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ALKALOIDS OF TISSUE CULTURES OF *NANDINA DOMESTICA*

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Key Word Index—*Nandina domestica*; Berberidaceae; callus tissue; protoberberine alkaloids; berberine; jatrorrhizine; dehydrodiscretamine; chemotaxonomy.

Abstract—From tissue cultures of *Nandina domestica*, 12 protoberberine alkaloids and magnoflorine were identified. The alkaloids were compared with the alkaloids obtained from other tissue cultures from the chemotaxonomic point of view

INTRODUCTION

We previously have shown that callus tissues of the Ranunculaceous plants *Coptis japonica* (Japanese name Owren) [1] and *Thalictrum minus* (Japanese name, Akikaramatsu), [2] produced protoberberine alkaloids including berberine and the aporphine alkaloid magnoflorine as the main alkaloidal components. It was very interesting from the chemotaxonomic point of view that these two kinds of callus tissues produced both protoberberine and aporphine type alkaloid components. Now, we wish to report on the alkaloids of the callus tissue of *Nandina domestica* (Berberidaceae, Japanese name, Nanten). Moreover, we have compared the presence of the alkaloid components with that obtained from other callus tissues from the chemotaxonomic point of view.

Nandina is a monotypic genus, represented by *N. domestica* and is an evergreen shrub which grows wild in Japan and China [3]. The fruits are used as a folk remedy for coughs. The fruits, seeds, roots, and stem bark contain alkaloids and there are many reports about the alkaloid components, which include nandinine, nandazurine, protopine, berberine and jatrorrhizine from the plant [4–6]. Recently, Shoji *et al.* [6] reported nantenine as a serotonergic receptor antagonistic active substance from a methanolic extract of the fruit.

RESULTS AND DISCUSSION

Callus was derived from the stem of *N. domestica* and grown in the dark on Murashige and Skoog's medium containing 2,4-D (1.0 and 0.1 mg/l) with kinetin 0.1 mg/l.

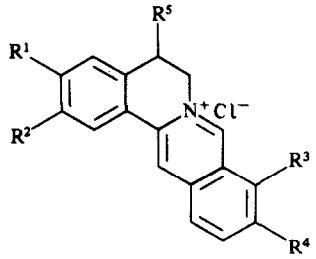
The quaternary base solution from the methanol extract of the callus was fractionated by Sephadex LH-20 and each fraction was submitted to preparative TLC to give 13 compounds (**1**–**13**) (Table 1). The main alkaloid **4** (20.4 mg) gave orange needles and its identity was confirmed by direct comparison with an authentic sample of jatrorrhizine chloride. Compound **6** (6.6 mg) also crystallized as the chloride and it was identified as thalifendine. Compound **7** (4.5 mg) crystallized as the chloride and its spectral data (^{1}H NMR and mass spectrum) were assigned by comparison with those of compounds **4** and **6**. It was identified as 3,10-dihydroxy-2,9-dimethoxyprotoberberine and confirmed by comparison (^{1}H NMR and R_f value) with an authentic sample obtained from *Corydalis tashiroi* [7]. (Papaveraceae) as dehydrodiscretamine. Other compounds, berberine **1** (4.5 mg), palmatine **2** (3.9 mg), columbamine **5** (2.7 mg) and magnoflorine **13** (15.4 mg) were identified from their ^{1}H NMR, mass spectra and R_f values. The minor compounds coptisine **3**, thalidastine **8**, groenlandicine **9**, epiberberine **10**, berberastine **11** and desoxythalidastine **12** were identified from their TLC R_f values. Twelve protoberberine type and one aporphine type alkaloids were thus identified from callus tissue of *N. domestica*.

Several chemical investigations of callus tissues from *Nandina* [8], *Mahonia* [8], *Coptis* [1, 8], *Berberis* [9], *Dioscorea* [10] and *Tinospora* (*crispa*) [15] have shown jatrorrhizine **4** to be the main alkaloid component, except for *Thalictrum* callus tissues [2] which produced mainly berberine **1** (Table 2). These 12 types of callus tissues which belonged to the families Ranuculaceae, Berberidaceae and Menispermaceae produced the protoberberine type alkaloids jatrorrhizine and palmatine. Moreover, the results are very similar to those described in a previous paper [11] in which Papaveraceous tissue callus produced the benzophenathridine, protopine, and aporphine type alkaloids in common. Such findings are interesting from the chemotaxonomic and phylogenetic point of view. In addition, the accumulation of jatrorrhizine is very interesting in the relation to Rueffer's jatrorrhizine biosynthesis experiments using *Berberis* spp tissue cultures, [16] and also the results suggested the biochemical differences between the intact plants and the callus tissues derived from them.

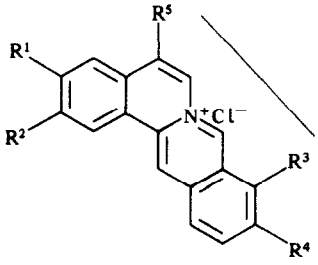
EXPERIMENTAL

UV spectra were recorded (in 95% EtOH), ^{1}H NMR spectra at 100 MHz in $CDCl_3$ (tertiary base) and CD_3OD (quaternary base) with TMS as int. standard. MS were measured at 70 eV.

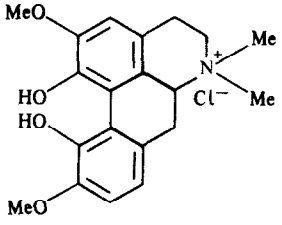
Table 1. Alkaloids from callus tissue of *Nandina domestica*



		R^1	R^2	R^3	R^4	R^5
Berberine	(1)	O—CH_2—O		OMe	OMe	H
Palmatine	(2)	OMe	OMe	OMe	OMe	H
Coptisine	(3)	O—CH_2—O		O—CH_2—O		H
Jatrorrhizine	(4)	OH	OMe	OMe	OMe	H
Columbamine	(5)	OMe	OH	OMe	OMe	H
Thalifendine	(6)	O—CH_2—O		OMe	OH	H
Thalidastine	(8)	O—CH_2—O		OMe	OH	OH
Epiberberine	(10)	OMe	OMe	O—CH_2—O		H



		R^1	R^2	R^3	R^4	R^5
Berberastine	(11)	O—CH_2—O		OMe	OMe	OH
Groenlandicne	(9)	OH	OMe	O—CH_2—O		H
Dehydrodiscretamine	(7)	OH	OMe	OMe	OH	H
Desoxythalidastine	(12)	O—CH_2—O		OMe	OH	OH



Magnoflorine (13)

Table 2. Alkaloids of callus tissues

Species	Type of alkaloids: Protoberberine											Aporphine
	1	2	3	4	5	6	7	8	9	10	11	13
Ranunculaceae												
Coptis japonica [1, 8]	++	+	+	++	+	−	−	+	+	+	+	+
Thalictrum minus [2]	++	+	+	+	+	+	−	+	−	+	+	+
Berberidaceae												
Nandina domestica	+	++	+	++	+	+	+	+	+	+	+	+
Mahonia japonica [8]	+	+	+	++	+	−	−	−	−	−	−	+
Berberis stolinifera [9]	+	+		++	+							
B. wilsonea [13]	+	+		++	+							
Jeffersonia dubia [14]				+								
Menispermaceae												
Dioscoreophyllum comminsii [10]	−	+		++	+							+
Tinospora caffra [12]		+		+	+							+
T. crispa [15]		+		+								
Chasmanthera dependens [12]		+		+								
Stephania japonica [12]		+		+								+

+ Present; ++ present (large amount); − absent.

Silica gel F_{254} (Merck) was used for prep. and analytical TLC using the following solvent systems EtOAc–C_6H_6–*n*-PrOH–MeOH–$EtNH_2$ (A) (4:8:2:1:1); (B) (1:8:2:2:1.5), MeOH–H_2O–NH_4OH (C) (8:1:1).

Material. N. domestica used for the induction of the callus as cultivated at the Medicinal Plant Garden of this college. The callus was derived from the stem in 1976 on Murashige and Skoog's (MS) medium containing 2,4-D (1 and 0.1 mg/l) and kinetin 0.1 mg/l and the callus was subcultured onto MS medium containing 2,4-D, 1 mg/l and kinetin 0.1 mg/l. The callus was subcultured every 5–6 weeks at 27° in dark.

Extraction and isolation of alkaloids. Fr. callus tissue (542) g, dry wt 28.4 g) was extracted with MeOH in a Waring blender and then refluxed with hot MeOH and hot EtOAc. The exts were combined and evapd *in vacuo*. The residue of the H_2O sol fraction extd with EtOAc to remove the organic solvent soluble fraction. The aq sol fraction was chromatographed on a Sephadex LH-20 column using MeOH–H_2O (4:1), and its alkaloid fraction rechromatographed on a column of Amberlight XAD-2 using H_2O–MeOH (1:1) and H_2O–MeOH–NH_4OH [5 ml:5 ml:1 drop (28%)]. The former fraction was submitted to prep. TLC using solvent C and compounds **4**, **5**, **7**, **9** and **13** were obtained. The latter fraction was submitted to prep. TLC using solvent B and compounds **1**, **2**, **3**, **6**, **10**, **11**, and **12** were isolated.

Dehydrodiscretamine **7**. Yield 4.5 mg, amorphous from MeOH–$CHCl_3$; UV (MeOH λ_{max}) nm 432, 346, 277, 224, 206, (MeOH + 0.1 N NaOH), 504, 382, 298 (sh), 272, 249. ^{1}H NMR (CD_3OD, δppm), 4.02 (3H, *s*), 4.15 (3H, *s*), 6.86 (1H, *s*), 7.66 (1H, *s*), 7.82 (1H, *d*, $J = 8$ Hz), 7.88 (1H, *d*, 8 Hz), 8.76 (1H, *s*), 9.64 (1H, *s*).

Discretamine **15**. ^{1}H NMR ($CDCl_3$, δppm), 3.86 (3H, *s*), 3.91 (3H, *s*), 6.71 (1H, *s*), 6.81 (1H, *d*), 6.86 (1H, *d*). MS *m/z* 327.1460 (calcd 327.1451 for $C_{19}H_{21}O_4N$) $[M]^+$ (100%). 296 (10), 178 (86), 176 (28), 150 (24), 149 (14), 135 (22).

Identification of minor alkaloids. Small amounts of palmatine, berberine, columbamine and magnoflorine were purified by CC and prep. TLC using solvents A, B and C [2] and identified by direct comparison with authentic samples.

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