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One New Pregnane Glycoside from the Seeds of Cultivated *Brucea javanica*

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A new pregnane glycoside, named (20*R*)-*O*-(3)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-pregn-5-en-3β,20-diol (**1**), and seven known compounds, brusatol (**2**), bruceine B (**3**), bruceine D (**4**), yadanziolide A (**5**), bruceine E (**6**), yadanzioside G (**7**), and yadanzioside B (**8**), were isolated from the cultivated dry seeds of *Brucea javanica*. The structure of **1** was elucidated on the basis of 1D- and 2D-NMR spectroscopic analyses. Their inhibitory effects on tumor cells were also tested. Compound **1** was slightly active against HL-60, SMMC-7721, A-549, and MCF-7 tumor cells. Compounds **2** and **3** demonstrated significant inhibitory activities against all tested cells. These results indicate that cultivated *B. javanica* could replace the wild plant as an antitumor plant resource.

Key words: *Brucea javanica*, Pregnane glycoside, Antitumor activity, Brusatol

INTRODUCTION

Brucea Javanica (L.) Merr. is distributed mainly in south China and exhibits bio-activities including anti-tumor and anti-malaria effects (Sharma and Agarwal, 1993). In previous phytochemical research, the plant material of *B. javanica* used was mainly the wild plant. As a potential crude drug with anti-pancreatic cancer agents (Lau et al., 2008), the plant of *B. javanica* was widely cultivated in south of China. However, the differences in the phytochemistries of cultivated and wild material are not clear.

In order to study the differences in the chemical constituents of wild and cultivated *B. javanica*, the constituents of cultivated *B. javanica* were investigated. In this paper, we describe the isolation and structure elucidation of a new pregnane glycoside (**1**) from the cultivated plant of *B. javanica*, together with 7 known compounds, brusatol (**2**) (Harigaya et al., 1989), bruceine B (**3**) (Anderson et al., 1987), bruceine D (**4**) (Lee et al., 1979), yadanziolide A (**5**) (Yoshimura et al., 1984), bruceine E (**6**) (Li and Tso, 1980), yadanzioside G (**7**) and yadanzioside B (**8**) (Saka et al., 1984). In addition, their inhibitory effects on tumor cells were also investigated. This study showed that cultivated *B. javanica* could replace the wild plant as an antitumor plant resource.

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MATERIALS AND METHODS

General procedures

Glucose and arabinose were purchased from Sigma. Column chromatography (CC): silica gel (SiO_2; 200-300 mesh; Qingdao Marine Chemical Products Industry Factory); Sephadex LH-20 (Pharmacia). TLC: silica gel G precoated plates (Qingdao Haiyang Chemical Co.); spots were visualized by spraying with 10% aq. H_2SO_4 solvent, followed by heating. GC: Shimadzu GC-17A gas chromatograph equipped with an H_2 flame ionization detector; column: TC-1 capacity column (30 m × 0.25 mm); detector, FID. Optical rotations: Horiba SEAP-300 spectropolarimeter. IR Spectra: Shimadzu IR-450 instrument, with KBr pellets; in cm^{-1}. NMR Spectra: Bruker AV-400 or DRX-500 instruments; chemical shifts δ in ppm relative to Me_4Si, coupling constants *J* in Hz. FAB-MS (negative-ion mode;

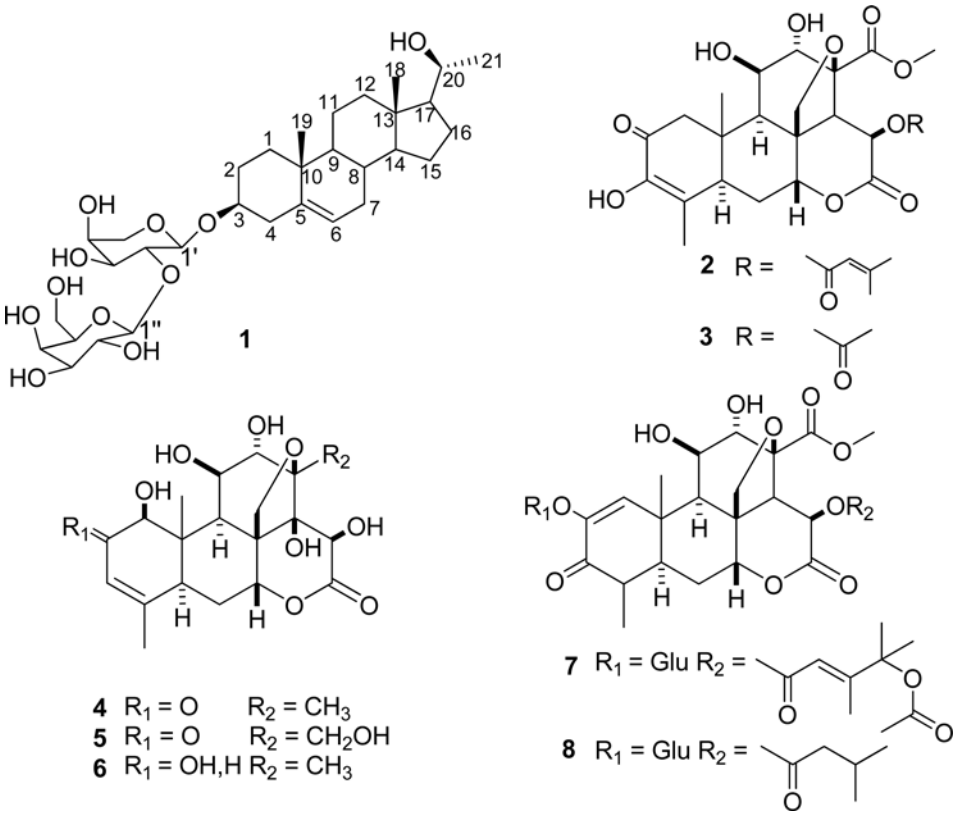

Fig. 1. Structures of **1**-**8**

glycerol matrix) and HR-ESI-MS: VG Auto-Spec 3000 and Thermo-Finnigan LCQ Advantage spectrometer; in *m/z* (rel. int. in % of the base peak).

Plant material

The seeds of *Brucea javanica* were purchased from XiShuangBanNa, Yunnan Province, China, in August 2009, and identified by Prof. Shukun Chen, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The dry fruits of *B. javanica* (5 kg) were extracted three times with MeOH under reflux. After removal of solvent, the extract (300 g) was suspended in water and then successively partitioned with petroleum ether (PE) and ethyl acetate (EtOAc). After evaporation of the solvent, the EtOAc extract (80 g) was subjected to column chromatography over silica gel with gradient elution with CH_3Cl-MeOH (60:1-1:1) to obtain fractions I-VIII. Fraction II (10 g) was separated over silica gel with CH_3Cl-MeOH (30:1) to yield **2** (520 mg) and **3** (83 mg). Fraction IV (12 g) was separated over silica gel with CH_3Cl-MeOH (25:1) and then over Sephadex LH-20 column eluted with MeOH to obtain compounds **4** (20 mg), **5** (18 mg), and **6** (30 mg). Fraction VI (13 g) was separated over silica gel with CH_3Cl-MeOH (18:1) and then over Sephadex LH-20 column eluted with MeOH to yield compounds **7** (30 mg), **8** (25 mg), and **1** (10 mg).

(20R)-*O*-(3)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-pregn-5-en-3β, 20-diol (1)

White amorphous powder; $[\alpha]_D^{25}$ + 82.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.08) nm; IR (KBr) υ_{max}: 3411, 2927, 1464, 1358, 1076 cm^{-1}; ^1H- and ^{13}C-NMR see Table I; negative FABMS *m/z* 611 $[M - H]^-$; HRFABMS (*m/z* calcd. 612.3510, found at 612.3501 $[M]^-$).

Sugar analysis

Compound **1** (2 mg) in 2 N HCl/1,4-dioxane 1 : 1 (2 mL) was heated at 90°C for 4 h in a water bath. The mixtures were neutralized with $AgCO_3$, filtered, and then extracted with $CHCl_3$ (3 × 1 mL). After concentration, the H_2O layer (monosaccharide portion) was dissolved in 0.6 mL of pyridine, then 0.4 mL of hexamethyl disilazane and 0.2 mL trimethyl chlorosilane were added successively. The mixture was kept at 60°C for 10 min in a water-bath. Next, the mixture was centrifuged for 20 min at 1.0×10^4 rpm. The supernatant was subjected to GC analysis under the following conditions: Shimadzu GC-17A gas chromatog-

raph equipped with an H_2 flame ionization detector. Column: TC-1 capacity column (30 m × 0.25 mm). Column temp. 200-260°C, programmed increase: 38/min; injection temp. 250°C; carrier gas: N_2 (1 mL/min); injection volume: 1 mL; split ratio: 1/50; glucose and arabinose 11.99 min and 9.13 min, respectively.

Cytotoxicity assay

A panel of human tumor cell lines was used: breast cancer MCF-7, hepatocellular carcinoma SMMC7721, human myeloid leukemia HL-60, colon cancer SW480 and lung cancer A549. The cells lines were obtained from Shanghai Cell Bank in China. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone), supplemented with 10% fetal bovine serum (Hyclone), at 37°C in a humidified atmosphere with 5% CO_2. The cytotoxicity assay was performed by the MTT (2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method in 96-well microplates (Mosmann, 1983). After compound treatment, cell viability was assessed and the cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method (Reed and Muench, 1938).

RESULTS AND DISCUSSION

Chemical structure elucidation of compound 1

Compound **1** was obtained as white powder. Its molecular formula was $C_{32}H_{52}O_{11}$ on the basis of HRFABMS (*m/z* calcd. 612.3510, found at 612.3501 $[M]^-$). The FABMS spectrum of **1** showed the $[M - H]^-$ peak at *m/z* 611 and the $[M - glu - H]^-$ peak at *m/z* 449. Hydrolysis of **1** yielded D-glucose and L-arabinose on GC and in its ^{1}H- and ^{13}C-NMR spectra. The two anomeric H-atom signals of H-1' at 5.10 (d, J = 5.6 Hz) and of H-1" at 5.16 (d, J = 7.6 Hz), respectively, suggested the presence of an α-arabinopyranoside and a β-glucopyranosyl moiety, respectively.

The ^{1}H-NMR spectrum of **1** (Table I) contained two methyl singlets at δ 0.97 (s) and δ 0.70 (s) and one methyl doublet at 1.42 (d, J = 6.1 Hz), suggesting a pregnane skeleton. This was supported by the ^{13}C-NMR (3 × CH_3, 8 × CH_2, 7 × CH and 3 × C for the aglycone). The chemical shift of the two olefinic carbons (a quaternary carbon at 141.0 and a tertiary one at 121.9) were in good agreement with the pregn-5-en-3-*O*-glycosides (Srivastar et al., 1994). The ^{13}C-NMR spectral features of **1** and (20*R*)-*O*-(3)-α-L-arabinopyranosyl-pregn-5-en-3β,20-diol (Christine et al., 1995) were nearly identical to each other, except for one additional glucosyl unit in **1**.

The linkage sites and sequences of the saccharides and the aglycone were determined by HMBC experiments. Long-range correlations between H-3 (δ 3.77) and C-1' (δ 103.2), together with correlations between H-2' (δ 4.56-4.60) and C-1', C-3' (δ 74.7), and C-1" (δ 105.8), were observed (Fig. 2). Meanwhile, some of the signals were observed in the H-H COSY experiments (Fig. 2), and these data further illustrated the structure of **1**. The configuration of **1** at C-20 was determined by comparison with the methyl proton shifts of the 20-epimers of pregn-5-en-3β,20-diol (Cooley et al., 1977), whereas good correspondence was found for the values of the (20*R*)-configuration. Thus, the structure of **1** was determined as (20*R*)-*O*-(3)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-pregn-5-en-3β,20-diol.

Table I. ^{1}H, ^{13}C-NMR spectral data of **1** (in pyridine-d_5, at 500 MHz and 125 MHz, respectively)

Position	δ_C	δ_H (ppm) (J in Hz)	Position	δ_C	δ_H (ppm) (J in Hz)
1	37.6 t	1.74-1.78, 1.00-1.03 2m	17	59.4 d	1.50-1.54 m
2	30.3 t	2.16-2.20, 1.78-1.82 2m	18	12.6 q	0.70 s
3	78.6 d	3.77 (d, J = 10.5)	19	19.5 q	0.97 s
4	39.1 t	2.73 (dd, J = 12.6, 2.5); 2.55 (t, J = 12.1)	20	69.0 d	3.93 m
5	141.0 s		21	24.8 q	1.42 (d, J = 6.1)
6	121.9 d	5.34 br s	1'	100.9 d	5.10 (d, J = 5.6)
7	32.2 t	1.82-1.86, 1.44-1.49 2m	2'	81.7 d	4.56-4.60 m
8	31.8 d	1.32-1.36 m	3'	73.1 d	4.38-4.42 m
9	50.4 d	0.85-0.89 m	4'	68.1 d	4.37-4.41 m
10	37.0 s		5'	65.0 t	4.28-4.31, 3.70-3.75 2m
11	21.1 t	1.36-1.41 m	1"	106.2 d	5.16 (d, J = 7.6)
12	39.1 t	1.87-1.90, 1.03-1.13 2m	2"	76.4 d	4.11 (t, J = 8.8)
13	41.6 s		3"	78.7 d	3.88-3.91 m
14	56.8 d	0.92-0.96 m	4"	71.5 d	4.22-4.28 m
15	24.5 t	1.58-1.61, 1.12-1.10 m	5"	78.2 d	4.21 (t, J = 8.8)
16	26.8 t	2.21-2.16, 1.91-1.89 2m	6"	62.7 t	4.50-4.53, 4.40-4.45 2m

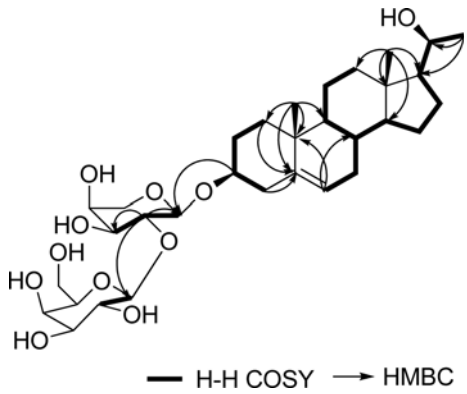

Fig. 2. Selected HMBC and H-H COSY correlations of **1**

Table II. Cytotoxic activity of compounds **1**-**8** (IC_{50} values: μM)

Compounds	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	30.22	22.67	34.56	19.80	>40
2	0.06	<0.064	<0.064	0.08	0.10
3	0.27	0.15	0.24	0.54	0.30
4	1.14	0.88	3.30	6.75	7.78
5	4.48	4.27	7.62	17.77	28.48
6	26.32	12.35	17.05	14.61	>40
7	>40	>40	>40	>40	>40
8	>40	>40	>40	>40	>40
Cis-platin (MW300)	3.17	11.27	16.46	18.70	8.00
Taxol	<0.008	0.02	0.70	0.02	0.96

The isolates were evaluated for their cytotoxicity against the human HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines using the MTT assay. Compound **1** was slightly active, with IC_{50} values around 20 μM against HL-60, SMMC-7721, A-549, and MCF-7. Compounds **2** and **3** demonstrated significant inhibitory activities against all tested cells. Other compounds also had cytotoxic activities, except compounds **7** and **8** (Table II).

In our research, we found that the main compounds, including brusatol, bruceine B, bruceine D, and yandanziolide A, from cultivated *B. javanica* were similar to those from wild *B. javanica*. Furthermore, these main compounds had significant antitumor effects in the cytotoxicity assay. Therefore, in our opinion, this cultivated *B. javanica* could replace the wild plant as an antitumor plant resource.

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