

BIOSYNTHESIS OF THE PROTOBERBERINE ALKALOID JATRORRHIZINE

M. Rueffer, O. Ekundayo, N. Nagakura[†], and M.H. ZenkLehrstuhl Pharmazeutische Biologie, Universität München,
8000 München 2, W-Germany[†]Kobe Women's College of Pharmacy, Kobe 658, Japan

Abstract: Feeding experiments with distant single or doubly labelled precursors show that the methylene dioxy group of berberine is opened in the formation of jatrorrhizine.

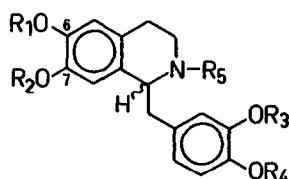
Jatrorrhizine, the major alkaloid of *Berberis* cell cultures¹, contains an unusual 2-O-methylation pattern which makes it difficult to deduce its biosynthesis from reticuline, the common precursor of isoquinoline alkaloids. A recent publication² prompts us to present here some of our work on this topic. Partially purified norlaudanosoline-6-O-methyltransferase from *Papaveraceae* cell cultures catalyses the formation of 6-O-methylnorlaudanosoline ($R_1=Me$; $R_2-R_5=H$) along with a smaller amount of 7-O-methylnorlaudanosoline ($R_2=Me$; $R_1=R_3-R_5=H$) exactly like the mammalian catechol-O-methyltransferase³. Therefore, the possibility existed that the 2-O-methylation pattern of jatrorrhizine is established already at the norlaudanosoline level. Further methylation reactions, cyclisation and oxidation could lead to jatrorrhizine via protosinomenine (IV), which should be a good substrate for the cyclising berberine-bridge enzyme⁴.

In order to test this possibility, (S)-reticuline (III, $-N-^{14}CH_3$) and (S)-protosinomenine (IV, $-N-^{14}CH_3$) were fed to callus of *Berberis stolonifera*¹. A predominant incorporation of (S)-reticuline (4.4%) over (S)-protosinomenine (0.73%) into jatrorrhizine was observed, indicating a preference for the reticuline pathway. To study even more distant precursors (R,S)6-O-Me-laundanosoline (I) and its 7-O-Me isomer (II) were synthesized with a $-N-^{14}CH_3$ label and applied to callus. Again, derivative I, with the methyl group in the "wrong" position, showed better (4%) incorporation than the 7-O-Me isomer (0.6%). Reduction of the labelled jatrorrhizine with BH_4^- followed by demethylation⁵ showed that no transfer of label to the methoxyl groups had occurred. These results indicate that transformation of precursors I and III into jatrorrhizine must involve an internal transfer of the methyl group from the C-6-position in (S)reticuline to the C-2-position in jatrorrhizine.

To provide experimental proof for this intramolecular methyl transfer doubly labelled precursor I was prepared using purified enzymes and S-adenosyl-

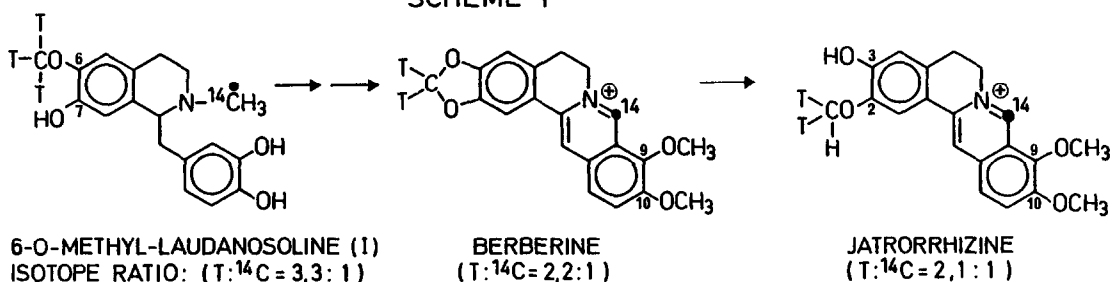
[methyl- ^3H]- and [methyl- ^{14}C]-methionine to label the 6-O- and the N-methyl groups respectively (Scheme 1). This compound (1.5 nmol; 6.78×10^5 dpm ^3H ; 2.05×10^5 dpm ^{14}C ; $^3\text{H}:^{14}\text{C}=3.3:1$) was supplied to 1 g *B. stolonifera* callus and allowed to metabolize for 48h (23°C). Incorporation into jatrorrhizine was 0.6% ($^3\text{H}:^{14}\text{C}=2.1:1$). This isotope ratio corresponds exactly to a loss of 1/3 of the tritium label in the original 6-O- CH_3 of I. Berberine, which was also isolated from this callus showed 0.6% incorporation of I and the same isotope ratio ($^3\text{H}:^{14}\text{C}=2.2$) as found in jatrorrhizine. This finding suggested that jatrorrhizine is formed from berberine by reopening of the methylene dioxy group. Indeed, berberine- ^{14}C (produced from L-tyrosine-2- ^{14}C by *Thalictrum minus* callus) was incorporated to an extent of 1.6% into jatrorrhizine (24h feeding). These experiments provide proof that the major biosynthetic route to jatrorrhizine is through berberine (Scheme 1). A minor route can be envisaged through (S)-protosinomenine which already carries the methoxy group in the "correct" 7-position.

The precursor role of berberine is in absolute agreement with the demonstration by Beecher and Kelleher² of the *in vivo* transformation of berberine (9-O- $^{14}\text{CH}_3$) into jatrorrhizine. We fully agree with their proposed mechanism for the conversion of berberine into jatrorrhizine.



- I $\text{R}_1=\text{R}_5=\text{Me}; \text{R}_2=\text{R}_3=\text{R}_4=\text{H}$ (6-O-METHYL-LAUDANOSOLINE)
 II $\text{R}_2=\text{R}_5=\text{Me}; \text{R}_1=\text{R}_3=\text{R}_4=\text{H}$ (7-O-METHYL-LAUDANOSOLINE)
 III $\text{R}_1=\text{R}_4=\text{R}_5=\text{Me}; \text{R}_2=\text{R}_3=\text{H}$ (RETICULINE)
 IV $\text{R}_2=\text{R}_4=\text{R}_5=\text{Me}; \text{R}_1=\text{R}_3=\text{H}$ (PROTOSINOMENINE)

SCHEME 1



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