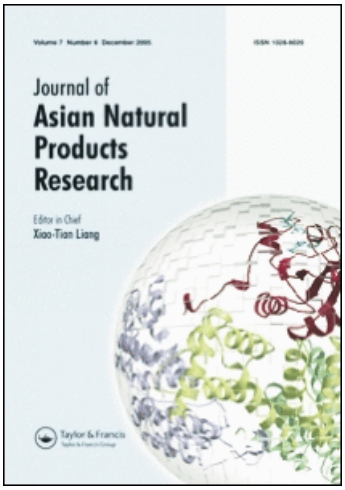

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Iridoid glucosides from leaves and stem barks of *Parkia javanica*

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Two new iridoid glucosides, javanicosides A (**1**) and B (**2**) along with the known compounds, ursolic acid and β-sitosterol were isolated from the leaf and stem bark of *Parkia javanica* and the structures were established on the basis of detailed spectroscopic analysis (MS, 1D, and 2D NMR experiments). The new compounds were identified as 8-*O*-*p*-hydroxybenzoyl-6′-*O*-*p*-coumaroyl-mussaenosidic acid (**1**) and 7-*O*-*E*-3,4-dimethoxycinnamoyl-6′-*O*-β-D-glucopyranosylloganic acid (**2**).

Keywords: *Parkia javanica*; Mimosaceae; iridoid glucosides

1. Introduction

Parkia javanica (Lamk.) Merr. syn., *Parkia roxburghii* G. Don., and *Parkia timoriana* (DC.) Merr. (local name: Sapota, Long-chak, Kuki-Tetai; Mimosaceae) are medium-sized trees and are widely distributed in different parts of India [1]. The tender fruits are eaten by the local people as vegetables. Our screening study on the methanolic extracts of both the leaf and the stem bark indicated significant anti-neoplastic activity against K 562 and EAC/Dox cell lines. For the search of biologically active compounds, we have examined both these methanol extracts and isolated two new iridoid glucosides **1** and **2** along with the known compounds, ursolic acid (**3**) and β-sitosterol. This paper describes the isolation and structure elucidation of the new compounds, named javanicosides A (**1**) and B (**2**).

2. Results and discussion

The leaves of *P. javanica* were extracted with MeOH. After solvent removal, the extract was successively partitioned between H_2O and benzene, H_2O and $CHCl_3$, and H_2O and *n*-BuOH. After the evaporation of solvent, the benzene fraction on column chromatography (CC; silica gel) gave β-sitosterol. The BuOH fraction after removal of solvent was further fractionated by CC (Diaion HP-20). The fractions eluted with H_2O and H_2O/MeOH 75:25 were mixed together, concentrated and subjected to CC (silica gel) to get javanicoside A (**1**) and ursolic acid (**3**). The stem bark of *P. javanica* was extracted similarly with MeOH. After the removal of solvent, the extract was fractionated into benzene-, $CHCl_3$-, and *n*-BuOH-soluble fractions. The *n*-BuOH-soluble fraction after removal of solvent was further fractionated by CC

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(Diaion HP-20 and silica gel) to get javanicoside B (**2**). The known compounds, β-sitosterol [2] and ursolic acid (**3**) [3], were identified by the comparison of their physical and spectral data with those reported in the literature. These known compounds were isolated from the title plant for the first time.

Javanicoside A (**1**), a white amorphous powder, had a molecular formula $C_{32}H_{34}O_{14}$ as determined by HR-FAB-MS at *m/z* 665.1840 $[M + Na]^+$ as well as ^{13}C and DEPT NMR spectral data. The negative-ion FAB-MS displayed a mass peak at *m/z* 641 $[M - H]^-$ also supporting its molecular weight of 642 Da. The IR spectrum showed the absorption bands for hydroxyl (3423 cm^{-1}), enol ether (1634 cm^{-1}), α,β-unsaturated ester (1703 cm^{-1}), and aromatic (1608 and 1508 cm^{-1}) functions. The EI-MS showed mass peaks at *m/z* 376 and 496 suggesting the presence of mussaenosidic acid and 8-*O*-*p*-hydroxybenzoylmussaenosidic units in the molecule. The negative-ion FAB-MS recorded also a mass ion at *m/z* 521 suggested the presence of *p*-coumaroylmussaenosidic unit in the molecule. The detailed analysis of 1D and 2D NMR data of **1** and **1a** (pentaacetate of **1**; Table 1) resulted to assign the structures of aglycone, acyl, and sugar moieties of compound **1**. The ^1H NMR spectrum of **1** showed the

Table 1. ^{13}C NMR (100 MHz) and ^1H NMR (400 MHz) spectral data of **1** in CD_3OD and **1a** in $CDCl_3$ (δ in ppm).

	1		**1a**	
Carbon no.	δ (C)[a]	δ (H)[b]	δ (C)[a]	δ (H)[b]
1	95.4	5.16 (d, $J = 2.5$)	96.0	5.24 (d, $J = 2.5$)
3	153.3	7.02 (brs)	152.0	7.20 (brs)
4	114.0	–	112.0	–
5	31.8	2.99 (m)	30.1	2.96 (m)
6	29.7	2.10–2.24 (m), 1.63–1.78 (m)	29.7	2.08–2.18 (m), 1.54–1.62 (m)
7	40.5	1.93–1.98 (m), 1.63–1.78 (m)	40.6	1.93–1.98 (m), 1.67–1.72 (m)
8	88.2	–	85.6	–
9	52.2	2.16 (dd, $J = 9.0, 2.5$)	50.2	2.14 (dd, $J = 9.0, 2.5$)
10	22.7	1.58 (s)	23.2	1.62 (s)
11	168.8	–	168.9	–
1′	96.4	4.74 (d, $J = 8.0$)	97.4	4.73 (d, $J = 8.0$)
2′	74.1	3.23–3.56	70.3	4.97 (dd, $J = 9.0, 8.0$)
3′	76.5		72.5	5.16 (dd, $J = 9.0, 9.0$)
4′	70.8		68.8	5.08 (dd, $J = 9.0, 9.0$)
5′	75.6		72.7	3.82 (m)
6′	63.8	4.40 (dd, $J = 12.0, 4.5$), 4.48 (dd, $J = 12.0, 2.5$)	62.8	4.31 (dd, $J = 12.0, 4.5$), 4.37 (dd, $J = 12.0, 2.5$)
1″	127.3	–	127.4	–
2″, 6″	130.6	7.41 (d, $J = 8.5$)	130.4	7.41 (d, $J = 8.5$)
3″, 5″	116.2	6.79 (d, $J = 8.5$)	116.3	6.79 (d, $J = 8.5$)
4″	160.2	–	161.2	–
7″	146.2	7.62 (d, $J = 16.0$)	146.8	7.62 (d, $J = 16.0$)
8″	116.3	6.39 (d, $J = 16.0$)	116.2	6.38 (d, $J = 16.0$)
9″	169.2	–	169.3	–
1‴	122.4	–	122.5	–
2‴, 6‴	132.9	7.85 (d, $J = 8.5$)	133.2	7.86 (d, $J = 8.5$)
3‴, 5‴	118.0	6.79 (d, $J = 8.5$)	118.4	6.88 (d, $J = 8.5$)
4‴	163.4	–	164.1	–
7‴	168.1	–	168.8	–

[a] Multiplicities were assigned from DEPT spectra.
[b] Overlapped ^1H NMR signals are reported without designating expected multiplicity.

presence of one β-glucopyranoside unit [anomeric proton at δ 4.74 (d, $J = 8.0$ Hz)] in addition to two A_2B_2 systems [δ 7.41 (2H, d, $J = 8.5$ Hz), 6.79 (4H, d, $J = 8.5$ Hz), and 7.85 (2H, d, $J = 8.5$ Hz)] together with one set of *trans* olefinic protons [δ 7.62 (1H, d, $J = 16.0$ Hz) and 6.39 (1H, d, $J = 16.0$ Hz)] suggesting the presence of one β-D-glucopyranosyl unit, one *p*-coumaroyl unit, and one *p*-hydroxybenzoyl unit in the molecule. The ^{1}H NMR spectrum of **1** also showed the presence of a *tert* methyl group (δ 1.58, 3H, s, H_3-10), attached to an oxygen-bearing carbon, a trisubstituted olefinic proton (δ 7.02, 1H, brs, H-3), and a methine proton (δ 5.16, 1H, d, $J = 2.5$ Hz, H-1), characteristic of an iridoid nucleus of mussaenosidic acid skeleton [4]. The ^{1}H and ^{13}C NMR signals of **1** and **1a** corresponding to mussaenosidic acid (**4**) were very similar to the previously reported data [4,5]. The attachment of *p*-hydroxybenzoyl moiety at C-8 position was indicated by the downfield chemical resonances of C-8 carbon and C-10 methyl protons [6]. Similarly, the linkage of *p*-coumaroyl moiety to C-6′ carbon of glucose moiety was ascertained by the downfield chemical shifts of C-6′ carbon and C-6′ methylene protons [4,7]. The HMBC correlation of H-1 proton (δ 5.16) with C-1′ carbon (δ 96.4) and of H-1′ proton (δ 4.74) with C-1 carbon (δ 95.4) in 2D NMR spectrum of **1** clearly indicated the presence of glycosidic linkage of glucopyranosyl moiety to C-1 position of the iridoid aglycone. Similarly, HMBC correlation of H-6′ methylene protons with C-9″ carbon in the HMBC spectrum of **1** indicated the attachment of *p*-coumaroyl unit at C-6′ position of the glucose moiety. The correlation of H-7 β-proton signal (δ 1.63 ppm) with the H-2‴ proton signal (δ 7.85 ppm) in the NOESY spectrum of **1** suggested the attachment of *p*-hydroxybenzoyl unit to C-8 position of the iridoid aglycone as well as the presence of both 7β-H and acyl moiety on the same site (β) to the cyclopentane ring. Similar correlation of H-1 proton to H-10 methyl protons in the NOESY spectrum of **1** also supported their location on the same site, i.e., α-orientation with respect to the plane of aglycone carbon skeleton. The correlation of H-5 signal (δ 2.99) with H-9 signal (δ 2.16) in the NOESY spectrum of **1** supported the *cis*-fused ring juncture of the cyclopentane ring with six-membered enol ether ring. The ^{1}H—^{1}H COSY cross-peaks in the COSY spectra of **1** and **1a** also supported the assigned chemical shift values of sugar and aromatic protons. Consequently, the structure of javanicoside A was concluded to be 8-*O*-*p*-hydroxybenzoyl-6′-*O*-*p*-coumaroyl-mussaenosidic acid (**1**; Figure 1). It is a new natural compound.

Javanicoside B (**2**) was obtained as a white amorphous solid and had a molecular formula $C_{33}H_{44}O_{18}$ assigned from its quasi-molecular ion peak at *m*/*z* 729.2600 $[M+H]^+$ in the positive-ion HR-FAB-MS as well as ^{13}C and DEPT NMR spectral data. This molecular formula was also supported by the positive-ion FAB-MS of its heptaacetate (**2a**) which showed a quasi-molecular ion peak at *m*/*z* 1045 $[M+Na]^+$. The IR spectrum of **2** showed absorptions for hydroxyl (3420 cm^{-1}), enol ether (1633 cm^{-1}), α,β-unsaturated ester (1699 cm^{-1}), and aromatic (1610 and 1508 cm^{-1}) functions. The positive-ion FAB-MS of **2** also recorded a mass ion peak at *m*/*z* 539 [glucosylloganic acid + H]$^+$ suggesting the presence of glucosylloganic acid moiety in the molecule. The detailed analysis of ^{1}H and ^{13}C NMR spectral data of **2** and **2a** (Table 2) led to establish the structures of aglycone, sugar, and acyl moieties of the compound. The ^{1}H and ^{13}C NMR spectral data for the loganic acid moiety of **2** and **2a** were similar to that of 7-*O*-*E*-feruloylloganic acid (**5**) [8] and that of sugar moiety were similar to that of loganic acid-6′-*O*-β-D-glucoside (**6**) [9]. The ^{1}H and ^{13}C NMR data for the 3,4-dimethoxycinnamoyl unit of **2** and **2a** were similar to that of the previously reported data [10]. The attachment of 3,4-dimethoxycinnamoyl moiety at C-7 carbon of the aglycone was established by the downfield chemical shifts of C-7 carbon and H-7 proton. The linkage of glucose moiety to C-6′ carbon of glucose was determined by the

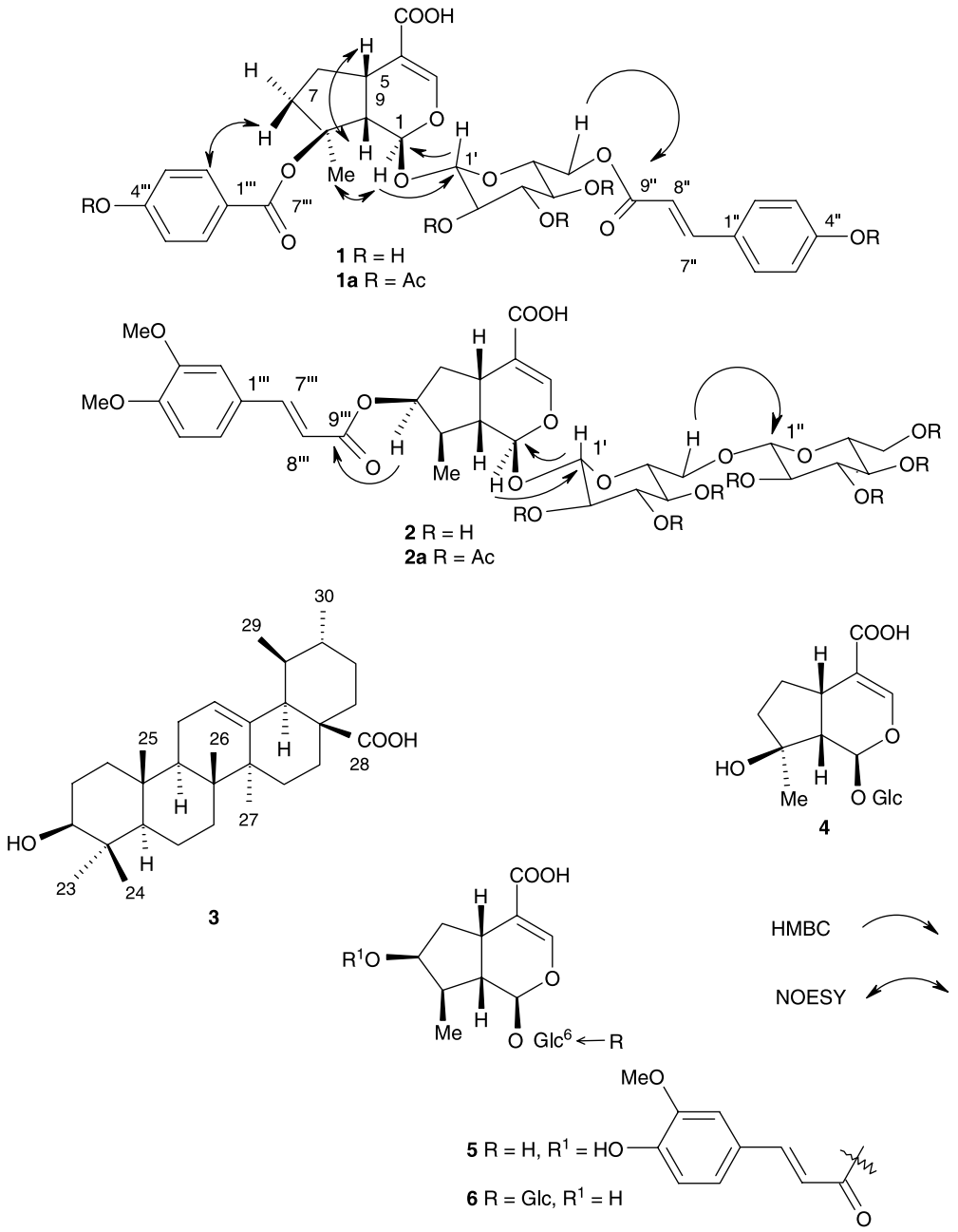

Figure 1. Structures and significant HMBC and NOESY correlations of **1** and **2**.

HMBC correlation of C-6′ methylene protons with C-1″ carbon as well as downfield chemical shifts of both C-6′ and C-1″ carbons. The $^{1}H—^{1}H$ COSY spectrum also corroborated the chemical shift values of sugar and aromatic protons. Thus, the structure of javanicoside B was formulated as 7-*O*-*E*-3,4-dimethoxycinnamoyl-6′-*O*-β-D-glucopyranosylloganic acid (**2**; Figure 1). This is a new natural compound.

Table 2. ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) spectral data of **2** in CD_3OD and **2a** in $CDCl_3$ (δ in ppm).

	2		**2a**	
Carbon no.	δ (C)[a]	δ (H)[b]	δ (C)[a]	δ (H)[b]
1	99.4	5.41 (d, $J = 3.5$)	99.6	5.26 (d, $J = 3.5$)
3	152.0	7.40 (brs)	152.6	7.39 (brs)
4	112.6	–	114.1	–
5	33.5	3.09 (m)	31.9	3.15 (m)
6	40.7	2.18 (m), 1.61–1.70 (m)	40.6	2.18 (m), 1.82–1.96 (m)
7	78.4	5.25 (t-like)	78.7	5.24 (t-like)
8	42.6	2.26–2.30 (m)	42.9	2.26–2.30 (m)
9	47.6	2.32–2.40 (m)	47.2	2.32–2.40 (m)
10	13.9	0.98 (d, $J = 7.0$)	14.1	1.04 (d, $J = 7.0$)
11	175.2	–	174.8	–
1′	100.6	4.68 (d, $J = 7.5$)	100.8	4.85 (d, $J = 7.5$)
2′	74.8	3.28 (dd, $J = 9.2, 7.5$)	70.2	4.94 (dd, $J = 9.0, 7.5$)
3′	78.0	3.38 (t, $J = 9.0$)	72.4	5.14 (t, $J = 9.0$)
4′	71.6	3.34 (t, $J = 9.2$)	68.7	5.06 (t, $J = 9.0$)
5′	76.1	3.54 (m)	73.0	3.84 (m)
6′	67.6	3.73 (dd, $J = 12.0, 5.5$), 3.93 (dd, $J = 12.0, 2.0$)	67.9	4.33 (dd, $J = 12.0, 5.5$), 4.46 (dd, $J = 12.0, 2.0$)
1″	102.6	4.51 (d, $J = 7.5$)	102.8	4.59 (d, $J = 8.0$)
2″	74.1	3.33 (t)	71.1	5.01 (dd, $J = 9.0, 7.5$)
3″	75.8	3.58 (dd, $J = 9.5, 9.0$)	72.4	5.16 (t, $J = 9.0$)
4″	71.8	3.38 (t, $J = 9.0$)	69.1	5.08 (t, $J = 9.0$)
5″	74.4	3.60 (m)	72.9	3.82 (m)
6″	62.2	3.63 (dd, $J = 12.0, 5.5$), 3.84 (dd, $J = 12.0, 2.5$)	61.6	4.28 (dd, $J = 12.0, 5.5$), 4.36 (dd, $J = 12.0, 2.5$)
1‴	128.0	–	128.1	–
2‴	117.8	7.36 (d, $J = 2.0$)	118.0	7.36 (d, $J = 2.0$)
3‴	150.6	–	150.8	–
4‴	154.2	–	154.1	–
5‴	112.3	7.00 (d, $J = 8.5$)	112.7	7.00 (d, $J = 8.5$)
6‴	124.4	7.20 (dd, $J = 8.5, 2.0$)	123.9	7.20 (dd, $J = 8.5, 2.0$)
7‴	145.0	7.64 (d, $J = 16.0$)	146.7	7.64 (d, $J = 16.0$)
8‴	115.4	6.40 (d, $J = 16.0$)	115.2	6.40 (d, $J = 16.0$)
9‴	168.2	–	168.8	–
3‴	55.9	3.84 (s)	55.9	3.83 (s)
4‴	56.2	3.86 (s)	56.2	3.85 (s)

[a] Multiplicities were assigned from DEPT spectra.
[b] Overlapped ^{1}H NMR signals are reported without designating expected multiplicity.

The anti-neoplastic activity of the isolated iridoids will be studied after isolation in more quantities.

3. Experimental

3.1 General experimental procedures

The UV spectra were recorded on a Perkin-Elmer kappa 18 UV–VIS instrument. IR spectra were recorded on a Shimadzu 8100 spectrometer. NMR spectra were measured on a Varian XL-400 spectrometer using TMS as the internal standard. EI-MS, FAB-MS, and HR-FAB-MS data were obtained on a Jeol JMS-AX50HA and JMS-700 MStation mass spectrometers. For CC, silica gel (60–120 mesh; Merck, Mumbai, India) and Diaion HP-20 (Mitsubishi Chemical Industries, Tokyo, Japan) were used. TLC was performed using silica gel G (Merck) coated plates and spots of TLC were detected by spraying 10% H_2SO_4/EtOH followed by

heating for glycosides and iodine chamber for steroid and terpenoid.

3.2 Plant material

The leaves and stem barks of *P. javanica* were collected in Ambassa, Dhalai District, Tripura, in January 2005 and were identified by Dr B.K. Datta, Department of Botany, Tripura University. A voucher specimen (No. BD-01/06) has been deposited in the Central National Herbarium, Govt. of India, Shibpur, Howrah.

3.3 Extraction and isolation

The dried leaves of *P. javanica* (5 kg) were extracted with MeOH (15 l) at room temperature. The extract was concentrated to a gummy mass, diluted with a little H_2O, and partitioned sequentially with benzene, $CHCl_3$, and *n*-BuOH (3× 300 ml). The benzene-soluble fraction was subjected to CC (silica gel). The fractions eluted with hexane/EtOAc 98:2 afforded β-sitosterol (10 mg). The BuOH-soluble fraction was subjected to CC (Diaion HP-20). The fractions eluted with H_2O, and H_2O/MeOH 75:25 were concentrated and subjected to TLC. Both these fractions were almost identical in composition on TLC. These were mixed together and concentrated to a residue (**A**, 180 g). A part of this residue **A** (60 g) was separated by CC (silica gel). The fraction eluted with EtOAc/MeOH 70:30 afforded a residue which on further CC (silica gel) gave **1** (12.5 mg) and ursolic acid (15 mg). Another part of residue **A** (20 g) was acetylated with Ac_2O and pyridine at room temperature for 24 h and usual work up of the acetylated mixture and CC (silica gel) afforded **1a** (30 mg).

The dried stem bark of *P. javanica* (4 kg) was extracted at room temperature with MeOH (12 l). The extract was concentrated to a gummy mass, diluted with a little H_2O, and partitioned sequentially with benzene, $CHCl_3$, and *n*-BuOH (3× 250 ml). The *n*-BuOH-soluble fraction was subjected to CC (Diaion HP-20). The fractions eluted with H_2O and H_2O/MeOH 75:25 and 50:50 afforded residues of almost similar composition on TLC. These were mixed together and concentrated to a residue (**B**, 160 g). A part of the residue **B** (50 g) was separated by CC (silica gel). The fraction eluted with EtOAc/MeOH 50:50 gave a residue, which was further separated by CC (silica gel) to get **2** (14.4 mg). Another part of residue **B** (15 g) was acetylated with Ac_2O and pyridine at room temperature for 24 h. The acetylated mixture obtained after usual work up was separated by CC (silica gel) to get **2a** (18 mg).

3.3.1 *Javanicoside A (= 8-O-(4-hydroxybenzoyl)-6′-O-(4-E-coumaroyl)-mussaenosidic acid;* **1**)

White powder; TLC (SiO_2; EtOAc/MeOH 70:30): $R_f = 0.65$. UV (MeOH) λ_{max} (nm): 230, 300, and 318. $[\alpha]_D^{24} - 52.4$ ($c = 0.6$, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3423, 1703, 1634, 1608, 1508. ^1H and ^{13}C NMR spectral data: see Table 1. EI-MS *m/z* (rel. int. %): 642 $[M^+]$ (7), 496 $[M - \text{coumaroyl} + H]^+$ (24), 522 $[M - \text{hydroxybenzoyl} + H]^+$ (16), 376 $[M - (\text{hydroxybenzoyl} + \text{coumaroyl}) + 2H]^+$ (64), 213 [mussaenosidic acid–glucosyl]$^+$ (100). HR-FAB-MS *m/z* (pos.): 665.1840 $[M + Na]^+$ (calcd for $C_{32}H_{34}O_{14}Na$, 665.1846).

3.3.2 *Javanicoside A pentaacetate (***1a***)*

Yellowish-white powder; TLC (SiO_2; $CHCl_3$/EtOAc 9.5:0.5): $R_f = 0.4$. IR (KBr) ν_{max} (cm^{-1}): 1751, 1700, 1634, 1608, 1508, 1371, 1225, 1051. ^1H and ^{13}C NMR spectral data: see Table 1. EI-MS *m/z* (rel. int. %): 852 $[M^+]$ (13), 810 $[M - CH_2CO]^+$ (37), 768 $[810 - CH_2CO]^+$ (35), 663 $[M - \text{acetoxybenzoyl}]^+$ (10), 647 $[768 - \text{hydroxybenzoyl}]^+$ (11), 478 [(2,3,4-triacetyl-6 (acetyl coumaroyl)–glucosyl)]$^+$ (63), 436 $[478 - CH_2CO]^+$ (100).

3.3.3 *Javanicoside B (= 7-O-(E-3,4-dimethoxycinnamoyl)-6′-O-(β-D-glucopyranosyl) loganic acid;* **2**)

Pale-white powder; TLC (SiO_2; EtOAc/MeOH 70:30): $R_f = 0.35$. UV (MeOH) λ_{max} (nm):

238 and 328. $[\alpha]_D^{24} - 68.6$ ($c = 0.3$, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3420, 1699, 1633, 1610, 1508. ^1H and ^{13}C NMR spectral data: see Table 2. EI-MS *m/z* (rel. int. %): 728 $[M^+]$ (7), 637 [M − 3,4-dimethoxy-cinnamoyl]$^+$ (89), 565 [M − glucosyl]$^+$ (45), 376 [M − (3,4-dimethoxycinnamoyl + glucosyl) + 2H]$^+$ (15), 326 [bisglucosyl]$^+$ (15), 163 [glucosyl]$^+$ (100). HR-FAB-MS (pos.) *m/z*: 729.2600 $[M+H]^+$ (calcd 729.2605).

3.3.4 *Javanicoside B heptaacetate (**2a**)*

Pale-brown powder; TLC (SiO_2; $CHCl_3$/EtOAc 30:20): $R_f = 0.40$. IR (KBr) ν_{max} (cm^{-1}): 1751, 1698, 1633, 1610, 1225, 1052. ^1H and ^{13}C NMR spectral data: see Table 2. FAB-MS (pos.) *m/z* (rel. int. %): 1045 $[M+Na]^+$ (5), 833 [M − (3,4-dimethoxy-cinnamoyl) + 2H]$^+$ (4), 620 [heptaacetyl-bisglucosyl]$^+$ (6), 331 [tetraacetylglucosyl]$^+$ (100), 289 [triacetylglucosyl]$^+$ (21).

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