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BERBERINE AND OTHER PROTOBERBERINE ALKALOIDS IN CALLUS TISSUE OF *THALICTRUM MINUS*

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Key Word Index—*Thalictrum minus*; *Coptis japonica*; Ranunculaceae; callus tissue; berberine; thalifendine; thalidastine; desoxythalidastine; jatrorrhizine; palmatine; magnoflorine; chemotaxonomy.

Abstract—A large amount of berberine, six protoberberine-type alkaloids and one aporphine-type alkaloid were isolated from callus tissue derived from the stem of *Thalictrum minus*. These alkaloids were compared with those of *Coptis japonica* callus tissue from the chemotaxonomic point of view.

INTRODUCTION

Thalictrum minus L. var. *hypoleucum* (*T. thunbergii* DC., Japanese name Akikaramastsu) is a perennial herb which grows wild in fields or hilly districts. It has been used in folk medicine as a bitter stomachtonic etc. in Japan and other countries[1] and there are many reports of its analysis for berberine[2], magnoflorine[3], takatonine[4] and bisbenzyl types[5] of alkaloids.

We now report on the alkaloid components of callus tissue of this plant, and compare them with those reported to be present in *Coptis japonica*[6].

RESULTS AND DISCUSSION

Callus was derived from the stem of the plant and was cultured in the dark on Murashige and Skoog's medium containing 2,4-D (0.1, 1.0 and 5.0 ppm) with kinetin (0.1 ppm).

The quaternary base solution from the methanol extract of the callus was fractionated by CC (Sephadex LH-20) followed by prep. TLC (Si gel) to give eight compounds (**1–8**). The chloride of the main alkaloid (**1**) gave yellow needles, mp 192–194° (decomp.), and its identity was confirmed by direct comparison (UV, IR, NMR, MS) with an authentic sample of berberine chloride. Desoxythalidastine (**7**) was identified by mp, UV, NMR, MS and co-chromatography with an authentic sample. Thalifendine[7] (**5**) was converted to tetrahydrothalifendine with sodium borohydrate and identified by direct comparison with an authentic sample derived from desoxythalidastine. The UV and NMR spectral data for thalidastine (**6**) were identical with those reported in the literature[8]. Furthermore, the mass spectrum of tetrahydrothalidastine formed by reduction of **6** with sodium borohydride was identical with the spectrum of tetrahydrothalidastine. The minor compounds jatrorrhizine (**3**) and columbamine (**4**) were identified by comparison with the FT-^{1}H NMR, UV, MS and R_f data in the literature. Palmatine (**2**) and magnoflorine (**8**) were identified by comparing their fluorescence under UV light and their colours with Dragendorff's reagent with those given authentic samples in three TLC systems.

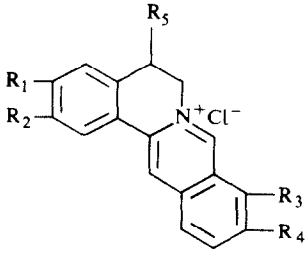
All the alkaloids except berberine were present in small amounts (*ca* 1–2 mg). Coptisine (**9**), a compound which has not been reported in this genus, was not detected. Alkaloid production was regulated by the concentration of 2,4-D. Thus, all the alkaloids (**1–8**) were detected at 0.1 ppm, **1–6** and **8** at 1.0 ppm and **1–3** and **8** at 5.0 ppm of 2, 4-D (Table 1). It is very interesting that the alkaloid berberine was present in callus tissue in much greater amounts (0.67% dry wt of callus (5.2 g), 5.0 ppm 2,4-D, 0.1 ppm kinetin; 0.25% dry wt of callus (23 g), 1.0 ppm 2, 4-D, 0.1 ppm kinetin) than reported in the stem and leaves of *T. thaunbergii* (Kochi) (0.0019%)[2].

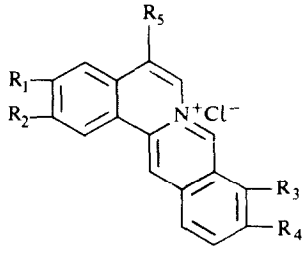
In the time course study of this callus, the growth index increased up to 6–8 weeks, and **1**, **8** and **5** (1.0 ppm 2,4-D only) were detected at all stages of growth. The content of **1** increased up to 6–8 weeks and was the main spot on TLC at both concentrations of 2,4-D (1.0 and 5.0 ppm). Spots of **6** and **3** were detected at 7 weeks.

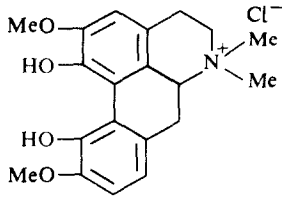
In *C. japonica* callus **1–3**, **8** and **9** are present, **3** being the main alkaloid[6].. It is very interesting from a chemotaxonomic point of view that these two kinds of callus tissue produce protoberberines and aporphine-type alkaloids as the main alkaloid components (Table 1).

Table 1. Regulation of alkaloid production by 2,4-D concentration

2,4-D (ppm)	Kinetin (ppm)	1	2	9	3	4	5	6	7	8
T. thunbergii callus										
0.1	0.1	+++	+	−	+	+	+	+	+	+
1.0	0.1	+++	+	−	+	+	+	+	−	+
5.0	0.1	++	+	−	+	−	−	−	−	+
Coptis japonica callus[6]										
1.0	0.1	+	+	+	++					+







		R_1	R_2	R_3	R_4	R_5
1	Berberine	$-O-CH_2-O-$		OMe	OMe	H
2	Palmatine	OMe	OMe	OMe	OMe	H
9	Coptisine	$-O-CH_2-O-$		$-O-CH_2-O-$		H
3	Jatrorrhizine	OH	OMe	OMe	OMe	H
4	Columbamine	OMe	OH	OMe	OMe	H
5	Thalifendine	$-O-CH_2-O-$		OMe	OH	H
6	Thalidastine	$-O-CH_2-O-$		OMe	OH	OH
7	Desoxythalidastine	$-O-CH_2-O-$		OMe	OH	H

8 Magnoflorine

EXPERIMENTAL

Mps are uncorr. FT-^{1}H NMR: 100 MHz (JEOL-FX-100) at room temp., CD_3OD or CF_3COOD, TMS as int. standard.

Plant material. T. thunbergii was collected in September 1974, at Torisawa, Yamanashi prefecture, Japan.

Derivation and culture of callus tissue. The callus tissue from stalk was derived in October 1974. Murashige and Skoog's medium containing 2,4-D (5 mg/l., 1 mg/l.) and kinetin (0.1 mg/l.) as plant growth regulators was used for the induction of callus tissue. The callus tissue was subcultured every 4–5 weeks onto fresh Murashige and Skoog's medium (minus glycine) containing 2,4-D (0.1 mg/l., 1 mg/l. and 5 mg/l.) and kinetin (0.1 mg/l.) at 26° ± 1 in the dark for 3 years.

Extraction and isolation. The fresh callus tissue was extracted with cold MeOH in a Waring blender and then refluxed with hot MeOH and hot C_6H_6. The extracts were combined, concd under red. pres. and acidified with conc. HCl. The acidic soln was extracted with C_6H_6 to remove the neutral and acidic fractions. The aq. soln was made basic (pH 8–9) with conc. NH_4OH and extracted repeatedly with $CHCl_3$. The quarternary base soln was chromatographed over a column Sephadex LH-20 using MeOH containing increasing proportions of H_2O and H_2O–MeOH–NH_4OH [10 drops/100 ml (H_2O–MeOH)]. **1**, **2** and **8** were eluted with MeOH and **3**–**7** with H_2O–MeOH–NH_4OH. The compounds were finally purified by prep. TLC (Si gel) using the following solvent systems; EtOAc–C_6H_6–*n*-PrOH–MeOH–$EtNH_2$ [(a) 1:8:2:2:1.5; (b) 4:8:2:1:1], MeOH–H_2O–NH_4OH [(c) 8 ; 1 ; 1].

Identification of other minor alkaloids. Small amounts of palmatine, jatrorrhizine, columbamine and magnoflorine were isolated as pure compounds, and were identified by comparison with authentic samples on TLC (Si gel 60 F_{254} Merck) using three solvent systems. (Compound, R_f in a, b and c): **1**, 0.07, 0.64, 0.36; **2**, 0.05, 0.43, 0.24; **3**, 0.68, 0.24, 0.06; **4**, 0.56, 0.22, 0.06; **5** 0.77, 0.53, 0.25; **6**, 0.72, 0.35, 0.12; **7**, 0.77, 0.52, 0.16; **8**, 0.40, 0.06,—; **9**, 0.06, 0.97, 0.91.

Time course study. Each sample (5 × 50 ml flask each) was collected every week for 9 weeks. It was extracted with cold MeOH and the extract subjected to TLC. (2,4-D, 5.0 and 1.0 mg/l., kinetin 0.1 mg/l.)

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