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Production of emetic alkaloid by in vitro culture of *cephaelis ipecacuanha* A. Richard

Daisuke Teshima [1], Kayo Ikeda [2], Motoyoshi Satake [2], Toshinobu Aoyama [1], and Koichiro Shimomura [2]

[1] Department of Hospital Pharmacy, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812, Japan
[2] Tsukuba Medicinal Plant Research Station, National Institute of Hygienic Sciences, 1 Hachimandai, Tsukuba, Ibaraki, 305, Japan

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Abstract

Callus and adventitious roots were induced on leaf segments from shoot culture of Cephaelis ipecacuanha A. Richard on Murashige-Skoog medium containing 2,4-dichlorophenoxyacetic acid, indole-3-acetic acid, 1-naphthaleneacetic acid and kinetin. The contents of emetic alkaloids in calli, roots and root suspension cultures were quantified by HPLC. Roots cultured in solid and liquid Murashige-Skoog media yielded emetine and cephaeline. The amount of the two alkaloids in the root suspension culture was very similar to that of roots from ipecac mother plant grown in a greenhouse. In contrast, calli subcultured on Murashige-Skoog media containing combinations of 2,4-dichlorophenoxyacetic acid and kinetin produced only trace amounts of emetic alkaloids.

Abbreviations

2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indole-3-acetic acid; NAA, 1-naphthaleneacetic acid; Kin, kinetin; MS, Murashige-Skoog; EM, emetine; CP, cephaeline; DW, dry weight.

Introduction

The Amazonian medicinal plant ipecac, Cephaelis ipecacuanha A. Richard and a related species C. acuminata Karsten, have been reported in the United States (1985) and British (1980) Pharmacopoeias and are well known for the emetic properties of their roots. The former species has appeared in the Japanese Pharmacopoeia (1986).

Ipecac contains emetine (EM) and cephaeline (CP) as main alkaloids, on the other hand, psychotrine, o-methylpsychotrine and emetamine as minor alkaloids (Janot 1953). It is said that the emetic action of ipecac is caused by EM and CP, and the action of CP is twice as strong as that of EM (Japanese Pharmacopoeia [Comentary book] 1966). Administration of these emetics to the patients is a common procedure in the U.S. and Europe after oral exposure to an excess of medicine or in toxicants. In recent years, infant and child poisonings have increased significantly in Japan (Naito 1984 and Tsuda et al. 1979). However, the use of ipecac as an emetic in Japan is not common, because the Japanese Pharmacopoeia has this species as an expectorant (Japanese Pharmacopoeia 1986). Therefore, the quality of ipecac as related to emetic alkaloid content in Japan is not known (Teshima et al. 1984).

Tissue culture was carried out for the purpose of evaluating the emetic components (EM, CP) of Ipecac. Although production of various types of alkaloids by tissue culture has been reported (Endo and Yamada 1985, Rhodes et al. 1986 and Kamada et al. 1986), to our knowledge no tissue culture work has been done on ipecac. This paper describes the production of EM and CP in calli and adventitious roots from leaf segments of C. ipecacuanha A. Richard cultured in vitro.

Materials and methods

Induction of callus and adventitious roots

Sterile plants of C. ipecacuanha were established via shoot tip culture from greenhouse grown plants and maintained on MS solid medium containing 0.01 mg/l NAA and 0.1 mg/l Kin at 25°C, under 16h light/8h dark (4000 lux) for 8 weeks. Leaf segments (ca. 5x5mm) of axenic shoots were cultured on MS solid medium supplemented with auxin or combinations of auxin and cytokinin at 25°C under dark. The medium was adjusted to pH 5.7 before addition of 0.2% Gelrite and then was autoclaved at 121°C for 15 minutes. Twenty five ml of medium was aseptically dispensed into 90x20 mm petri dishes. After 4 to 6 weeks, callus and adventitious roots appeared.

Culture of adventitious roots

Segments (1-2 cm in height) of adventitious roots were cut off and subcultured on MS solid medium containing auxins. Segments of the growing adventitious roots (20-100 mg Fresh weight) grown on MS solid medium were inoculated into 100 ml Erlenmeyer flasks containing MS liquid medium supplemented with auxin to establish root suspension cultures at 25°C under dark on a rotary shaker (100 rpm) for 10-12 weeks. The media were adjusted pH 5.7 before autoclave and 50 ml of MS liquid media were dispensed into 100 ml Erlenmeyer flasks, then autoclaved at 121 °C for 15 minutes.

Alkaloid extraction

Extraction of the emetic alkaloid was done following the method reported by Teshima et al. (1984).

Offprint requests to: K. Shimomura

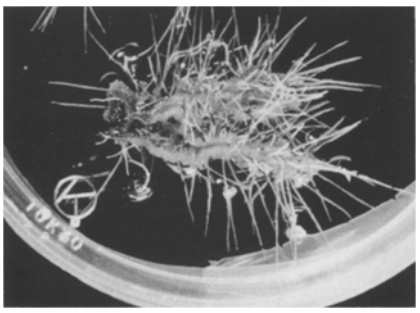

Fig. 1. Adventitious roots grown on MS solid medium containing 3 mg/l IAA.

Table 1. Effects of phytohormones on alkaloid production in ipecac roots cultured on MS solid media

hormone (mg/l)			1*		2*		3*	
			content(% DW)		content(% DW)		content(% DW)	
IAA	NAA	Kin	EM	CP	EM	CP	EM	CP
1.0	0	0	0.082	0.636	0.078	0.550	0.046	0.355
3.0	0	0	0.176	1.101	0.115	0.791	0.076	0.474
0	1.0	0	0.138	0.631	0.116	0.654	0.045	0.408
0	1.0	0.1	0.075	ND	0.009	0.029	0.059	0.460

ND: not detected; *: number of passages

Calli and adventitious roots were lyophilized, and further dried *in vacuo* over $CaCl_2$ at room temperature for 5 hours. Dried samples were ground to a fine powder prior to extraction. A powdered sample (10 mg) was mixed with 200 µl of 10% NH_4OH for 1 min, then extracted with 6 ml of ether for 5 min using a voltex mixer. Two ml of the upper layer was accurately withdrawn and concentrated *in vacuo* at room temperature. The sample was dissolved in an appropriate volume of methanol and analyzed by HPLC.

High performance liquid chromatography

The extracts were quantitatively analyzed by HPLC using a Shimadzu Liquid Chromatography (model LC-3A) equipped with a variable wavelength Shimadzu UV spectrophotometer (model SPD-2A). Analyses were carried out using a Zorbax silicagel column (4.6mm i.d. x 25 cm) with chloroform- methanol-diethylamine (273:27:0.01) as the mobile phase and monitering absorbance at 285nm. The flow rate was 1.8 ml/min throughout the analysis. The Rt (retention time) values obtained from the extracts were identified with those obtained by authentic standards. The Rt values obtained were as follows:EM, 2.8 min;CP, 3.6min. Identification of the compounds determined as EM and CP was confirmed by mass spectrometry. EM;m/z(rel. int.) 480[M^+](33), 288(22), 272(39), 258(7), 246(12), 206(49), 192(100). CP;466[M^+](46), 451(4), 437(4), 288(32), 274(24), 273(27), 272(58), 246(26), 244(28), 206(9), 205(5), 192(76), 178(100).

Results and Discussion

In a preliminary survey, the effects of 2,4-D and Kin concentrations and combinations on callus induction were investigated.

Callus growth was relatively satisfactory even after the third passage in culture. Callus tissues produced only trace amounts of alkaloids when cultured in the presence of 1mg/l 2,4-D though they were detected by using TLC and HPLC at the first and second passage. By the third passage EM and CP were not detected. Therefore the addition of 2,4-D, which favored callus formation and growth, was unsuitable for alkaloid production.

Adventitious roots were induced from leaf segments on MS solid media supplemented with IAA, NAA and Kin in various combinations (Fig.1), and the contents of EM and CP were determined (Table 1). After 3 culture passages, cultures formed 20-40 regenerated adventitious roots per segment on the solid medium. With regard to the contents of alkaloids, the addition of IAA (3 mg/l) resulted in significant production of EM (0.176 % DW) and CP (1.101 % DW); addition of NAA (1 mg/l) also yielded EM (0.138 % DW) and CP (0.631 % DW). Therefore IAA (3 mg/l) was shown to be more effective than that of NAA (1 mg/l) for growth and alkaloid production. However, upon subculture of roots on MS solid media, alkaloid contents tended to decrease.

Root suspension culture was established in the presence of IAA, NAA and Kin (Fig.2). As compared to root culture on solid media, liquid root culture was better for root growth rate and alkaloid production (Table 2). The results of alkaloid production were summarized in Table 2. In contrast, to the decrease in alkaloids observed for roots cultured on solid media, root suspensions showed a stable level of alkaloids even after 3 culture passages.

The addition of IAA (3 mg/l) and NAA (1 mg/l) resulted in alkaloid contents of EM (0.412 % DW) and CP (1.319 % DW) for IAA and EM (0.366 % DW) and CP (1.043 % DW) for NAA.

Roots from ipecac mother plant grown in a greenhouse were divided into roots, rootlets and rhizomes, and these were used to extract for emetic alkaloids analysis (Data will be shown in elsewhere). Contents of the emetic alkaloids of rootlets were only slightly higher than those of roots, while those of rhizomes had only 1/6 of EM and 1/3 of CP levels in root. Alkaloid content in the root of the mother plant was very similar to that of root suspension cultures (Table 2).

In summary, root and root suspension cultures with NAA and IAA may be useful for the production of ipecac alkaloids. In addition, root suspension cultures were shown to give considerable alkaloid production. Thus, this method is considered appropriate for production of the emetic alkaloids. While callus cultured on MS medium containing 2,4-D dose not appear to be a good source of alkaloids. In order to increase the amount of the emetic alkaloids

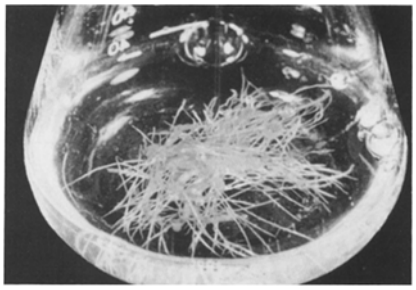

Fig. 2. Adventitious roots cultured in MS liquid medium containing 3mg/l IAA.

Table 2. Effects of phytohormones on alkaloid Production in ipecac roots cultured in MS liquid media

hormone (mg/l)			1* content(% DW)		2* content(% DW)		3* content(% DW)	
IAA	NAA	Kin	EM	CP	EM	CP	EM	CP
1.0	0	0	0.349	1.281	0.514	1.142	0.281	1.182
3.0	0	0	0.412	1.319	0.308	0.821	0.359	1.000
0	1.0	0	0.366	1.043	0.210	0.750	0.272	0.945
0	1.0	0.1	0.259	1.082	0.385	1.319	0.164	0.625

* : number of passages

in root suspension culture, the culture conditions including phytohormones and basal media are currently being investigated.

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