Tetrahedron Letters,Vol.26,No.2,pp 201-202,1985
Printed in Great Britain

0040-4039/85 $3.00 + .00

BERBERINE SYNTHASE, THE METHYLENEDIOXY GROUP FORMING ENZYME IN BERBERINE SYNTHESIS

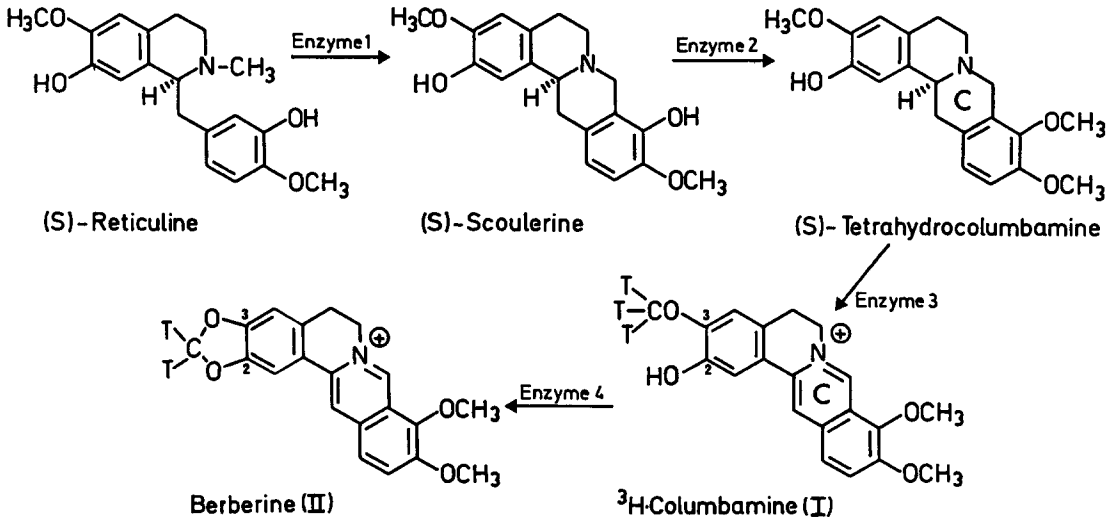
M. Rueffer and M.H. Zenk *

Lehrstuhl Pharmazeutische Biologie, Universität München, Karlstrasse 29, D-8000 München 2, W-Germany

Abstract: An Fe^{++} containing enzyme responsible for the formation of the methylenedioxy group in berberine was discovered, purified to homogeneity and characterized; it acts on columbamine as a substrate rather than on (S)-tetrahydrocolumbamine as previously assumed.

Methylenedioxy groups are frequently found in isoquinoline alkaloids[1]. Feeding experiments with plants using labelled precursors demonstrated that the methylenedioxy group is formed from an o-methoxy phenol precursor[2]. The biosynthesis of this group can be visualized in terms of either an ionic or a free radical mechanism[3]. As part of our continuing work directed toward elucidating the complete sequence of reactions in berberine biosynthesis, we investigated the formation of the methylenedioxy group. We report here on a new enzyme which catalyses the formation of the methylenedioxy group from columbamine the immediate precursor of berberine. The enzyme was assayed for its catalytic activity by using 3-O-C^3H_3-columbamine (I) as a substrate. The enzyme reaction was followed by monitoring the release of tritium into the aqueous phase of the incubation mixture. Exactly 1/3 of the radioactivity of the 3-O-methyl group was lost during the transformation of compound I to berberine (II). The enzyme was isolated from *Berberis stolonifera* cell cultures which produce considerable amounts of protoberberine alkaloids and were grown as described[4], and it was purified to homogeneity (purification factor 160; yield 8%). The UV absorption spectrum of the pure protein shows the usual peak at 280 nm, and an additional one at 408 nm. The enzyme has a molecular weight of 32 000, a sharp pH-optimum for maximal catalytic activity at pH 8.9 and a surprisingly high temperature optimum at 70°C. The catalytic activity is inhibited by cyanide and o-phenanthroline (50% inhibition at 1 μM and 22 μM respectively). Dialysis of the protein against o-phenanthroline renders the enzyme completely inactive. Full activity could be restored by the addition of Fe^{++} but not Fe^{+++}. None of 10 other heavy metal ions tested (including Ni^{++}) was able to reactivate the inactive enzyme form. The enzyme is specific for columbamine, with a K_M of 2 μM. Neither (R)- nor (S)-tetrahydrocolumbamine acted as substrates, contrary to what has been assumed [3,5]. In the light of

these findings, the biosynthetic sequence for berberine starting with (S)-reticuline is shown in the scheme:



The enzymology of (S)-reticuline formation is known in detail[6]; four additional enzymes are involved in the biosynthesis of berberine starting from (S)-reticuline. Enzyme 1 is the highly stereospecific berberine bridge enzyme[7]. (S)-Scoulerine is methylated by the highly stereo- and regiospecific S-adenosyl-L-methionine:(S)-scoulerine -9-O-methyltransferase[8] (enzyme 2). (S)-Tetrahydroprotoberberine oxidase[9] (enzyme 3) catalyses only the aromatisation of ring C of the (S)-tetrahydroprotoberberine molecule, thus giving rise to the last intermediate columbamine (I). This protoberberine is further modified by enzyme 4, the berberine synthase described in this paper. The biosynthesis of berberine, which is used pharmaceutically, is now completely known at the enzyme level. All of the 8 enzymes[6] involved have now been discovered, highly purified and characterized.

Acknowledgement: Supported by SFB 145 of Deutsche Forschungsgemeinschaft, Bonn, and Fonds der Chemischen Industrie.

References:

1. Cordell, G.A. Introduction to Alkaloids. John Wiley Interscience. New York (1981).
2. Barton, D.H.R., D.S. Bhakuni, G.M. Chapman, and G.W. Kirby. J.Chem.Soc. 2134 (1967).
3. See Shamma, M.: The Isoquinoline Alkaloids. Academic Press. New York (1972).
4. Hinz, H., and M.H. Zenk: Naturwissenschaften 67, 620 (1981).
5. Beecher, C.W.W., and W.J. Kelleher. Tetrahedron Letters 24, 469 (1983).
6. Zenk, M.H. in: The Chemistry and Biology of Isoquinoline Alkaloids (J.D. Phillipson et al. eds.). Springer Verlag (Heidelberg). In press.
7. Steffens, P., N. Nagakura, M.H. Zenk. Tetrahedron Letters 25, 951 (1984).
8. Mümmler, S., M. Rueffer, N. Nagakura, and M.H. Zenk. Plant Cell Reports. In press.
9. Amann, M., N. Nagakura, and M.H. Zenk. Tetrahedron Letters 25, 953 (1984).

(Received in Germany 16 October 1984)