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Three novel quassinoids, javanicolides A and B, and javanicoside A, from seeds of *Brucea javanica*

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Abstract—Two novel quassinoids, javanicolides A and B, and one novel quassinoid glucoside, javanicoside A were isolated from the seeds of *Brucea javanica* Merr. (Simaroubaceae), along with four known quassinoids, yadanziolides A and D, and bruceins D and E, and two known quassinoid glucosides, yadanziosides D and L. Their structures were elucidated by the analysis of spectral data and chemical evidence.

1. Introduction

Brucea javanica 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,[1,2] are also known as a rich source of quassinoids.[3–6] In the present study, from seeds of this plant we isolated three novel quassinoids **1**, **2** and **3** possessing unique structural features along with six known quassinoids, yadanziolides A and D (**4**),[3,7] bruceins D (**5**) and E,[8] and yadanziosides D and L.[4,9] The three new quassinoids are javanicolide A (**1**), a C_{19}-quassinoid characterized by an isomeric 1,2-seco-1-nor-6(5→10)*abeo*-picrasane skeleton, javanicolide B (**2**), a C_{20}-quassinoid characterized by a hemiacetal linkage at C-20, and javanicoside A (**3**), a C_{20}-quassinoid glucoside characterized by an A/B *cis* ring juncture. This paper describes their isolation and structure elucidation (Fig. 1).

2. Results and discussion

By Diaion HP-20 resin column chromatography of the *n*-BuOH-soluble portion from a hot MeOH extract of the seeds of *B. javanica*, a quassinoid-rich H_2O/MeOH (1:1) eluate was obtained. By preparative reversed-phase HPLC of this eluate using MeOH/H_2O (26:74 and 1:0) afforded nine fractions. When left standing, the first fraction produced crude crystals, which upon subsequent reversed-phase HPLC gave known quassinoids, bruceins D (**5**) and E. Reversed-phase HPLC of the mother liquid afforded two novel quassinoids, javanicolides A (**1**) and B (**2**), and known quassinoids, bruceins D (**5**) and E, and yadanziolides A and D (**4**). The seventh fraction of the preparative reversed-phase HPLC gave javanicoside A (**3**) and yadanziosides D and L. Identification of those known compounds was made by the comparison of their spectral data with those in the literature.

Javanicolide A (**1**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{19}H_{24}O_9$ by the $[M+Na]^+$ ion peak at *m/z* 419.1297 (calcd for $C_{19}H_{24}O_9Na$ 419.1318) in the HRESIMS. Its IR spectrum showed the presence of hydroxyl groups (3408 cm^{-1}), δ- and conjugated γ-lactones (1737 cm^{-1}) and an olefinic (1640 cm^{-1}) bond. The ^1H NMR spectrum showed the presence of two tertiary methyls (δ 1.35 and 1.77), one olefinic methyl (δ 1.92), a pair of protons giving AB-quartet signals (δ 4.46 and 4.84, *J*=6.9 Hz) and one olefinic proton (δ 5.91) (Table 1). Analysis of the ^{13}C NMR and HMBC spectra suggested the presence of a β-methyl-α,β-unsaturated-γ-lactone group attached to ring B (Table 2). These observations and general similarity of the ^1H and ^{13}C NMR spectra of **1** and **4** suggested that **1** possessed the 1,2-seco-1-nor-6(5→10)*abeo*-picrasane skeleton as **4**. A long-range coupling observed between H-12 and C-16 in the HMBC spectrum indicated that a lactone linkage exists between C-12 and C-16 in **1**. The stereochemistry of **1** was determined by the analysis of NOESY spectrum (Fig. 2). The NOESY correlations between H_3-19 and H_a-20, and H_α-6 and H-9 indicated a *trans* B/C ring juncture, that between H_3-19 and OH-11 revealed that the configuration of C-11 hydroxyl group to be β, and the correlations between H-7 and H_b-20, and H-15 and H_3-21 indicated that the hydroxyl groups at C-7 and C-15 were both of α-configuration. The stereochemistry at C-5 was

Keywords: *Brucea javanica*; quassinoid; javanicolides A and B; javanicoside A; Simaroubaceae.
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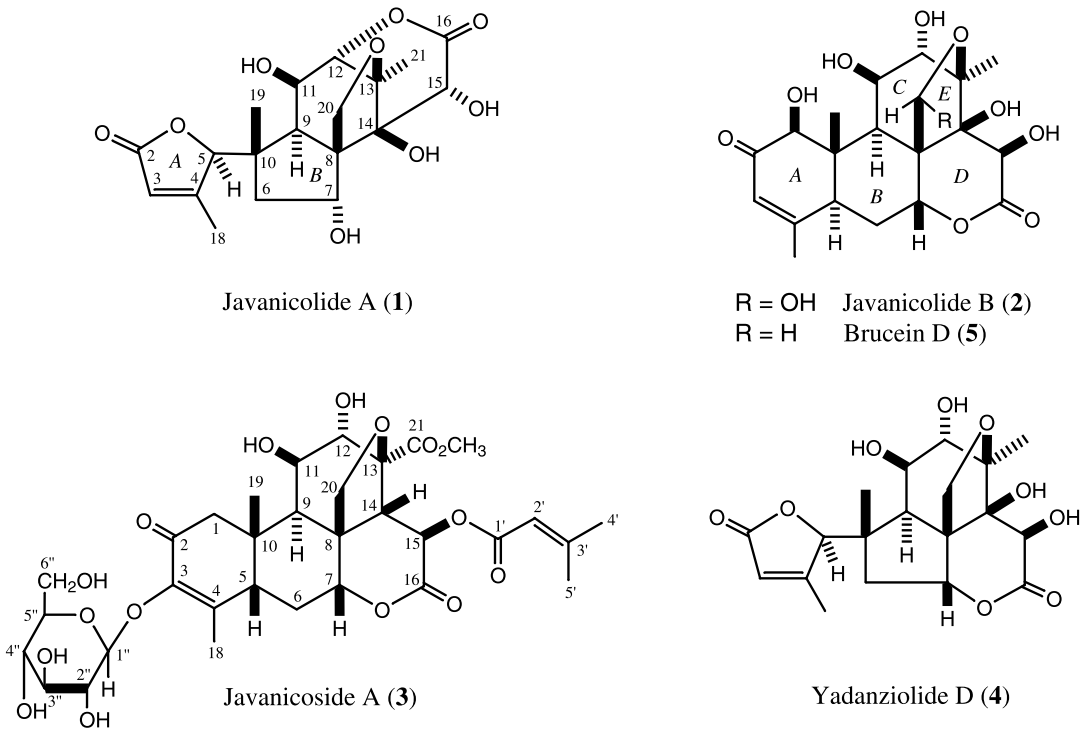

Figure 1.

determined by the observation of correlations between the ring A and ring B protons. Correlations were observed between H-5 and H_{α}-6, H-5 and H-9, and H_3-18 and H_{α}-6. We did not particularly investigate the absolute configurations at 5 and 10, but on the basis of these observations and biosynthetic considerations, we concluded that compound **1** possessed 5*S*, 10*S* configurations as in yadanziolide D (**4**). Accordingly, javanicolide A (**1**) was shown to have the structure given in Figure 1.

Several quassinoids having the 1,2-seco-1-nor-6(5⟶10)*abeo*-picrasane skeleton are reported in several Simaroubaceous plants.[7,10–15] However, all the reported compounds having this skeleton possess a lactone linkage between C-7 and C-16, whereas javanicolide A (**1**) has a lactone unit formed between C-12 and C-16. Javanicolide A (**1**) is the first example of this type of quassinoids.

Javanicolide B (**2**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{20}H_{26}O_{10}$ by the $[M+Na]^+$ ion peak at *m/z* 449.1438 (calcd for $C_{20}H_{26}O_{10}Na$ 449.1424) in the HRESIMS, which was equivalent to that of brucein D (**5**) ($C_{20}H_{26}O_9$) plus one oxygen atom. Comparison of the NMR spectra with those of **5** revealed that **2** had A, B, C and D rings of the same structure as in **5** and that, accordingly, the structural differences between these two quassinoids resided only in the E ring. The most distinguished features observed in the NMR spectra were that C-20 of **2** gave a methine carbon resonance (δ 98.1) (Table 2), and the proton attached to C-20 gave a broad doublet signal of one proton at very low field of δ 6.64 (Table 1), suggesting that two oxygen atoms were connected to C-20. Thus, **2** was determined to be an analogue of **5** in which one of the C-20 methylene protons of **5** was replaced by a hydroxyl group. The stereochemistry of **2** was confirmed by the analysis of NOESY spectrum (Fig. 3). Correlations were observed between H-1, H-5 and H-9, between H-9 and H-15, H_{β}-6 and H_3-19, H_3-19 and H-20 and H-7 and OH-20. On the basis of these observations and biosynthetic considerations, the stereochemistry at C-20 was assigned to *S* and the stereochemistry at the rest of the stereocenters to be the same as those in **5**. From these observations, javanicolide B (**2**) was determined to have the structure shown in Figure 1. This is the first example of quassinoids having a hemiacetal linkage at C-20.

Javanicoside A (**3**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{32}H_{42}O_{16}$ as it gave the $[M+Na]^+$ ion peak at *m/z* 705.2313 (calcd for $C_{32}H_{42}O_{16}Na$ 705.2371) in the HRESIMS. Its ^1H NMR spectrum showed the presence of a tertiary methyl (δ 1.80), three olefinic methyls (δ 1.59, 2.11 and 2.50) and a carbomethoxy (δ 3.64) group (Table 1). The IR spectrum showed the presence of hydroxyl (3347 cm^{-1}), δ-lactone and ester (1746 cm^{-1}), and α,β-unsaturated ketone (1681 cm^{-1}) groups. The ESIMS showed a fragment ion peak $[MH-C_6H_{10}O_5]^+$ at *m/z* 521, which suggested that **3** was a glycoside. Its ^{13}C NMR and HMBC spectra demonstrated the presence of a β-D-glucose unit (δ 104.9, 78.1, 77.9, 75.6, 71.1 and 62.4), a ketone group at C-2 (δ 193.8), and a senecioyl group (δ 165.8, 158.5, 116.0, 26.9 and 20.1) (Table 2). An HMBC correlation between H-1″ and C-3 revealed that the sugar moiety was linked to the C-3 oxygen atom. The sugar component was identified as D-glucose by acid hydrolysis of **3** 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 (7.7 Hz) of the anomeric proton of the glucosyl moiety indicated a β anomeric orientation. In the ROESY spectrum, H-2′, H_3-4′ and H_3-5′ were

Table 1. ^{1}H NMR spectral data for compounds **1**–**3** in C_5D_5N[a]

Position	**1**[b]	**2**[c]	**3**[c,d]
1 α		4.35 (s)	3.33 (d, 15.5)
β			2.57 (d, 15.5)
3	5.91 (s)	6.12 (s)	
5	5.25 (s)	3.13 (d, 13.5)	2.49 (–[e])
6 α	2.26 (dd, 13.8, 3.1)	2.39 (dt, 14.7, 2.8)	2.46 (–[e])
β	1.79 (dd, 13.8, 4.9)	2.03 (ddd, 14.7, 13.5, 2.2)	2.14 (–[e])
7	4.60 (br m)	5.87 (s-like)	4.96 (–[e])
9	3.01 (d, 4.0)	2.96 (d, 5.2)	3.01 (d, 3.7)
11	5.14 (m)	5.37 (m)	4.91 (br s)
12	4.71 (d, 2.0)	4.63 (d, 4.0)	5.13 (br s)
14			4.01 (br d, 11)
15	4.99 (s)	6.05 (s)	6.48 (br s)
18	1.92 (s)	1.74 (s)	2.50 (s)
19	1.35 (s)	1.49 (s)	1.80 (s)
20 a	4.84 (d, 6.9)	6.64 (br d, 6)	5.04 (d, 7.3)
b	4.46 (d, 6.9)		3.85 (d, 7.3)
21	1.77 (s)	2.18 (s)	
OMe			3.64 (s)
2′			5.68 (s)
4′			1.59 (s)
5′			2.11 (s)
1″			5.43 (d, 7.7)
2″			4.20 (dd, 8.7, 7.7)
3″			4.30 (dd, 9.2, 8.7)
4″			4.37 (t, 9.2)
5″			4.18 (m)
6″			4.46 (dd, 11.7, 4.0)
			4.57 (dd, 11.7, 2.7)
1-OH		6.80 (s)	
7-OH	7.02 (br s)		
11-OH	6.94 (d, 6.2)	5.07 (d, 5.8)	6.64 (br s)
12-OH		7.48 (d, 4.0)	8.23 (br s)
14-OH	7.52 (s)	7.14 (s)	
15-OH	–[f]	–[f]	
20-OH		7.68 (br d, 6)	

[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 not determined due to overlapping of the signals.
[f] Signals not identified because of extreme broadening.

demonstrated to be correlated with 21-OCH_3 (Fig. 4). Therefore, the senecioyl group was determined to link to the oxygen atom at C-15, as those ROESY correlations were possible only when the senecioyloxy group was present at C-15 with β-configuration. 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. The analysis of ROESY spectrum afforded further information about the stereochemistry of **3** (Fig. 4). Correlations observed between H_β-1 and H-5, H-5 and H_3-19, H_β-6 and H_3-19 suggested that these protons and the methyl group involved were all of β-configuration, and that the A/B rings were *cis* fused, whereas those between H-7 and H-14, H-14 and H_b-20, H_3-19 and H_a-20 suggested that the methyleneoxy bridge between C-8 and C-13 was of β-configuration. Accordingly, javanicoside A (**3**) was determined to have the structure shown in Figure 1. Quite a few natural C_{20}-quassinoids have been reported, but when they have a hydrogen atom at 5, the A/B ring juncture is always *trans*. This is the first example of the C_{20}-quassinoids having an A/B *cis* ring juncture with a hydrogen at 5.

Table 2. ^{13}C NMR spectral data for compounds **1**–**3** in C_5D_5N

Position	**1**[a]	**2**[b]	**3**[b]
1		82.7	51.0
2	172.6	198.2	193.8
3	119.9	125.0	146.6
4	167.9	163.4	147.7
5	94.0	43.4	48.1
6	46.0	27.6	27.6
7	73.1	77.6	83.1
8	63.1	52.9	46.2
9	48.7	46.0	31.9
10	47.1	48.7	39.7
11	71.9	75.4	73.3
12	87.7	81.1	76.3
13	83.9	85.9	82.7
14	82.9	82.4	48.8
15	72.0	70.5	68.4
16	172.3	174.8	167.0
18	16.2	22.1	16.7
19	19.5	11.6	26.5
20	73.4	98.1	73.3
21	17.2	20.1	170.8
OMe			52.3
1′			165.8
2′			116.0
3′			158.5
4′			26.9
5′			20.1
1″			104.9
2″			75.6
3″			78.1
4″			71.1
5″			77.9
6″			62.4

[a] Measured at 100 MHz.
[b] Measured at 125 MHz.

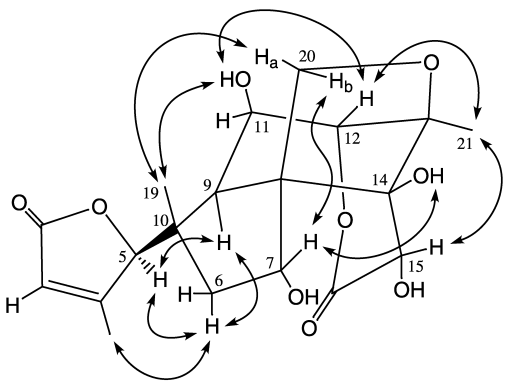

Figure 2. Selected NOESY correlations for **1**.

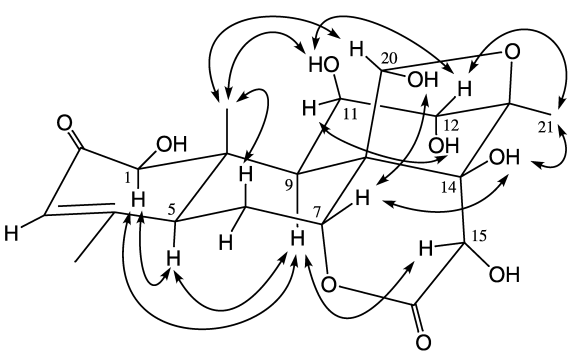

Figure 3. Selected NOESY correlations for **2**.

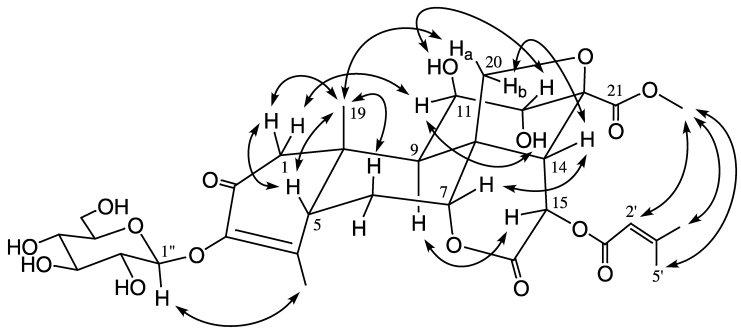

Figure 4. Selected ROESY correlations for **3**.

Javanicolides A (**1**) and B (**2**), and javanicoside A (**3**) showed a weak cytotoxic activity on P-388 murine leukemia cells with IC_{50} values of 17, 8.0, and 60 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 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, Inertsil PREP-ODS (10 μm, 20 mm i.d.×250 mm) or Mightysil RP 18 GP (5 μm, 20 mm i.d.×250 mm) (MeOH/H_2O or MeCN/H_2O, flow rate 10 mL/min). Analytic 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).

3.2. Plant material

The seeds of *Brucea javanica* Merr. were purchased in China in 2000, and identified by Dr Koichi Takeya, Professor 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. javanica* (20 kg) were extracted with hot MeOH (4×18 L). The solvent was removed in vacuo to give a residue (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 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) and finally with acetone to give five fractions. The 1:1 H_2O/MeOH 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. 1–9). Those fractions were evaporated to dryness. MeOH was added to Fr. 1 (2.3 g). When the solution was left standing, it produced precipitate of 451.8 mg of crude crystals, which was separated and further subjected to reversed-phase HPLC using MeOH/H_2O (17:83) to afford bruceins D (**5**) (95.0 mg) and E (253.8 mg). From the mother liquid (1.8 g) by repeated reversed-phase HPLC using either MeOH/H_2O or MeCN/H_2O mixture afforded compounds **1** (4.3 mg) and **2** (5.1 mg), bruceins D (**5**) (28.9 mg) and E (287.4 mg), and yadanzioldides A (197.2 mg) and D (**4**) (7.7 mg). Fr. 7 (349.4 mg) gave compound **3** (27.0 mg), yadanziosides D (121.8 mg) and L (95.4 mg) when separated by using reversed-phase HPLC using MeCN/H_2O (15:85).

3.3.1. Javanicolide A (1). Amorphous powder; $[\alpha]_D=-52.3°$ (*c* 0.09, MeOH); UV (MeOH) λ_{max} nm (log ε): 220 (4.08), 279 (3.19); IR (film) ν_{max} cm^{-1}: 3408, 2950, 1737, 1640. ^1H and ^{13}C NMR: refer to Tables 1 and 2; HRESIMS *m/z* 419.1297 $[M+Na]^+$, calcd for $C_{19}H_{24}O_9Na$ 419.1318.

3.3.2. Javanicolide B (2). Amorphous powder; $[\alpha]_D=+63.7°$ (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε): 239 (4.17); IR (film) ν_{max} cm^{-1}: 3364, 2946, 1734, 1663. ^1H and ^{13}C NMR: refer to Tables 1 and 2; HRESIMS *m/z* 449.1438 $[M+Na]^+$, calcd for $C_{20}H_{26}O_{10}Na$ 449.1424.

3.3.3. Javanicoside A (3). Amorphous powder; $[\alpha]_D=-41.3°$ (*c* 0.54, MeOH); UV (MeOH) λ_{max} nm (log ε): 224 (4.33); IR (film) ν_{max} cm^{-1}: 3347, 2945, 1746, 1681. ^1H and ^{13}C NMR: refer to Tables 1 and 2; HRESIMS *m/z* 705.2313 $[M+Na]^+$, calcd for $C_{32}H_{42}O_{16}Na$ 705.2371.

3.3.4. Acid hydrolysis of 3. A solution of **3** (5.3 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 the aglycon (2.1 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 component was identified as D-glucose by the comparison of the HPLC retention time, t_R 11.80 (D-glucose t_R 11.55) and the sign (positive) of optical rotations.

Data for aglycon. ^{1}H NMR (500 MHz, C_5D_5N) δ 5.73 (1H, s, H-2′), 5.09 (1H, br s, H-12), 5.09 (1H, d, J=7.3 Hz, H_a-20), 4.98 (1H, s, H-7), 4.88 (1H, br s, H-11), 3.99 (1H, br s, H-14), 3.90 (1H, d, J=7.3 Hz, H_b-20), 3.66 (3H, s, OMe), 3.38 (1H, d, J=16.0 Hz, H_α-1), 3.10 (1H, br s, H-9), 2.55 (1H, m, H_α-6), 2.53 (1H, d, J=16.0 Hz, H_β-1), 2.45 (1H, m, H-5), 2.34 (3H, s, H_3-18), 2.17 (1H, m, H_β-6), 2.11 (3H, s, H_3-5′), 1.85 (3H, s, H_3-19), 1.60 (3H, s, H_3-4′); HRESIMS m/z 521.2018 $[M+H]^+$, calcd for $C_{26}H_{33}O_{11}$ 521.2023.

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