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A Piperidine Alkaloid and Limonoids from *Arisaema decipiens*, a Traditional Antitumor Herb Used by the Dong People

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A new piperidine alkaloid and three known tetranortriterpenoids were isolated from the methanol extracts of the rhizomes of *Arisaema decipiens* Schott (Araceae) and their chemical structures were identified as (-)-(2*R**,3*S**,6*S**)-*N*,2-dimethyl-3-hydroxy-6-(9-phenylnonyl) piperidine (**1**), 6-deacetylnimbin (**2**), 28-deoxonimbolide (**3**) and nimbin (**4**). The *N*-methylated derivative (**1a**) of **1** was synthesized. Compound **1** exhibited weak inhibitory activity against the MCF-7 cell line, while compound **1a** showed potential inhibitory activity against the MCF-7 cell line with an IC_{50} value of 4.6 μM and weak inhibitory activity against K562 and SK-OV-3 cells. This plant in genus *Arisaema* is firstly reported as the source of limonoids that are considered a natural antitumor herbal medicine.

Key words: Araceae, *Arisaema decipiens* Schott, (-)-(2*R**,3*S**,6*S**)-*N*,2-Dimethyl-3-hydroxy-6-(9-phenylnonyl), Limonoids, Antitumor activity

INTRODUCTION

Arisaema is a large genus in the Araceae family, which comprises about 150 species worldwide; almost 100 of these species are found in China (Li and Long, 1998). The rhizomes or tubers of *A. calcareum* H. Li, *A. serratum* (Thunb.) Schott, *A. asperatum* N. E. Brown, *A. heterophyllum* Blume, and *A. amurense* Maxim. are used as analgesic, antitumor and pesticide agents in traditional Chinese medicine (Chinese Pharmacopoeia Committee, 2005). Previous studies identified cytotoxic diacylglycerylgalactosides against murine leukemia P388 and human colon adenocarcinoma DLD-1 cells and antihepatotoxic cerebrosides from *A. amurense* (Jung et al., 1996a, 1996b); insecticidal (*Bactrocera cucurbitae*) and *in vitro* antiproliferative [HOP-62 (lung), HCT-15 (colon) and so forth] lectins from *A. hellebori-folium* Schott (Kaur et al., 2006; Dhuna et al., 2008); and antiproliferative lectins against J774 and P388D1 murine macrophage cancer cell lines from *A. flavum* (Singh et al., 2004). Additionally, some 2-alkylpyrrolidine and 2-alkylpiperidine alkaloids, which significantly interact with DNA, were isolated from *A. vulgare* Targ (Melhaoui and Belouali, 1998). Such activity is considered to be closely related to the mechanism of several antitumor agents (Pezzuto et al., 1991). Irnigaine and *N*-methylirnigaine were toxic in the brine shrimp bioassay, showing LC_{50} values of 2.5 and 0.25 μg/mL, respectively (Melhaoui et al., 1992; Melhaoui and Bodo, 1995).

The Dong ethnic group is the eleventh largest among China's 56 ethnic minorities. The Dong people primarily reside in twenty counties where Guizhou, Hunan and Guangxi provinces meet. A relatively small number emigrated to and live scattered throughout Hubei Province. The national census of 2000 revealed that the population of the Dong minority was 2.96 million and that 1.09 million or 43% resided in eight counties of Southeast Guizhou. Our recent ethnobotanical survey, which mainly focused on the traditional uses of medicinal plants, especially antitumor herbs, in Dong

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ethnic communities, was implemented in Liping County, Qiandongnan Miao & Dong Ethnic Autonomous Prefecture of Guizhou Province, Southwest China, in April and September, 2009. We found that the rhizome of *A. decipiens* is used externally to treat human breast cancer in local communities. The rhizomes are generally collected and dried during January or February by the Dong healers. The rhizome is considered toxic, so the dried rhizomes are baked with calcareous substances to decrease its virulence. One preparation method used by the Dong people for increased efficacy is to grind/crush the rhizome (into a powder) and mix it with sticky rice wine.

A. decipiens is a perennial herbaceous plant that grows in evergreen forests, mossy forests, bamboo thickets and grasslands throughout Hunan, Southeast Tibet, Yunnan, Sichuan, Guizhou, and Guangxi (Li, 1979). The chemical constituents and bioactivity of *A. decipiens* have not yet been reported. This study was carried out to isolate the chemical constituents from the rhizomes of *A. decipiens* and evaluate their antitumor activities. Four compounds, including a new piperidine alkaloid (**1**) and three known tetranortriterpenoids (**2**-**4**), were isolated. The cytotoxic activity of the new compound (**1**) and its *N*-methyl derivative (**1a**) against three cell lines was evaluated. The structure elucidation and bioassay results are reported.

MATERIALS AND METHODS

General experimental procedures

Optical rotations were determined on a JASCO DIP-370 automatic digital polarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as an internal standard. MS data were measured on a VG Auto Spec-3000 mass spectrometer. Column chromatography was performed over silica gel G (80-100 and 300-400 mesh), silica gel H (10-40 μm), D_{101} resin (Qingdao Marine Chemical Ltd.), and Sephadex LH-20 (40-70 μm; Amersham Pharmacia Biotech AB). TLC was conducted on precoated silica gel plates GF_{254}. HPLC separations were performed using an Agilent 1200 series pump equipped with a diode array detector and a semi-preparative Zorbax SB-C_{18} (5 μm, ϕ 9.4 × 250 mm) column.

Plant material

Arisaema decipiens rhizomes used in this study were collected from Liping County, Qiandongnan Miao & Dong Ethnic Autonomous Prefecture, Southeast Guizhou, in April 2009. The plant was identified by Dr. Long Chun-Lin, who works in the Kunming Institute of Botany, Chinese Academic of Sciences, and a voucher specimen (DS09064) is preserved in the Institute's herbarium.

Extraction and isolation

The dried and powdered rhizomes (2.5 kg) of *A. decipiens* were extracted with MeOH at 70°C. After removal of the solvent, the crude extracts (154 g) were partitioned into 6 fractions (Fr. 1 to Fr. 6) by silica gel column chromatography with a $CHCl_3$-MeOH gradient [1:0, 10:1, 5:1, 2:1, 1:1, 0:1 (v/v)].

Fraction 2 was separated into Fr. 2.1 and Fr. 2.2. Fr.2.1 was subjected to D_{101} resin with H_2O, 80% MeOH, 100% MeOH and Me_2CO. The partition, which was eluted by 80% MeOH, was submitted to reversed-phase [MeOH-H_2O, 80:20 (v/v)] and Sephadex LH-20 (MeOH) column chromatography, and then semi-preparative HPLC [MeOH-H_2O, 75:25 (v/v)] to obtain compounds **2** (11.1 mg), **3** (3.2 mg) and **4** (6.1 mg).

Fraction 2.2 was subjected to reversed-phase [MeOH-H_2O, 80:20 (v/v)], Sephadex LH-20 (MeOH), and silica gel [EtOAc-Me_2CO, 5:1 to 2:1 (v/v)] column chromatography, and then preparative TLC [$CHCl_3$-Me_2CO-Et_2NH, 80:16:1 (v/v/v)] to yield compound **1** (22.8 mg).

(-)-(2*R**,3*S**,6*S**)-*N*,2-Dimethyl-3-hydroxy-6-(9-phenylnonyl) piperidine (1)

Colorless amorphous solid (MeOH); $[\alpha]_D^{21.2}$ −19.4 (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log ε): 208 (3.54) nm; IR (KBr) ν_{max}: 3356, 1629, 1604 and 1467 cm^{-1}; ^1H and ^{13}C NMR, see Table I; EI-MS: *m/z* (%) = 330 ($[M - H]^+$, 2), 316 ($[M - CH_3]^+$, 3), 128 (1,2-dimethyl-3-hydroxy-piperidine, 100); HR-ESI-MS: *m/z* = 332.2946 [M + H]$^+$ (calcd for $C_{22}H_{38}NO^+$, 332.2953).

6-Deacetylnimbin (2)

Colorless, needle-like crystalloid ($CHCl_3$), $C_{28}H_{34}O_8$. ESI-MS: *m/z* = 499 [M + H]$^+$, 521 [M + Na]$^+$, 1019 [2M + Na]$^+$. ^1H and ^{13}C NMR data are similar to those in the literature (Bokel et al., 1990).

28-Deoxonimbolide (3)

Colorless, needle-like crystalloid ($CHCl_3$), $C_{27}H_{32}O_6$. EI-MS: *m/z* (%) = 452 (M^+, 75), 335 (66), 259 (85), 185 (91). ^1H and ^{13}C NMR data are consistent with those in the literature (Bokel et al., 1990).

Nimbin (4)

Colorless, needle-like crystalloid ($CHCl_3$), $C_{30}H_{36}O_9$. EI-MS: *m/z* (%) = 540 (M^+, 83), 509 (44), 498 (18), 480

Table I. ^{1}H (500 MHz) and ^{13}C NMR (100 MHz) data of compounds **1** and **1a** in $CDCl_3$

Position	**1**		**1a**	
	δ_H (m; J_{Hz})	δ_C	δ_H (m; J_{Hz})	δ_C
2	2.32 (m)	65.5	3.87 (m)	74.2
3	3.44 (ddd; 10.0, 10.0, 4.7)	70.3	3.96 (m)	66.3
4a	1.69 (m)	33.1	2.23 (m)	31.5
4b	1.37 (m)		1.85 (m)	
5a	2.06 (m)	33.3	1.90 (m)	29.7
5b	1.37 (m)		1.41 (m)	
6	2.32 (m)	63.5	3.68 (m)	74.1
1'	1.79 (m), 1.48 (m)	27.0	1.41 (2H, m)	27.1
2'	1.34 (m), 1.25 (m)	26.1	2.13 (2H, d; 14.2)	25.0
3'-7'	1.29 (10H, m)	29.3-29.8	1.29 (10H, m)	29.3-29.8
8'	1.60 (2H, m)	31.5	1.59 (2H, m)	31.4
9'	2.60 (2H, t; 7.7)	35.9	2.59 (2H, t; 7.7)	35.9
1''		142.9		142.9
2'',6''	7.17 (2H, m)	128.4	7.17 (2H, m)	128.4
3'',5''	7.26 (2H, m)	128.2	7.26 (2H, m)	128.2
4''	7.16 (m)	125.5	7.17 (m)	125.5
2-CH_3	1.33 (3H, d; 6.0)	15.6	1.66 (3H, d; 6.4)	12.2
N-CH_3	2.36 (3H, s)	34.8	2.83 (3H, s)	38.9
N -CH_3			3.27 (3H, s)	50.8

(50), 421 (31), 383 (55), 340 (47), 273 (71), 259 (53), 231 (100), 215 (32), 187 (41), 174 (52), 159 (45), 147 (56), 112 (32), 91 (45), 81 (39) and 59 (42). ^{1}H and ^{13}C NMR data are consistent with those in the literature (Bokel et al., 1990; Luo et al., 2001).

N-Methylation of 1

To determine the relative configuration of **1**, its *N*-methylation was executed as follows (Morita et al., 1999; Garrido et al., 2003): a solution of **1** (7.2 mg), MeI (0.1 mL), and Na_2CO_3 (6.7 mg) in acetone (5 mL) was heated under reflux for 4 h at 60°C and then concentrated under reduced pressure. The residue was dissolved in $CHCl_3$, washed with saturated NaCl (aq), and then dried over anhydrous Na_2SO_4. The removal of the solvent afforded **1a** (10.0 mg; 97.2%).

(-)-(2*R**,3*S**,6*S**)-*N*,*N*,2-Trimethyl-3-hydroxy-6-(9-phenylnonyl) piperidine iodide (**1a**): Colorless amorphous solid ($CHCl_3$); $[\alpha]_D^{21.4}$ −16.9 (*c* 0.38, MeOH); ^{1}H and ^{13}C NMR, see Table I; ESI-MS: *m/z* = 346 $[M]^+$.

In vitro cytotoxic assay

K562 human chronic myelogenous leukemia cell line was purchased from the China Center for Type Culture Collection, and MCF-7 human breast cancer cell line and SK-OV-3 human ovarian carcinoma cell line were bought from Shanghai Cell Bank, CAS. K562 cells in RPMI1640 medium (Sigma) and MCF-7 and SK-OV-3 cells in MEM (Hyclone) were maintained at 37°C in an atmosphere of humidified 5% CO_2; the media were supplemented with 10% fetal bovine serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd.).

Cytotoxic assays were carried out in quintuplicate in 96-well microplates (Corning), and the amount of viable cells at the end of the incubation period was measured in the MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] and SRB (sulforhodamine B) assays (Vichai and Kirtikara, 2006; Lai et al., 2010). The MTT assay was used to measure the cytotoxicity of compound **1** and **1a** on K562 cells; the SRB method was used in MCF-7 and SK-OV-3 cells. K562, MCF-7 and SK-OV-3 cells were also exposed to the solution of test compounds for 24 h. Non-treated culture cells were used as a negative control. IC_{50} was calculated using the GWBASIC software as the concentration (μM) of compound causing a 50% inhibition of cell viability. Adriamycin was used as a positive control.

RESULTS AND DISCUSSION

The molecular formula of compound **1** was identified as $C_{22}H_{37}NO$ by HR-ESI-MS (obsd $[M + H]^+$ at *m/z* 332.2946, calcd 332.2953). The IR spectrum of **1** showed absorption peaks at 3356, 1629, 1604 and 1467 cm^{-1}, which indicate the presence of hydroxy and aromatic protons. The ^{1}H NMR spectrum of **1** (Table I) revealed monosubstituted phenyl [δ_H 7.16-7.17 (3H, m) and 7.26 (2H, m)], *N*-methyl [δ_H 2.36 (3H, s)] and methyl [δ_H 1.33 (3H, d, *J* = 6.0 Hz)] groups. Careful comparison of the NMR data of **1** with those of *N*-methylirnigaine (Melhaoui and Bodo, 1995) revealed that the two compounds share the same planar structures, as confirmed by the ^{1}H-^{1}H COSY and HMBC spectra of **1**.

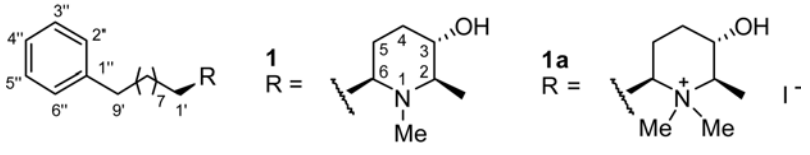

Fig. 1. Structures of **1** and **1a**

It was deduced that H-2 and H-3 are in *trans* diaxial relationship by the coupling constant ($J_{2,3}$ = 10.0 Hz) between H-2 and H-3 (Table I). H-3 was arbitrarily assigned to the β-orientation, while H-2 was α-oriented. However, the orientation of H-6 was still ambiguous due to the overlapping of H-2 (δ_H 2.32, m) and H-6 (δ_H 2.32, m). Therefore, the *N*-methylated derivative (**1a**) of **1** was prepared and then the α-orientation of both H-2 (δ_H 3.87, m) and H-6 (δ_H 3.68, m) was proved by the ROESY correlation between H-2 and H-6 in **1a**. The structure of **1** was consequently determined as (-)-(2*R**,3*S**,6*S**)-*N*,2-dimethyl-3-hydroxy-6-(9-phenylnonyl) piperidine, a C-3 epimer of *N*-methylirnigaine.

In the present study, compound **1a**, (-)-(2*R**,3*S**,6*S**)-*N*,*N*,2-trimethyl-3-hydroxy-6-(9-phenylnonyl) piperidine iodide, exhibited significant inhibitory activity against the human breast cancer cell lines with an IC_{50} value of 4.6 μM. But the same bioactivity was weak for **1**, (-)-(2*R**,3*S**,6*S**)-*N*,2-dimethyl-3-hydroxy-6-(9-phenylnonyl) piperidine. Both compounds showed stronger activity against MCF-7 cells than against K562 and SK-OV-3 cells (Table II).

In a study of the DNA affinity of the isomer of **1**, (-)-(2*S*,3*S*,6*R*)-*N*-methylirnigaine, the isomer elicited an important positive response in the HPLC detection system (90%), and it was surmised that the isomer might have antitumor, antibiotic and antimalarial bioactivities (Melhaoui and Belouali, 1998) because the mechanism of several antitumor, antibiotic and antimalarial agents involves interaction with DNA (Pezzuto et al., 1991). The stereochemistry of these kinds of alkaloids may be significant for their biological activity. It is therefore worthwhile to further study the structure-activity relationships of 2-alkylpiperidine alkaloids against breast cancer cells and other tumor cells.

The NMR data of compounds **2**-**4** are consistent with previous reports (Bokel et al., 1990; Luo et al., 2001); therefore, compounds **2**-**4** were identified as 6-deacetylnimbin, 28-deoxonimbolide, and nimbin, respectively.

6-Deacetylnimbin (**2**), 28-deoxonimbolide (**3**) and nimbin (**4**) belong to the well-known family of limonoids, which exhibit a range of biological activities, including insecticidal, insect antifeedant and insect growth regulating activities. Antibacterial, antifungal, antimalarial, anticancer, antiviral and other pharmacological activities have also been recorded (Roy and Saraf, 2006). The antitumor activities of 6-deacetylnimbin and nimbin are very weak (Cohen et al., 1996a, 1996b; Takgi et al., 2009). Meanwhile, some research has shown that 28-deoxonimbolide has strong cytotoxic potential against human breast cancer BC-1 cells, human lung cancer LU-1 cells, hormone-dependent human prostate cancer LNCaP cells, and lymphocytic leukemia P-388 cells (Kigodi et al., 1989; Cui et al., 1998).

Remarkably, nimbolide is the best potential antitumor constituent among the known limonoids (Cohen et al., 1996b). Nimbolide was not obtained in the present study, but it is thought to be biologically synthesized and present in the plant, because 28-deoxonimbolide is derived from the reduction of the carbonyl at C-28 in nimbolide during biogenesis in a unidirectional reaction (Akhila and Rani, 1999, 2002).

In conclusion, we found a new natural product, (-)-(2*R**,3*S**,6*S**)-*N*,2-dimethyl-3-hydroxy-6-(9-phenylnonyl) piperidine (**1**), which is a C-3 epimer of *N*-methylirnigaine and shows little inhibitory activity towards the tested cancer cell lines. Moreover, this study also indicated that some antitumor limonoids found in plants of Rutales, especially in Meliaceae, Rutaceae, and less frequently in Cneoraceae and *Harrisonia* spp. of Simaroubaceae (Roy and Saraf, 2006), are also distributed in the genus *Arisaema*. All of these findings could partially explain the traditional use of *A. decipiens* in the Dong ethnic community.

Table II. IC_{50} (μM) of *in vitro* cytotoxic activity assay of **1** and **1a**

Compounds	MCF-7	SK-OV-3	K562
1	62.1	261.9	inactive
1a	4.6	17.8	49.7
Adriamycin	0.35	0.13	0.22

K562: Human chronic myelogenous leukemia cell line. SK-OV-3: Human ovarian carcinoma cell line. MCF-7: Human breast cancer cell line. Adraimycin as positive control.

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