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COMPARISON OF BISBENZYLISOQUINOLINE ALKALOIDS IN ROOT CULTURES OF *STEPHANIA CEPHARANTHA* WITH THOSE IN THE PARENT PLANT

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We have established optimum culture conditions for bisbenzylisoquinoline alkaloid production by root cultures of *Stephania cepharantha* Hayata (Menispermaceae) and have demonstrated the production of five bisbenzylisoquinoline alkaloids: aromoline, homoaromoline, berbamine, isotetrandrine, and cycleanine (1,2). Aromoline has been identified in many plant sources: *Abuta splendida, Pycnarrhena longifolia, Triclisia patens* (Menispermaceae), *Daphnandra aromatica, Daphnandra tenuipes, Doryphora aromatica* (Monimiaceae), *Thalictrum lucidum, Thalictrum rugosum, Thalictrum thunbergii* (Ranunculaceae), and *Berberis orthbotrys* (Berberidaceae) (3,4). For *S. cepharantha* we identified aromoline in root cultures (1), and Akasu *et al.* (5) identified it in callus, but there has been no report on the identification of aromoline in the parent plant.

Aromoline is a probable precursor of homoaromoline, cepharanoline, and cepharanthine, all of which have been identified in the parent *S. cepharantha* plant (3). Aromoline also is the main constituent in *Stephania* root cultures; therefore, we expected to find it in the parent plant, specifically in the roots.

We here describe the bisbenzylisoquinoline alkaloids present in root cultures of *S. cepharantha* and compare them to those in the roots and tuber of the parent plant. We also discuss the biogenesis of bisbenzylisoquinoline alkaloids in *S. cepharantha*.

In the identification of the bisbenzylisoquinoline alkaloids present in the basic fraction of cultured roots of *S. cepharantha*, slightly yellowish crystals formed when MeOH was added to the dried root residue. These crystals were identified as aromoline by their chromatographic behavior (hplc and tlc), melting point, and ^{1}H-nmr spectroscopy. The absolute configuration was determined by measuring the optical rotation. The yield was 1.2% on a dry wt basis.

Isolation of crystalline aromoline by simple treatment depended mainly on there being a high content of aromoline in the cultured roots. After 3 months of culture, the aromoline content in 50 root cultures ranged from 0.12 to 1.08%, dry wt (average 0.462%). The optimal medium for culture was investigated and SB5 medium developed (2). Use of this culture medium increased the aromoline content to 2.25%. As reported elsewhere (1) the main alkaloids in root cultures of *S. cepharantha* are aromoline and berbamine, and there is a high positive correlation between their contents.

Changes in aromoline and berbamine contents in 10 root cultures that had been maintained for 2 years from induction are shown in Figure 1. The high positive correlation was maintained, but the ratio changed. After 3 months of culture, the ratio of aromoline to berbamine was about 1.3:1, and it had increased to about 2:1 one year later. A rough estimation made by hplc showed that aromoline accounted for approximately 60% of the basic fraction from root cultures prepared as described in the Experimental section.

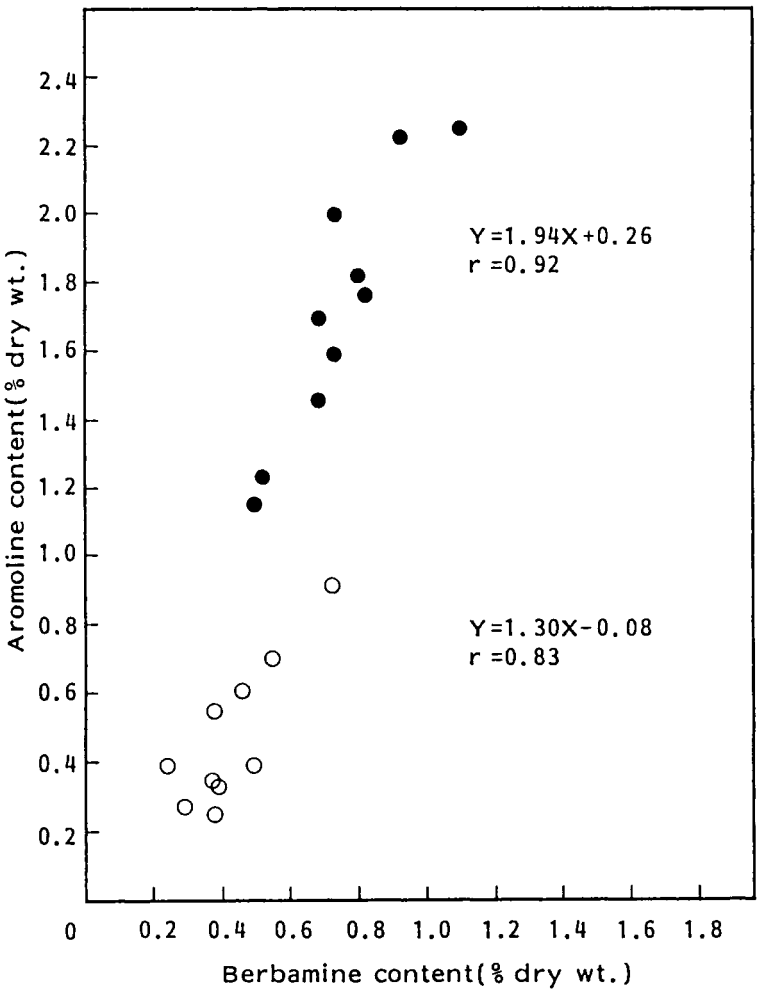

FIGURE 1. Changes in aromoline and berbamine contents during culture period after root induction. Subcultured roots were harvested at 3 months (○) and 15 months (●) for the content analysis.

Aromoline is the probable precursor of homoaromoline, cepharanoline, and cepharanthine. These three alkaloids derived from aromoline have been identified in the parent *S. cepharantha* plant (3), but aromoline has not. Because our root cultures produced aromoline as their main constituent, we expected to find this compound in the parent plant, in particular in the roots. Therefore, we reinvestigated what bisbenzylisoquinoline alkaloids are present in the tuber, roots, and aerial parts of *S. cepharantha* (Table 1).

The aerial parts contained no alkaloids, whereas the underground parts contained several. We confirmed the presence of aromoline in *S. cepharantha* roots by hplc, tlc, and ms. Previous investigations of bisbenzylisoquinoline alkaloids in *S. cepharantha* mainly dealt with its tubers. During harvest (from October to November in Japan), very few roots are present on *Stephania* tubers. For that reason, aromoline, a main alkaloid in the roots, might not have been found in the parent plant.

The alkaloid composition of the cultured *S. cepharantha* roots was similar to that of the parent roots but differed markedly from that of the tuber. A comparison of the alkaloid composition of

TABLE 1. Comparison of Bisbenzylisoquinoline Alkaloids in *Stephania cepharantha*.

Source	Alkaloid Content (% dry wt)				
	Aromoline	Berbamine	Homoaromoline + Cepharanoline	Isotetrandrine + Cycleanine	Cepharanthine
Tuber[a]	n.d.[d]	0.262	0.115	0.532	0.300
Tuber[b]	trace	0.351	0.119	0.582	0.219
Roots[b]	0.191	0.466	0.118	0.186	0.029
Root cultures[c] . . .	2.326	0.980	0.256	0.071	n.d.

[a]Harvested in October.
[b]Harvested in May.
[c]Harvested after 30 days of culture.
[d]n.d. = not detected.

the roots with that of the tuber of *S. cepharantha* showed aromoline and berbamine were present mainly in the roots, whereas the further modified cepharanthine and isotetrandrine were present in the tuber. Such a skewed distribution of alkaloids is evidence that in *S. cepharantha* the early steps of bisbenzylisoquinoline alkaloid biosynthesis take place in the roots, after which the alkaloids formed are transported to the tuber where they are further modified and stored. The tuber showed very little seasonal change in the quality and quantity of the bisbenzylisoquinoline alkaloids.

Tracer experiments done on whole plants or cut branches (6–8) have produced supporting evidence that intermolecular oxidative coupling of coclaurines and their *N*-methyl derivatives gives bisbenzylisoquinoline alkaloids. Bisbenzylisoquinoline alkaloid formation produced by the enzymatic coupling of two coclaurine units is being investigated with *S. cepharantha* cultured roots as the enzyme source.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Optical rotation was determined for solutions in $CHCl_3$. The ^1H-nmr spectrum was recorded at 400 MHz for solutions in $CHCl_3$ with TMS as the internal standard. Mass spectra were determined at 70 eV with a direct inlet system. The Si gel plates (Merck Art. 5715) used for tlc were treated with a solvent system of $CHCl_3$-MeOH-aqueous NH_3 (200:50:1). Alkaloids were made visible by spraying the plates with modified Dragendorff's reagent. Hplc was done on Develosil ODS-3 (150 × 4.6 mm) with 80% MeOH that contained 0.2% aqueous NH_3 as the mobile phase. Alkaloids were detected by the uv absorption at 282 nm. Root cultures of *S. cepharantha* were established as described previously (1,2). Parent *S. cepharantha* plants were harvested in October 1984 and May 1987.

ISOLATION OF AROMOLINE FROM *S. CEPHARANTHA* ROOT CULTURES.—Roots were cultured for 30 days in SB5 medium (modified B5 medium) (2), then harvested and freeze-dried. A 5-g sample (dry wt) of the freeze-dried roots was powdered and sonicated for 10 min in MeOH, then centrifuged for 5 min at 3000 rpm. Treatment with MeOH was repeated once more, after which the combined MeOH extracts were dried completely by evaporation. The dry residue was dissolved in 60 ml of 3% citric acid; then the acidic aqueous solution was passed through filter paper. The filtrate was made alkaline with aqueous NH_3. This solution was loaded on an Extrelut column (Merck Art. 15371), after which 400 ml of $CHCl_3$ was passed through the column. The $CHCl_3$ extracts obtained were dried completely by evaporation. The basic residue (158.8 mg) yielded 59.6 mg of aromoline as slightly yellowish crystals, mp 205–206° [lit. (9) 198–202°], $[\alpha]^{22.5}D$ +350° ($c = 0.1$) [lit. (10) +320°]. ^1H-nmr and mass spectra matched literature values (11).

IDENTIFICATION OF BISBENZYLISOQUINOLINE ALKALOIDS IN THE *S. CEPHARANTHA* PLANT.—The tuber, roots, and aerial parts of *S. cepharantha* were harvested and freeze-dried, after which the basic fractions were extracted from each material as described above. The alkaloids contained in these fractions were analyzed by hplc as described elsewhere (1).

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LITERATURE CITED

1. Y. Sugimoto, Y. Sugimura, and Y. Yamada, *Phytochemistry*, **27**, 1379 (1988).
2. Y. Sugimoto, Y. Sugimura, and Y. Yamada, *Agric. Biol. Chem.*, **52**, 1495 (1988).
3. K.P. Guha, B. Mukherjee, and R. Mukherjee, *J. Nat. Prod.*, **42**, 1 (1979).
4. P.L. Schiff, Jr., *J. Nat. Prod.*, **46**, 1 (1983).
5. M. Akasu, H. Itokawa, and M. Fujita, *Phytochemistry*, **15**, 471 (1976).
6. D.H.R. Barton, G.W. Kirby, and A. Wiechers, *J. Chem. Soc. C*, 2313 (1966).
7. A.K. Sidjimov and N.L. Marekov, *Phytochemistry*, **21**, 871 (1982).
8. D.S. Bhakuni, S. Gupta, and S. Jain, *Tetrahedron*, **39**, 4003 (1983).
9. D. Dwuma-Badu, J.S.K. Ayim, A.N. Tackie, J.E. Knapp, D.J. Slatkin, and P.L. Schiff, Jr., *Phytochemistry*, **14**, 2524 (1975).
10. J.M. Saá, M.V. Lakshmikantham, M.J. Mitchell, and M.P. Cava, *J. Org. Chem.*, **41**, 317 (1976).
11. H. Guinaudeau, A.J. Freyer, and M. Shamma, *Nat. Prod. Rep.*, **3**, 477 (1986).

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