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Quassinoid glucosides from seeds of *Brucea amarissima*

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Abstract

Quassinoid glucosides, javanicosides I, J, K and L, were isolated from the seeds of *Brucea amarissima* (Lour.) Desv. ex B. A. Gomes (Simaroubaceae), along with two known quassinoids, i.e. bruceins D and E, and seven known quassinoid glucosides, yadanziosides B, C, E, I and K, bruceoside B and yadanzigan. Their structures were elucidated by analysis of the spectral data and chemical evidence.

Keywords: *Brucea amarissima*; *Brucea javanica*; Simaroubaceae; Quassinoid; Javanicosides I, J, K and L

1. Introduction

Brucea amarissima (Lour.) Desv. ex B. A. Gomes (syn. *Brucea javanica* (L.) Merr.) is a shrub which is distributed from Southeast Asia to northern Australia. Its seeds, having been used for the treatment of dysentery, malaria and cancer (Lin et al., 1990; Anderson et al., 1991), are known also as a rich source of quassinoids (Okano et al., 1981; Sakaki et al., 1984, 1985, 1986a,b; Yoshimura et al., 1984; Ohnishi et al., 1995). As regards its bioactive components, some quassinoids from *B. amarissima* are reported to have interesting biological effects, such as antimalarial (Pavanand et al., 1986), antitumor (Lee et al., 1979) and antiamoebic (Wright et al., 1988) activities. In our previous paper (Kim et al., 2003), we reported the isolation, structural elucidation, and stereochemistry of three quassinoids, javanicolides A and B, and javanicoside A. In our further studies on the seeds of this plant, we have isolated four new quassinoid glucosides, javanicosides I (**1**)–L (**4**), along with two known quassinoids, bruceins D and E (Lee et al., 1979), and seven known quassinoid glucosides, yadanziosides B (**5**), C (**6**), E (**7**) (Sakaki et al., 1985), I (Yoshimura et al., 1985) and K (Sakaki et al., 1986a), bruceoside B (Lee et al., 1979) and yadanzigan (Zhang et al., 1983). This paper describes the isolation and structural elucidation of the new quassinoid glucosides.

2. Results and discussion

By Diaion HP-20 resin column chromatography (H_2O/MeOH 1:0, 1:1, 1:4 and 0:1), the *n*-BuOH-soluble portion from a hot MeOH extract of the seeds of *B. amarissima* gave five fractions. The H_2O/MeOH (1:1) eluate gave nine fractions when subjected to reversed-phase HPLC using MeOH/H_2O (26:74 and 1:0). On standing, the first fraction produced a crystalline precipitate, which, by reversed-phase HPLC, gave two known quassinoids, bruceins D and E. Reversed-phase HPLC of the mother liquor afforded two known quassinoid glucosides, yadanzioside I and yadanzigan. Silica gel column chromatography ($CHCl_3$/MeOH 10:1, 5:1, 3:1 and 0:1) of the H_2O/MeOH (1:4) eluate, and subsequent repeated reversed-phase HPLC of its $CHCl_3$/MeOH

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(3:1) eluate afforded four new quassinoid glucosides, javanicosides I (**1**), J (**2**), K (**3**) and L (**4**), and five known quassinoid glucosides, yadanziosides B (**5**), C (**6**), E (**7**) and K, and bruceoside B. Identification of the known compounds was accomplished by the comparison of their spectral data with those in the literature.

Javanicoside I (**1**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{32}H_{42}O_{16}$ by the $[M + Na]^+$ ion peak at *m/z* 705.2334 (calcd for $C_{32}H_{42}O_{16}Na$ 705.2371) in the HRESIMS. The ESIMS showed a fragment ion peak $[M + H-C_6H_{10}O_5]^+$ at *m/z* 521, which suggested that **1** was a glycoside. The IR spectrum showed the presence of hydroxyl (3398 cm^{-1}), δ-lactone and ester (1741 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **1** were very similar to those of yadanzioside N (**8**) (Sakaki et al., 1986b), suggesting that **1** was an analogue of **8**. Its ^1H NMR spectrum indicated the signals of one secondary methyl (δ 0.86), one tertiary methyl (δ 1.85), one carbomethoxy group (δ 3.74), one olefinic proton (δ 6.08) and signals due to a hexose unit as in that of **8** (Table 1), thus implying that the difference between **1** and **8** was only in the ester side chain at C-15. The ester side chain at C-15 of **1** was shown to be a senecioyloxy group by analysis of the ^{13}C NMR (δ 165.4, 158.5, 115.9, 26.9 and 20.1), H–H COSY and HMBC spectra (Table 2). In the ROESY spectrum, H-2′, H_3-4′, and H_3-5′ were demonstrated to be correlated with CH_3O-21 (Fig. 2). Those ROESY correlations were possible only when the senecioyloxy group was present at C-15 with a β-orientation, so the

Table 1
^1H NMR spectral data for compounds **1**–**4** in C_5D_5N[a]

	1[b,d]	**2**[b,d]	**3**[c,d]	**4**[b,d]
1α		2.51 (*d*, 16.2)	3.98 (–[e])	2.01 (*br d*, 14)
1β		3.27 (*d*, 16.2)		2.62 (*dd*, 11.6, 3.9)
2			4.93 (*br s*)	4.33 (*m*)
3	6.08 (*d*, 2.2)		5.62 (*br s*)	4.25 (*br s*)
4	2.23 (*m*)			1.56 (–[e])
5	1.87 (–[e])	3.06 (*br d*, 12)	2.58 (*br d*, 13)	2.26 (–[e])
6α	2.01 (*d*, 15.0)	2.28 (*d*, 14.6)	2.09 (*dt*, 14.7, 2.6)	2.01 (*d*, 14.6)
6β	1.54 (*t*, 12.8)	1.73 (*ddd*, 14.6, 12.8, 2.1)	1.63 (*ddd*, 14.7, 13.5, 2.6)	1.41 (*ddd*, 14.6, 13.8, 2.2)
7	4.87 (*br s*)	5.11 (*br s*)	4.95 (*br s*)	4.98 (–[e])
9	2.92 (*d*, 4.1)	2.62 (*d*, 4.2)	2.51 (*d*, 4.6)	2.31 (*d*, 4.2)
11	6.16 (*d*, 4.0)	4.79 (*br s*)	5.48 (*t*, 4.9)	4.82 (*t*, 4.7)
12	5.10 (–[e])	5.06 (*br s*)	5.09 (*br s*)	5.02 (–[e])
14	3.97 (–[e])	4.10 (*br d*, 12.7)	3.94 (–[e])	3.92 (–[e])
15	6.90 (*br s*)	7.17 (–[e])	6.92 (*br s*)	6.74 (*br s*)
18	0.86 (*d*, 6.8)	2.07 (*s*)	1.45 (*s*)	1.07 (*d*, 6.7)
19	1.85 (*s*)	1.73 (*s*)	1.45 (*s*)	1.56 (*s*)
20a	5.08 (*d*, 7.4)	5.12 (*d*, 7.5)	5.22 (*d*, 7.3)	5.13 (*d*, 7.2)
20b	3.87 (*d*, 7.4)	3.98 (*d*, 7.5)	3.89 (*d*, 7.3)	3.87 (*d*, 7.2)
OMe	3.74 (*s*)	3.56 (*br s*)	3.71 (*s*)	3.74 (*s*)
2′	5.86 (*s*)		6.73 (*br s*)	5.81 (*s*)
3′		8.23 (*d*, 7.5)		
4′	1.66 (*s*)	7.50 (*t*, 7.7)		1.64 (*s*)
5′	2.14 (*s*)	7.37 (*t*, 7.4)	1.42 (*s*)[f]	2.12 (*s*)
6′		7.50 (*t*, 7.7)	1.44 (*s*)[f]	
7′		8.23 (*d*, 7.5)	2.40 (*s*)	
1″	5.43 (*d*, 7.4)	5.46 (*d*, 7.0)	5.20 (*d*, 7.8)	4.99 (*d*, 7.9)
2″	4.27 (*t-like*, 7.5)	4.25 (–[e])	4.12 (*t*, 8.1)	4.01 (*t*, 8.0)
3″	4.30 (*d*, 8.3)	4.28 (–[e])	4.23 (–[e])	4.21 (*t*, 8.5)
4″	4.22 (*t*, 9.3)	4.29 (–[e])	4.24 (–[e])	4.23 (–[e])
5″	3.98 (*m*)	3.89 (*m*)	4.00 (*m*)	3.91 (*m*)
6″	4.52 (*dd*, 12.0, 2.1)	4.46 (*d*, 10.2)	4.56 (*d*, 11.7)	4.53 (*br d*, 8)
	4.33 (–[e])	4.34 (*m*)	4.40 (*dd*, 11.7, 5.0)	4.37 (*m*)
1-OH			6.52 (*br s*)	
3-OH				5.07 (*br s*)
11-OH	6.31 (*d*, 5.6)	6.57 (*br s*)	5.09 (*d*, 5.5)	6.35 (*d*, 5.6)
12-OH	7.87 (*br s*)	7.97 (*br s*)	7.39 (*br s*)	7.60 (*br s*)
4′-OH			6.37 (*s*)	

[a] Multiplicity and *J* values in Hz are given in parentheses.
[b] Measured at 400 MHz.
[c] Measured at 500 MHz.
[d] The hydroxyl protons of the sugar moiety were not assigned due to broadening and overlapping of the signals.
[e] Multiplicity was not determined due to overlapping of the signals.
[f] The assignments of these signals may be interchangeable.

Table 2
^{13}C NMR spectral data for compounds **1**–**4** in C_5D_5N[a]

	1[b]	**2**[b]	**3**[c]	**4**[b]
1	199.7 *s*	51.0 *t*	81.3 *d*	37.8 *t*
2	146.2 *s*	193.6 *s*	84.9 *d*	77.6 *d*
3	124.8 *d*	146.6 *s*	124.3 *d*	73.5 *d*
4	31.4 *d*	147.9 *s*	135.9 *s*	33.7 *d*
5	44.0 *d*	43.3 *d*	43.1 *d*	38.4 *d*
6	28.6 *t*	29.3 *t*	28.4 *t*	29.4 *t*
7	82.9 *d*	83.7 *d*	84.3 *d*	84.3 *d*
8	46.6 *s*	46.2 *s*	46.7 *s*	46.5 *s*
9	36.8 *d*	42.2 *d*	42.9 *d*	43.6 *d*
10	48.8 *s*	40.8 *s*	44.4 *s*	38.8 *s*
11	75.0 *d*	73.0 *d*	75.7 *d*	73.2 *d*
12	76.2 *d*	75.7 *d*	75.7 *d*	76.1 *d*
13	82.9 *s*	82.8 *s*	82.5 *s*	82.7 *s*
14	50.5 *d*	50.5 *d*	50.5 *d*	49.9 *d*
15	68.7 *d*	69.2 *d*	68.7 *d*	68.7 *d*
16	168.2 *s*	168.0 *s*	168.3 *s*	168.2 *s*
18	18.8 *q*	15.3 *q*	20.8 *q*	16.5 *q*
19	14.4 *q*	15.8 *q*	12.2 *q*	15.8 *q*
20	73.5 *t*	73.7 *t*	74.2 *t*	74.1 *t*
21	171.1 *s*	171.2 *s*	171.5 *s*	171.5 *s*
OMe	52.2 *q*	52.3 *q*	52.3 *q*	52.3 *q*
1′	165.4 *s*	165.3 s	166.5 *s*	165.3 *s*
2′	115.9 *d*	130.3 s	112.9 *d*	116.0 *d*
3′	158.5 *s*	130.1 *d*	168.0 *s*	158.2 *s*
4′	26.9 *q*	128.8 *d*	73.2 *s*	26.9 *q*
5′	20.1 *q*	133.6 *d*	28.9 *q*	20.1 *q*
6′		128.8 *d*	28.9 *q*	
7′		130.1 *d*	15.6 *q*	
1″	100.6 *d*	104.9 *d*	107.0 *d*	103.8 *d*
2″	74.5 *d*	76.1 *d*	76.1 *d*	75.2 *d*
3″	78.9 *d*	78.7 *d*	78.6 *d*	78.3 *d*
4″	71.3 *d*	71.5 *d*	71.7 *d*	71.6 *d*
5″	78.0 *d*	78.4 *d*	78.8 *d*	78.4 *d*
6″	62.3 *t*	62.8 *t*	62.8 *t*	62.7 *t*

[a] The multiplicities of carbon signals were determined by the distortionless enhancement by polarization transfer (DEPT) method and are indicated as *s*, *d*, *t* and *q*.
[b] Measured at 100 MHz.
[c] Measured at 125 MHz.

senecioyl group was determined to be linked to the oxygen atom at C-15. The HMBC spectrum, however, was not able to give information about the location of the senecioyl group, as the H-15 resonance was extremely broad. Its ^{13}C NMR spectrum and the HMBC correlations between H_3-19/C-1 and between H-1″/C-2 demonstrated the presence of a ketone group (δ 199.7) at C-1 and a hexose unit (δ 100.6, 78.9, 78.0, 74.5, 71.3 and 62.3) connected to the C-2 oxygen atom, respectively (Table 2). The sugar component was identified as D-glucose by the 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 (7.4 Hz) of the anomeric proton of the glucosyl moiety indicated that the glucoside linkage was β. Analysis of the ROESY spectrum afforded further information about the stereochemistry of **1** (Fig. 2). Correlations observed between H-4/H_3-19, H-5/H-9, H_β-6/H_3-19, H-7/H-14, H-7/H_b-20 and H_3-19/H_a-20 indicated that the A/B and B/C ring junctures were both *trans*, whereas the B/D and C/D ring junctures were both *cis*, and that the methyleneoxy bridge between C-8 and C-13 was of β-orientation. The ROESY correlations between OH-11/H_3-19, OH-11/H_a-20, OH-11/H-12 and OH-12/H-11 indicated that the hydroxyl group at C-11 was of β-orientation, whereas that at C-12 was of α-orientation. Accordingly, javanicoside I (**1**) was determined to have the structure shown in Fig. 1.

Javanicoside J (**2**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{34}H_{40}O_{16}$ by the $[M + H]^+$ ion peak at *m*/*z* 705.2459 (calcd for $C_{34}H_{41}O_{16}$ 705.2395) in the HRESIMS. The ESIMS showed a fragment ion peak $[M + H-C_6H_{10}O_5]^+$ at *m*/*z* 543, which suggested that **2** was a glycoside. The IR spectrum showed the presence of hydroxyl (3371 cm^{-1}), δ-lactone and ester (1737 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of yadanzioside B (**5**), with all the resonances caused by the basic structure of **2** had corresponding signals in the spectra of **5**, suggesting that **2** had basically the same structure as **5** and that the difference was only in the side chain at C-15. Its ^{1}H NMR spectrum indicated signals due to one benzoyl (δ 8.23, 7.50 and 7.37) group, and on the basis of analysis of the ^{13}C NMR (δ 165.3, 133.6, 130.3, 130.1 × 2 and 128.8 × 2), H–H COSY and HMBC spectra, the ester side chain at C-15 of **2** was shown to be a benzoyloxy group (Tables 1 and 2). The ROESY correlations observed between CH_3O-21/H-3′, H-4′, H-6′ and H-7′ and between H-9/H-15 indicated that the benzoyloxy group at C-15 was of β-orientation (Fig. 3). Its ^{13}C NMR spectrum and the HMBC correlations between H-1″/C-3 demonstrated that the hexose unit (δ 104.9, 78.7, 78.4, 76.1, 71.5 and 62.8) was connected to the C-3 oxygen atom (Table 2). The sugar component was identified as D-glucose by the acid hydrolysis followed by the HPLC analysis. The relatively large *J* value (7.0 Hz) of the anomeric proton of the glucosyl moiety indicated that the glucoside linkage was β. The analysis of the ROESY spectrum afforded further information about the stereochemistry of **2** (Fig. 3). Correlations observed between H_β-6/H_3-19, H-7/H-14, and OH-11/H_3-19 implied that these protons, the hydroxyl group and the methyl group involved were all of β-orientation, whereas the correlations between H_α-1/H-5/H-9, and H-11/OH-12 suggested that these protons and the hydroxyl group involved were all of α-orientation. Accordingly, javanicoside J (**2**) was determined to have the structure shown in Fig. 1.

Javanicoside K (**3**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{34}H_{48}O_{17}$ by the $[M + Na]^+$ ion peak at *m*/*z* 751.2767 (calcd for $C_{34}H_{48}O_{17}Na$ 751.2789) in the HRESIMS. The ESIMS showed a fragment ion peak [M + H

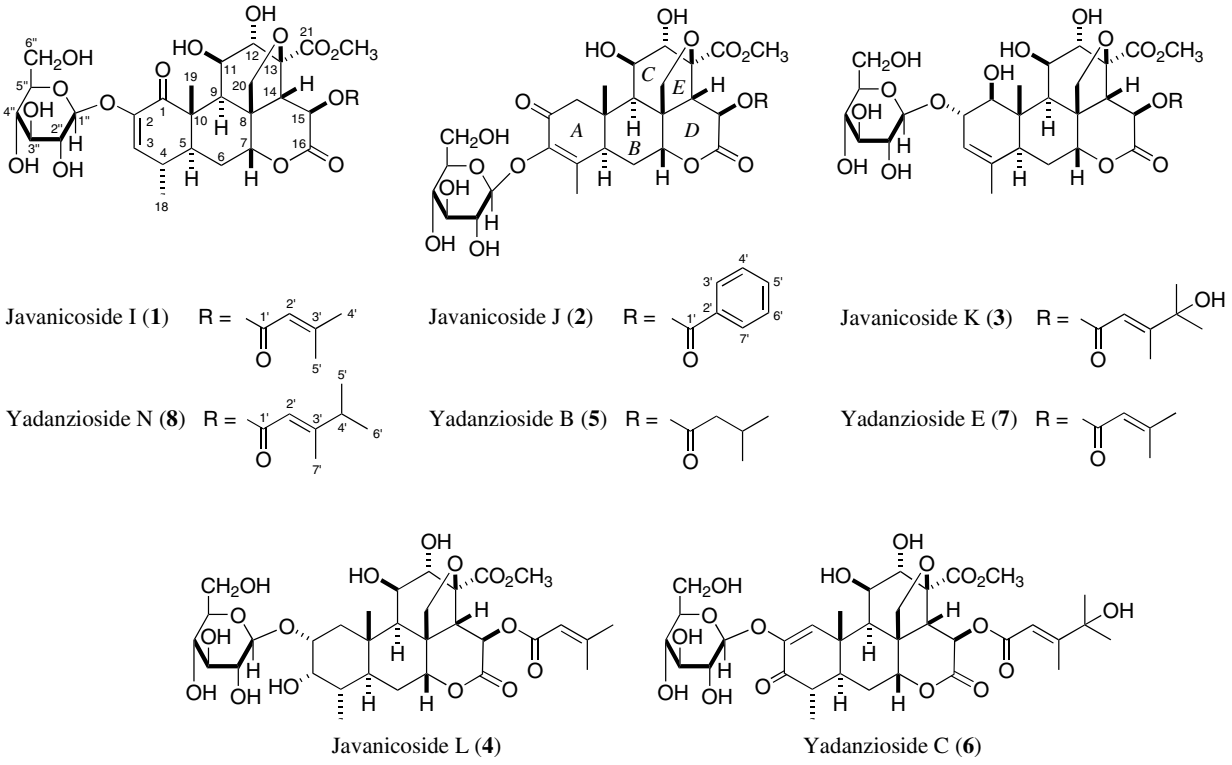

Fig. 1. Structures of javanicosides I (**1**)–L (**4**) and related compounds.

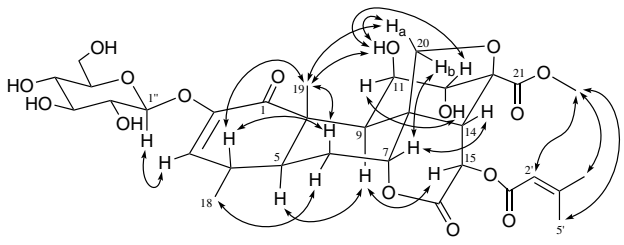

Fig. 2. Selected ROESY correlations for **1**.

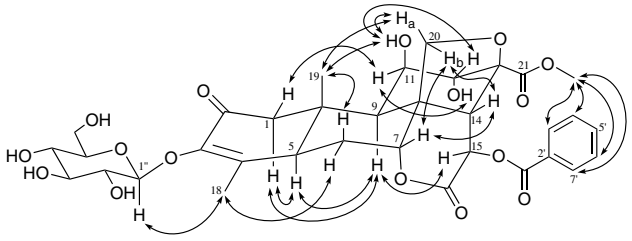

Fig. 3. Selected ROESY correlations for **2**.

$-H_2O-C_6H_{10}O_5]^+$ at *m/z* 549, which suggested that **3** was a glycoside. The IR spectrum showed the presence of hydroxyl (3381 cm^{-1}), δ-lactone and ester (1734 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **3** showed all the signals corresponding to those of the quassinoid skeleton of yadanzioside E (**7**) while the remainder, by comparison with those of yadanzioside C (**6**), were assigned to those of an (*E*)-4-hydroxy-3,4-dimethyl-2-pentenoyloxy group, placed at C-15β by correlation in the NOESY spectrum between CH_3O-21/H-2′, H_3-5′, H_3-6′ and H_3-7′ (Fig. 4). Thus, javanicoside K (**3**) had the structure shown in Fig. 1.

Javanicoside L (**4**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{32}H_{46}O_{16}$ by the $[M + Na]^+$ ion peak at *m/z* 709.2720 (calcd for $C_{32}H_{46}O_{16}Na$ 709.2684) in the HRESIMS. The fragment ion peak at *m/z* 525, $[M + H-C_6H_{10}O_5]^+$, in the ESIMS and the characteristic six resonances (δ 103.8, 78.4, 78.3, 75.2, 71.6 and 62.7) in the ^{13}C NMR spectrum indicated that **4** was a glycoside. The sugar component was shown to be D-glucose by acid hydrolysis followed by HPLC. The IR spectrum showed the presence of hydroxyl (3388 cm^{-1}), δ-lactone and ester (1737 cm^{-1}) groups. Its ^1H NMR spectrum generally resembled that of **1** showing that **4** was a quassinoid glucoside with a picrasane skeleton (Table 1).

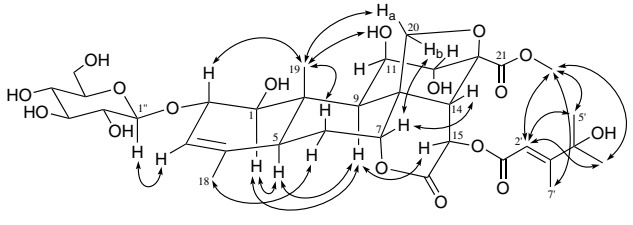

Fig. 4. Selected NOESY correlations for **3**.

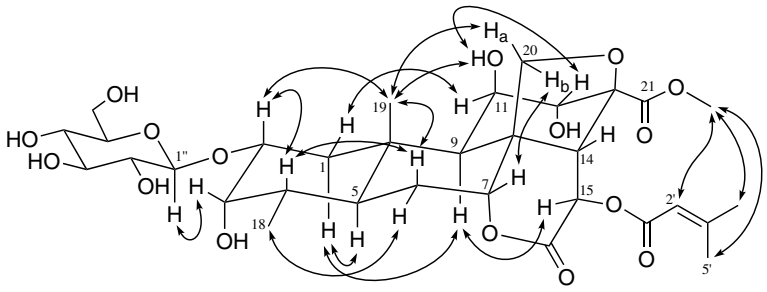

Fig. 5. Selected ROESY correlations for **4**.

However, the ^{1}H and ^{13}C NMR spectra showed that **4** had no signals ascribable to the olefinic proton at C-3 and the carbonyl carbon at C-1 in ring A observed in the spectra of **1**, and instead the spectra of **4** showed the signals of one methylene (δ_H 2.62 and 2.01) at C-1 (δ_C 37.8) two methines at C-2 (δ_H 4.33; δ_C 77.6) and C-3 (δ_H 4.25; δ_C 73.5), and one hydroxyl group (δ_H 5.07) at C-3. The HMBC correlation between H-1″ and C-2 revealed that the sugar moiety was linked to the C-2 oxygen atom. The glucoside linkage was determined to be β by the relatively large *J* value (7.9 Hz) of the anomeric proton as in **1**. Its ^{13}C NMR (δ 165.3, 158.2, 116.0, 26.9 and 20.1) and HMBC spectra showed that the senecioyloxy group was at C-15 with a β-orientation, as demonstrated also by the ROESY correlations between CH_3O-21/H-2′, H_3-4′ and H_3-5′, and between H-9/H-15 (Fig. 5). The ROESY correlations observed between H-2/H-4, H-4/H_β-6, H_β-6/H_3-19, OH-11/H-12 and OH-11/H_3-19 implied that these protons, the hydroxyl group and the methyl group involved were all of β-orientation, whereas those between H_α-1/H-5, H_α-1/H-9 and H_α-6/H_3-18 suggested that these protons and the methyl group involved were all of α-orientation. The orientation of the hydroxyl group at C-3 was determined to be α by the small coupling constant (<1 Hz) between H-2/H-3 and H-3/H-4. Thus, javanicoside L (**4**) was determined to have the structure shown in Fig. 1.

Javanicosides I (**1**), J (**2**), K (**3**) and L (**4**) showed moderate cytotoxic activity on P-388 murine leukemia cells with IC_{50} values of 7.5, 2.3, 1.6 and 2.9 μg/ml, respectively.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-360 digital polarimeter, UV spectra on a Hitachi U-2001 spectrophotometer and IR spectra on a Perkin–Elmer 1710 spectrophotometer. NMR spectra were measured on Bruker DRX-500 and DPX-400 spectrometers. The chemical shifts of ^{1}H NMR in C_5D_5N were referenced to the residual C_5D_4HN resonance at 7.21 ppm, and those of ^{13}C NMR to the solvent resonance at 135.5 ppm. Mass 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, Inertsil PREP-ODS (10 μm, 250 × 20 mm) or Mightysil RP 18 GP (5 μm, 250 × 20 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, 250 × 4.6 mm) (MeCN/H_2O (85:15), flow rate 1 ml/min).

3.2. Plant material

The seeds of *B. amarissima* were purchased in China in 2000, and the botanical origin of seeds was confirmed 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.

3.3. Extraction and isolation

Dried and ground seeds of *B. amarissima* (20 kg) were extracted with hot MeOH (4 × 18 l). 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. A part (40 g) of the *n*-BuOH-soluble portion was placed on a Diaion HP-20 (110 g) column and eluted sequentially with H_2O/MeOH mixtures (1:0, 1:1, 1:4 and 0:1, each 1 l) and finally with acetone to give five fractions.

The H_2O/MeOH (1:1) eluate (9.7 g) was further separated by reversed-phase HPLC using MeOH/H_2O (26:74 and 1:0) to afford nine fractions (frs. A–I), each of which was evaporated to dryness. MeOH (2 ml) was added to fr. A (2.3 g). When the solution was left

standing, it produced a precipitate of crude crystals (451.8 mg), which was separated and further subjected to reversed-phase HPLC using MeOH/H_2O (17:83) to afford bruceins D (95.0 mg) and E (253.8 mg). The mother liquor was concentrated to dryness (1.8 g), and subjected to reversed-phase HPLC using MeOH/H_2O (15:85 and then 1:0) to afford twelve fractions (frs. 1–12). Fr. 10 (154.8 mg) was further subjected to reversed-phase HPLC using MeCN/H_2O (13:87 and 1:0) to afford five sub-fractions (frs. 10A–10E). After removal of the solvent, reversed-phase HPLC using MeCN/H_2O (7:93), fr. 10D (78.1 mg) afforded yadanzioside I (20.0 mg) and yadanzigan (16.3 mg).

The H_2O/MeOH (1:4) eluate (12.8 g) was placed on a silica gel (250 g) column and eluted sequentially with $CHCl_3$ containing an increasing amount of MeOH (10:1, 5:1, 3:1 and 0:1) to give seven fractions. The $CHCl_3$/MeOH (3:1) eluate (1.4 g) was further separated by reversed-phase HPLC using MeOH/H_2O (37:63 and 1:0) into ten fractions (frs. a–j), which were evaporated to dryness. Fr. b (299.8 mg) was pure bruceoside B. By reversed-phase HPLC using MeCN/H_2O (20:80), fr. e (69.8 mg) afforded compounds **1** (19.6 mg) and **2** (19.6 mg), whereas fr. f (370.4 mg) gave compound **4** (13.3 mg) and yadanziosides B (**5**) (172.3 mg) and C (**6**) (139.3 mg); fr. g (36.0 mg) yadanzioside K (16.5 mg), and fr. h (37.7 mg) gave bulk compound **3** (15.3 mg) and yadanzioside E (**7**) (11.9 mg), respectively.

3.4. Javanicoside I (*1*)

Amorphous powder; $[\alpha]_D^{26} - 2.0°(c\ 0.65, \text{MeOH})$; UV (MeOH) λ_{max} nm (log ε): 221 (4.11), 256 (3.76); IR (film) ν_{max} cm^{-1}: 3398, 2922, 1741, 1684, 1646; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively; HRESIMS *m/z* 705.2334 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_{16}Na$ 705.2371).

3.5. Identification of sugar component by acid hydrolysis of *1*

A solution of **1** (4.8 mg) in 0.1 M H_2SO_4 (1 ml) was heated at 90 °C for 30 min under an argon 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 aglyco fraction (1.4 mg). The H_2O layer was passed through a short Amberlite IRA-400 column and evaporated to dryness to give a sugar fraction (1.4 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-glucose by the HPLC retention time, t_R 11.57 min (D-glucose, t_R 11.55 min) and the optical rotation (positive).

3.6. Javanicoside J (*2*)

Amorphous powder; $[\alpha]_D^{26} + 1.3°(c\ 0.24, \text{MeOH})$; UV (MeOH) λ_{max} nm (log ε) 233 (4.21), 255 (3.95); IR (film) ν_{max} cm^{-1}: 3371, 2924, 1737, 1660; for ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 705.2459 $[M + H]^+$ (calcd for $C_{34}H_{41}O_{16}$ 705.2395).

3.7. Identification of sugar component by acid hydrolysis of *2*

Compound **2** (5.1 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction (1.8 mg) and a sugar fraction (1.5 mg). The sugar component was identified as D-glucose by its HPLC retention time, t_R 11.42 min, and the positive optical rotation measured.

3.8. Javanicoside K (*3*)

Amorphous powder; $[\alpha]_D^{26} + 31°(c\ 0.12, \text{MeOH})$; UV (MeOH) λ_{max} nm (log ε): 220sh (4.10); IR (film) ν_{max} cm^{-1}: 3381, 2932, 1734, 1658; for ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 751.2767 $[M + Na]^+$ (calcd for $C_{34}H_{48}O_{17}Na$ 751.2789).

3.9. Identification of sugar component by acid hydrolysis of *3*

Compound **3** (4.2 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-glucose by the HPLC retention time, t_R 11.45 min, and the positive optical rotation measured.

3.10. Javanicoside L (*4*)

Amorphous powder; $[\alpha]_D^{26} - 5.8°(c\ 0.31, \text{MeOH})$; UV (MeOH) λ_{max} nm (log ε): 222sh (4.05); IR (film) ν_{max} cm^{-1}: 3388, 2923, 1737, 1646; for ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; HRESIMS *m/z* 709.2720 $[M + Na]^+$ (calcd for $C_{32}H_{46}O_{16}Na$ 709.2684).

3.11. Identification of sugar component by acid hydrolysis of *4*

Compound **4** (7.7 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction (2.1 mg) and a sugar fraction (1.5 mg). The sugar component was identified as D-glucose by the HPLC retention time, t_R 11.70, min and the measured positive optical rotation.

3.12. Cytotoxic activity assay

The assay was performed in the same manner as described previously (Ozeki et al., 1998).

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