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QUASSINOID XYLOSIDES, JAVANICOSIDES G AND H, FROM SEEDS OF *BRUCEA JAVANICA*

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Abstract – Two new quassinoid xylosides, javanicosides G and H, were isolated from the seeds of *Brucea javanica* (L.) Merr. (Simaroubaceae). Their structures were elucidated by the analysis of spectral data and chemical evidence.

INTRODUCTION

Brucea javanica (L.) Merr. (Simaroubaceae) is a shrub which is distributed from Southeast Asia to northern Australia. Its seeds, rich in quassinoids,[1-4] have been used for the treatment of dysentery, malaria and cancer,[5,6] and indeed some of the quassinoids from this plant have been shown to be responsible for antiamoebic,[7] antimalarial[8] and antitumor[9] activities. In the present study, from seeds of this plant we isolated two new quassinoid glycosides, javanicosides G (**1**) and H (**2**), which are unusual in that their sugar component is D-xylose. This paper describes their isolation and structure elucidation.

RESULTS AND DISCUSSION

Silica gel column chromatography ($CHCl_3$/MeOH 1:0, 20:1, 5:1 and 0:1) of the $CHCl_3$-soluble portion from a hot MeOH extract of the seeds of *B. javanica* gave six fractions. Of them, the $CHCl_3$/MeOH (5:1) eluate gave, after Diaion HP-20 column chromatography and subsequent repeated reversed-phase HPLC, two new quassinoids, javanicosides G (**1**) and H (**2**).

Javanicoside G (**1**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{31}H_{40}O_{15}$ by the $[M+Na]^+$ ion peak at *m/z* 675.2211 (calcd for $C_{31}H_{40}O_{15}Na$ 675.2265) in the HRESIMS. The ESIMS showed a fragment ion peak $[MH-C_5H_8O_4]^+$ at *m/z* 521, which suggested that **1** was a pentoside. The IR spectrum showed the presence of hydroxyl (3427 cm^{-1}), δ-lactone and ester (1732 cm^{-1}), and α,β-unsaturated ketone (1645 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **1** were very similar to those of bruceoside A (**3**) (the structure shown in Figure 1 for reference), also isolated from this plant source,[9] except for the signals caused by the sugar moiety. Its ^1H NMR spectrum showed the

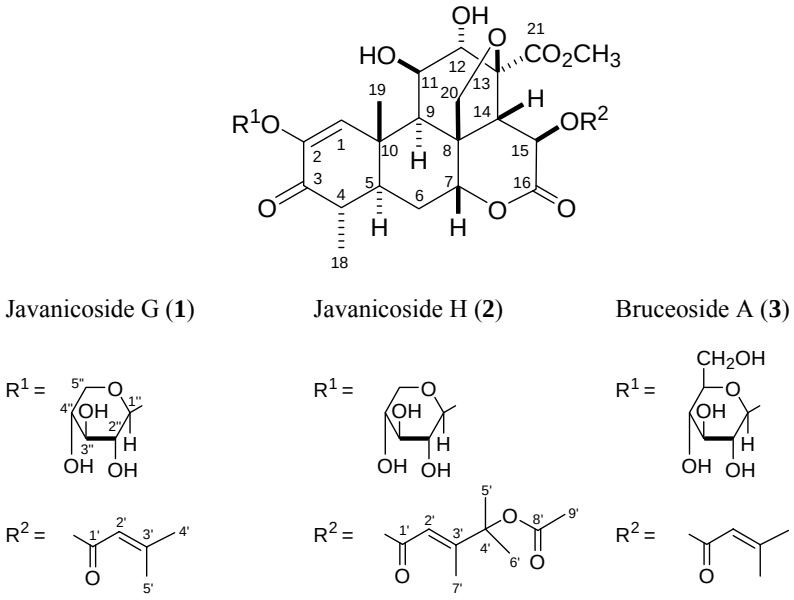

Figure 1

presence of one secondary methyl (δ 1.15), one tertiary methyl (δ 1.83), two olefinic methyls (δ 2.13 and 1.66), one carbomethoxy group (δ 3.76) and two olefinic protons (δ 7.19 and 5.85) (Table 1). As to the side chain, its ^{13}C NMR (δ 165.2, 158.4, 115.9, 27.0 and 20.1) and HMBC spectra showed the presence of a senecioyloxy group connected to C-15 with β-configuration, as demonstrated by the NOESY correlations between CH_3O-21/H-2', H_3-4', and H_3-5', and between H-9/H-15 (Figure 2). Its ^{13}C NMR spectrum and the HMBC correlations between H_3-18/C-3 and between H-1″/C-2 demonstrated that an α,β-unsaturated ketone carbonyl group (δ 194.7) was at C-3 and that the pentose unit (δ 102.4, 77.8, 74.3, 70.6 and 66.9) was connected to the C-2 oxygen atom, respectively, as in bruceoside A (**3**) (Table 1). Thus, **1** and **3** were shown to have the same aglycone moiety. The NOESY correlations between H-1″, H-3″ and $H_α$-5″ and the chemical shift values in its ^{13}C NMR spectrum indicated that the sugar component was D-xylose, which was confirmed by acid hydrolysis of **1** followed by the HPLC analysis of the hydrolysate using an aminopropyl-bonded silica gel column and an optical rotation detector. The relatively large *J* value (7.3 Hz) of the anomeric proton of the xylosyl moiety indicated that the xyloside linkage was β. The analysis of NOESY spectrum afforded further information about the stereochemistry

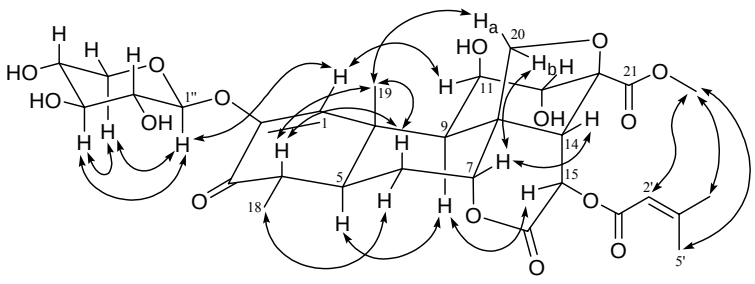

Figure 2 Selected NOESY correlations for **1**.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds (**1**) and (**2**) in C_5D_5N

Position		Javanicoside G (**1**)		Javanicoside H (**2**)	
		δ_C	δ_H[a]	δ_C	δ_H[a]
1		128.8	7.19 (s)	129.6	7.21 (s)
2		148.8		148.9	
3		194.7		194.7	
4		41.3	2.48 (m)	41.3	2.50 (m)
5		43.3	2.13 ([b])	43.8	2.17 ([b])
6	α	30.0	2.04 (d, 14.8)	30.0	2.07 (d, 14.7)
	β		1.70 (ddd, 14.8, 13.8, 2.5)		1.72 (ddd, 14.7, 13.8, 2.1)
7		83.4	5.01 (br s)	83.5	5.06 (br s)
8		46.7		46.7	
9		40.4	2.58 (d, 4.4)	40.4	2.60 (d, 4.3)
10		39.8		39.8	
11		73.4	5.17 ([b])	73.5	5.18 (d, 4.3)
12		76.2	5.17 ([b])	76.1	5.19 ([b])
13		82.6		82.6	
14		50.4	4.04 ([b])	50.0	4.10 ([b])
15		68.3	6.92 ([b])	69.0	[c]
16		168.3		168.0	
18		12.5	1.15 (d, 6.7)	12.5	1.17 (d, 6.7)
19		18.1	1.83 (s)	18.1	1.84 (s)
20	a	73.8	5.13 (d, 7.4)	73.8	5.15 (d, 7.4)
	b		3.95 (d, 7.4)		3.96 (d, 7.4)
21		171.2		171.2	
OMe		52.3	3.76 (s)	52.6	3.89 (s)
1'		165.2		165.7	
2'		115.9	5.85 (s)	113.6	6.10 (s)
3'		158.4		163.4	
4'		27.0	1.66 (s)	82.3	
5'		20.1	2.13 (s)	25.7	1.43 (s)
6'				26.3	1.39 (s)
7'				14.5	2.25 (s)
8'				169.5	
9'				21.4	1.94 (s)
1"		102.4	5.29 (d, 7.3)	102.5	5.30 (d, 7.2)
2"		74.3	4.22 (dd, 8.0, 7.3)	74.3	4.23 (dd, 8.1, 7.2)
3"		77.8	4.15 (t-like, 8.4)	77.8	4.14 (t-like, 8.5)
4"		70.6	4.18 (m)	70.7	4.19 (m)
5"	α	66.9	3.58 (dd, 11.3, 8.7)	67.0	3.60 (t, 10.7)
	β		4.10 (dd, 11.3, 4.7)		4.11 (dd, 11.0, 4.8)
11-OH			6.92 (br s)		6.98 (br s)
12-OH			7.90 (br s)		8.03 (br s)

[a] Multiplicity and *J* values in Hz given in parentheses. Hydroxyl proton signals of the sugar moiety not assigned due to broadening and overlapping. [b] Multiplicity not determined due to overlapping of the signals. [c] Signal not detected due to broadening.

of **1** (Figure 2). Correlations observed between H-4/H_β-6, H-4/H_3-19, H_β-6/H_3-19 and H-7/H-14 implied that these protons and the methyl group involved were all of β-configuration, whereas those between H-5/H-9 and H_α-6/H_3-18 suggested that these protons and the methyl group involved were all of α-configuration. Accordingly, javanicoside G (**1**) was determined to have the structure shown in Figure 1.

Javanicoside H (**2**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{35}H_{46}O_{17}$ by the $[M+Na]^+$ ion peak at *m/z* 761.2640 (calcd for $C_{35}H_{46}O_{17}Na$ 761.2633) in the HRESIMS. The ESIMS showed a fragment ion peak $[MNa-C_5H_8O_4]^+$ at *m/z* 629, which indicated that **2** was a pentoside. Its ^1H NMR spectrum showed the presence of one secondary methyl (δ 1.17), three tertiary methyls (δ 1.84, 1.43 and 1.39), one olefinic methyl (δ 2.25), one acetyl (δ 1.94), one carbomethoxy group (δ 3.89) and two olefinic protons (δ 7.21 and 6.10) (Table 1). Comparison of the NMR spectra of **1** and **2** revealed that they had the same ring system and sugar moiety, with a different ester side chain moiety at C-15. Analysis of the ^{13}C NMR (δ 169.5, 165.7, 163.4, 113.6, 82.3, 26.3, 25.7, 21.4 and 14.5), ^1H-^1H COSY and HMBC spectra revealed that the side chain at C-15 of **2** was an (*E*)-4-acetoxy-3,4-dimethyl-2-pentenoyloxy group (Table 1). The ^{13}C NMR spectrum (δ 102.5, 77.8, 74.3, 70.7 and 67.0) suggested that the pentose component was D-xylose, which was confirmed by acid hydrolysis of **2** followed by HPLC. The NOESY correlations showed that **2** had the same stereochemistry as **1**. On the basis of these data, javanicoside H (**2**) was determined to have the structure shown in Figure 1.

Although quite a few quassinoid glycosides have been reported from plants of the Simaroubaceae family,[2-4,10-16] they all contain D-glucose as the sugar component. Javanicosides G (**1**) and H (**2**) are the first examples of natural quassinoid xylosides.

Some of the quassinoids from this plant are known to have a strong cytotoxic activity.[7,17] However, present javanicosides G (**1**) and H (**2**) showed a rather weak cytotoxic activity against P-388 murine leukemia cells with IC_{50} values of 7.2 and 19 μg/mL, respectively.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 digital polarimeter, UV spectra on a Hitachi U-2000A spectrophotometer and IR spectra on a Perkin-Elmer 1710 spectrophotometer. NMR spectra were measured in C_5D_5N on a Bruker DRX-500 spectrometer. The ^1H chemical shifts were referenced to the residual C_5D_4HN resonance at 7.21 ppm, and the ^{13}C chemical shifts to the solvent resonance at 135.5 ppm. ESI MS spectra were obtained on a Micromass LCT spectrometer. Preparative HPLC was performed on a Tosoh CCPP-D system equipped with a JASCO 875-UV detector (at 220 nm) and a reversed-phase column, TSK-Gel ODS-80 TM (10 μm, 20 mm i.d. × 300 mm) or Inertsil PREP-ODS (10 μm, 20 mm i.d. × 250 mm) (MeOH/H_2O or MeCN/H_2O, flow rate 10 mL/min). Analytical HPLC was performed on a Tosoh CCPM system equipped with a Tosoh CCP

PX-8010 controller, a Tosoh RI-8010 detector, a Shodex OR-2 optical rotation detector and a CAPCELL PAK column, NH_2 UG80 (5 μm, 4.6 mm i.d. × 250 mm) (MeCN/H_2O (85:15), flow rate 1 mL/min).

Plant material. The seeds of *Brucea javanica* (L.) Merr. were purchased in China in 2000, and the botanical origin of seeds was identified by Dr. K. Takeya, Prof. of Medicinal Plant Chemistry of Tokyo University of Pharmacy and Life Science. A voucher specimen was deposited in the herbarium of this university.

Extraction and isolation. Dried and ground seeds of *B. javanica* (20 kg) were extracted four times with MeOH (18 L) under reflux for 24 h. The solvent was removed *in vacuo* to give a residue (*ca*. 1 kg), which was suspended in H_2O (2 L). Then the suspension was extracted with *n*-hexane (2 × 1 L), $CHCl_3$ (2 × 1 L), and *n*-BuOH (2 × 1 L), successively, and the solvent was removed *in vacuo* to afford *n*-hexane-soluble (439 g), $CHCl_3$-soluble (105 g), and *n*-BuOH-soluble (363 g) portions, respectively. The $CHCl_3$-soluble portion was placed on a silica gel column (1 kg) and eluted sequentially with $CHCl_3$ containing an increasing amount of MeOH (1:0, 20:1, 5:1 and 0:1) to give six fractions.

The $CHCl_3$/MeOH (5:1) eluate (21.6 g) of the silica gel column was placed on a Diaion HP-20 (315 g) column and eluted successively with MeOH (2 L) and acetone (1 L) to give two fractions. After removal of the solvent, the MeOH fraction (19.5 g) was further subjected to reversed-phase HPLC using MeOH/H_2O (40:60 and then 1:0) to afford thirteen fractions (frs. 1–13). After removal of the solvent to dryness, by reversed-phase HPLC using MeCN/H_2O (20:80), fr. 8 (64.8 mg) afforded compound (**1**) (17.6 mg).

By analogous reversed-phase HPLC using MeOH/H_2O (45:55 and 1:0), fr. 13 (3.2 g) afforded seven sub-fractions (frs. 13A–13G), which were evaporated to dryness. By reversed-phase HPLC using MeOH/H_2O (38:62), fr. 13C (107.6 mg) afforded compound (**2**) (12.5 mg).

Javanicoside G (1): Amorphous powder; $[\alpha]^{26}_D$ +13.8° (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.16), 250 (3.90) nm; IR (film) ν_{max} 3427, 2927, 1732, 1645 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 675.2211 $[M+Na]^+$ (calcd for $C_{31}H_{40}O_{15}Na$, 675.2265).

Identification of sugar component by acid hydrolysis. A solution of **1** (6.1 mg) in 0.1 M H_2SO_4 (1 mL) was heated at 90 °C for 30 min under an Ar atmosphere. After cooling, H_2O (5 mL) was added to the mixture, and the whole was extracted with $CHCl_3$ (3 × 5 mL). The combined $CHCl_3$ layers were washed with brine, dried over Na_2SO_4, and evaporated to give an aglycon fraction (2.3 mg). The H_2O layer was passed through a short Amberlite IRA-400 column and evaporated to dryness to give a sugar fraction (1.1 mg). The sugar fraction was dissolved in MeOH/H_2O (2:8) and after passing through a Sep-Pak C_{18} cartridge, it was analyzed by HPLC using MeCN/H_2O (85:15). The sugar component was identified as D-xylose by the HPLC retention time, t_R 8.01 min (D-xylose, t_R 7.90 min), and the sign (positive) of optical rotation.

Javanicoside H (2): Amorphous powder; $[\alpha]^{26}_{D}$ +9.6° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.22), 253sh (3.89) nm; IR (film) ν_{max} 3446, 1728, 1643 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 761.2640 $[M+Na]^{+}$ (calcd for $C_{35}H_{46}O_{17}Na$, 761.2633).

Identification of sugar component by acid hydrolysis. Compound (**2**) (3.8 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction (1.8 mg) and a sugar fraction (1.6 mg). The sugar component was identified as D-xylose by the HPLC retention time, t_R 7.90 min, and positive optical rotation.

Cytotoxic activity assay. The assay was performed in the same manner as described previously.[18]

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