Arch Pharm Res Vol 24, No 6, 518-521, 2001

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

Cytotoxic Alkaloids from *Houttuynia cordata*

Seong-Kie Kim, Shi Yong Ryu, Jaesung No, Sang Un Choi, and Young Sup Kim

Korea Research Institute of Chemical Technology, Yusung P.O. Box 107, Taejeon 305-606, Korea

(Received September 1, 2001)

Six bioactive alkaloids, aristolactam B(**1**), piperolactam A(**2**), aristolactam A(**3**), norcepharadione B(**4**), cepharadione B(**5**) and splendidine(**6**) were isolated by bioactivity-guided fractionation of a methanolic extract of the aerial part of *Houttuynia cordata*. Several of them exhibited significant cytotoxicity against five human tumor cell lines (A-549, SK-OV-3, SK-MEL-2, XF-498 and HCT-15) *in vitro*.

Key words: *Houttuynia cordata*, Cytotoxicity, Alkaloids, Aristololactams, 4,5-Dioxoaporphine, Oxoaporphin

INTRODUCTION

In the continuing search for cytotoxic compounds from various plant sources, *Saururaceae* was found to possess reproducible activity against human cancer cell lines *in vitro*. The *Saururaceae* family consists of five genera (*Anemopsis, Saururus, Houttuynia, Gymnotheca* and *Circaeocarpus*) and seven species (*A. californica, S. cernus, S. chinensis, H. cordata, G. involucrata* and *C. saururoides*). Of these seven species, only two, *S. chinensis* and *H. cordata,* are found in Korea. *H. cordata* is native to Asia and grows well in moist to soggy soil. In Korea it grows mainly in Ulleung-do and its Korean name is "YAKMOMIL". These perennials produce a unique bad smell and with white flowers blooming in early summer. This plant is a traditional medicinal plant in Korea, Japan, India and China, and is used as a anti-pyretic, detoxicant, anti-ulcer remedy family and as an anti-inflammatory agent (Chang *et al.*, 1986). It is also used for promoting pus drainage and for insect bites. Here, we report the isolation and identification the cytotoxic components from this plant.

MATERIALS AND METHODS

Plant materials

Dried aerial parts of *H. cordata* were obtained from a crude drug market in Daejon and identified taxonomically with respect to the morphology. A voucher specimen of this material is deposited at the Korea Research Institute of Chemical Technology.

Instruments and materials

The melting points reported are uncorrected. The low-resolution mass spectrum was determined on a JMS-DX 303 mass spectrometer (JEOL), UV-visible spectra were recorded in either MeOH or EtOH. ^{1}H, ^{13}C, 2D-NMR spectra were recorded in $CDCl_3$, pyridine-d_5 or DMSO-d_6 on either a Brucker AM-300 or an AMX-500 spectrometer. The chemical shifts are expressed as parts per million (ppm) downfield from TMS as an internal standard. Silica gel (230-400 mesh, Merck) was used for flash column chromatography. TLC was performed on a silica gel 60 F_{254} plate (Merck). HPLC (LKB) was performed with YMC C_{18}(ID 10 mm × 250 mm, mobile phase, MeCN, 0.01 mM, H_3PO_4 buffer(pH 3.0), 30:70 →90:10) (V/V); flow rate, 2.0 ml/min; detector, 360nm)

Cytotoxic assays by sulforhodamin B (SRB) method
(Skehan *et al.* 1990)

Each test material was dissolved in dimethylsulfoxide (DMSO) then diluted with the medium solution until the final DMSO concentration in the medium was <0.5%. All experimental procedures were followed according to the NCI protocol based on the SRB method. Each isolate was evaluated in a test for *in vitro* cytotoxicity against the human tumor cell lines, A-549 (human lung adenocarcinoma), SK-OV-3(human ovarian adenocarcinoma), SK-MEL-2(human malignant melanoma), XF-498(human CNS carcinoma) and HCT-15(human colon adenocarcinoma). The cells were inoculated over a series of standard 96-

Correspondence to: Korea Research Institute of Chemical Technology, Yusung P.O. Box 107, Taejeon 305-606, Korea
E-mail: kims@plaza.snu.ac.kr

well flat bottom microtiter plates. These cells were then preincubated for attachment on the microtiter plate for 24 h. The test compounds were added to the wells in serial dilutions beginning from the highest concentrations. At the end of incubation with each drug for 72 h, the culture medium in each well was removed and the cells were fixed with cold 10% TCA at 4°C for 1 h. After washing the TCA, a 0.4% SRB solution was added and incubated for 30 min at room temperature. The cells were washed again, and the bound stain was dissolved with a 10 mM Tris-base solution (pH 10.5) and the absorbance was measured spectrophotometrically at 520 nm in a microtiter plate reader.

Extraction and isolation

The dried aerial parts of *Houttuynia cordata* Thunb. (*Saururaceae*) (2 Kg) were extracted twice with methanol at room temperature for two weeks and the methanolic extract was evaporated under reduced pressure. The residue (117 g) was diluted with H_2O then partitioned successively with CH_2Cl_2 (61 g), EtOAc (14 g) and BuOH (29 g). Since the cytotoxic activity emerged mainly in the methylene chloride fraction, it was chromatographed on a silica gel column eluted with a stepwise gradient of CH_2Cl_2 and MeOH. This yielded five fractions (CH_2Cl_2: MeOH=100:1, 20:1, 10:1, 5:1, 2:1). Fraction 2 (20.3 g) was further separated by RP-18 column chromatography with a H_2O-MeCN gradient system to give five fractions. The second fraction (H_2O:MeCN=9:1 →7:3) was purified on a silica gel column with a benzene:tetrahydrofuran (THF) (benzene:THF=20:1→2:1) mixture affording six alkaloids, which tested positive to dragendorff reagent. Finally, all compounds were purified by reverse phase HPLC (**1**: 25 mg, **2**: 13 mg, **3**: 7 mg , **4**: 42 mg , **5**: 82 mg, **6**: 3 mg).

Aristololactam B(Cepharanone B) [10-amino-3,4-dimethoxyphenanthrene-1-carboxylic acid lactam]

pale yellow needles (from chloroform-methanol). mp: 257-258°C. HR-MS m/z 279.0899($[M]^+$, calcd. for $C_{17}H_{13}O_3N$: 279.0895). EI-MS m/z (rel. int.): 279 $[M]^+$ (100), 264$[M-CH_3]^+$(24), 236$[M-CO-NH]^+$(34), 193$[M-2COCH_3]^+$ (33), 164(51), 138(22), 82(62). UV λ_{max} (MeOH) nm (log ε): 231(3.87), 261(3.67), 275(3.69), 286(3.67), 374(3.00). ^{1}H-NMR(300 MHz, $CDCl_3$): 4.01(s, OCH_3, C-3), 4.07(s, OCH_3, C-4), 7.11(s, H-9), 7.53(m, H-6, H-7), 7.76 (s, H-2), 7.76(m , H-8), 8.82(br s, NH), 9.18(m, H-5). ^{13}C-NMR(75 MHz, pyridine-d_5): 56.8(C-3 methoxy), 60.2(C-4 methoxy), 105.1(C-9), 110.2(C-2), 121.2(C-1b), 122.8 (C-4a), 124.9(C-1a), 125.8(C-6), 127.3(C-5a), 127.9(C-5), 128.0(C-7), 129.3(C-8), 131.4(C-8a), 136.6(C-9a), 151.4 (C-4), 154.9(C-2), 169.9(C-1).

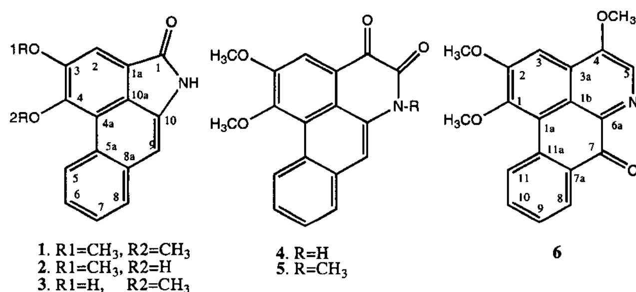

Fig. 1. Chemical structure of the alkaloids isolated from *Houttuynia cordata*

Piperolactam A [10-amino-4-hydroxy-3-methoxyphenanthrene-1-carboxylic acid lactam]

Brown amorphous solid (from EtOAc). mp:268-271°C. EI-MS m/z (rel.int.):265 $[M]^+$(96), 250$[M-CH_3]^+$(59), 222 $[M-CO-NH]^+$(24), 149(100), 79(50). UV λ_{max}(MeOH) nm (log ε):233(4.01), 243(3.99), 265(3.62), 275(3.57), 290 (3.50), 303(3.58), 315(3.60), 445(3.38). ^{1}H-NMR(300 MHz, DMSO-d_6): 4.01(s, OCH_3-4), 7.08(m, H-9), 7.56 (m, H-6, H-7), 7.61 (m, H-2), 7.93 (m, H-8), 9.10 (m, H-5), 10.30(s, NH), 10.77(s, OH).

Aristololactam A [10-amino-3-hydroxy-4-methoxyphenanthrene-1-carboxylic acid lactam]

Dark brown solid (from acetone). mp:270-271°C. CI-MS m/z (rel. int.): $C_{16}H_{11}O_3N$, 266 $[M+1]^+$ (46), 250 (86), 222(44), 166(100), 139(66). UV λ_{max}(MeOH) nm (log ε): 232(3.86), 262(3.75), 274(3.85), 285(3.79), 311 (3.31), 379(3.21). ^{1}H-NMR (300 MHz, DMSO-d_6): 4.03 (s, OCH_3, C-3), 7.48(s, H-9), 7.65(m, H-6, H-7), 7.90(m, H-8), 8.08(s, H-2), 9.45(m, H-5), 10.75(s, H-5), 12.0(s, NH).

Norcepharadione B [1,2-dimethoxy-4H-dibenzo [de,g] quinolin-4,5(6H)-dione]

yellow crystal(from DMF). mp:304-305°C (decomp.). CI-MS m/z (rel. int.): $C_{18}H_{13}O_4N$, 308$[M+1]^+$(100), 279 $[M-CO]^+$(50), 257(19), 197(54), 179(41), 85(61). UV λ_{max} (MeOH) nm(log ε):232(3.59), 262(3.19), 272(3.13), 301 (3.20), 313(3.21), 442(3.10). ^{1}H-NMR(500 MHz, DMSO-d_6): 4.05(s, OCH_3, C-1), 4.09 (s, OCH_3, C-2), 7.53(s, H-7), 7.64 (m, H-9, H-10), 7.61(m, H-2), 7.92 (m, H-8), 8.16 (s, H-3), 9.43(m, H-11), 12.1(s, NH). ^{13}C-NMR (125 MHz, DMSO-d_6): 152.8(C-1), 155.6(C-2), 112.6(C-3), 127.3(C-3a), 176.9(C-4), 153.9(C-5), 132.5(C-6a), 112.9 (C-7), 128.1(C-7a), 130.3(C-8), 126.9(C-9), 124.7(C-10), 128.6(C-11), 123.6(C-11a), 126.0(C-1a), 118.3(C-1b), 60.2(C-1 methoxy), 56.5(C-2 methoxy).

Cepharadione B [1,2-dimethoxy-6-methyl-4H-dibenzo [de,g]quinolin-4,5(6H)-dione]

Orange needles (from EtOH). mp: 263-264°C. HR-MS m/z 321.1008 ($[M]^+$, calcd. for $C_{17}H_{13}O_3N$: 321.1001). EI-MS m/z (rel. int.): 321 $[M]^+$(57), 293$[M-CO]^+$(78), 278

$[M-COCH_3]^+$(22), 250$[M-2COCH_3]^+$(24), 222(33), 194 (31), 84(100). UV λ_{max}(EtOH) nm (log ε): 235(3.36), 241 (3.36), 272(2.86), 301(2.93), 313(2.94). ^{1}H NMR(500 MHz, $CDCl_3$): 3.80 (s, N-CH_3), 4.09 (s, OCH_3, C-1), 4.09 (s , OCH_3, C-2), 7.42 (s, H-7), 7.65 (m, H-9, H-10), 7.84 (dd, J=9, 2.2, H-8), 8.16 (s, H-3), 9.48(dd, J=9, 2.2, H-11). ^{13}C-NMR (125 MHz, $CDCl_3$): 154.9(C-1), 156.3(C-2), 112.6(C-3), 131.7(C-3a), 175.5(C-4), 152.8(C-5), 132.3 (C-6a), 114.3(C-7), 127.6(C-7a), 129.0(C-8), 127.6(C-9), 124.6(C-10), 128.0(C-11), 123.6(C-11a), 126.9(C-1a), 119.5(C-1b), 60.4(C-1 methoxy), 56.5(C-2 methoxy), 30.5 (N-methyl)

Splendidine [1,2,4-trimethoxy-7*H*-dibenzo [de,g]quinolin-7-one]

Bright yellow needles (from acetone). mp: 234-236°C. CI-MS m/z (rel. int.): $C_{19}H_{15}O_4N$, 322 $[M+1]^+$(100), 307 (12), 292 (13), 278(65). UV λ_{max}(MeOH) nm (log ε): 233 (3.49), 237(3.49), 270(3.38), 290(*sh*, 3.06), 414(3.05). ^{1}H-NMR (500 MHz, $CDCl_3$): 4.01 (s , OCH_3, C-2), 4.08 (s, OCH_3, C-1), 4.20 (s, OCH_3, C-4), 7.56 (s, H-3), 7.57 (t, J=8.4, H-9), 7.76 (t , J=8.4, H-10), 8.50 (s, H-5), 8.59 (d, J=8.4, H-8), 9.19(d, J=8.4, H-11). ^{13}C-NMR (125 MHz, $CDCl_3$): 152.3(C-1), 119.6(C-1a), 122.9(C-1b), 152.1(C-2), 101.6(C-3), 132.2(C-3a), 155.8(C-4), 126.5(C-5), 139.1 (C-6a), 181.6(C-7), 134.5(C-7a), 128.4(C-8), 128.5(C-9), 133.9(C-10), 128.7(C-11), 125.4(C-11a), 60.5(C-1 methoxy), 56.2(C-2 methoxy), 56.5(C-4 methoxy).

RESULTS AND DISCUSSION

The extraction of the aerial part of *H. cordata* and the activity-guided chromatographic fractionation led to the isolation and characterization of three aristololactams, two 4,5-dioxoaporphines and a oxoaporphine.

Compound 1-3 exhibited a UV spectrum characteristic of a phenanthrene chromophore (λ_{max}, nm=230, 260, 275 and 285)(Sanster *et al.* 1965). The ^{1}H-NMR spectra showed the NH signals. These signals disappeared upon the addition of D_2O, which further indicated the presence of one or two methoxyl groups. The aromatic region of those spectra closely resembled those of other 5,6,7,8-unsubstituted aristololactams. Two singlets corresponding to uncoupled aromatic protons were ascribed to H-2 and H-9 while the signals of H-5, H-6, H-7 and H-8 appeared as a complex ABCX coupling pattern. Their structures were also supported by their mass fragmentation pattern showing the ion peak corresponding to the elimination of one methyl and one lactam group, respectively (Dyke *et al.*, 1978). By comparing their physical and spectroscopic properties, compounds **1-3** were identified as aristolactam B(**1**), piperolactam A(**2**), aristolactam A(**3**)(Crohare *et al.*, 1962; Sun *et al.*, 1987).

Compound **5** exhibited a UV maximum, in methanol, closely resembling that of compound **4**. The mass spectrum of compounds **4** and **5** showed a $[M]^+$ and an $[M-28]^+$ indicating the facile loss of a carbonyl group. Further fragment ions $[M-CO-Me]^+$, $[M-CO-Me-CO]^+$ were also observed. This particular fragmentation pattern is characteristic of a 4,5-dioxoaporphine type compound (Acasu *et al.*,1974; Achari *et al.*, 1982). The ^{1}H-NMR spectra of **4** and **5** exhibited six aromatic protons, which were similar to each other. The structures of these compounds were identified as norcepharadion and cepharadion B by comparing their spectral data with the reported spectra (Desai *et al.*, 1988; Rao *et al.*, 1990; Achenbach *et al.*, 1991; Acasu *et al.*, 1974; Acasu *et al.*, 1975).

Compound **6**, mp 234-236°C, ($[M+1]^+$ m/z 322), had a UV absorption unlike those of the 4,5-dioxoaporphines. The UV spectrum displayed absorptions at 237, 270, 290 (*sh*) and 414nm associated with an oxoaphorphine chromophore(Shakya *et al.*, 1972). Its ^{1}H-NMR spectrum exhibited signals of six protons consisting of four vicinal aromatic protons at δ 8.59(d, J=8.4), δ 7.57(t, J=8.4), δ 7.76(t, J=8.4), δ 9.19(d, J=8.4) and two singlets of uncoupled aromatic protons at δ 7.56 and δ 8.50. In addition, three methoxy signals at δ 4.08, δ 4.01 and δ 4.20 were observed. The main difference observed in the ^{1}H-NMR spectrum, compared to that of **4** and **5**, was due to signal of a singlet aromatic proton at δ 8.50, which moved further downfield. Furthermore, the absence of the ortho-coupling protons of H-5 suggested that this compound might be a 4-oxygenated 7-oxoaporphine. With the ^{13}C-NMR spectra, most of the protonated carbons could be assigned using the 2D-C, H correlation technique. The assignments of the quarternary carbons were obtained by COLOC (^2J and ^3J) NMR experiments. Assigning the ^{13}C-NMR data was done for the first time in this study, although Skiles *et al.* first isolated this compound from *Abuta rufescens* (*Menispermaceae*). From the spectral data, **6** was identified as splendidine (Skiles *et al.*, 1979).

All the isolates exhibited moderate cytotoxicity against the five human cancer cell lines examined *in vitro*.

Table I. Cytotoxicities of the compounds isolated from *H. cordata*

compound	ED_{50} (μg/ml)				
	A-549	SK-OV-3	SK-MEL-2	XF-498	HCT-15
1	23.2	8.3	20.9	0.84	5.5
2	27.1	23.2	36.8	29.6	31.7
3	>40	>40	>40	>40	>40
4	40	26.4	40	14.2	38.2
5	40	6.3	12.1	42.7	47.5
6	3.4	2.6	5.8	2.6	1.4
Adriamycin	0.1	0.2	0.1	0.2	2.4

The ED_{50} value of compounds against each cancer cell line was defined as a concentration (μg/ml)that caused 50% inhibition of cell growth, and adriamycin was used as a reference.

Among them, 6 was found to exhibit significant activity against each cell line and **1** exhibited selective activity against XF-498 (central nerve system cell)(ED_{50}, 0.84 μg/ml).

REFERENCES

Acasu, M., Itogawa, H., and Fujita, M., Oxoaporphine alkaloids from the callus tissue of *Stephania cepharantha*. *Phytochemistry,* 14, 1673-1674 (1975).

Acasu, M., Itogawa, H., and Fujita, M., Four new fluorescent components isolated from the Callus tissue of *Stephania cepharantha*. *Tetrahedron Lett.,* 41, 3609-3612 (1974).

Achari, B., Chakrabarty, S., Bandyopadhyay, S., and Pakrashi, S. C., Studies on Indian medicinal plants. Part 69. A new 4,5-dioxoaporphine and other constituents of *Aristolochia indica Heterocycles,* 19, 1203-1206 (1982).

Achenbach, H., Frey, D., and Waibel, R., 6a,7-dehydro-2-hydroxy-4,5-dioxonoraporphine and other alkaloids from *Monocyclanthus vignei*:^{13}C-NMR studies on 4,5-dioxoaporphines. *J. Nat. Prod.,* 54(5), 1331-1336 (1991).

Chang, H. and But, P., Pharmacology and applications of chinese materia Medica, World Scientific, Singapore (1986).

Crohare, R., Priestap, H. A., Farine, M., and Ruveda, E., Aristololactams of *Aristolochia argentina*. *Phytochemistry,* 13, 1957-1962 (1962).

Desai, S. J., Prabhu, B. R., and Mulchandani, N. B., Aristololactams and 4,5-dioxoaporphines from *Piper longum*. *Phytochemisry,* 27(5), 1511-1515 (1988).

Dyke, S. F. and Gellert, E., The structure of alkaloids Y from *Scherffromitra subaequalis, Phytochemistry,* 17, 599 (1978).

Hansel, R. and Leuschke, A., Aporphine-type alkaloids from *Piper auritum*, *J. Nat. Pro.,* 38(6), 529-530 (1975).

Sanster, A. W. and Stuart, K. L. Ultraviolet spectra of alkaloids. *Chem. Rev.,* 65, 69-130 (1965).

Priestap, H. A., Seven aristololactams from *Aristolochia argentina*. *Phytochemistry,* 24(4), 849-852 (1985).

Shamma, M., The isoquinoline alkaloids. Academic press inc., p. 245 (1972).

Skan, P., Streng, R., Scudiero, D., Monks, A., Mcmahon, J., Kenney, S., and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer. Inst.,* 82, 1107-1112 (1990).

Skiles, J. W., Saa, J. M., and Cava, M. P., Splendidine, a new oxoaporphine alkaloid from *Abuta rufescens* Aublet. *Can. J. Chem.,* 57, 1642-1646 (1979).

Sun, N. J., Antoun, M. Chang, C. J., and Cassady, J. M., New cytotoxic aristololactams from *Pararistolochia flos-avis*. *J. Nat. Prod.,* 50(5), 843-846 (1987).

Rao, K. V. and Reddy G.C.S, Chemistry of *Saururus cernuus*, v. Sauristolactam and other nitrogeneous constituents. *J. Nat. Prod.,* 53(2), 309-312 (1990).