[Chem. Pharm. Bull. 36(4)1575—1576(1988)]

Production of Swertiamarin in Cultured Tissues of *Swertia pseudochinensis*

YOSHIE KITAMURA,* MIKA DONO, HIROSHI MIURA and MICHIYASU SUGII

Faculty of Pharmaceutical Sciences, Nagasaki University, Bunkyo-machi 1–14, Nagasaki 852, Japan

(Received September 3, 1987)

Production of swertiamarin in various cultured tissues of *Swertia pseudochinensis* was investigated. This bitter glucoside was not detected in callus, root or leaf cultures, whereas regenerated plants from callus contained it. Swertiamarin contents in the regenerated plants were compared with those in the original plant.

Keywords——*Swertia pseudochinensis*; Gentianaceae; cultured tissue; cultured root; cultured leaf; callus; regenerated plant; secoiridoid glucoside; swertiamarin

Swertia species (Gentianaceae, biennial herb) contains the secoiridoid glucoside swertiamarin as a constituent bitter stomachic.[1] There has been no report on the bitter glucoside production by tissue cultures. Here we describe the ability of various cultured tissues of *Swertia pseudochinensis* HARA to produce swertiamarin, in comparison with that of the original plant.

Materials and Methods

Plant Materials——Seeds of *S. pseudochinensis* were collected from 2-year-old wild plants indigenous to Nomozaki Peninsula (Nagasaki, Japan). The seeds were treated with 400 mg/l of gibberellic acid for 24 h for germination.[2] Then they were sterilized with 5% calcium hypochlorite for 10 min followed by washing three times with sterile deionized water. The seeds germinated on agar medium without nutrients in the light. After 5—7 d, the seedlings were used for callus induction, root and leaf cultures. Twenty-day-old seedlings were used for analysis.

Isolated root tips were incubated in half-strength B5 basal medium[3] containing naphthaleneacetic acid (NAA) (1 μg/l) and 3% sucrose under shaking (120 rpm) and subcultured at 6- to 8-week intervals in dim light.[4] Isolated buds were allowed to develop leaves without any root on Murashige–Skoog basal agar (MS) medium[5] containing 6-benzyladenine (BA) (1 mg/l) and 3% sucrose in the light. Callus was induced from seedlings on MS medium containing NAA (0.1—10 mg/l) with 0—0.1 mg/l BA in the light and subcultured on medium containing 1 mg/l NAA at one-month intervals.

Embryoids were produced from calluses induced with 10 mg/l NAA with or without BA and subcultured with 1 mg/l NAA. When embryoids having cotyledonary leaves were transferred onto MS medium without plant hormone, they grew into plantlets which were successfully transferred onto soil.[6]

Harvested roots, leaves, calluses and regenerated plants (5- and 8-month-old stages) were used for analysis of bitter glucosides.

Extraction and Analysis of Swertiamarin——Wild plants and cultured tissues were lyophilized and then powdered. One gram of a sample was extracted overnight with 20 ml of MeOH twice. The combined extract was evaporated to dryness under reduced pressure, and the residue was dissolved in 20 ml of hot water followed by filtration. The filtrate was extracted with 10 ml of $CHCl_3$ and then with 10 ml of AcOEt three times each. The aqueous layer was extracted with 10 ml of BuOH three times, the combined BuOH layer was evaporated to dryness under reduced pressure, and the residue was redissolved in 5 ml of MeOH for analysis.

Identification of swertiamarin was performed by thin-layer chromatography (TLC) in comparison with an authentic sample. The glucoside was separated on a Silica gel F_{254} plate (Merck, 20 × 20 cm) with AcOEt–*n*-PrOH–H_2O (6 : 1 : 3) (upper layer) and with $CHCl_3$–MeOH–H_2O (10 : 3 : 1) (lower layer); its *Rf* values were 0.29 and 0.15,

TABLE I. Swertiamarin Content in Callus, Cultured Organs, Regenerated Plants and the Original Plant of *S. pseudochinensis*

Tissue examined		Swertiamarin content (%)	Tissue examined		Swertiamarin content (%)
Cultured tissue			Original plant tissue		
Callus		n.d.	20-Day-old seedling	Root	0.02
Organ culture	Root	n.d.		Leaf	2.70
	Leaf	n.d.	2-Year-old wild plant	Root	0.29
5-Month-old RP[a)]	Root	n.d.		Stem	0.34
	Leaf	4.17 ± 2.44		Leaf	0.49
8-Month-old RP[b)]	Root	0.87 ± 0.52		Flower	3.33
	Leaf	2.42 ± 0.93		Seed	0.02

RP = regenerated plant. n.d. = not detected. *a*) $n = 15$, *b*) $n = 5$.

respectively. The spot of swertiamarin was detected under ultraviolet (UV) light and by spraying with anisaldehyde–H_2SO_4 reagent. Quantitative estimation of swertiamarin was performed by dual-wavelength densitometry (Shimadzu CS-901) according to Hayashi *et al.*[1]; sampling wavelength, 240 nm; reference wavelength, 310 nm. Authentic swertiamarin was purchased from Nakarai Chemicals.

Results and Discussion

Swertiamarin contents in various cultured tissues of *S. pseudochinensis* were compared with those in the original plant tissues (Table I). Two-year-old wild plant and 20-day-old seedlings germinated from seeds were analyzed. All tissues of the original plant contained swertiamarin as the main bitter glucoside, though the contents of seeds and roots of 20-day-old seedlings were extremely low.

On the other hand, no bitter glucoside was detected in cultured tissues of callus, root or leaf. However, 5- and 8-month-old regenerated plants contained swertiamarin, though it was not detected in roots of 5-month-old plants. The contents of leaves of 5-month-old regenerated plants varied greatly, and one plant contained 11% swertiamarin.

Further study is needed to clarify the mechanism of bitter glucoside formation in cultured tissues of *S. pseudochinensis*.

References

1) T. Hayashi, Y. Tsuji and T. Matuda, *Yakugaku Zasshi*, **96**, 498 (1976).
2) K. Inoue, T. Ikenaga, S. Ookubo and H. Oohashi, *Shoyakugaku Zasshi*, **31**, 75 (1977).
3) O. L. Gamborg, R. A. Miller and K. Ojima, *Exp. Cell Res.*, **50**, 151 (1968).
4) Y. Kitamura, M. Nakano, H. Miura and M. Sugii, *Shoyakugaku Zasshi*, **41**, 58 (1987).
5) T. Murashige and F. Skoog, *Physiol. Plant.*, **15**, 473 (1962).
6) Y. Kitamura, H. Miura and M. Sugii, *J. Plant Physiol.* (submitted).