New Quassinoids, Javanicolides C and D and Javanicosides B−F, from Seeds of *Brucea javanica*

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Two new quassinoids, javanicolides C and D, and five new quassinoid glucosides, javanicosides B−F, were isolated from the seeds of *Brucea javanica*, along with eight known quassinoids, i.e., yadanziolides A, C, D, and S, bruceins D and E, brusatol, and the aglycone of yadanzioside D, and 19 known quassinoid glucosides, i.e., yadanziosides A−G, I, and K−P, bruceosides A−C and E, and bruceantinoside A. Their structures were elucidated by analysis of spectroscopic data and chemical evidence.

Brucea javanica (L.) Merr. is a shrub that is widely distributed in Southeast Asia and northern Australia. Its seeds have been used for the treatment of dysentery, malaria, and cancer[1,2] and are known to be a rich source of quassinoids.[3−7] Some quassinoids from *B. javanica* exhibit such interesting biological activities as antimalarial,[8] antitumor,[9] and antiamoebic[10] activities. In the present study, we isolated from the seeds of this plant two new quassinoids, javanicolides C (**1**) and D (**2**), and five new quassinoid glucosides, javanicosides B−F (**3**−**7**), along with eight known quassinoids, i.e., yadanziolides A, C,[3] D,[11] and S,[12] bruceins D and E,[9] brusatol,[13] and the aglycone of yadanzioside D (**8**),[4] and 19 known quassinoid glucosides, i.e., yadanziosides A−G,[5] I,[14] K−N (**9**),[15] O (**11**), and P,[16] bruceosides A, B,[9] C,[17] and E,[18] and bruceantinoside A (Figure 1).[19] We describe herein 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 of the hot MeOH extract of *B. javanica* seeds gave six fractions. The $CHCl_3$/MeOH (20:1) eluate gave, after Diaion HP-20 column chromatography and subsequent preparative reversed-phase HPLC using MeOH/H_2O (33:67 and 1:0), 14 fractions. The third fraction of the preparative reversed-phase HPLC gave the aglycone of yadanzioside D (**8**), whereas the 12th fraction afforded brusatol on crystallization from EtOAc. The $CHCl_3$/MeOH (5:1) eluate of the first silica gel column chromatography gave, after Diaion HP-20 column chromatography and subsequent repeated reversed-phase HPLC, seven new quassinoids, **1**−**7**, along with yadanziolides A, C, D, and S, bruceins D and E, yadanziosides A−G, I, K−N (**9**), O (**11**), and P, bruceosides A−C and E, and bruceantinoside A. Identification of the known compounds was accomplished by comparing their spectroscopic data with those in the literature.

Javanicolide C (**1**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{26}H_{36}O_{11}$ from the $[M + H]^+$ ion peak at *m/z* 525.2309 (calcd for $C_{26}H_{37}O_{11}$ 525.2336) in the HRESIMS. The IR spectrum showed bands indicative of hydroxyl (3427 cm^{-1}) as well as δ-lactone and ester (1724 cm^{-1}) groups. Its ^1H NMR spectrum showed resonances of one secondary methyl (δ 1.17), one tertiary methyl (δ 1.64), two olefinic methyls (δ 2.13 and 1.64), one carbomethoxy group (δ 3.74), and one olefinic proton (δ 5.81) (Table 1) and generally resembled that of **8**, thereby indicating that **1** was a quassinoid having a picrasane skeleton. The ^{13}C NMR spectrum gave resonances of C-2 (δ 68.3), C-3 (δ 74.8), C-11 (δ 73.5), and C-12 (δ 76.0), indicating that hydroxyl groups were attached to those carbons (Table 2). ^{13}C NMR (δ 165.3, 158.2, 116.0, 27.0, and 20.1) and HMBC spectra revealed the presence of a senecioyloxy group that was connected to C-15 with a β-orientation, as demonstrated by NOESY correlations between OCH_3-21/H-2′, H_3-4′, and H_3-5′ and between H-9/H-15 (Figure 2). Analysis of the NOESY spectrum afforded further information about the configuration of **1** (Figure 2). The correlations 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 NOESY correlations between H-2/H-4, H-2/H_3-19, H-4/H_3-19, OH-11/H_3-19, OH-11/H_a-20, and OH-11/H-12 indicated that the hydroxyl groups at C-2 and C-12 and the methyl group at C-4 were α-oriented, whereas the hydroxyl group at C-11 was in a β-orientation. The orientation of the hydroxyl group at C-3 was α based on the small coupling constant (<1 Hz) between H-2/H-3 and H-3/H-4. Accordingly, **1** possessed the structure shown in Figure 1.

Javanicolide D (**2**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{28}H_{38}O_{12}$ from the $[M + H]^+$ ion peak at *m/z* 567.2461 (calcd for $C_{28}H_{39}O_{12}$ 567.2442) in the HRESIMS. The IR spectrum showed bands indicative of hydroxyl (3424 cm^{-1}) as well as δ-lactone and ester (1724 cm^{-1}) groups. Its ^1H NMR spectrum was very similar to that of the aglycone of yadanzioside D (**8**), except for the resonances of the ester side chain at C-15, and showed signals ascribable to three tertiary methyls (δ 1.55, 1.42, and 1.40), two olefinic methyls (δ 2.39 and 1.57), one carbomethoxy group (δ 3.70), and two olefinic protons (δ 6.75 and 5.75) (Table 1). Analysis of the ^{13}C NMR, H−H COSY, and HMBC spectra revealed that **2** possessed an (*E*)-4-hydroxy-3,4-dimethyl-2-pentenoyloxy group (δ 168.1, 166.6, 112.9, 73.1, 28.9, 28.8, and 15.5) (Table 2) at C-15. The orientation of the B, C, D, and E ring substituents was the same as that of **1**, as demonstrated by NOESY correlations (Figure 3). The NOESY correlations between H-1/H-5 and H-2/H_3-19 indicated that the hydroxyl groups at C-1 and C-2 were β-

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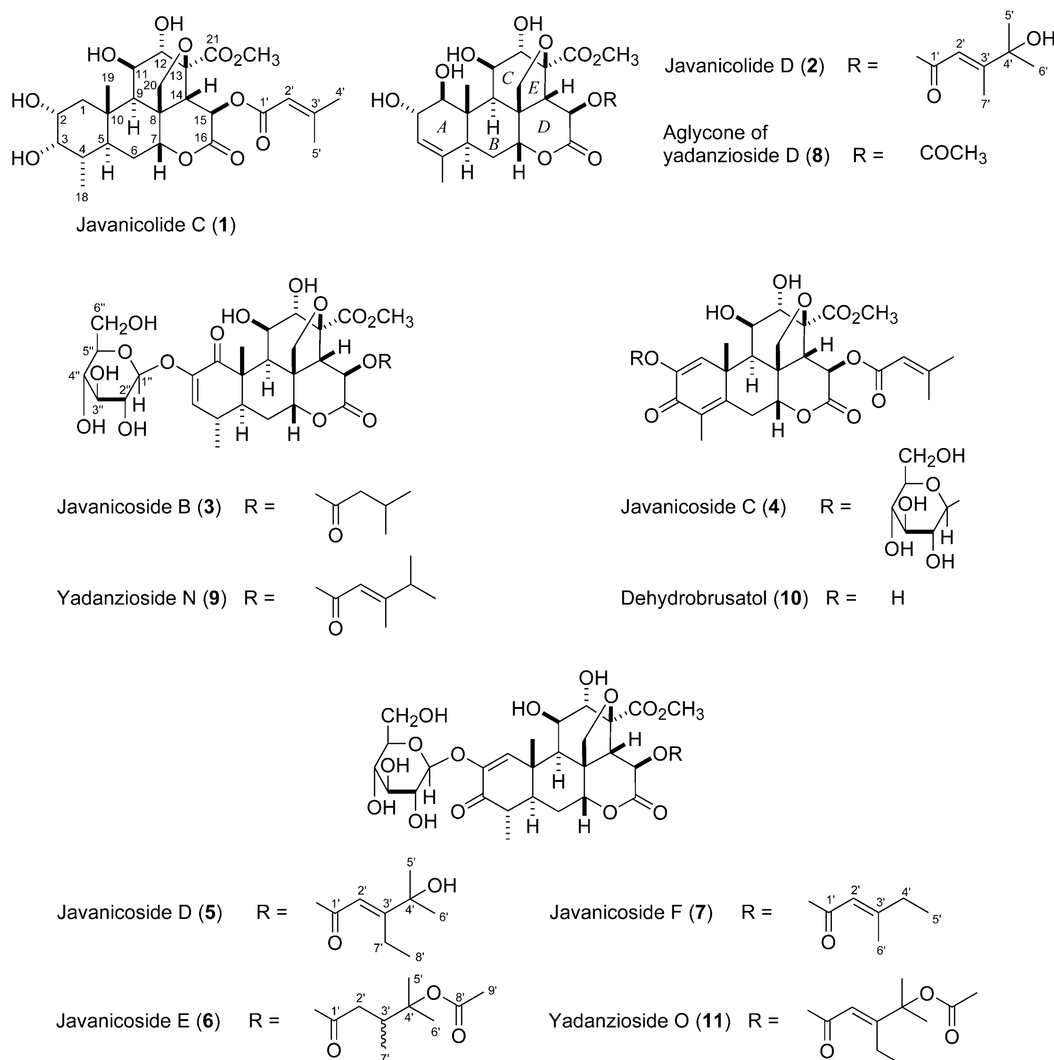

Figure 1.

and α-oriented, respectively. Thus, **2** possessed the structure shown in Figure 1.

Javanicoside B (**3**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{32}H_{44}O_{16}$ from the $[M + Na]^+$ ion peak at *m/z* 707.2556 (calcd for $C_{32}H_{44}O_{16}Na$ 707.2527) in the HRESIMS. The ESIMS showed a fragment ion $[MH - C_6H_{10}O_5]^+$ at *m/z* 523 that suggested that **3** was a glycoside. The IR spectrum showed bands indicative of hydroxyl (3389 cm^{-1}), δ-lactone and ester (1744 cm^{-1}), and α,β-unsaturated ketone (1681 cm^{-1}) groups. The ^1H NMR spectrum showed resonances ascribable to one tertiary methyl (δ 1.84), three secondary methyls (δ 0.99, 0.96, and 0.86), one carbomethoxy group (δ 3.82), and one olefinic proton (δ 6.07) (Table 1). The ^1H and ^{13}C NMR spectra of **3** were very similar to those of yadanzioside N (**9**) except for the resonances ascribable to the ester side chain at C-15. This side chain was shown to be a 3-methylbutanoyloxy group based on analysis of the ^{13}C NMR (δ 171.9, 43.3, 25.9, 22.5, and 22.4), H−H COSY, and HMBC spectra (Table 2). The ^{13}C NMR spectrum and the HMBC correlations between H_3-19/C-1 and H-1″/C-2 demonstrated the presence of a ketone group (δ 199.6) at C-1 and a hexose unit (δ 100.5, 78.9, 78.6, 74.6, 71.4, and 62.4) connected to the C-2 oxygen atom (Table 2). The sugar component was identified as D-glucose by acid hydrolysis of **3**, followed by HPLC analysis of the hydrolysate using an aminopropyl-bonded silica gel column and an optical rotation detector. The relatively large *J* value (7.2 Hz) of the anomeric proton of the glucosyl moiety indicated that the glucoside linkage was β. The stereochemistry of the B, C, D, and E rings and the substituents on those rings was the same as that of **1**, as demonstrated by NOESY correlations (Figure 4). The correlation between H-4/H_3-19 implied that the methyl group at C-4 was α-oriented. Accordingly, **3** possessed the structure shown in Figure 1.

Javanicoside C (**4**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{32}H_{40}O_{16}$ from the $[M + Na]^+$ ion peak at *m/z* 703.2258 (calcd for $C_{32}H_{40}O_{16}Na$ 703.2214) in the HRESIMS. The IR spectrum showed bands indicative of hydroxyl (3430 cm^{-1}) as well as δ-lactone and ester (1726 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **4** and dehydrobrusatol (**10**)[5] were very similar, and all the resonances in the spectra of **10** corresponded to those of **4**, suggesting that **4** and **10** had the same carbon framework and ester side chain. The fragment ion at *m/z* 519, $[MH - C_6H_{10}O_5]^+$, in the ESIMS and the six characteristic resonances (δ 101.8, 78.9, 78.5, 74.7, 71.7, and 62.3) in the ^{13}C NMR spectrum indicated that **4** was a glycoside. Acid hydrolysis afforded **10** and the sugar component that was identified as D-glucose by HPLC. The HMBC correlation between H-1″ and C-2 revealed that the sugar moiety was linked to the C-2 oxygen atom. The relatively large *J* value (7.8 Hz) of the anomeric proton of the glucosyl moiety confirmed its β-glucoside linkage. Accordingly, the structure of **4** was determined as shown in Figure 1.

Table 1. ^{1}H NMR Data for Compounds **1**−**7** in C_5D_5N[a]

position	**1**[b]	**2**[b]	**3**[c,d]	**4**[b,d]	**5**[b,d]	**6**[b,d]	**7**[c,d]
1α	2.09 (—[e])	4.08 (d, 7.1)		7.39 (s)	7.30 (s)	7.28 (s)	7.29 (s)
1β	2.61 (dd, 11.9, 4.5)						
2	4.23 (br m)	4.56 (br s)					
3	4.06 (br s)	5.75 (s)	6.07 (d, 2.2)				
4	1.70 (m)		2.21 (—[e])		2.41 (m)	2.41 (m)	2.42 (m)
5	2.36 (br t, 12)	2.69 (br m)	1.79 (—[e])		2.12 (br m)	2.04 (—[e])	2.10 (—[e])
6α	2.09 (—[e])	2.15 (d, 14.6)	1.99 (dd, 11.9, 3.0)	3.28 (dd, 14.8, 3.0)	2.03 (d, 14.7)	2.02 (d, 14.8)	2.04 (d, 14.8)
6β	1.50 (ddd, 13.9, 13.8, 2.5)	1.71 (ddd, 14.6, 13.3, 2.4)	1.53 (t, 12.8)	2.72 (d, 14.8)	1.67 (dd, 14.7, 2.3)	1.67 (dd, 14.8, 2.3)	1.68 (dd, 14.8, 3.3)
7	5.00 (s)	5.01 (—[e])	4.83 (s)	5.22 (s)	4.97 (—[e])	4.99 (—[e])	5.01 (—[e])
9	2.44 (br s)	2.68 (d, 4.6)	2.90 (d, 4.1)	2.50 (br s)	2.54 (d, 4.4)	2.53 (d, 4.5)	2.54 (d, 4.4)
11	4.91 (—[e])	5.58 (br t, 5)	6.16 (br t, 5)	5.10 (d, 4.7)	5.20 (br s)	5.18 (br s)	5.20 (d, 4.6)
12	5.05 (br s)	5.15 (br s)	5.07 (—[e])	5.16 (s)	5.16 (br s)	5.13 (br s)	5.16 (s)
14	3.97 (br s)	4.02 (br s)	3.84 (—[e])	4.08 (br s)	4.03 (br s)	3.91 (—[e])	4.00 (br s)
15	6.79 (br s)	6.89 (br s)	6.96 (br s)	6.55 (br s)	6.81 (br s)	6.82 (br s)	6.80 (br s)
18	1.17 (d, 6.7)	1.57 (s)	0.86 (d, 6.8)	2.16 (s)	1.17 (d, 6.7)	1.16 (d, 6.7)	1.17 (d, 6.6)
19	1.64 (s)	1.55 (s)	1.84 (s)	1.81 (s)	1.62 (s)	1.61 (s)	1.62 (s)
20a	5.17 (d, 7.2)	5.27 (d, 7.3)	5.09 (d, 7.3)	5.26 (d, 7.3)	5.07 (d, 7.3)	5.08 (d, 7.3)	5.09 (d, 7.3)
20b	3.90 (d, 7.2)	3.92 (d, 7.3)	3.86 (—[e])	4.01 (d, 7.3)	3.90 (—[e])	3.91 (—[e])	3.91 (—[e])
OMe	3.74 (s)	3.70 (s)	3.82 (s)	3.72 (s)	3.73 (s)	3.88 (s)	3.77 (s)
2′	5.81 (s)	6.75 (s)	2.37 (d, 6.6) 2.23 (—[e])	5.80 (s)	6.70 (s)	2.88 (br d, 15) 2.28 (m)	5.83 (s)
3′			2.20 (—[e])			2.78 (m)	
4′	1.64 (s)		0.99[f] (d, 6.6)	1.65 (s)			1.93 (q, 7.3)
5′	2.13 (s)	1.42[f] (s)	0.96[f] (d, 6.6)	2.13 (s)	1.47[f] (s)	1.41 (s)	0.82 (t, 7.4)
6′		1.40[f] (s)			1.45[f] (s)	1.45 (s)	2.51 (s)
7′		2.39 (s)			2.88 (m) 2.82 (br m)	1.10 (d, 6.8)	
8′					1.33 (br s)		
9′						1.92 (s)	
1"			5.45 (d, 7.2)	5.42 (d, 7.8)	5.36 (d, 7.4)	5.33 (d, 7.3)	5.36 (d, 7.3)
2"			4.28 (—[e])	4.31 (—[e])	4.30 (—[e])	4.28 (—[e])	4.28 (—[e])
3"			4.30 (—[e])	4.19 (—[e])	4.25 (—[e])	4.25 (—[e])	4.25 (—[e])
4"			4.22 (—[e])	4.26 (—[e])	4.24 (—[e])	4.23 (—[e])	4.24 (—[e])
5"			3.98 (m)	3.83 (m)	3.93 (—[e])	3.91 (—[e])	3.91 (—[e])
6"			4.53 (br d, 12) 4.32 (—[e])	4.49 (dd, 12.0, 2.1) 4.28 (—[e])	4.49 (dd, 11.8, 1.9) 4.22 (—[e])	4.50 (dd, 11.7, 1.7) 4.21 (—[e])	4.49 (dd, 11.9, 2.1) 4.22 (—[e])
1-OH		7.67 (br s)					
2-OH	5.51 (br s)	6.31 (br s)					
3-OH	5.64 (br s)						
11-OH	6.21 (d, 5.1)	5.43 (d, 5.0)	6.34 (d, 5.8)	7.02 (br s)	6.53 (br s)	6.54 (br s)	6.53 (br s)
12-OH	7.70 (br s)	7.61 (br s)	7.86 (br s)	7.95 (br s)	7.87 (br s)	7.96 (br s)	7.90 (br s)
4′-OH		6.50 (s)			6.46 (s)		

[a] Multiplicity and *J* values in Hz are given in parentheses. [b] Measured at 500 MHz. [c] Measured at 400 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 reversed in each column.

Javanicoside D (**5**) was obtained as an amorphous powder. Its molecular formula was established as $C_{35}H_{48}O_{17}$ from the $[M + Na]^+$ ion peak at *m/z* 763.2731 (calcd for $C_{35}H_{48}O_{17}Na$ 763.2789) in the HRESIMS. Its ^{1}H NMR spectrum showed resonances ascribable to three tertiary methyls (δ 1.62, 1.47, and 1.45), one secondary methyl (δ 1.17), one primary methyl (δ 1.33), one carbomethoxy group (δ 3.73), and two olefinic protons (δ 7.30 and 6.70) (Table 1). The NMR spectra of **5** were very similar to those of yadanzioside O (**11**), thereby indicating that the two compounds had the same basic structure. Analysis of the ^{13}C NMR, H−H COSY, and HMBC spectra revealed that **5** possessed an (*E*)-3-ethyl-4-hydroxy-4-methyl-2-pentenoyloxy side chain (δ 168.3, 166.0, 112.6, 73.5, 29.3, 29.2, 22.9, and 15.0) (Table 2), whereas **11** had an (*E*)-4-acetoxy-3-ethyl-4-methyl-2-pentenoyloxy group. The sugar component was identified as D-glucose by acid hydrolysis followed by HPLC. The HMBC correlation between H-1″ and C-2 indicated that the glucose unit (δ 102.1, 78.9, 78.6, 74.7, 71.3, and 62.3) was linked to the C-2 oxygen atom, and the *J* value (7.4 Hz) of the anomeric proton of the sugar moiety confirmed the β glucoside linkage. ROESY correlations showed that **5** had the same stereochemistry of the B, C, D, and E rings as **1** (Figure 5). The methyl group at C-4 was α-oriented based on the observation of the ROESY correlation between H-4/H_3-19. Accordingly, **5** had the structure shown in Figure 1.

Javanicoside E (**6**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{36}H_{50}O_{18}$ from the $[M + Na]^+$ ion peak at *m/z* 793.2950 (calcd for $C_{36}H_{50}O_{18}Na$ 793.2895) in the HRESIMS. Its ^{1}H NMR spectrum showed resonances ascribable to three tertiary methyls (δ 1.61, 1.45, and 1.41), two secondary methyls (δ 1.16 and 1.10), one acetyl (δ 1.92), one carbomethoxy (δ 3.88) group, and one olefinic proton (δ 7.28) (Table 1). Comparison of its NMR spectra with those of **5** revealed that **6** and **5** had the same ring system and sugar moiety, but a different ester side chain moiety at C-15. Analysis of the ^{13}C NMR (δ 170.0, 167.5, 83.8, 39.5, 36.8, 23.5, 22.2, 22.1, and 14.5), H−H COSY, and HMBC spectra revealed that the side chain at C-15 of **6** was a 4-acetoxy-3,4-dimethylpentanoyloxy group (Table 2). The sugar component of **6** was identified as D-glucose by acid hydrolysis followed by HPLC. The NOESY correlations revealed that **6** had the same stereochemistry as **5**. On the basis of these data, **6** had the structure shown in Figure 1.

Javanicoside F (**7**) was obtained as an amorphous powder. Its molecular formula was established as $C_{33}H_{44}O_{16}$

Table 2. ^{13}C NMR Data for Compounds **1**−**7** in C_5D_5N

position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[b]
1	41.1	82.3	199.6	128.6	129.3	129.1	129.3
2	68.3	73.2	146.2	148.9	148.9	148.9	148.9
3	74.8	126.5	124.8	180.2	194.5	194.5	194.5
4	34.2	134.3	31.4	131.9	41.4	41.4	41.4
5	38.5	43.3	44.0	155.6	43.8	43.7	43.8
6	29.6	28.6	28.6	32.7	30.0	30.0	30.0
7	84.4	84.3	83.1	84.8	83.5	83.8	83.5
8	46.5	46.7	46.7	46.7	46.6	46.7	46.6
9	43.7	43.2	37.1	41.5	40.4	40.6	40.6
10	39.0	44.6	48.8	44.3	39.6	39.6	39.5
11	73.5	75.9	75.1	75.8	73.6	73.6	73.5
12	76.0	75.7	76.2	76.2	76.1	75.9	76.0
13	82.7	82.5	83.0	83.0	82.6	82.7	82.6
14	50.4	50.9	50.8	49.4	50.4	50.2	50.3
15	68.3	68.4	68.9	68.4	68.4	68.2	68.5
16	168.3	168.4	168.1	167.6	168.2	168.2	168.3
18	16.6	21.0	18.8	11.2	12.5	12.5	12.6
19	16.0	12.3	14.4	24.0	17.9	17.9	17.9
20	74.1	74.1	71.4	72.9	73.7	73.7	73.7
21	171.5	171.4	171.2	170.9	171.2	171.2	171.2
OMe	52.3	52.3	52.3	52.4	52.4	52.4	52.3
1′	165.3	166.6	171.9	165.6	166.0	167.5	165.8
2′	116.0	112.9	43.3	115.9	112.6	36.8	114.3
3′	158.2	168.1	25.9	158.6	168.3	39.5	163.7
4′	27.0	73.1	22.5[c]	27.0	73.5	83.8	33.6
5′	20.1	28.9[c]	22.4[c]	20.1	29.3[c]	22.2	11.7
6′		28.8[c]			29.2[c]	23.5	18.8
7′		15.5			22.9	14.5	
8′					15.0	170.0	
9′						22.1	
1″			100.5	101.8	102.1	102.0	102.0
2″			74.6	74.7	74.7	74.7	74.7
3″			78.6	78.5	78.6	78.5	78.5
4″			71.4	71.7	71.3	71.3	71.4
5″			78.9	78.9	78.9	79.0	78.9
6″			62.4	62.3	62.3	62.3	62.3

[a] Measured at 125 MHz. [b] Measured at 100 MHz. [c] The assignments of these signals may be reversed in each column.

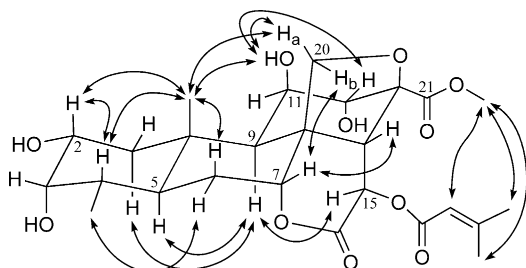

Figure 2. Selected NOESY correlations for **1**.

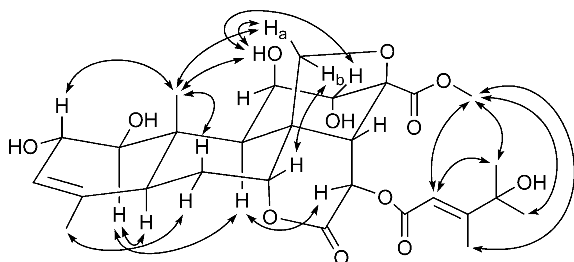

Figure 3. Selected NOESY correlations for **2**.

from the $[M + Na]^+$ ion peak at m/z 719.2485 (calcd for $C_{33}H_{44}O_{16}Na$ 719.2527) in the HRESIMS. Its ^1H NMR spectrum showed resonances ascribable to one tertiary methyl (δ 1.62), one secondary methyl (δ 1.17), one primary methyl (δ 0.82), one olefinic methyl (δ 2.51), one carbomethoxy group (δ 3.77), and two olefinic protons (δ 7.29 and 5.83) (Table 1). The ^1H and ^{13}C NMR spectra of **7** were very similar to those of **5** except for the resonances due to

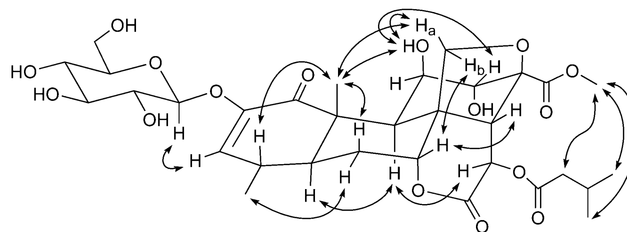

Figure 4. Selected NOESY correlations for **3**.

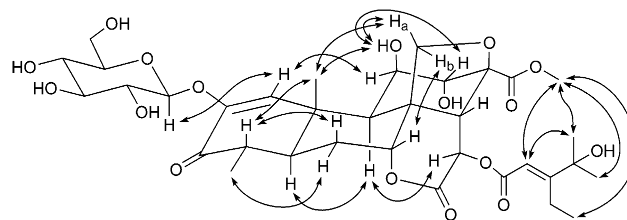

Figure 5. Selected ROESY correlations for **5**.

the ester side chain moiety, suggesting that **7** was a congener of **5** having a different ester side chain. Analysis of the ^{13}C NMR (δ 165.8, 163.7, 114.3, 33.6, 18.8, and 11.7), H−H COSY, and HMBC spectra revealed that the side chain at C-15 of **7** was an (*E*)-3-methyl-2-pentenoyloxy group (Table 2). The sugar moiety of **7** was identified as D-glucose by acid hydrolysis followed by HPLC. The stereochemistry of **7** was the same as that of **5** by the analysis of its NOESY spectrum. Thus, **7** had the structure shown in Figure 1.

Javanicoside B (**3**) showed moderate cytotoxicity against P-388 murine leukemia cells with an IC_{50} value of 5.6 μg/mL, whereas javanicolides C (**1**) and D (**2**) and javanicosides C (**4**), D (**5**), E (**6**), and F (**7**) had weak or no activity, with IC_{50} values of >100, 18, 18, 89, 16, and 50 μg/mL, respectively.

Experimental Section

General Experimental Procedures. 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 spectrometer. NMR spectra were measured on Bruker DRX-500 and DPX-400 spectrometers. The ^1H chemical shifts in C_5D_5N 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. 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, 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 was identified by Dr. K. Takeya, a professor of Medicinal Plant Chemistry at Tokyo University of Pharmacy and Life Science. A voucher specimen was deposited in the herbarium of the university.

Extraction and Isolation. Dried and ground seeds of *B. javanica* (20 kg) were extracted with hot MeOH (4 × 18 L). The solvent was removed in vacuo to give a residue (ca. 1 kg) that was suspended in H_2O (2 L). Then, the suspension was extracted successively with *n*-hexane (2 × 1 L), $CHCl_3$ (2 × 1 L), and *n*-BuOH (2 × 1 L), 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 (20:1) eluate (24.0 g) was placed on a Diaion HP-20 (100 g) column and eluted with MeOH (3 L) and then with acetone (1 L) to give two fractions. The MeOH eluate (21.3 g) was further separated by reversed-phase HPLC using MeOH/H_2O (33:67 and 1:0) into 14 fractions, and those fractions were evaporated to dryness. The third fraction (415.0 mg) was further subjected to reversed-phase HPLC using MeCN/H_2O (13:87) to afford the aglycone of yadanzioside D (**8**) (111.2 mg), and the 12th fraction (3.7 g) was, after removal of the solvent, crystallized from EtOAc to afford brusatol (1.7 g).

The $CHCl_3$/MeOH (5:1) eluate (21.6 g) of the first silica gel column chromatography 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 eluate (19.5 g) was further subjected to reversed-phase HPLC using MeOH/H_2O (40:60 and then 1:0) to afford 13 fractions (fractions 1−13), all of which were evaporated to dryness. Fraction 1 (2.0 g) was subjected to reversed-phase HPLC using MeOH/H_2O (30:70 and 1:0) to afford eight subfractions (fractions 1A−1H), which were evaporated to dryness. Fraction 1B (224.6 mg) was brucein E. By reversed-phase HPLC using MeOH/H_2O (20:80), fraction 1A (1.2 g) afforded yadanziolides A (35.0 mg), C (2.9 mg), and D (24.6 mg), yadanzioside I (19.7 mg), and brucein D (244.7 mg), and by reversed-phase HPLC using MeCN/H_2O (15:85), fraction 1D (298.8 mg) afforded yadanziolide S (14.7 mg) and yadanzioside F (225.6 mg). By reversed-phase HPLC using MeCN/H_2O (17:83), fraction 1F (167.5 mg) afforded yadanziosides D (77.1 mg) and L (8.4 mg) and bruceoside C (8.3 mg), and fraction 1G (28.5 mg) afforded bruceoside B (12.7 mg).

Fractions 2 (811.0 mg) and 4 (114.2 mg) were subjected to reversed-phase HPLC using MeOH/H_2O (20:80) to afford bruceoside B (492.3 mg) and javanicoside C (**4**) (5.2 mg), respectively.

Fraction 5 (4.5 g) was crystallized from MeOH to afford crystals of bruceoside A (2.7 g) and the mother liquid (1.9 g), which, by repeated reversed-phase HPLC using either MeCN/H_2O (18:82) or MeOH/H_2O (35:65), gave yadanziosides B (140.6 mg) and C (26.3 mg) and bruceoside A (1.3 g).

By reversed-phase HPLC using MeCN/H_2O (20:80), fraction 6 (245.3 mg) afforded yadanzioside K (102.4 mg), fraction 7 (342.2 mg) afforded yadanzioside E (216.0 mg), fraction 8 (64.8 mg) afforded javanicolide C (**1**) (3.7 mg) and javanicoside B (**3**) (7.5 mg), and fraction 9 (285.8 mg) afforded yadanzioside M (114.4 mg) and **3** (16.9 mg).

Fraction 10 (2.7 g) was subjected to reversed-phase HPLC using MeCN/H_2O (22:78 and 1:0) to afford 12 subfractions (fractions 10A−10L), all of which were evaporated to dryness. Yadanzioside A was obtained from fractions 10H (375.2 mg) and 10I (1.8 g). By reversed-phase HPLC using MeOH/H_2O/AcOH (40:60:1), fraction 10A (213.6 mg) afforded bruceoside E (38.2 mg). Analogously, by reversed-phase HPLC using MeOH/H_2O (38:62), fraction 10B (17.4 mg) afforded javanicolide D (**2**) (10.0 mg), and by similar reversed-phase HPLC using MeCN/H_2O (20:80), fraction 10D afforded javanicoside D (**5**) (11.8 mg), fraction 10J (45.9 mg) afforded javanicoside F (**7**) (11.9 mg), and fraction 10K (75.4 mg) afforded yadanzioside P (57.4 mg).

Fraction 12 (3.7 g) was crystallized from MeOH to afford crystals of yadanzioside G (3.1 g).

Fraction 13 (3.2 g) was subjected to further separation by reversed-phase HPLC using MeOH/H_2O (45:55 and 1:0) to afford seven subfractions (fractions 13A−13G), all of which were evaporated to dryness. Fraction 13E (502.6 mg) comprised bruceantinoside A. By reversed-phase HPLC using MeOH/H_2O (38:62), fraction 13C (107.6 mg) afforded javanicoside E (**6**) (21.9 mg) and yadanzioside N (**9**) (12.3 mg), and by reversed-phase HPLC using MeCN/H_2O (28:72), fraction 13F (232.8 mg) afforded yadanzioside O (**11**) (95.7 mg).

Javanicolide C (1): amorphous powder; $[\alpha]^{26}_D$ +60.0° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.39), 261 (3.41), 277 (3.43) nm; IR (film) ν_{max} 3427, 2965, 1724, 1644 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 525.2309 $[M + H]^+$ (calcd for $C_{26}H_{37}O_{11}$ 525.2336).

Javanicolide D (2): amorphous powder; $[\alpha]^{26}_D$ +60.0° (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.15) nm; IR (film) ν_{max} 3424, 2971, 1724, 1643 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 567.2461 $[M + H]^+$ (calcd for $C_{28}H_{39}O_{12}$ 567.2442).

Javanicoside B (3): amorphous powder; $[\alpha]^{26}_D$ −20.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 254 (3.88) nm; IR (film) ν_{max} 3389, 2960, 1744, 1681 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 707.2556 $[M + Na]^+$ (calcd for $C_{32}H_{44}O_{16}Na$ 707.2527).

Javanicoside C (4): amorphous powder; $[\alpha]^{26}_D$ −34.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 256 (3.54) nm; IR (film) ν_{max} 3430, 2934, 1726, 1638 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 703.2258 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_{16}Na$ 703.2214).

Javanicoside D (5): amorphous powder; $[\alpha]^{24}_D$ +4.2° (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.07), 254sh (3.78) nm; IR (film) ν_{max} 3365, 2977, 1736, 1679 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 763.2731 $[M + Na]^+$ (calcd for $C_{35}H_{48}O_{17}Na$ 763.2789).

Javanicoside E (6): amorphous powder; $[\alpha]^{24}_D$ −2.3° (*c* 0.44, MeOH); UV (MeOH) λ_{max} (log ϵ) 255 (3.87) nm; IR (film) ν_{max} 3412, 2980, 1739, 1676 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 793.2950 $[M + Na]^+$ (calcd for $C_{36}H_{50}O_{18}Na$ 793.2895).

Javanicoside F (7): amorphous powder; $[\alpha]^{24}_D$ +10.4° (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.11), 252sh (3.80) nm; IR (film) ν_{max} 3349, 2924, 1739, 1678 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 719.2485 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{16}Na$ 719.2527).

Acid Hydrolysis of 3. A solution of **3** (4.5 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 mixture was extracted with $CHCl_3$ (3 × 5 mL). The combined $CHCl_3$ layer was washed with brine, dried over Na_2SO_4, and evaporated to give an aglycone fraction (1.5 mg). The H_2O layer was passed through a short Amberlite IRA-400 column and evaporated to dryness to give a sugar fraction (1.7 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 was identified as D-glucose based on the HPLC retention time, t_R, of 11.70 min (D-glucose t_R, 11.55 min) and the optical rotation (positive).

Acid Hydrolysis of 4. Compound **4** (3.2 mg) was subjected to acid hydrolysis as described for **3** to give an aglycone fraction (1.9 mg) and a sugar fraction (1.3 mg). HPLC analysis of the sugar fraction under the conditions described above showed that the sugar was D-glucose (t_R, 11.70 min, and positive optical rotation). The aglycone was identified as dehydrobrusatol from the ^{1}H NMR spectrum.

Acid Hydrolysis of 5. Compound **5** (3.2 mg) was subjected to acid hydrolysis as described for **3** to give an aglycone fraction (0.9 mg) and a sugar fraction (1.2 mg). Subsequent HPLC analysis of the sugar fraction demonstrated that the sugar in **5** was D-glucose (t_R, 11.74 min, and positive optical rotation).

Acid Hydrolysis of 6. Compound **6** (3.8 mg) was subjected to acid hydrolysis as described for **3** to give an aglycone fraction (1.1 mg) and a sugar fraction (1.0 mg). HPLC analysis under the conditions described for **3** showed that the sugar was D-glucose (t_R, 12.10 min, and positive optical rotation).

Acid Hydrolysis of 7. Compound **7** (3.2 mg) was subjected to acid hydrolysis as described for **3** to give an aglycone fraction (0.9 mg) and a sugar fraction (1.2 mg). HPLC analysis of the sugar fraction showed that it was D-glucose (t_R, 11.74 min, and positive optical rotation).

Cytotoxicity against P-388 Cells. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed on a 96-well plate. Murine P-388 leukemia cells (3 × 10^3 cells) in 100 μL of RPMI-1640 medium

(Nissui Pharmaceutical Company, Ltd., Tokyo, Japan) supplemented with 5% fetal calf serum (Mitsubishi Chemical Industry Co., Ltd., Tokyo, Japan) and kanamycin (100 μg/mL) were inoculated into each well and incubated at 37 °C in a humidified atmosphere of 7% CO_2. Test samples of various concentrations (10 μL) were added to the cultures 24 h after incubation. The medium was incubated for 48 h at 37 °C, and then 20 μL of the MTT solution (5 mg/mL) was added to each well. After a further incubation for 4 h, 100 μL of 10% sodium dodecyl sulfate/0.01 N HCl solution was added to each well, and the formazan crystals that were formed in each well were dissolved by stirring with a pipet. Optical density was recorded on a microplate reader (Tosoh MPR-A4i) at 550 nm. In the assay for cytotoxicity, each data point represents the average of three replicate measurements.

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