Research

Bisbenzylisoquinoline Biosynthesis in Cultured Roots of *Stephania cepharantha*

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Little attention had been paid to the biosynthesis of compounds which accumulate in roots, as compared to those found in other plant organs. One reason for this state of affairs is most certainly the fact that roots are buried in the ground and difficult to have an assessment for them. Another major reason, I believe, is the lack of appropriate experimental systems in which to study the secondary metabolism of roots.

Recently, however, we have become aware that some excised roots as well as roots transformed with *Agrobacterium rhizogenes* can grow very rapidly in liquid medium and produce secondary metabolites at levels higher than those of whole plant, though root culture is an old technique. With respect to the production of the secondary metabolites in the roots, this article deals with our recent studies using ^{13}C-labelled tracers on bisbenzylisoquinoline biosynthesis in cultured roots of *Stephania cepharantha*.

Bisbenzylisoquinoline alkaloids are composed of two benzylisoquinoline units linked by ether bridges. We have been interested in the bisbenzylisoquinolines because of their diverse formulations and varied pharmacological effects. We established cultured roots of *S. cepharantha*, an excellent source of bisbenzylisoquinolines containing more than 2% aromoline and more than 1% berbamine [1]. Although tracer molecules labelled with ^{13}C are less sensitive than ^{14}C-labelled tracers, they have the advantage that the location of the labelled position is easy to identify by ^{13}C NMR spectroscopy. In our cultures, it was possible to study the alkaloid formation with ^{13}C-labelled tracers because roots produced very large amounts of aromoline and berbamine, which are isolated through a simple procedure.

Roots were cultured for 25 days in SB5 medium [2] containing 200 ppm [3-^{13}C] tyrosine or [2-^{13}C] tyramine, which was prepared from [3-^{13}C] tyrosine by decarboxylation with tyrosine decarboxylase from *Streptococcus faecalis*. Freeze-dried roots were subjected to the isolation procedure and

yielded ^{13}C-labelled aromoline and isotetrandrine (berbamine methyl ether) as crystals [3, 4, 5]. The ^{13}C-NMR spectra of ^{13}C-enriched aromoline and isotetrandrine are shown in Figures 1 and 2, respectively. In each alkaloid, four signals (C-4, C-α, C-4' and C-α') were specifically enhanced when [3-^{13}C] tyrosine was fed while only two signals (C-4 and C-4') were enhanced when [2-^{13}C] tyramine was fed as compared with the spectra of the unlabelled compounds. These results unequivocally show that tyrosine was incorporated in approximately equal amounts into the isoquinoline and benzyl portions of aromoline and berbamine. On the other hand, the results show that tyramine was specifically incorporated into the isoquinoline portion.

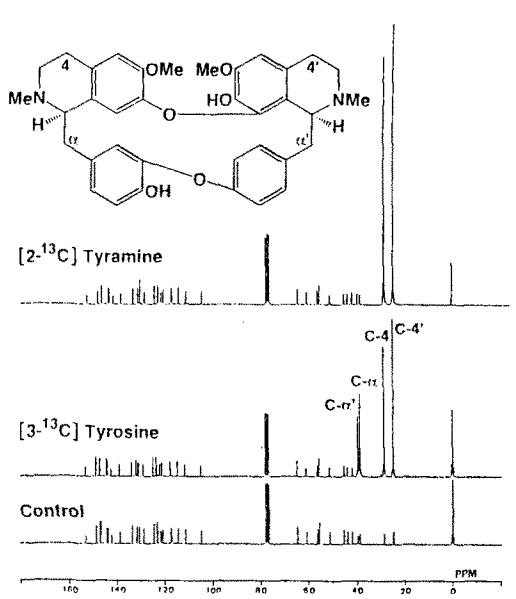


Figure 1. ^{13}C-NMR spectra of aromoline.

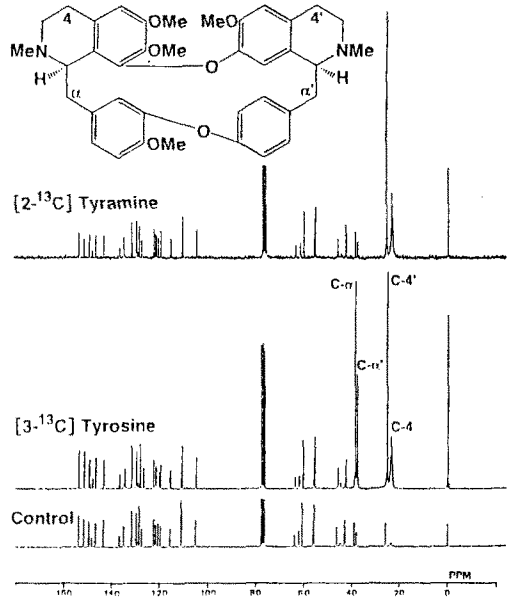
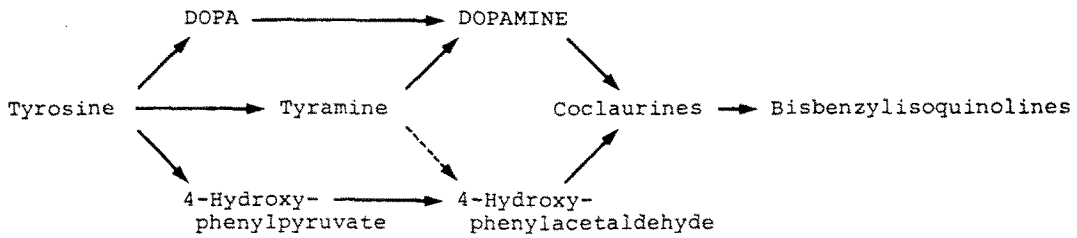


Figure 2. ^{13}C-NMR spectra of isotetrandrine.

When 200 ppm of tyrosine or tyramine was fed to *S. cepharantha* root cultures and allowed to be metabolized for one month, millimolar quantities of tyramine were detected in the roots, approximately 4 mM for tyrosine and 6 mM for tyramine. However none of [2-^{13}C] tyramine administered exogeneously was incorporated into the benzyl moiety of aromoline and berbamine as shown in Figures 1 and 2, respectively. These results suggest that the hydroxylation of tyramine proceeds more rapidly than its oxidation

or that tyrosine conversion to the benzyl moiety proceeds via its corresponding α-keto acid in *Stephania* root cultures (Scheme 1). Because the ratios of the ^{13}C enrichments of C-4, C-α, C-4' and C-α' in aromoline and berbamine were the same within the experimental limits, both alkaloids are believed to be derived from a common intermediate. According to Stadler *et al.* [6], this intermediate is berbamunine. Recently they isolated a new cytochrome P-450 enzyme which catalyzed the oxidative coupling of *(R)*- and *(S)*-*N*-methylcoclaurine to afford berbamunine [7].



Scheme 1. Biosynthetic sequence leading from tyrosine to the bisbenzylisoquinolines.

These results clearly indicate that root cultures are useful experimental systems for studying the biosynthesis of plant secondary products, not only for tracer-feeding studies but for the isolation of enzymes that function in biosynthesis.

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