Arch Pharm Res Vol 29, No 9, 757-761, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Protoberberine Alkaloids and their Reversal Activity of P-gp Expressed Multidrug Resistance (MDR) from the Rhizome of *Coptis japonica* Makino

Yong Deuk Min, Min Cheol Yang, Kyu Ha Lee, Kyung Ran Kim, Sang Un Choi[1], and Kang Ro Lee

Natural Products Laboratory, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea and [1]Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea

(Received March 15, 2006)

Six protoberberine alkaloids were isolated from the chloroform layer of the rhizome of *Coptis japonica* Makino (Ranunculaceae). The structures of the isolated compounds were determined to be 6-([1,3]dioxolo[4,5-g]isoquinoline-5-carbonyl)-2,3-dimethoxy-benzoic acid methyl ester (**1**), oxyberberine (**2**), 8-oxo-epiberberine (**3**), 8-oxocoptisine (**4**), berberine (**5**) and palmatine (**6**) by physicochemical and spectroscopic methods. The compound **3** (8-oxo-epiberberine) was first isolated from natural sources. The compounds were tested for cytotoxicity against five tumor cell lines *in vitro* by SRB method, and also tested for the MDR reversal activities. Compound **4** was of significant P-gp MDR inhibition activity with ED50 value 0.018 μg/mL in MES-SA/DX5 cell and 0.0005 μg/mL in HCT15 cell, respectively.

Key word : *Coptis japonica*, Ranunculaceae, Protoberberine alkaloid, 8-Oxo-epiberberine, Multidrug resistance

INTRODUCTION

The rhizome of *Coptis japonica* Makino (Ranunculaceae) has been widely used in traditional medicine as anxiolytic, antibacterial, antihypertensive and CNS depressant activities (Tang *et al.*, 1992). Isoquinoline alkaloid, lignan and phenolic compounds have been isolated from *C. japonica* (Otsuka *et al.*, 1981; Cho *et al.*, 2000).

In a continuing study on the secondary metabolites of korean medicinal plants, we have examined *C. japonica*, since the methanol extract of the rhizome of *C. japonica* showed significant effect for the P-gp mediated MDR reversal activity in human cancer cells. As a result, we have isolated six protoberberine alkaloids, from the chloroform fraction from *C. japonica*. Their structures were determined to be 6-([1,3]dioxolo[4,5-g]isoquinoline-5-carbonyl)-2,3-dimethoxy-benzoic acid methyl ester (**1**), oxyberberine (**2**), 8-oxo-epiberberine (**3**), 8-oxocoptisine (**4**), berberine (**5**) and palmatine (**6**) by physicochemical and spectroscopic methods. The compound **3**, 8-oxo-epiberberine, was first isolated from the natural source. The compounds were tested for cytotoxicity against five tumor cell lines *in vitro* by SRB method. The marginal or non cytotoxic compounds were tested for the MDR reversal activities. This paper describes the isolation, structural determination and the P-gp expressed MDR reversal activities of the compounds isolated from *C. japonia*.

Correspondence to: Kang Ro Lee, Natural Products Laboratory, College of Pharmacy, Sungkyunkwan University, 300 Chonchon-dong, Jangan-ku, Suwon 440-746, Korea
Tel: 82-031-290-7710, Fax: 82-031-292-8800
E-mail: Krlee@skku.ac.kr

MATERIAL AND METHODS

General experimental procedures

Melting points were determined on an Gallenkamp melting point apparatus and uncorrected. Optical rotations were determined with JASCO P-1020 polarimeter. IR spectra were recorded as KBr discs on Bruker Vector 22 FT-IR spectrometer. UV spectra were obtained with Shimadzu UV-1601 UV/Visuble (Japan) and PDA detector (Waters Co.). NMR spectra were recorded on Varian VXR-500 and JNM-LA400. EI-MS data were obtained using JMS700 spectrometer (Jeol Co.). LC-ESI-MS/MS data were obtained using Quattro micro (Waters Co.). prep-HPLC was performed with Prep Nova-Pak HR C18 (6 μm, 19×300 mm) column using PDA detector (Waters Co., model 2996) and RI detector (Waters Co., model 2414). Silica gel 60 (0.063-0.200 mm, Merck Co.) was

used for column chromatography. TLC used Kiesel gel 60 F_{254} precoated plates (Merck Co.) and RP-18 F_{254s} precoated plates (Merck Co.). Packing material of molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.).

Analytical condition of HPLC : detector = PDA; column =XTerra RP18, 5 um, 4.6×150 mm (Waters); flow rate = 1 ml/min.; eluent = gradient 20% MeOH → 90%MeOH (15 min.) → 90% MeOH (10 min.).

Plant material

The rhizome of *C. japonica* was purchased in January, 2003, Seoul, Korea and voucher speciemen was deposited in the college of Pharmacy at Sungkyunkwan University.

Extraction and isolation

The dried, chopped rhizome (3 kg) were extracted three times with methanol (6 L × 3) at room temperature. The resultant extracts (180 g) were suspended with distilled water (3 L), followed by fractionated with n-hexane (9.2 g), chloroform (8.2 g), and n-butanol (39 g). The chloroform layer (8 g) was subjected to silica gel column chromatography with solvent system of methylene chloride : methanol (50:1→5:1) as eluents to give five sub-fractions (C-1~C-8). The subfraction C-2 was chromatographed with Sephadex LH-20 column (CH_2Cl_2:methanol=1:1) to give five fractions (C-21~C-25). The subfraction C-22 (410 mg) was purified with silica Lobar-A column (n-hexane: ethylacetate:methanol=10:10:1) and RP prep. HPLC (80% methanol) to afford compounds **1** (5 mg), **2** (15 mg) and **3** (3 mg). The subfraction C-23 (69 mg) was purified with silica Lobar-A column (n-hexane:ethylacetate : methanol= 10:10:1) and RP prep. HPLC (80% methanol) to afford compound **4** (2 mg). The subfraction C-4 (1.4 g) was purified with silica prep. TLC (n-butanol:ethyl acetate:formic acid:water=3:5:1:1) and RP prep. HPLC (gradient, 70%→ 90% methanol, 0.1% TFA) to afford compounds **5** (26 mg) and **6** (270 mg).

6-([1,3]Dioxolo[4,5-g]isoquinoline-5-carbonyl)-2,3-dimethoxybenzoic acid methyl ester (1)

Colorless powder, UV λ_{max}^{MeOH} nm : 239, 312; LC-ESI-MS/MS *m/z* (rel. int.) : 396 ($[M+H]^+$, 50), 364 (100), 218, 200, 190, 172; EI-MS *m/z* (rel. int.) : 395 ($[M]^+$, 0.8), 336 (100) 223, 172, 59; ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 3.78 (3H, s, $-OCH_3$), 3.94 (3H, s, $-OCH_3$), 3.96 (3H, s, $-OCH_3$), 6.17 (2H, s), 6.95 (1H, d, *J*=8.1 Hz), 7.20 (1H, s), 7.42 (1H, d, *J*=8.1 Hz), 7.70 (1H, s), 7.72 (1H, d, *J*=5.3 Hz), 8.46 (1H, d, *J*=5.3 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 52.83 ($-OCH_3$), 56.47 ($-OCH_3$), 62.16 ($-OCH_3$), 102.55, 102.94, 103.10, 112.31, 123.08, 124.40, 128.45, 130.11, 130.71, 136.80, 138.69, 147.11, 150.49, 152.37, 157.63, 167.48, 191.93.

Ooxyberberine (2)

Colorless powder, mp : 198~199°C; IR ν_{max}^{KBr} cm^{-1} : 2937, 1645 (C=O), 1492 (C=C), 1274, 1036; UV nm : 226, 255, 330, 341, 370; EI-MS *m/z* (rel. int.) : 351 (M^+, 100), 336 (60), 322 (36) and 308 (24); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 2.90 (2H, t, *J*=6.2 Hz, H-5), 3.96 (3H, s, $-OCH_3$), 4.03 (3H, s, $-OCH_3$), 4.31 (2H, t, *J*=6.2 Hz, H-6), 6.02 (2H, s, $-O-CH_2-O-$), 6.71 (1H, s, H-4), 6.73 (1H, s, H-1), 7.23 (1H, s, H-13), 7.28 (1H, d, *J*=8.7 Hz, H-12) and 7.32 (1H, d, *J*=8.7 Hz, H-11); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 28.96 (C-5), 39.61 (C-6), 57.16 (C-10, $-OCH_3$), 61.85 (C-9, $-OCH_3$), 101.53 (C-13), 101.65 ($-O-CH_2-O-$), 104.94 (C-1), 108.15 (C-4), 119.35 (C-11), 119.65 (C-14), 122.50 (C-12), 124.02 (C-8a), 130.29 (C-4a), 132.64 (C-12a), 135.90 (C-14a), 147.60 (C-3) 148.69 (C-2), 149.86 (C-10), 151.68 (C-9), 160.34 (C-8).

8-Oxo-epiberberine (3)

Colorless powder, UV λ_{max}^{MeOH} nm : 226, 256, 331, 345, 377; EI-MS *m/z* (rel. int.) : 351 (M^+, 100), 336 (60), 322 (36), 308 (24), 256 (15), 84 (10); HR-EI-MS : *m/z* 351.1108 (calcd for $C_{20}H_{17}NO_5$ 351.1107); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 2.94 (2H, t, *J*=6.2 Hz, H-5), 3.96 (3H, s, $-OCH_3$), 4.00 (3H, s, $-OCH_3$), 4.32 (2H, t, *J*=6.2 Hz, H-6), 6.24 (2H, s, $-O-CH_2-O-$), 6.75 (1H, s, H-4), 6.80 (1H, s, H-1), 7.08 (1H, d, *J*=8.7 Hz, H-12), 7.18 (1H, d, *J*=8.7 Hz, H-11) and 7.24 (1H, s, H-13); ^{13}C-NMR (125 MHz, CDCl3) : δ 28.41 (C-5), 39.45 (C-6), 56.29 (C-9, $-OCH_3$), 56.53 (C-10, $-OCH_3$), 101.90, (C-13), 102.80 ($-O-CH_2-O-$), 107.95 (C-1), 110.84 (C-4), 114.16 (C-11), 119.27 (C-12), 122.63 (C-4a), 128.70 (C-14a), 132.19 (C-12a), 132.49 (C-8a), 135.69 (C-14), 146.42 (C-10) 146.98 (C-9), 148.74 (C-2), 150.40 (C-3), 160.09 (C-8).

8-Oxocoptisine (4)

Colorless powder, UV λ_{max}^{MeOH} nm : 226, 258, 330, 347, 377; LC-ESI-MS/MS *m/z* (rel. int.) : 336 ($[M+H]^+$, 50), 334, 308 (100), 293; ^{1}H-NMR (500 MHz, CDCl3) : δ 2.90 (2H, t, *J*=6.1 Hz, H-5), 4.28 (2H, t, *J*=6.1 Hz, H-6), 6.03 (2H, s, $-O-CH_2-O-$), 6.23 (2H, s, $-O-CH_2-O-$), 6.72 (1H, s, H-4), 6.76 (1H, s, H-1), 7.06 (1H, d, *J*=8.5 Hz, H-12), 7.18 (1H, d, *J*=8.5 Hz, H-11), 7.23 (1H, s, H-13); ^{13}C-NMR (125 MHz, CDCl3) : δ 28.85 (C-5), 39.38 (C-6), 102.81 ($-O-CH_2-O-$), 101.68 ($-O-CH_2-O-$), 102.34 (C-13), 104.99 (C-1), 108.21 (C-4), 110.88 (C-4a), 114.16 (C-11), 119.42 (C-12), 123.98 (C-14a), 130.10 (C-12a), 130.20 (C-8a), 135.65 (C-14), 146.45 (C-2) 146.91 (C-3), 147.61 (C-10), 148.68 (C-9), 159.95 (C-8).

Berberine (5)

Yellow crystal, mp : 158~160°C; IR ν_{max}^{KBr} cm^{-1} : 1686 (C=N), 1636 (aromatic C=C), 1203 (C-O); UV λ_{max}^{MeOH} nm : 229, 235, 265, 275, 340, 348; LC-ESI-MS/MS *m/z* (rel. int.) :

336 (M^+, 62), 321 (85), 320 (100), 306 (30), 304 (18), 292 (85), 278 (7); ^{1}H-NMR (500 MHz, CD_3OD) : δ 3.26 (2H, t, *J*=6.4 Hz, H-5), 4.11 (3H, s, -OCH_3), 4.21 (3H, s, -OCH_3), 4.92 (2H, t, *J*=6.4 Hz, H-6), 6.11 (2H, s, -O-CH_2-O-), 6.96 (1H, s, H-4), 7.66 (1H, s, H-1), 8.00 (1H, d, *J*=8.8 Hz, H-12), 8.11 (1H, d, *J*=8.8 Hz, H-11), 8.70 (1H, s, H-13), 9.76 (1H, s, H-8); ^{13}C-NMR (125 MHz, CD_3OD) : δ 28.20 (C-5), 57.20 (C-6), 57.68 (C-10, -OCH_3) 62.53 (C-9, -OCH_3), 103.67 (-O-CH_2-O-), 106.54 (C-1), 109.38 (C-4), 121.50 (C-1a), 121.87 (C-13), 123.35 (C-8a), 124.46 (C-12), 128.17 (C-11), 131.89(C-4a), 135.23 (C-12a), 139.73 (C-13a), 145.83 (C-8), 146.37 (C-9), 149.96 (C-2), 152.02 (C-10), 152.21 (C-3).

Palmatine (6)

Yellow crystal, mp : 208°C (decomp.); IR ν_{max}^{KBr} cm^{-1} : 1686 (C=N), 1606 (aromatic C=C), 1197 (C-O); UV λ_{max}^{MeOH} nm : 226, 235, 260, 274, 330, 346; LC-ESI-MS/MS m/z (rel. int.) : 352 (M^+), 337, 322, 308; ^{1}H-NMR (500 MHz, CD_3OD) : δ 3.29 (2H, t, *J*=6.4 Hz, H-5), 3.95 (3H, s, 3-OCH_3), 4.00 (3H, s, 2-OCH_3), 4.11 (3H, s, 10-OCH_3), 4.22 (3H, s, 9-OCH_3), 4.94 (2H, t, *J*=6.4 Hz, H-6), 7.06 (1H, s, H-4), 7.67 (1H, s, H-1), 8.02 (1H, d, *J*=8.8 Hz, H-12), 8.12 (1H, d, *J*=8.8 Hz, H-11), 8.80 (1H, s, H-13), 9.76 (1H, s, H-8); ^{13}C-NMR (125 MHz, CD_3OD) : δ 27.78 (C-5), 56.64 (C-6), 56.92 (-OCH_3), 57.32 (-OCH_3), 57.60 (-OCH_3), 62.49 (-OCH_3), 109.85 (C-1), 112.16 (C-4), 120.47 (C-8a), 121.27 (C-13), 123.28 (C-1a), 124.43 (C-11), 128.00 (C-12), 130.06 (C-4a), 135.26 (C-12a), 139.83 (C-13a), 145.73 (C-10), 146.42 (C-8), 150.89 (C-2), 151.91 (C-3), 153.80 (C-9).

Cytotoxicity test *in vitro*

Sulforhodamin B Bioassay (SRB) was used for cytotoxicity test. The activity of a compound was tested at several concentrations against five cultured human tumor cells in vitro (Skehan *et al.*, 1990); A549 (non small cell lung adenomcarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon).

MDR reversal activity

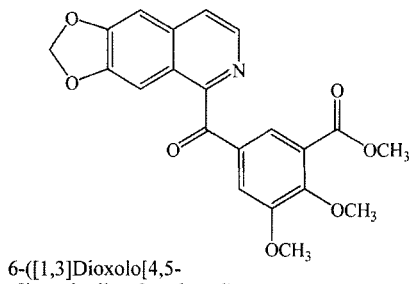
The human ovarian cancer cell line SK-OV-3, the human colorectal cancer cell line HCT15 cell, the human uterine sarcoma cell line MES-SA, the human MDR uterine sarcoma cell line MES-SA/DX5 were provided by the National Cancer Institute (NCI), and maintained in the KRICT. SK-OV-3 cells and MES-SA cells were P-gp non-expressed and non-multidrug resistant cancer cells. Meanwhile, HCT15 cells and MES-SA/DX5 cells were revealed high level P-gp expression. Cell cultures were conducted with RPMI1640 medium supplied with 5% FBS previously reported (Choi *et al.*, 1996). The cells were incubated with serial diutions of paclitaxel in the absence or presence of each isolated compound (10 μM) or verapamil (10 μM) for 72 h. The procedure for calculation of survival fractions was identical to that of cytotoxicity assay. In this assay, the controls were wells that contained each isolated compound or verapamil without paclitaxel.

RESULTS AND DISCUSSION

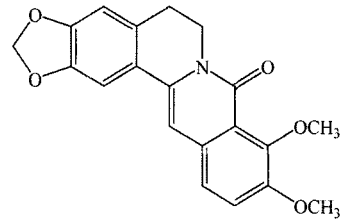
Compound **1** (Cheng *et al.*, 1980; Wu *et al.*, 1980; Elango *et al.*, 1983), compound **2** (Pinho *et al.*, 1992), compound **4** (Li *et al.*, 2001; Rahman *et al.*, 1995), compound **5** (Moriyasu *et al.*, 1992; Lee *et al.*, 1997; Kim *et al.*, 2000) and compound **6** (Moriyasu *et al.*, 1992; Lee *et al.*, 1997; Kim *et al.*, 2000) were identified by comparison of their spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with those reported literatures. Compounds **1** and **2** were first isolated from *C. japonica*.

Compound **3** was obtained as colorless powder and positive against dragendorff reagent. The UV spectrum pattern of **3** was similar to that of berberine standard. The molecular ion peak of **3** in EI-MS spectrum showed at m/z 351 as base peak. High-resolution mass spectrum showed molecule ion at m/z 351.1108, suggesting that the molecular formula of **3** to be $C_{20}H_{17}NO_5$. The ^{1}H-NMR spectrum showed H-11 and H-12 signals of protobeberberine D-ring at δ 7.18 and 7.08 (d, *J*=8.7 Hz), H-13 signal of C-ring at δ 7.24 (s), H-1 and H-4 signals of A-ring at δ 6.80 (s) and 6.75 (s) and H-5 and H-6 signals of isoquinoline B-ring at δ 2.94 and 4.32 (2H, t, *J*=6.2 Hz). On the basis of spectral data, **3** was deduced to be 8-oxo-protoberberine derivative (Patra *et al.*, 1987). The ^{1}H- and ^{13}C-NMR spectral data suggested that **3** had two methoxyl groups, a amide carbonyl and a dioxymethylene group. In the ^{1}H-NMR spectrum, H-11 (δ 7.18) and H-12 (δ 7.08) signals of **3** appeared at the same position with those of **4**, but shifted 0.2 ppm upfield in comparing with those of **2**. In the ^{13}C-NMR spectrum, the C-9 and C-10 signals of **3** and **4** showed at δ 146. 9 and 146.4, respectively. These data suggested that a dioxymethylene group was connected with C-9 and C-10 of D-ring and two methoxyl groups were substituted at C-2 and C-3 of A-ring. The retention times of **2**, **3** and **4** were also quite different (Rt=17.84 min. in **2**; Rt=16.94 min. in **3**; Rt=18.36 min. in **4**) in the analytical HPLC chromatography profile column=RP18, 5 um, 4.6×150 mm; eluent=gradient 20% MeOH → 90% MeOH (15 min.) → 90% MeOH (10 min.). On the above mentioned data, the structure of **3** was suggested to be 8-oxo-epiberberine. Epiberberine was isolated as quaternary alkaloid from *Coptis trifolia*. (Mizuno *et al.*, 1992). The compound **3,** 8-oxo-epiberberine, was first isolated from the natural sources.

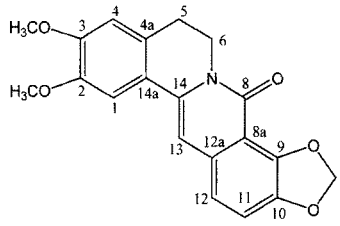
The compound **2** was significant cytotoxic against five tumor cell lines with ED_{50} values of ranging from 1.07 to



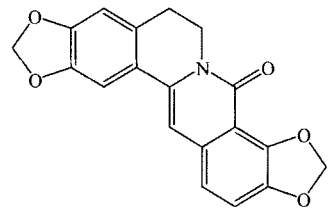
6-([1,3]Dioxolo[4,5-g]isoquinoline-5-carbonyl)-2,3-dimethoxy-benzoic acid methyl ester (**1**)



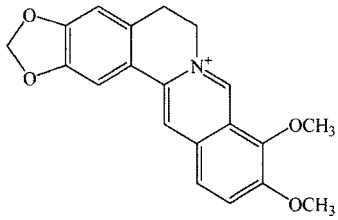
Oxyberberine (**2**)



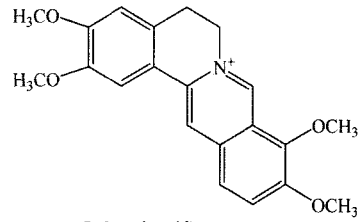
8-Oxo-epiberberine (**3**)



8-Oxocoptisine (**4**)



Berberine (**5**)



Palmatine (**6**)

Fig. 1. Structures of compounds **1-6** isolated from *C. japonica*

Table I. Cytotoxic activities of compounds (**1~6**) isolated from *C. japonica*

Compounds	ED_{50} (μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	>30.0	>30.0	>30.0	>30.0	>30.0
2	1.32	1.56	1.07	2.96	1.47
3	14.18	6.54	15.11	26.83	v34.05
4	7.58	7.73	7.46	>30.0	>30.0
5	2.95	15.29	18.47	1.94	27.20
6	22.31	>30.0	21.44	28.71	>30.0
Doxorubicin	0.16	0.38	0.04	0.04	0.82

Table II. MDR reversal activities of compounds **1**, **4** and **6** from *C. japonica*

Compounds	ED_{50} (μg/mL)		
	SK-OV-3	HCT15	MES-SA/DX5
Paclitaxel (P)	0.0004	0.1130	0.817
P + **1**	0.0003	0.0020	0.046
P + **4**	0.0003	0.0005	0.018
P + **6**	0.0009	0.0350	0.414
P + verapamil	0.0007	0.0003	0.022

2.96 μg/mL. The compounds **1**, **4** and **6** were marginal or noncytotoxic in the five human cancer cell lines (Table I). The noncytotoxic compounds were tested for the MDR reversal activities in the P-gp nonexpressed (non-MDR) cell line SK-OV-3 cell, and P-gp expressed MDR cell line MES-SA/DX5 cell and HCT15 cell. Compounds **1** and **4** showed P-gp MDR inhibition and their cytotoxicity to MDR cells were very weak. Especially, P-gp MDR inhibition activity of compound **4** was similar to verapamil, having ED_{50} value 0.018 μg/mL in MES-SA/DX5 cell and 0.0005 μg/mL in HCT15 cell, respectively (Table II).

REFERENCES

Cheng H. Y. and Doskotch, R. W., Alkaloids of Thalictrum XXXI, Synthesis of Rugosinone. *J. Nat. Prod.*, 43, 151-156 (1980).

Cho, J. Y., Baik, K. U., Yoo, E. S., Yoshikawa, K., and Park, M. H., *In vitro* antiinflammatory effects of woorenosides from the rhizomes of *Coptis japonica*. *J. Nat. Prod.*, 63, 1205-1209 (2000).

Choi, S. U., Kim, N. Y., Choi, E. J., Kim, K. H., and Lee, J. O., Establishment of doxorubicin-resistant subline derived from HCT15 human colorectal cancer cells. *Arch. Pharm. Res.*, 19, 342-347 (1996).

Elango, V. and Shamma, M., A pyrolytic route to the phthalide-isoquinolines. *J. Org. Chem.*, 48, 4879-4881 (1983).

Kim, M. S., Hwang, B. Y., Choe, S. G., Lee, M. K., Ro, J. S., and Lee, K. S., Alkaloidal components of Chelidonii Fructus. *Kor. J. Pharmacogn.*, 31, 390-393 (2000).

Lee, H. Y. and Kim, C. W., Studies on the constituents of *Berberis amurensis* Ruprecht. *Kor. J. Pharmacogn.*, 28, 257-263 (1997).

Li, M., Chen, X., Tang, Q. M., and Zhang, J. S., Isoquinoline alkaloids from *Thalictrum delavayi*. *Planta Med.*, 67, 189-190 (2001).

Mizuno, M., Kojima, H., Inuma, M., Yanaka, T., and Goto, K., Coumarin derivatives in Coptis Trifolia. *Phytochemistry*, 31, 717-719 (1992).

Moriyasu, M., Ichimaru, M., Sawada, Y., Izutsu, K., Nishiyama, Y., and Kato, A., Analysis of alkaloids in *Nandina domestica* by means of HPLC and TLC-densitometry. *Shoyakugaku Zassi*, 46, 143-149 (1992).

Otsuka, H., Fujimura, H., Sawada, T., and Goto, M., Studies on Anti-inflammatory Agent II. Anti-inflammatory constituents from Rhizome of *Coptis japonica* MAKINO. *Yakugaku zasshi*, 101, 883-890 (1981).

Pinho, P. M. M., Pinto, M. M. M., Kijioa, A., Pharadai, K., Diaz, J., and Herz, W., Protoberberine alkaloids from *Coscinium fenestratum*. *Phytochemistry*, 31, 1403-1407 (1992).

Rahman, A. U., Ahmad, S., Bhatti, K., and Choudhary, M. I., Alkaloidal constitients of *Fumaria indica*. *Phytochemistry*, 40, 593-596 (1995).

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, 82, 1107-1112 (1990).

Tang, W. and Eisenbrand, G., *Coptis supp.* In: Chinese drugs of plant origin. Springer Verlag, New York, pp. 362-371 (1992).

Wu, W. N., Beal, J. L., and Doskotch, R. W., Alkaloids of Thalictrum XXX, eleven minor alkaloids from *Thalictrum rugusum*. *J. Nat. Prod.*, 43, 143-150 (1980).