Phytochemistry, 1981, Vol. 20, pp. 545–547. © Pergamon Press Ltd. Printed in England. 0031-9422/81/030545-03 $02.00/0

PRODUCTION OF BERBERINE IN CULTURED CELLS OF *COPTIS JAPONICA*

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(*Received* 2 *July* 1980)

Key Word Index—*Coptis japonica*; Ranunculaceae; cultured cells; berberine production.

Abstract—A culture of small fragments of tissues (rootlets) of *Coptis japonica* on a solid medium followed by successive liquid culture produced friable cell lines with high growth rates and a high berberine content. Light inhibited growth and berberine production in these cell lines whereas high aeration stimulated both.

INTRODUCTION

Berberine, which can be obtained from *Coptis japonica* roots and *Phellodendoron amurense* bark, is a useful antibacterial, antimalarial and stomach drug in the Orient. However it takes 5–6 years to produce *Coptis* roots as the raw material for berberine production and we have tried, therefore, to produce berberine from a cell culture of *Coptis*. Furuya *et al.* [1] succeeded in culturing the callus of *Coptis*, but their cells grew slowly and their berberine content was low. This paper describes the establishment of a cell line of *Coptis japonica* Makino var. *dissecta* (Yatabe) Nakai (Seribaworen) that has a higher growth rate and higher berberine content than has been reported previously [1, 2]. The effect of light and of aeration on the growth and berberine production of these cells is also described.

RESULTS

Callus induction

Segments of the petiole and leaf of young *Coptis japonica* were cultured in the dark on solid Linsmaier and Skoog basal medium containing 10^{-5} M α-naphthaleneacetic acid and 10^{-8} M benzyladenine. At first only roots formed. We repeatedly subcultured these roots on fresh solid medium, but still no callus formed. The increase in the fresh weight of roots was only 30–40% after 3 weeks of culture. We also cultured the cells left on the original medium after removal of the rootlets. After 3–4 months of continuous culture, small calluses appeared and these were transplanted into liquid medium. In the liquid culture, some cells formed rootlets. After sedimenting the large cell aggregates and roots, we obtained a cell line from the upper layer of the liquid medium containing small cell aggregates which grew and dispersed well and had a yellow colour. The increase in fresh weight of these cells in liquid culture was 300–400% after 3 weeks of incubation.

Identification of berberine in the cultured cells

The substances that produced the yellow colour in cultured *Coptis* cells were fluorescent under UV and gave a positive test with Dragendorff reagent. They could be extracted completely from freeze-dried, cultured cells with 90% MeOH, and three yellow fluorescent substances (A, B, C) were separated by silica gel TLC. The R_f value of the main spot, A, was identical with authentic berberine hydrochloride on two-dimensional TLC with BuOH–HOAc–H_2O (7:1:2) then $CHCl_3$–MeOH–HOAc (25:10:1) [3]. The lemon-yellow fluorescent zone on preparative TLC (silica gel G) that corresponded to berberine was eluted with 90% MeOH and, after addition of 2 N HCl, berberine hydrochloride was twice recrystallized from water, yielding yellow needles (mp 192–193°; decomp.), which were identified as authentic berberine hydrochloride. UV λ_{max}^{EtOH} nm: 432, 351, 266, 230; IR ν_{max}^{KBr} cm^{-1}: 1599 (benzene C=C), 1501 (benzene C=C), 1357, 1266 (=C—O—), 1226 (=C—O—), 1032 (—C—O—); MS *m/e* (rel. int.): 337 (8), 322 (29), 321 (100%), 320 (45), 307 (19), 306 (37), 304 (33), 292 (30), 278 (56). The berberine content in the cultured cells of *Coptis* cells was 2–4% on a dry weight basis.

Effects of light and aeration on the growth and berberine content of cultured Coptis *cells*

Light inhibited both the growth and berberine production of cultured *Coptis* cells (Table 1). The increase in the dry weight of *Coptis* cells cultured under light was 74% of that in the dark and the amount of berberine was also lower. After successive cultures in the light, the cells became white and grew slowly. We also investigated the effect of aeration and showed that changing the type of plug aided the growth and berberine production of cultured *Coptis* cells (Table 2).

DISCUSSION

We have described a method for obtaining a well dispersed cell line of *Coptis* with a good growth rate from organized tissue with a low growth rate. Adventitious rootlets derived from segments of the petiole or leaf of *Coptis* remained in an organized form during successive transplants on fresh, callus-induction medium. Highly dispersed cells appeared when the small tissues that

Table 1. Effect of light on the growth and berberine production of cultured *Coptis* cells

	Growth Dry wt (mg)	Berberine content (mg/flask)		
		Total amount	Cells	Medium
Light	404	5.5	3.9	1.6
Dark	545	8.4	6.5	1.9

Each flask was inoculated with 84 mg (dry wt) of *Coptis* cells and cultured for 3 weeks in the dark. Fluorescent light of 4000 lx was used.

Table 2. Effect of the type of flask plug used on the growth and berberine production of cultured *Coptis* cells

Type of plug	Growth Dry wt (mg)	Berberine content (mg/flask)		Aeration*
		Cells	Medium	
Aluminium	490	10.4	0.06	+
Silicone sponge	680	28.4	0.31	++
Cotton	780	31.0	0.17	++

Each flask was inoculated with 140 mg (dry wt) of *Coptis* cells containing 4.8 mg of berberine hydrochloride and cultured for 3 weeks in the dark.

* ++ = 80–90%; + = 5–10%.

The figures show values based on the ratio of the aeration to the aeration in no plug.

remained on the old medium after rootlets had been subcultured, and which usually were not visible to the naked eye, were cultured on this same medium for a longer period. These cultured *Coptis* cells grew better and produced more berberine in liquid culture than in solid culture which should be most useful for mass production of the alkaloid. The main alkaloid contained in these cells was shown to be berberine and its analogues (TLC, mp, UV, IR, NMR, MS). The amount found was higher than any previously reported in the literature [1] and is comparable to the content in the whole root [2].

In trying to obtain cell lines from plants with special characteristics (e.g. photosynthetic activity [4], alkaloid production [5, 6] and UQ_{10} [7] production) we must take into account genetic factors (selection) and environmental (physiological and chemical) regulation. We must not only, therefore, continue to carefully select cell lines, but also investigate the effects of environmental factors on the production of the desired constituents. Light inhibited the growth and berberine production of cultured *Coptis* cells whereas a high degree of aeration stimulated both growth and berberine production. In cultured *Coptis japonica* cells high cell growth is thus not inhibitory to secondary metabolite production.

EXPERIMENTAL

Callus culture. Adventituous roots were derived from the petiole and leaf of *Coptis japonica* Makino var. *dissecta* (Yatabe) Nakai (Seribaworen in Japanese) in May 1977, and cultured cells were established in 1978 in the dark at 26° on Linsmaier and Skoog's medium containing 10^{-5} M α-naphthaleneacetic acid and 10^{-8} M benzyladenine. Cells were subcultured at intervals of 3 weeks.

Identification of berberine. The freeze-dried cultured cells were homogenized with a mortar and pestle, then 90% MeOH was used repeatedly to extract the yellow pigment until all colour had been removed from the material. The MeOH extract then was evapd to a small vol. and the conc extract was separated by TLC [Si gel G 0.25 mm thick, with BuOH–HOAc–H_2O (7:1:2), then with $CHCl_3$–MeOH–HOAc (25:10:1)]. The lemon-yellow fluorescent fraction was collected and eluted with 90% MeOH, after which it was evapd to dryness. Crude berberine hydrochloride was obtained after addition of 2 N HCl to the dried extract. Yellow needles were recrystallized from H_2O and identified by comparing R_f values on TLC, mp and UV, IR, NMR and MS spectra with the respective values of authentic berberine hydrochloride. The berberine content was determined by densitometry with a dual wavelength chromatoscanner,

containing an authentic sample of berberine, after separation of the MeOH extract by TLC [Si gel G, solvent BuOH–HOAc–H_2O (7:1:2).

Acknowledgements—We thank Mr. Y. Koizumi and Miss M. Akamatsu for their technical advice on the IR and NMR measurements and Dr. T. Ueno for his technical advice on the MS measurement.

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