, and a temperature optimum at 40°C. The enzyme is specific for (S)-canadine ($R^1 = R^2 = CH_3$; 100%) and (S)-stylopine ($R^1 + R^2 = CH_2$; 77%); the (R)-enantiomers as well as (S)-scoulerine, (R,S)-tetrahydropalmatine, (R,S)-tetrahydrojatrorrhizine, (R,S)-columbamine, (S)-norreticuline and (S)-reticuline are not metabolized. The enzyme therefore acts on tetrahydroprotoberberines of (S)-configur-

ation containing a methylenedioxy bridge at carbon atoms 2 and 3. Feeding experiments of N-($^{14}CH_3$)-stylopine obtained by enzymatic catalysis showed good incorporation into protopine (0.6%) and sanguinarine (7.7%) in Fumaria capreolata callus cultures. This demonstrated that the product formed by the new enzyme is biologically active. Since Takao et al. [3] have unequivocally demonstrated that only the cis-N-methyl derivatives of tetrahydroprotoberberines can be stereospecifically metabolized into the benzophenanthridine and protopine type skeleton - while the trans-N-methyl derivatives are absolutely inactive - we can assign the cis-configuration to the enzymatically formed product. The enzyme responsible for the formation of this important branch point metabolite is therefore termed: S-Adenosyl-L-methionine: (S)-7,8,13,14-tetrahydroberberine-cis-N-methyltransferase. The high substrate specificity of this enzyme is reflected by the fact that the vast majority of benzophenanthridine and protopine alkaloids in nature contain methylenedioxy groups biogenetically corresponding to the C_2 and C_3 position of the tetrahydroprotoberberine skeleton. Since the methylenedioxy group in berberine biosynthesis is formed at the quaternary stage (the substrate being columbamine and not tetrahydrocolumbamine) [6] and since quaternary alkaloids cannot diffuse or be transported across the membrane of the specific vesicle in which they are formed [7], the question that remains is how (S)-canadine and (S)-stylopine, both acceptors for the methyl group, are formed. There are at least two possibilities: either quaternary protoberberines are stereospecifically reduced to their tetrahydro counterparts with (S)-configuration, which serve as substrates for the N-methylating enzyme, or (S)-canadine is formed from (S)-tetrahydrocolumbamine by a yet to be discovered methylenedioxy bridge forming enzyme acting on this substrate rather than on the quaternary alkaloid columbamine [6].



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