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ALKALOIDS IN PLANTS REGENERATED FROM *COPTIS* CALLUS CULTURES*

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Key Word Index— *Coptis japonica*; Ranunculaceae; callus tissues; berberine type alkaloids; regeneration.

Abstract—All the main alkaloids in the rhizome of *Coptis japonica* were found to be present in callus cultures. Berberine and jatrorrhizine were the main alkaloids in the callus tissue, although the content was much less. The restoration of alkaloid content was observed in the rhizome of plants regenerated from callus cultures. The results indicated that plants regenerated from callus cultures were normal in both morphology and biosynthetic activity.

INTRODUCTION

Coptis japonica Makino var. *dissecta* (Yatabe) Nakai (Seribaworen in Japanese) is well-known as a berberine-bearing and pharmaceutically important plant in Japan. In previous papers, we briefly reported the successful isolation of berberine [1] and the regeneration of whole plants [2] from *Coptis* callus cultures. The present paper describes further detailed studies on alkaloid production in *Coptis* callus cultures and in plants regenerated from them.

RESULTS

The regeneration of whole plant from Coptis *callus*

Friable yellow calluses first derived from the petiole of *Coptis* plant were subcultured on the Murashige and Skoog's medium [3] (minus glycine) containing 1 mg/l of 2,4-D and 0·1 mg/l of kinetin. They consisted of both white and yellow cells; tests with Dragendorff's reagent suggested that yellow cells contained alkaloids. Numerous pale yellow nodules (0·5–3 mm dia) were occasionally observed, when the calluses were subcultured for more than 6 weeks. Some of them developed into both shoot- and root-bearing nodules. From the nodules transferred to a culture medium lacking growth regulators, green leaves expanded and yellow roots elongated during 1 month cultivation under fluorescent lamp (40 W) at 25–30°. The differentiated plantlets were transferred to soil in pots. They grew vigorously and normally flowered 5 years (January 1974) after transplantation to soil. The main axis of flower stalk had three short branches, each of which terminated in a flower. The flower had five white petals, five sepals and many stamens.

Alkaloid constituent in callus cultures

The callus tissues, subcultured for 6 weeks, were collected and extracted with methanol. After the removal of the acidic and neutral fractions with ether, at least 7 Dragendorff's reagent and iodoplatinate reagent-positive spots (alkaloids A–G) were separated by preparative TLC. Among them the hydrochloride of alkaloid B gave yellow needles, m.p. 192–193° (decomp.) and was identical with authentic sample of berberine hydrochloride by UV, IR and MS [1]. Alkaloid C gave orange needles, m.p. 205–206° (decomp.) and was identical with authentic sample of jatrorrhizine hydrochloride by UV, IR, mass and NMR spectra. Alkaloids A, D and G were identified as palmatine, coptisine and magnoflorine by TLC comparison with authentic samples. Alkaloids E and F remain to be identified.

* Part 24 in the series "Studies on Plant Tissue Cultures". For Part 23 see Ikuta, A., Syōno, K. and Furuya, T. (1974) *Phytochemistry* **13**, 2175.

Table 1. Alkaloid content of callus tissues, rhizome of the original and the regenerated *Coptis* plant

Culture periods (weeks)	Tissues	Growth regulators in culture medium	Alkaloid content Berberine (μg/g fr. wt)	Berberine (mg/g d. wt)	Jatrorrhizine (μg/g fr. wt)	Jatrorrhizine (mg/g d. wt)	Growth* (mg fr. wt/flask)
6	Callus	None	432	—	456	—	250
6	Callus	2,4-D (1 mg/l)	584	—	912	—	375
3	Callus	2,4-D (1 mg/l)+	425	—	434	—	325
6	Callus	Kinetin (0·1 mg/l)	774	9·0	816	10·0	500
	Rhizome of the regenerated plant†		—	42·6	—	19·9	—
	Rhizome of the original plant		—	50·4	—	11·5	—

* Initial fr. wt of callus tissues per flask was 160 mg.
† Alkaloid content was estimated 5 years after the regeneration from callus cultures.

Alkaloid content of the callus tissues and the rhizome of the regenerated plants

The major alkaloids in callus tissues, berberine and jatrorrhizine, were separated by PC and estimated by UV absorbance at 420 nm. Table 1 shows that the alkaloid content of callus tissues, cultured on the medium containing 2,4-D and kinetin, appreciably increases with prolonged culture periods (6 weeks). Growth regulators also affected on both growth and alkaloid content. Alkaloid content, especially jatrorrhizine, and callus growth reduced when 2,4-D was removed from the culture medium. On the other hand, kinetin, in the presence of 2,4-D, increased berberine but slightly decreased jatrorrhizine content.

Alkaloid content was also measured in the callus tissues, the rhizome of the regenerated plants and the rhizome of the original plants. The alkaloid content of the regenerated plants was much higher than that of callus tissue, especially in berberine, and was similar to that in the rhizome of the original plant.

DISCUSSION

In spite of many reports of the regeneration of whole plants from callus cultures (e.g. [4, 5]), we know little about chemical constituents of the regenerated plants, except in the case of the tobacco plant [6]. It may be important to determine the biosynthetic ability of the regenerated plants, since alkaloid production or increase associated with organized organ development has been reported [7–9] and the regenerated plant from callus cultures may not necessarily be normal in morphology and may be modified at least partially during subcultures [10]. From our results, the nearly complete restoration of alkaloid content was observed in the rhizome of the regenerated plant, although jatrorrhizine content in the rhizome of the regenerated plant was much higher than in that of the original plant.

EXPERIMENTAL

The methods of callus culture and alkaloid extraction and identification were as in previous papers [1, 2].

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REFERENCES

1. Furuya, T., Syōno, K. and Ikuta, A. (1972) *Phytochemistry* **11**, 175.
2. Syōno, K. and Furuya, T. (1972) *Experientia* **28**, 236.
3. Murashige, T. and Skoog, F. (1962) *Physiol. Plant.* **15**, 473.
4. Steward, F. C., Mapes, M. O. and Mears, K. (1958) *Am. J. Botany* **45**, 705.
5. Pillai, S. K. and Hildebrandt, C. (1969) *Am. J. Botany* **56**, 52.
6. Tabata, M., Yamamoto, H. and Hiraoka, N. (1968) *Jap. J. Genet.* **43**, 319.
7. Ikuta, A., Syōno, K. and Furuya, T. (1974) *Phytochemistry* **13**, 2175.
8. Bhandary, S. B. Rai, Collin, H. A., Thomas, E. and Street, H. E. (1969) *Ann. Botany* **33**, 647.
9. Tabata, M., Yamamoto, H., Hiraoka, N. and Konoshima, M. (1972) *Phytochemistry* **11**, 949.
10. Syōno, K. and Furuya, T. (1972) *Bot. Mag.* (Tokyo) **85**, 273.