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Phenolic glycosides from *Barnettia kerrii*

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Abstract

From the leaves and branches *Barnettia kerrii*, two verbascoside derivatives (6″-*O*-acetylverbascoside and 4‴-*O*-acetylverbascoside), as well as two phenolic glycosides (khaephuosides A and B) were isolated together with 15 known compounds. The structural elucidations were based on analyses of spectroscopic data. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Barnettia kerrii*; Bignoniaceae; Phenylethanoid glycoside; Verbascoside derivative; Phenolic glycoside; 6″-*O*-Acetylverbascoside; 4‴-*O*-Acetylverbascoside; Khaephuosides A and B

1. Introduction

As part of an ongoing study on Thai Bignoniaceous plants (Kanchanapoom et al., 2001), we investigated the constituents of *Barnettia kerrii* Santisuk (Thai name: Khae-Phu) collected from the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. *B. kerrii* is a tree, 6–15 m high, endemic to Thailand (Santisuk, 1987). No phytochemical investigation has been carried out on this genus. The present study deals with the structural determination of two new verbascoside derivarives (**4** and **5**), as well as two new phenolic glycosides (**11** and **12**), along with 12 known phenolic compounds (**1**–**3**, **6**–**10**, **13**–**16**), a megastigmane (**17**), a cyclohexyl-ethanoid (**18**) and a iridoid (**19**) from the leaves and branches of this plant. Also, the iridoids in Bignoniaceae was reviewed by von Poster et al. (2000).

2. Results and discussion

The methanolic extract of the leaves and branches of *B. kerrii* was suspended in H_2O and defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer resin of styrene and divinylbenzene, using H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH was repeatedly chromatographed on columns of silica gel, RP-18, or prep. HPLC to afford 19 compounds (**1**–**19**). Fifteen were identified as known compounds; verbascoside (**1**), isoverbascoside (**2**), leucosceptoside A (**3**) (Miyase et al., 1982), luteoside A (**6**), luteoside B (**7**) (Kernan et al., 1998), 2″-*O*-apiosylverbascoside (**8**) (Kanchanapoom et al., 2001), decaffeoylverbacoside (**9**) (Karasawa et al., 1986), seguinoside K (**10**) (Zhong et al., 1999), (+)-lyoniresinol 3a-*O*-β-glucopyranoside (**13**), (+)-5′-methoxyisolariciresinol 3a-*O*-β-glucopyranoside (**14**) (Achenbach et al., 1992), (−)-secoisolariciresinol-9′-*O*-β-glucopyranoside (**15**) (Inoshiri et al., 1987), (7*S*, 8*R*)-dehydrodiconiferyl alcohol 9-*O*-β-glucopyranoside (**16**) (Binns et al., 1987), (6*S*,9*R*)-roseoside (**17**) (Otsuka et al., 1995) rengyoside B (**18**) (Seya et al., 1989) and stegioside III (**19**) (Nass and Rimpler, 1996) by comparison of physical data with literature values and from spectroscopic evidence.

The molecular formula of compound **4** was determined as $C_{31}H_{38}O_{16}$ by HR–FAB mass spectrometry. The ^1H NMR spectral data showed the presence of two sets of ABX system for β-(3,4-dihydroxyphenyl) ethoxy moiety and caffeoyl moiety, as well as two *trans*-olefinic protons, two anomeric protons for β-glucopyranosyl and α-rhamnopyranosyl units, and an acetyl signal. The ^1H and ^{13}C NMR spectra were very similar to those of **1** except for the additional signal from an acetyl group. Comparison of the ^{13}C NMR spectral data of **4** with those of **1** revealed the downfield position of C-6″ (+1.3

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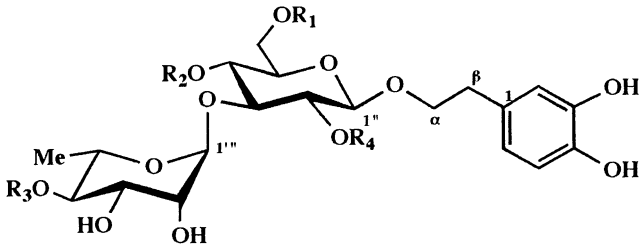

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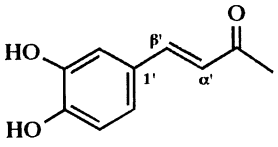
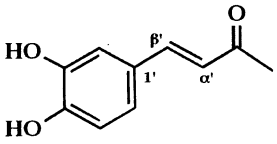
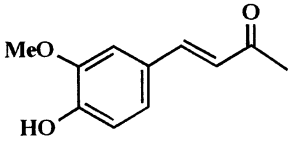
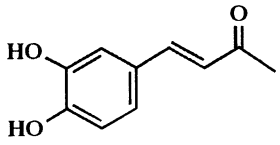
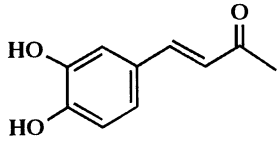
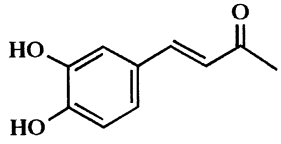
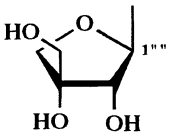
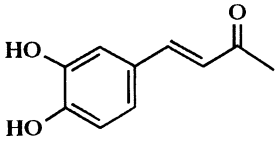
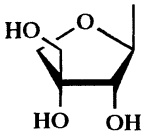
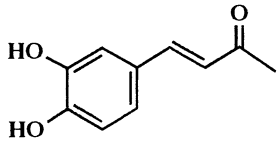
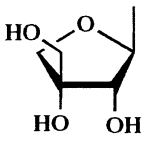
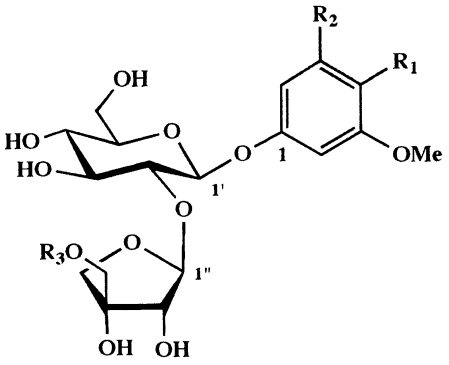
1	H		H	H
2		H	H	H
3	H		H	H
4	Ac		H	H
5	H		Ac	H
6	Ac		H	
7		H	H	
8	H		H	
9	H	H	H	H



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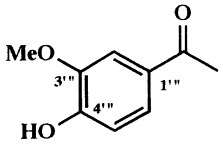
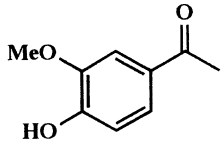
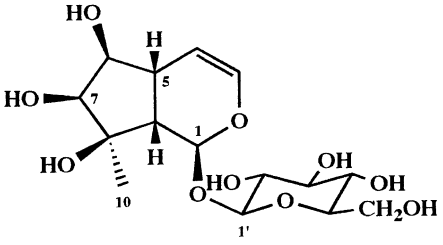
10	OH	H	
11	OMe	OMe	H
12	OMe	OMe	



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ppm) and upfield shift of C-5″ (−3.2 ppm) of the glucosyl moiety indicating that the acetyl group is at C-6″ of the glucosyl moiety. Furthermore, the HMBC spectrum showed the three-bond correlation between H-6″ (δ 4.03 and 4.12) of the glucosyl moiety and carbonyl carbon (δ 172.6) of the acetyl group. Therefore, the structure of compound **4** was elucidated as 6″-*O*-acetylverbascoside.

Compound **5** had the molecular formula $C_{31}H_{38}O_{16}$, the same as **4**, based on HR–FAB mass spectrometry. The ^1H and ^{13}C NMR spectra were very similar to those of **1**. In addition, the signals of an acetyl group were observed in the spectra. The acetyl group was assigned to be at C-4‴ of the rhamnosyl unit based on the downfield shift of C-4‴ (+1.6 ppm) together with the upfield shifts of C-3‴ (−2.0 ppm) and C-5‴ (−2.8 ppm), when compared to the signals of **1**. The assignments were confirmed by HMBC experiment, in which the HMBC correlations were found between H-4‴ (δ 4.78) and C-3‴, C-5‴, C-6‴ and a carbonyl carbon of the acetyl group (δ 172.6), and between H-1‴ and C-2‴, C-3‴, C-6‴ and C-3″. On the basis of these spectral data, the structure of compound **5** was identified as 4‴-*O*-acetylverbascoside.

The molecular formula of compound **11** was determined as $C_{20}H_{30}O_{13}$ by HR–FAB mass spectrometry. The ^1H and ^{13}C NMR spectra revealed the presence of one 1,3,4,5-tetrasubstituted symmetrical aromatic ring, one methoxyl (3H) and two equivalent methoxyl (6H) groups, indicating that compound **11** is an aromatic glycoside. The sugar moiety was identified as β-apiofuranosyl (1→2)-β-glucopyranose by comparison of the chemical shifts with the reported data (Zhong et al., 1999). Two equivalent methoxyl groups were assigned to be at C-3 and C-5 by a difference NOE experiment in the ^1H NMR spectrum. Irradiation of H-1′ (δ 4.86) gave rise to an NOE enhancement at H-2,6 (δ 6.44). Therefore, the structure of compound **11** was proposed as 3,4,5-trimethoxyphenyl 1-*O*-β-apiofuranosyl (1→2)-β-glucopyranoside, named khaephuoside A.

The molecular formula of compound **12** was determined as $C_{28}H_{36}O_{16}$ by HR–FAB mass spectrometry. The ^1H and ^{13}C NMR spectra were in part very similar to those of **11**. In addition, the signals of 1,3,4-trisubstituted aromatic ring, one methoxy signal and one ester carbonyl carbon were observed. Comparison of the ^{13}C NMR spectral data of **12** with those of **11** revealed the downfield shift of C-5″ (+1.3 ppm) together with the upfield shift of C-3″ (−1.6 ppm) of the apiosyl moiety indicating that the additional unit was assigned to be located at C-5″ of the apiosyl moiety. Moreover, the ^{13}C NMR spectral data showed signals due to two sugar moieties and an acyl moiety similar to those of seguinoside K (**10**) as shown in Table 3. Accordingly, the structure of compound **12** was elucidated as 3‴-methoxy-4‴-hydroxybenzoic acid ester of khaephuoside A, named khaephuoside B.

3. Experimental

3.1. General

NMR spectra were recorded in CD_3OD using a JEOL JNM A-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as internal standard. MS were recorded on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (20×150 mm i.d., YMC) and Diol-120 (8.0×300 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. The flow rates were 6 ml/min for ODS and 3 ml/min

Table 1
^{1}H NMR spectral data of compounds **4** and **5** (400 MHz, CD_3OD)

H	4	5
Aglycone		
2	6.66 (1H, *d*, *J* = 2.0 Hz)	6.65 (1H, *d*, *J* = 2.0 Hz)
5	6.66 (1H, *d*, *J* = 8.3 Hz)	6.63 (1H, *d*, *J* = 8.1 Hz)
6	6.51 (1H, *dd*, *J* = 8.3, 2.0 Hz)	6.52 (1H, *dd*, *J* = 8.1, 2.0 Hz)
α	3.90 (1H, *m*)	3.98 (1H, *m*)
	3.66 (1H, *m*)	3.61 (1H, *m*)
β	2.73 (2H, *td*, *J* = 7.0, 3.4 Hz)	2.75 (2H, *td*, *J* = 7.3, 2.0 Hz)
Caffeoyl moiety		
2′	7.03 (1H, *d*, *J* = 2.0 Hz)	7.02 (1H, *d*, *J* = 2.0 Hz)
5′	6.75 (1H, *d*, *J* = 8.3 Hz)	6.75 (1H, *d*, *J* = 8.3 Hz)
6′	6.90 (1H, *dd*, *J* = 8.3, 2.0 Hz)	6.92 (1H, *dd*, *J* = 8.3, 2.0 Hz)
α′	6.23 (1H, *d*, *J* = 15.9 Hz)	6.22 (1H, *d*, *J* = 15.9 Hz)
β′	7.55 (1H, *d*, *J* = 15.9 Hz)	7.55 (1H, *d*, *J* = 15.9 Hz)
Glucose		
1″	4.32 (1H, *d*, *J* = 7.8 Hz)	4.33 (1H, *d*, *J* = 7.8 Hz)
2″	3.37 (1H, *dd*, *J* = 8.8, 7.8 Hz)	3.39 (1H, *dd*, *J* = 9.0, 7.8 Hz)
3″	3.78 (1H, *dd*, *J* = 9.3, 8.8 Hz)	3.84 (1H, *dd*, *J* = 9.3, 9.0 Hz)
4″	4.96 (1H, *dd*, *J* = 9.5, 9.3 Hz)	4.92 (1H, *dd*, *J* = 9.5, 9.3 Hz)
5″	3.66 (1H, *m*)	3.52 (1H, *m*)
6″	4.12 (1H, *dd*, *J* = 12.2, 4.9 Hz)	3.60[a]
	4.03 (1H, *dd*, *J* = 12.2, 2.5 Hz)	3.47[a]
Rhamnose		
1‴	5.16 (1H, *d*, *J* = 1.3 Hz)	5.35 (1H, *d*, *J* = 1.7 Hz)
2‴	3.90[a]	3.85 (1H, *dd*, *J* = 3.4, 1.7 Hz)
3‴	3.56 (1H, *dd*, *J* = 9.5, 3.2 Hz)	3.62 (1H, *dd*, *J* = 10.0, 3.4 Hz)
4‴	3.28[a]	4.78[a]
5‴	3.54 (1H, *dq*, *J* = 9.5, 6.1 Hz)	3.67[a]
6‴	1.06 (3H, *d*, *J* = 6.1 Hz)	0.98 (3H, *d*, *J* = 6.1 Hz)
Ac	1.96 (3H, *s*)	1.64 (3H, *s*)

[a] Signal pattern unclear due to overlapping.

for Diol-120. For CC, silica gel G 60 (Merck), YMC-gel ODS (50 μm, YMC) and highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) EtOAc–MeOH–H_2O (4:1:0.1), (II) EtOAc–MeOH–H_2O (7:3:0.3), (III) EtOAc–MeOH–H_2O (6:4:1), (IV) 30–70% MeOH, (V) 20% MeCN, (VI) 20–50% MeCN, (VII) 6% MeCN, (VIII) 15% MeCN, (VIII) 15% MeCN, (IX) MeCN and (X) 25% MeCN. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2. Plant material

The leaves and branches of *Barnettia kerrii* Santisuk were collected in April 2000 from the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The identification of the plant was confirmed by Professor Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (KKU-0021) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried leaves and branches (2.0 kg) of *B. kerrii* were extracted with hot MeOH. After removal of the solvent by evaporation, the residue (109.0 g) was partitioned between H_2O and Et_2O. The aqueous layer was subjected to a column of highly porous copolymer of styrene and divinylbenzene and eluted with H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH (24.0 g) was applied to a column of silica gel (systems I, II and III, respectively) affording six fractions (monitored by TLC). Fraction 2 (3.4 g) was chromatographed on a column of RP-18 using system IV to provide eight fractions, with compound **1** (1.0 g) from fraction 2-3, and compound **4** (1.2 g) from fraction 2-6. Fractions 2-2, 2-4 and 2-5 were purified by prep. HPLC-ODS (system V) to give compounds **2** (223 mg), **3** (42 mg), **5** (42 mg), **10** (31 mg) and **12** (23 mg). Fraction 3 (8.5 g) was chromatographed on a column of RP-18 (system VI) to afford eight fractions, with compound **6** (2.3 g) from fraction 3-6. Fraction 3-1 was purified by prep. HPLC-ODS (systemVII) to provide compound **9** (12 mg). Fraction 3-3 was purified by prep. HPLC-ODS

Table 2
^{13}C NMR spectral data of compounds **1**, **4** and **5** (100 MHz, CD_3OD)

	1	4	5
Aglycone			
1	131.5	131.3	131.5
2	117.1	117.0	117.1
3	144.6	144.4	144.6
4	146.1	145.9	146.1
5	116.3	116.3	116.3
6	121.3	121.2	121.3
α	72.2	72.3	72.2
β	36.5	36.4	36.5
Caffeoyl moiety			
1′	127.7	127.5	127.5
2′	115.2	115.2	115.2
3′	149.7	149.5	149.9
4′	146.8	146.6	146.9
5′	116.5	116.5	116.6
6′	123.2	123.2	123.3
α′	114.7	114.5	114.5
β′	148.0	147.9	148.0
C=O	168.3	167.9	168.1
Glucose			
1″	104.2	104.1	104.1
2″	76.2	75.9	76.5
3″	81.6	81.3	79.0
4″	70.4	70.3	70.3
5″	76.0	72.8	75.8
6″	62.4	63.7	62.7
Rhamnose			
1‴	103.0	102.9	101.6
2‴	72.3	72.2	72.2
3‴	72.0	71.9	70.0
4‴	73.8	73.6	75.4
5‴	70.6	70.3	67.8
6‴	18.4	18.4	18.1
$\underline{C}H_3CO$		20.7	20.6
$CH_3\underline{C}O$		172.6	172.6

Table 3
^{13}C NMR spectral data of compounds **10**, **11** and **12** (100 MHz, CD_3OD)

C	10	11	12
1	152.4	134.2	134.2
2	103.1	96.1	95.4
3	149.2	154.8	154.6
4	142.7	156.0	155.4
5	116.0	154.8	154.6
6	109.4	96.1	95.4
1′	101.8	102.0	101.1
2′	78.8	78.7	78.8
3′	78.5	78.3	78.3
4′	71.7	71.8	71.7
5′	78.8	78.7	78.8
6′	62.6	62.7	62.6
1″	110.5	110.8	110.6
2″	78.0	78.0	78.2
3″	79.2	80.7	79.1
4″	75.4	75.5	75.2
5″	67.9	66.1	67.4
1‴	122.3		122.1
2‴	113.8		113.8
3‴	152.9		152.8
4‴	148.7		148.6
5‴	125.3		125.2
6‴	115.9		115.8
C=O	167.8		167.6
MeO-3	56.3	56.6	56.4
MeO-4		61.2	61.2
MeO-5		56.6	56.4
MeO-3‴	56.3		56.5

(system VIII) to afford compounds **11** (11 mg) and **17** (16 mg). Fraction 3-4 was purified by prep. HPLC-ODS (system VIII) and HPLC-Diol (system IX) to give compounds **13** (21 mg) and **14** (33 mg). Fraction 3-5 was similarly purified by prep. HPLC-ODS (system X) to afford compounds **15** (18 mg) and **16** (53 mg). Fraction 4 (3.2 g) was rechromatographed on a column of RP-18 (system VI) to afford nine fractions. Fraction 4-1 was purified by prep. HPLC-ODS (system VII) to provide compound **18** (27 mg). Fraction 4-7 was further purified by prep. HPLC-ODS (system X) to give compounds **7** (87 mg) and **8** (17 mg). Fractions 5 (5.4 g) was applied to a column of RP-18 (system IV), then followed by prep. HPLC-ODS (system IV) to provide compound **19** (15 mg).

3.4. 6″-O-Acetylverbascoside (*4*)

Amorphous powder, $[\alpha]_D^{30}$ −74.4° (MeOH, *c* 0.81); ^1H NMR (CD_3OD): Table 1; ^{13}C NMR (CD_3OD): Table 2; Negative HR–FAB–MS, *m/z*: 665.2117 $[M-H]^-$ ($C_{31}H_{37}O_{16}$ requires 665.2081).

3.5. 4‴-O-Acetylverbascoside (*5*)

Amorphous powder, $[\alpha]_D^{30}$ −106.8° (MeOH, *c* 2.82); ^1H NMR (CD_3OD): Table 1; ^{13}C NMR (CD_3OD): Table 2; Negative HR–FAB–MS, *m/z*: 665.2117 $[M-H]^-$ ($C_{31}H_{37}O_{16}$ requires 665.2081).

3.6. Khaephuoside A (*11*)

Amorphous powder, $[\alpha]_D^{30}$ −61.8° (MeOH, *c* 0.76); ^1H NMR (CD_3OD): δ 6.44 (2H, *s*, H-2,6), δ 3.80 (6H, *s*, MeO-3,5), δ 3.69 (3H, *s*, MeO-4), δ 4.86 (1H, *d*, *J* = 7.6 Hz, H-1′ Glc), δ 3.64 (1H, *dd*, *J* = 12.0, 6.6 Hz, H-6′ Glc), δ 3.90 (1H, *dd*, *J* = 12.0, 2.2 Hz, H-6′ Glc), δ 5.46 (1H, *d*, *J* = 1.6 Hz, H-1″ Api), δ 3.94 (1H, *d*, *J* = 1.6 Hz, H-2″ Api), δ 3.80 (1H, *d*, *J* = 9.5 Hz, H-4″ Api), δ 4.11 (1H, *d*, *J* = 9.5 Hz, H-4″ Api), δ 3.58 (2H, *s*, H-5″ Api); ^{13}C NMR (CD_3OD): Table 3; Negative HR–FAB–MS, *m/z*: 477.1634 $[M-H]^-$ ($C_{20}H_{29}O_{13}$ requires 477.1607).

3.7. Khaephuoside B (**12**)

Amorphous powder, $[\alpha]_D^{30}$ −73.4° (MeOH, *c* 1.55); ^{1}H NMR (CD_3OD): δ 6.28 (2H, s, H-2,6), δ 3.70 (6H, *s*, MeO-3,5), δ 3.63 (3H, *s*, MeO-4), δ 4.86 (1H, *d*, *J* = 7.6 Hz, H-1′ Glc), δ 3.68 (1H, *dd*, *J* = 12.0, 3.1 Hz, H-6′ Glc), δ 3.88 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-6′ Glc), δ 5.50 (1H, *d*, *J* = 1.2 Hz, H-1″ Api), δ 4.09 (1H, *d*, *J* = 1.2 Hz, H-2″ Api), δ 3.92 (1H, *d*, *J* = 9.8 Hz, H-4″ Api), δ 4.28 (1H, *d*, *J* = 9.8 Hz, H-4″ Api), δ 4.25 (1H, *d*, *J* = 10.2 Hz, H-5″ Api), δ 4.40 (1H, *d*, *J* = 10.2 Hz, H-5″ Api), δ 7.42 (1H, *d*, *J* = 2.0 Hz, H-2‴), δ 7.45 (1H, *dd*, *J* = 8.3, 2.0 Hz, H-6‴), δ 6.72 (1H, *d*, *J* = 8.3 Hz, H-5‴), δ 3.83 (3H, *s*, MeO-3‴); ^{13}C NMR (CD_3OD): Table 3; Negative HR–FAB–MS, *m*/*z*: 627.1903 $[M-H]^-$ ($C_{28}H_{35}O_{16}$ requires 627.1924).

3.8. Stegioside III (**19**)

Amorphous powder, $[\alpha]_D^{30}$ −153.9° (MeOH, *c* 1.02); ^{1}H NMR (CD_3OD): δ 5.44 (1H, *brs*, H-1), δ 6.11 (1H, *dd*, *J* = 6.3, 1.7 Hz, H-3), δ 4.80 (1H, *brd*, *J* = 6.3 Hz, H-4), δ 2.61 (1H, *brs*, H-5), δ 3.77 (1H, *m*, H-6), δ 3.57 (1H, *d*, *J* = 4.2 Hz, H-7), δ 2.61 (1H, *brs*, H-9), δ 1.22 (3H, *s*, H-10), δ 4.58 (1H, *d*, *J* = 7.8 Hz, H-1′ Glc), δ 3.15 (1H, *dd*, *J* = 9.0, 7.8 Hz, H-2′ Glc), δ 3.23–3.30 (3H, overlapping, H-3′,4′,5′ Glc), δ 3.84 (1H, *brd*, *J* = 12.0 Hz, H-6′ Glc), δ 3.60 (1H, *dd*, *J* = 12.0, 5.4 Hz, H-6′ Glc); ^{13}C NMR (CD_3OD): δ 93.8 (C-1), δ 140.4 (C-3), δ 106.1 (C-4), δ 36.8 (C-5), δ 78.3 (C-6 or C-7), δ 78.4 (C-7 or C-6), δ 79.3 (C-8), δ 49.8 (C-9), δ 22.7 (C-10), δ 99.3 (C-1′ Glc), δ 74.8 (C-2′ Glc), δ 78.0 (C-3′ Glc), δ 71.7 (C-4′ Glc), δ 78.2 (C-5′ Glc), δ 62.8 (C-6′ Glc); Negative HR–FAB–MS, *m*/*z*: 363.1260 $[M-H]^-$ ($C_{15}H_{23}O_{10}$ requires 363.1291).

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