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THE INCORPORATION OF BERBERINE INTO JATRORRHIZINE

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Abstract: The incorporation of berberine into jatrorrhizine is demonstrated.

The protoberberine alkaloids, one of the larger classes of isoquinoline alkaloids, exhibit much variability in their methylation/methylenation patterns. Considering only the more common 2,3,9,10 substituted protoberberines, all of the monohydroxy and most of the dihydroxy possibilities are known natural products. Biosynthetically, this presents a problem if a common biosynthetic pathway routed through the dimethoxy compound, scoulerine, is assumed. We wish to report here a possible reconciliation to a common pathway.

The biosynthetic origin of the protoberberine skeleton has been established in a series of studies, most of which focused on berberine. The results of these studies provided the basis for the currently accepted pathway depicted in Scheme 1. Spenser and co-workers[1,2] fed [2-^{14}C]-DL-tyrosine, [3-^{14}C]-DL-tyrosine and [1-^{14}C]-dopamine to Hydrastis canadensis and obtained incorporations of 0.124%, 1.83% and 0.11%, respectively, into berberine. Battersby et al.[3] fed doubly labeled [N-^{14}CH$_3$, 3-^{14}C]-(RS)-laudanosoline to Berberis japonica and obtained a 0.07% incorporation. Barton et al.[4] fed variously labeled (R)-, (S)-, and (RS)-reticulines to Hydrastis canadensis and obtained incorporations of 0.66%, 9.9% and 0.8% (ave.). Battersby et al. have shown the incorporation of scoulerine[5] (0.01%), tetrahydrocolumbamine[6] (0.06%) and tetrahydroberberine[6] into berberine. More recent studies[7] with enzymes obtained from callus cultures have shown that dihydroxyphenylacetaldehyde (and not dihydroxyphenylpyruvate as formerly believed) condenses with dopamine to give norlaudanosoline. Böhm and Rink[8] demonstrated the enzymatic cyclization of reticuline to scoulerine and, on the basis of testing three other potential substrates, concluded that the only requirement for this cyclization was a phenolic group at C-3' of the benzyltetrahydroisoquinoline.

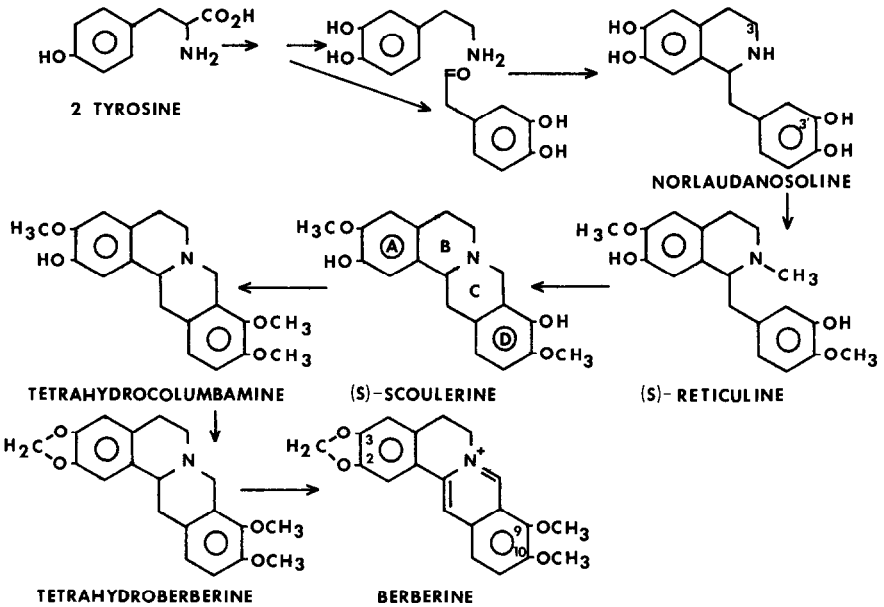
Beginning with reticuline, the reactions required for its conversion to berberine are, in sequence: cyclization, methylation, cyclization and oxidation. The substituent patterns of many other alkaloids in this class can be arrived at by invoking no more than these reactions. However, a number of alkaloids, jatrorrhizine among them, have methylation patterns that do not easily fit with the currently understood biosynthetic route. The methylation pattern of jatrorrhizine requires one of three possibilities: (i) reticuline is not the only point of entry to the class; (ii) a demethylation of palmatine (2,3,9,10-tetramethoxyprotoberberine); or (iii) a reopening of the methylenedioxy group of berberine.

Two lines of evidence led us to favor the third possibility. One was the very high incidence of co-occurrence of berberine and jatrorrhizine; the other was the results obtained with an enzymatic incubation mixture containing reticuline and [$^{14}CH_3$]-S-adenosylmethionine.[9] Among the four protoberberines[10] produced by the callus culture used as the source of the enzyme extract, palmatine and columbamine were formed in the cell-free incubation, but jatrorrhizine and berberine were not. We concluded that formation of the methylenedioxy group, an oxidative process, probably required the presence of stoichiometric amounts of an oxidation-reduction cofactor.[11] If berberine is an obligatory intermediate in jatrorrhizine biosynthesis, the failure to produce berberine would necessarily preclude formation of jatrorrhizine. On the basis of these considerations, and having a convenient method for labeling berberine, we chose to examine the third possibility experimentally.

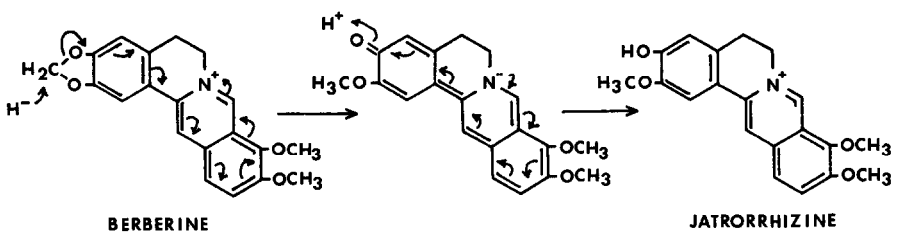
We wish to report that berberine is incorporated, in 12.8% yield, into jatrorrhizine. 9-O-($^{14}CH_3$)-Berberine (sp. act. 1.33×10^{10} dpm/mmole) was synthesized by converting berberine to berberrubine and then methylating with $^{14}CH_3I$ according to the procedure of Frerichs.[12] This was administered (2.8×10^6 dpm) to a 14-day-old Berberis aggregata callus culture.[9] After six days incubation, this tissue was harvested and exhaustively extracted with methanol. Chromatography in three systems[13] showed that the major radioactive peak corresponded with the jatrorrhizine band. One-fourth of the extract was partitioned between brine and dichloromethane. To the dried combined dichloromethane fractions was added 51.25 mg of authentic jatrorrhizine iodide, which was then crystallized (from ethanol) to constant specific activity. After a minor drop in activity between the 1st and 2nd crystallizations, the 2nd, 3rd, 4th and 5th crystallizations proved constant (1752, 1748, 1724 and 1730 dpm/mg, respectively). The recovery of radioactivity in jatrorrhizine represents 12.8% of the radioactivity fed and 26.4% of the activity that was in the methanol extract. This sample was reduced to tetrahydrojatrorrhizine and crystallized as the free base (from methanol) to constant specific activity: third and fourth crystallizations, 2424 and 2450 dpm/mg, respectively. Another sample was converted to jatrorrubine[14] which was shown to have no radioactivity associated with it. This is the expected result if the original label remained in place.

These results show that berberine is a precursor of jatrorrhizine, an observation not previously reported. They do not establish the mechanism of the conversion. In view of the high incorporation rate, we feel that a rather direct pathway is involved. Taking into account the known ease with which jatrorrhizine converts to a quinoid structure, we find it most attractive to propose the following mechanism (Scheme 2). Hydride attack at the methylenedioxy carbon of berberine promotes an electron shift into the quaternary nitrogen to give the stable quinone-tertiary amine (the tautomer of the zwitterionic form of jatrorrhizine). This then picks up a proton and undergoes a reversal of the electron flow to give the quaternary salt of jatrorrhizine. We are preparing berberine labeled in the methylenedioxy carbon in order to further clarify its transformation to jatrorrhizine.

SCHEME 1



SCHEME 2



We are unable to find any reports of methylenedioxy cleavage in higher plants, but did find examples in xenobiotic metabolism in insects,[15] mammals,[15-18] and microorganisms.[19] The most common products were o-diphenols or various conjugates of o-diphenols. In one instance, an o-methoxyphenol was one of the major products; the author however provided substantial arguments that this arose by methylation of the diphenol.

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10. The callus culture of Berberis aggregata used in this study produces 3% of its dry weight in quaternary protoberberine alkaloids distributed as follows: 76% jatrorrhizine, 14% columbamine, 6% palmatine, and 4% berberine.
11. The oxidative cyclization leading to the formation of ring C of the protoberberine skeleton occurs in this incubation mixture even though it is free of dissociable low molecular weight cofactors. This result suggests that this cyclization which has been considered to be chemically analogous to the cyclization leading to the methylenedioxy group, is biochemically different.
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