Three New Glycosides from *Sinopodophyllum emodi* (WALL.) YING

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Tow new aryltetralin-type lignan glycosides: methyl epipodophyllate 7′-*O*-*β*-D-glucopyranoyl-(1→6)-*β*-D-glucopyranoside (1), 4-demethylepipodophyllotoxin 7′-*O*-*β*-D-glucopyranoside (2), and a new phenyl ethanol glycoside: phenyl ethanol 4-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (3), along with three known compounds: junipetriolosides (4), 3,4-dihydroxy-phenyl ethanol (5), and 4-hydroxy-phenyl ethanol (6) were isolated and identified from the *n*-butanol extract of the roots and rhizomes of *Sinopodophyllum emodi* (WALL.) YING. The structures of the above were established by means of spectral data and chemical methods.

Key words *Sinopodophyllum emodi*; Berberidaceae; Lignan; methyl epipodophyllate 7′-*O*-*β*-D-glucopyranoyl-(1→6)-*β*-D-glucopyranoside; 4-demethyl-epipodophyllotoxin 7′-*O*-*β*-D-glucopyranoside; phenyl ethanol 4-*O*-*β*-D-xylosyl-(1→6)-*O*-*β*-D-glucopyranoside

The genus of *Podophyllum* (Berberidaceae) are well known due to a wide range of biological activities.[1] The constituents and activities of the major species of *Podophyllum* have been reported.[2] The plants of *Sinopodophyllum emodi* (WALL.) YING, which are mainly distributed over the western regions of the QinLing Mountains of China, are used as a folk medicine for treating cancer and various verrucosis in this locality. In our previous studies of this species, two new lignan glycosides, eight known lignans and five flavonoids were isolated and identified.[3] As part of our continued study of the constituents of this species, we new report the isolation and identification of two new lignan glycosides, one new phenethyl alcohol glucoside and three known compounds.

Results and Discussion

Dried roots and rhizomes of *S. emodi* were extracted with 95% ethanol. The ethanol extract was partitioned between water and ether, chloroform, ethyl acetate, and *n*-butanol, respectively. The *n*-butanol and ethyl acetate extracts were chromatographed on columns of silica gel, Lobar RP-18, Sephadex LH-20 and preparative HPLC to give compounds 1—6.

Compound **1** was obtained as a colorless powder. Its molecular formula was determined as $C_{35}H_{46}O_{19}$ by high-resolution (HR)-FAB-MS. The UV spectrum of **1** showed absorption bands at λ_{max} 210 and 280 nm, suggesting the presence of an aromatic ring. The ^{1}H-NMR spectrum of **1** revealed proton signals for four methyl groups at δ 3.76 (6H, s), 3.73 (3H, s), and 3.60 (3H, s), three aromatic signals at δ 7.03 (1H, s), 6.50 (2H, s), and 6.22 (1H, s), a pair of proton signals for $-CH_2-$ at δ 5.86 (1H, d, J=1.3 Hz), 5.88 (1H, d, J=1.3 Hz) and aliphatic proton signals at δ 2.67—4.80 ppm. The ^{13}C-NMR (distortionless enhancement by polarization transfer, DEPT) spectral data of the aglycone of **1** revealed the existence of nine quaternary carbons, eight methine carbons, two methylene carbons and four methyl carbons. By comparison with the literature[4] and analysis of the heteronuclear multiple bond spectroscopy (HMBC), nuclear Overhauser effect spctroscopy (NOESY), and correlation spectroscopy (COSY) spectra. The aglycone of **1** was identified as methyl epipodophyllate. The ^{1}H- and ^{13}C-NMR spectral data of **1** showed two anomeric signals at δ 4.58 (1H, d, J=7.6 Hz) and δ 4.44 (1H, d, J=7.6 Hz), and δ 105.2 and 103.7 ppm, respectively, indicating **1** should be a diglycoside. Acid hydrolysis of **1** gave glucose, which was compared by TLC with a standard sample. The absolute configuration of the glucose was shown to be D-glucose according to the method reported by Hara and co-workers.[5] The C-1 of the inner glucose was attached to the 7′-OH of the aglycone of **1**, as indicated by the C-7′ chemical shift (δ 76.9, CH), the correlation of H-1 of the inner glucose and C-7′ of the aglycone in HMBC experiment, and the correlation of H-1 of the inner glucose and H-7′ (δ 4.91, d, J=3.3 Hz) of the aglycone by NOESY. From the HMBC experiment, the correlation of H-1 of the terminal glucose and C-6 (δ 70.3) of the inner glucose indicated that C-1 of the terminal glucose was linked to 6-OH of the inner glucose. Based on these findings, the structure of **1** was identified as methoxyl epipotophyllate 7′-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

Compound **2** was obtained as white needles. A molecular formula of $C_{27}H_{30}O_{13}$ for **2** was determined by HR-FAB-MS. The ^{1}H-NMR spectrum of **2** exhibited four aromatic proton signals at δ 6.28 (2H, s), 7.02 (1H, s), 6.52 (1H, s), two methoxyl proton signal at δ 3.71 (6H, s), and seven aliphatic proton signals at δ 2.95—5.96. The ^{13}C-NMR (DEPT) spectral data of aglycone of **2** revealed the existence of nine quaternary carbons, eight methine carbons, two methylene carbons and two methyl carbons. The ^{1}H-, ^{13}C-NMR spectrum data for **2** suggested that the aglycone of **2** was a podophyllotoxin-type lignan. Comparison of the spectral data with those of 4-demethyl-podophyllotoxin and analysis of the HMBC and nuclear Overhauser effect (NOE) spectrum of **2**, suggested that the aglycone of **2** was 4-demethyl-epipodophyllotoxin.[6]

The ^{1}H- and ^{13}C-NMR spectral data showed one-anomeric signal at δ 4.49 (1H, d, J=8.0 Hz), and δ 101.8, respectively, indicating that **2** is a glycoside. Acid hydrolysis of **2** gave glucose is shown TLC comparison with a standard sample. The absolute configuration was shown to be D-glucose by the method reported by Hara and co-workers. C-1 of the glucose was attached to 7′-OH of the aglycone, as indicated by the C-7′ chemical shift (δ 72.9, CH), the correlation of H-1 of glucose and C-7′ of the aglycone in the HMBC experiment, and the correlation of H-1 of the inner glucose and H-7′ (δ 5.13, d, J=3.3 Hz) of the aglycone by NOESY. Based on these findings, the structure of compound **2** was identified as 4-

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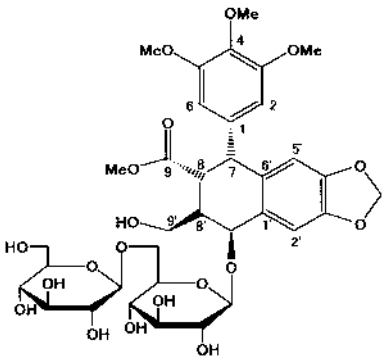

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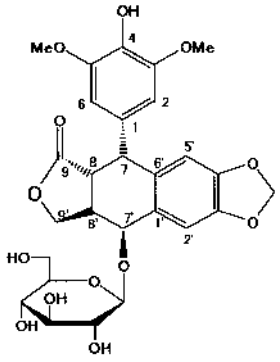

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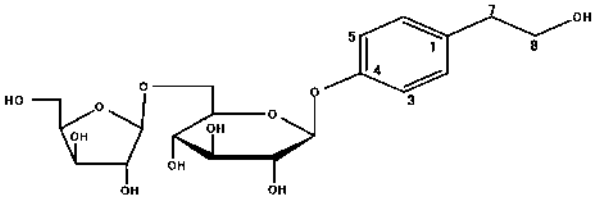

3

demethylepipodophyllotoxin 7′-*O*-*β*-D-glucopyranoside.

Compound **3** was obtained as an amorphous powder. A molecular formula of $C_{19}H_{28}O_{11}$ for **3** was determined by HR-FAB-MS. The ^{1}H-NMR spectrum of **3** showed two aromatic proton signals at δ 7.29 (2H, dd, J=7, 1.8 Hz) and δ 7.41 (2H, dd, J=7, 1.8 Hz), and two group aliphatic signals at δ 4.10 (2H, t, J=7.0 Hz) and 2.91 (2H, t, J=7.0 Hz). The ^{13}C-NMR spectrum (DEPT) of **3** revealed two quaternary aromatic signals at δ 133.9 (C) and δ 157.2 (C), two methine peaks at δ 130.6 (CH) and δ 117.2 (CH), and two aliphatic carbon signals at δ 63.7 (CH_2) and 39.6 (CH_2). Furthermore, analysis of the correlations in the COSY and HMBC spectra of **3**, revealed that the aglycone of **3** was 4-hydroxyl-phenyl-ethanol.

The ^{1}H- and ^{13}C-NMR spectral data of **3** showed two anomeric signals at δ 5.49 (1H, d, J=7.4 Hz) and δ 4.98 (1H, d, J=7.4 Hz), and δ 102.7 and 105.8, respectively, indicating **3** possesses two sugars. Acid hydrolysis of **3** gave glucose and xylose, which were analyzed by TLC in companion with standard samples and literature.[7] The configurations of glucose and xylose were determined by the same methods used for **1**. From the HMBC experiment, **3** was shown to have the inner sugar attached to C-4 of the aromatic ring, as indicated by the C-4 chemical shift (δ 157.2, quaternary carbon). The correlation of H-1 of terminal xylose and C-6 (δ 69.7 ppm) of the inner glucose indicated that C-1 of the terminal xylose was linked at 6-OH of the inner glucose. Based on these findings, the structure of **3** was identified as phenyl ethanol 4-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**3**).

The structures of known compounds **4**—**6** were characterized on the basis of their ^{1}H-, ^{13}C-NMR and two dimensional (2D)-NMR spectral, and by comparison with literature as junipetriolosides (**4**),[8] 3,4-dihydroxy-phenyl ethanol (**5**),[9] and 4-hydroxy-phenyl ethanol (**6**).[10]

Table 1. ^{13}C-NMR Spectral Data of Compounds 1—3[a]

C-position	Compounds 1	2	3
1	142.7	135.8	133.9
2	107.9	109.4	130.6
3	154.3	148.6	117.2
4	137.7	132.3	157.2
5	154.3	148.6	117.2
6	107.9	109.4	130.6
7	45.3	45.1	39.6
8	47.0	42.4	63.7
9	176.1	178.1	
1′	133.7	134.6	
2′	111.7	111.1	
3′	147.6	148.3	
4′	149.3	150.0	
5′	109.6	111.4	
6′	128.1	130.1	
7′	76.9	72.9	
8′	44.0	39.4	
9′	61.2	69.9	
3,5-OMe	56.7	56.8	
4-OMe	61.1		
9-OMe	52.3		
–OCH_2O–	102.3	102.9	
Glucose (inner)			Glucose (inner)
1	103.3	101.8	102.7
2	75.2	75.1	75.1
3	78.0	78.0	78.4
4	71.6	71.9	71.2
5	78.3	78.1	77.6
6	70.3	63.0	69.7
Glucose (terminal)			Xylose
1	105.2		105.8
2	75.1		74.9
3	77.9		78.2
4	71.6		71.2
5	78.2		67.1
6	62.8		

a) Compounds **1** and **2** were detected in CD_3OD and **3** was detected in C_5D_5N.

Experimental

General Procedures Instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous paper.[3a)]

Extraction and Isolation Dried roots and rhizomes of *Sinopodophyllum emodi* (WALL.) YING 2.47 kg were extracted three times with 95% ethanol (60 min at 50 °C each time). The ethanol solution was concentrated to afford an ethanol extract (240 g). The ethanol extract (40 g) was dissolved in water and partitioned with ether, chloroform, ethyl acetate and *n*-butanol, respectively. The ether extract (6.89 g), chloroform extract (1.33 g), ethyl acetate extract (3.75 g), *n*-butanol extract (18.6 g), and water layer (12.4 g) were obtained, respectively.

The *n*-butanol extract (18.6 g) was subjected to column chromatography on silica gel and by stepwise elution with a solvent mixture of chloroform, methanol and water (40 : 1 : 0.1→6 : 4 : 1), nine fractions (Fr. 1-9) were collected. Fraction 7 was chromatographed on a Lobar column (40% MeOH) to give three fractions (B-7-1 0.21 g, B-7-2 0.346 g, B-7-3 0.371 g). B-7-2 was chromatographed on a Sephadex column (MeOH) to give B-7-2-1 and B-7-2-2. The B-7-2-1 fraction was chromatographed by preparative HPLC (20% MeCN, 2 ml/min) to give compound **1** (8.4 mg). Fraction 4 was chromatographed on a Lobar column (40% MeOH) to give four fractions (B-4-1 to B-4-4). Fraction B-4-2 was chromatographed by preparative HPLC (40% MeCN, 2 ml/min) to give compound **2** (6.7 mg). Fraction 9 was chromatographed on a Lobar column (10%—20% MeOH) to give three fractions (B-9-1 to B-9-3). The B-9-2 fraction was chromatographed by preparative HPLC (2%—5% MeCN, 2 ml/min) to give compounds **3** (4.3 mg), **4** (5.2 mg), **5** (6.7 mg), and **6** (3.3 mg).

Compound **1**: Obtained as white powder. $[\alpha]_D^{15}$ −120° (c=0.34). HR-FAB-MS (positive): *m/z*: 771.2704 $[M]^+$ (Calcd for $C_{35}H_{46}O_{19}$: 771.2712). CD: (MeOH) $\Delta\varepsilon$: 288 (+22.2), 274 (−14.6), 258 (−4.6), 249 (−8.5), 238.5 (−0.5), 221.5 (−27.1). ^{1}H-NMR δ (ppm, CD_3OD): 6.5 (2H, s, H-2, 6), 4.19 (1H, d, J=4.6 Hz, H-7), 3.62 (1H, dd, J=11.5, 4.6 Hz, H-8), 7.03 (1H, s, H-2′), 6.22 (1H, s, H-5′), 4.91 (1H, d, J=3.3 Hz, H-7′), 2.70 (1H, m, H-8′), 3.39 (1H, d, J=10.7, 3.7 Hz, H-9′a), 3.65 (1H,d, J=10.7, 2.1 Hz, H-9′b), 3.60 (3H, s, 9-OCH_3), 3.76 (6H, s, 3,5-OCH_3), 3.73 (3H, s, 4-OCH_3), 5.86 (1H, d, J=1.1 Hz, –OCH_2O–Ha), 5.87 (1H, d, J=1.1 Hz, –OCH_2O–Hb), 4.44 (1H, d, J=7.6 Hz, H-1 of inner glucose), 4.58 (1H, d, J=7.6 Hz, H-1 of terminal glucose), 4.18 (1H, dd, J=12.0, 2.1 Hz, H-6a of inner glucose), 3.81 (1H, m, H-6b of inner glucose), 3.85 (1H,dd, J=11.3, 1.9 Hz, H-6a of terminal glucose), 3.65 (1H, dd, J=11.3, 5.4 Hz, H-6b of terminal glucose), 3.20—3.80 (m, H-2 to H-5 of inner glucose and terminal glucose). The ^{13}C-NMR spectral data are shown in Table 1.

Compound **2** was obtained as white needle, $[\alpha]_D^{15}$ −29.3° (c=0.63 MeOH). FAB-MS (positive): 563 $[M]^+$, 370 [M-glc]. HR-FAB-MS (positive): *m/z*: 563.1779 $[M]^+$. (Calcd for $C_{27}H_{30}O_{13}$: 563.1765). CD: $\Delta\varepsilon$ (MeOH): 287.5 (+11.4), 274.5 (−26.2), 251.5 (−9.0), 235 (+30.7), 224.5 (−2.8), 221.5 (+7.7). ^{1}H-NMR: δ: 6.28 (2H, s, H-2, 6), 4.57 (1H, d, J=5.4 Hz, H-7), 3.52 (1H, dd, J=13.9, 5.4 Hz, H-8), 7.02 (1H, s, H-2′), 6.52 (1H, s, H-5′). 5.13 (1H, d, J=3.3 Hz, H-7′), 2.95 (1H, m, H-8′), 4.34 (1H, t, J=8.5 Hz, H-9′a), 4.53 (1H, m, H-9′b), 3.70 (6H, s, H-3, 5-OMe), 5.95 (1H, d, J=0.9 Hz, Ha of –OCH_2O–), 5.96 (1H, d, J=0.9 Hz, Hb of –OCH_2O–), 4.49 (1H, d, J=8.0 Hz, H-1 of glucose), 3.95 (1H, dd, J=2.1, 11.5 Hz, H-6a of glucose), 3.71 (1H, dd, J=5.3, 11.5 Hz, H-6b of glucose), 3.20—3.45 (m, H-2 to H-5 of glucose). The ^{13}C-NMR (CD_3OD) spectral data are shown in Table 1.

Compound **3** was obtained as a colorless amorphous powder, HR-FAB-MS (positive): *m/z*: 455.1564 $[M+Na]^+$ (Calcd for $C_{19}H_{28}O_{11}Na$: 455.1529). ^{1}H-NMR (C_5D_5N) δ: 7.29 (2H, dd, J=7, 1.8 Hz, H-2, 6), 7.41 (2H, dd, J=7, 1.8 Hz, H-3, 5), 2.91 (2H, t, J=7.0 Hz, H-7), 4.10 (2H, t, J=7.0 Hz, H-8), 5.49 (1H, d, J=7.4 Hz, H-1 of glucose), 4.05 (1H, d, J=7.4, 10.5 Hz, H-2 of glucose), 4.14—4.16 (m, H-3, 4, 5 of glucose and H-2,3,4 of xylose), 4.78 (1H, dd, J=11.3, 6.0 Hz, H-6a of glucose), 4.18 (1H, dd, J=11.3, 2.0 Hz, H-6b of glucose), 4.98 (1H, d, J=7.4 Hz, H-1 of xylose), 3.58 (1H, dd, J=10, 12 Hz, H-5a of xylose), 4.14 (1H, m, H-5b of xylose). The ^{13}C-NMR spectral data are shown in Table 1.

Compounds **1**—**3** (1 mg each) were hydrolyzed (2 N HCl, 95 °C, 1 h). The sugars from **1** and **2** were detected as glucose, while the sugars from **3** were detected as glucose and xylose in TLC with a standard sample. TLC conditions: $CHCl_3$: MeOH : H_2O (65 : 35 : 10 below layer).

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