

COLUMBAMINE, THE CENTRAL INTERMEDIATE IN THE
LATE STAGES OF PROTOBERBERINE BIOSYNTHESIS

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Abstract: A specific methyltransferase which in the presence of S-adenosylmethionine [SAM] converts columbamine to palmatine has been demonstrated to occur in *Berberis* cell cultures. The enzyme acts only on the quaternary alkaloid as substrate, not on its tetrahydro derivative, which fact is in sharp contrast to previous claims.

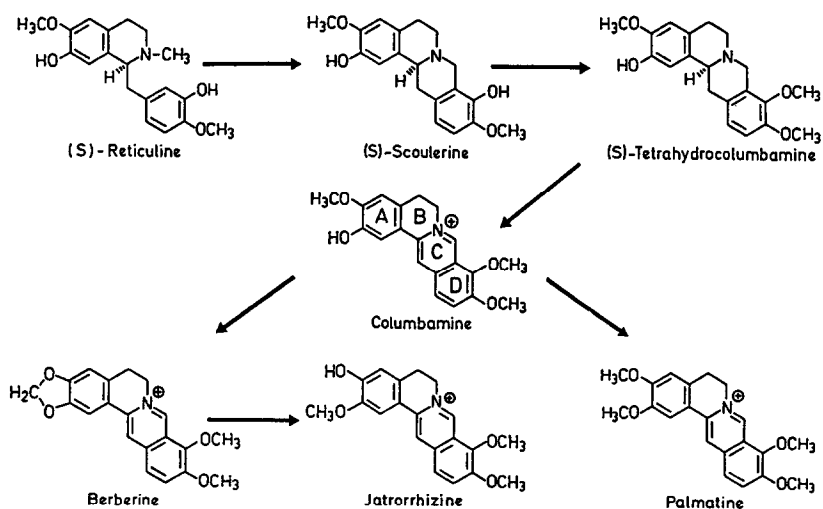
The biosynthesis of the protoberberine alkaloids has been essentially clarified both at the precursor feeding stage¹ and at the enzyme level². However, the modification of rings A and D of the protoberberine molecule during the late stages of the formation of these alkaloids are still not clear. Recently, utilizing crude extracts from *Berberis aggregata*, Beecher and Kelleher³ reported on the demonstration of a methyl transferase catalysing the methylation of tetrahydrocolumbamine to tetrahydropalmatine which should subsequently be oxidized by tetrahydroberberine oxidase (STOX⁴) to form palmatine. To prevent the oxidation by the STOX enzyme either the inhibitor morine⁴ was added or incubations were performed under nitrogen. Tetrahydropalmatine was the sole reaction product³ and as a consequence tetrahydrocolumbamine was claimed to be a triple branch point intermediate, from which the substitution pattern of the tetrahydroprotoberberines should be established.

Since we had previously shown that berberine is formed from columbamine⁵, both compounds being at the quaternary stage, we decided to re-investigate the sequence of methylation of palmatine as well. We report here on an enzyme which catalyzes the formation of the methyl group at position 2 of columbamine to yield palmatine. The enzyme was detected in and isolated from *Berberis wilsoniae* var. *subcaulialata* and *Berberis aggregata* cell cultures grown as described⁶. Considerable amounts (ca. 2 g protoberberines per liter of medium) of protoberberine alkaloids were produced by these cultures. The activity was monitored using either (R,S)-tetrahydrocolumbamine or columbamine as the substrate. Purification was achieved via (NH₄)₂SO₄ precipitation and DEAE cellulose chromatography followed by affinity chromatography utilizing an epoxy activated Sepharose 6B (Pharmacia) column which contained columbamine as the ligand. This final step ultimately eliminated STOX activity from the preparation. The enzyme has a molecular weight of 52 000, a pH optimum for maximum catalytic activity at 8.5, a

temperature optimum at 20°C and was specific for columbamine with a K_M of 14.3 μM (K_M SAM, 1.5 μM). A typical incubation mixture contained in a final volume of 300 μl : 5 nmols columbamine, 1 nmol (C^3H_3 -SAM, 15×10^3 cpm), 50 μmols of tris chloride buffer pH 8.0 and enzyme solution (200 μl purified enzyme = 30 μg). The incubation was conducted 2 h and yielded 11.7 pmols palmatine. Neither (R,S)-tetrahydrocolumbamine nor the separated (R)- and (S)-enantiomers acted as substrates, contrary to what has been assumed previously³. In the presence of SAM the enzyme did not catalyze the methylation of reticuline, scoulerine, tetrahydrocolumbamine, tetrahydrojatrorrhizine, dehydroscoulerine, desmethyleneberberine, dehydrocorydalmine, berberrubine or jatrorrhizine.

The previously³ observed methylation of tetrahydrocolumbamine in *Berberis aggregata* therefore must be incorrect. This error was possibly due to an insufficient concentration of morine used as STOX inhibitor in the crude extract, incomplete anaerobic conditions during the incubation, as well as cocrystallization of labelled palmatine with tetrahydropalmatine.

The methylation pattern of the A ring of protoberberines in the late stages of biosynthesis was determined at the quaternary state. Columbamine, not tetrahydrocolumbamine, is the central intermediate as shown in the Scheme.



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