Two New Podophyllotoxin Glucosides from *Sinopodophyllum emodi* (Wall.) Ying

Changqi Zhao,[a,b] Jing Huang,[a,c] Akito Nagatsu,[a] and Yukio Ogihara*,[a]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] 3–1 Tanabe-Dori, Mizuho-ku, Nagoya 467–8603, Japan, Biology Department, Northwest University,[b] Xi'an 710069, P. R. China, and Faculty of Pharmaceutical Sciences, West China University of Medical Sciences,[c] 3–7 Renmingnanlu, Chengdu, 610041, P. R. China.
Received January 19, 2001; accepted February 20, 2001

Two new aryltetralin-type lignans, isopodophyllotoxin 7′-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (1) and 4-demethyl-picropodophyllotoxin 7′-*O*-*β*-D-glucopyranoside (2), along with eight known podophyllotoxin derivatives: 4-demethyl-podophyllotoxin 7′-*O*-*β*-D-glucopyranoside (3), podophyllotoxin 7′-*O*-*β*-D-glucopyranoside (4), deoxypodophyllotoxin (5), picropodophyllotoxin (6), podophyllotoxin (7), 4-demethyl-picropodophyllotoxin (8), 4-demethyl-podophyllotoxin (9), and 4-demethyl-deoxypodophyllotoxin (10), were isolated from the roots and rhizomes of *Sinopodophyllum emodi* (Wall.) Ying (Berberidaceae). Their structures were identified based on NMR spectral data and chemical evidence.

Key words *Sinopodophyllum emodi*; Berberidaceae; lignan; isopodophyllotoxin 7′-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside; 4-demethyl-picropodophyllotoxin 7′-*O*-*β*-D-glucopyranoside

The plants of *Podophyllum*, which grow in the Himalaya region and in American, are traditional medicines, belonging to the Berberidaceae family, and were used by the natives of both continents as cathartics and anthelminthics.[1)] Podophyllotoxin, a main constituent of the genus, had already been described by Podwyssotzki[2)] in 1880. Renewed interest in this genus was generated in the 1940's, when Kaplan[3)] demonstrated the curative effect of podophyllin, isolated from the alcoholic extracts of the podophyllum rhizomes, in condylomata acuminata. Subsequently, a number of podophyllotoxin derivatives and their glycosides isolated from the plants of the *Podophyllum* species have shown a wide range of biological activities, such as antimitotic and antiviral. Some of them have also shown to be toxic to fungi, insects and vertebrates.[4)] The successful chemical conversion of a major constituent, podophyllotoxin, into the clinically useful anticancer drugs Etoposide and Teniposide, has also triggered further research in this area.[5)] However, the phytochemistry and pharmacology of the species of *Sinopodophyllum emodi* (Wall.) Ying, which were mainly distributed over the western region of the QinLing mountains of China, have not been investigated. *S. emodi* was a folk medicine and has been used to treat cancer and various verrucosis in this locality.[6)] This paper reports the isolation and structural elucidation of two new podophyllotoxin glucosides for the first time from this species: isopodophyllotoxin 7′-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**) and 4-demethyl-picropodophyllotoxin 7′-*O*-*β*-D-glucopyranoside (**2**), along with eight known podophyllotoxin derivatives.

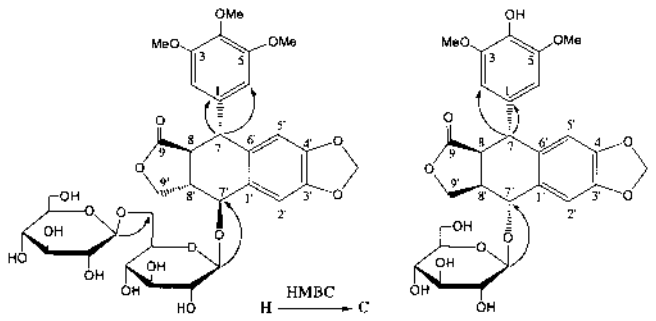
Results and Discussion

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 the columns of silica gel, Lobar RP-18 and Sephadex LH-20 to give compounds **1**—**10**.

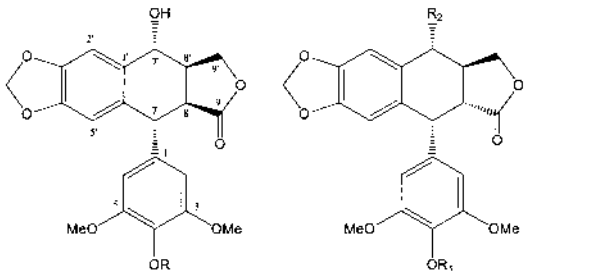
Compound **1** was obtained as colorless needles. Its molecular formula was determined as $C_{34}H_{42}O_{18}$ by high-resolution (HR)-FAB-MS. The UV spectrum of **1** showed the absorption bands at λ_{max} 208 and 280 nm, suggesting the existence of the benzene rings. The IR spectrum of **1** showed the absorption bands at ν 3550 (–OH), 3350 (–OH) and 1780 (C=O) cm^{-1}, suggesting the existence of hydroxyl and carbonyl groups, respectively. The ^{1}H-NMR spectrum of **1** revealed proton signals of three methoxyl groups and signals of four aromatic protons at δ 3.76 (6H, s), 3.89 (3H, s), and 7.03 (2H, s), 8.01 (1H, s), 6.56 (1H, s), a pair of proton signals of $-CH_2-$ at δ 6.00 (1H, d, J=1.2 Hz), 6.12 (1H, d, J=1.2 Hz) and the aliphatic proton signals at δ 3.11—5.30 ppm. The ^{13}C-NMR (distortionless enhancement by polarization transfer, DEPT) spectral data of **1** revealed the existence of nine quaternary carbons, eight methine carbons, two methylene carbons and three methoxyl carbons. The carbon chemical shifts of the aglycone of **1** were very similar to those of podophyllotoxin, except for the signals due to the sugar moieties. However, the ^{1}H-NMR spectral data of the aglycone of **1** was different from those of podophyllotoxin. In the ^{1}H-NMR spectrum, the signals of H-7, H-8, H-7′ and H-8′ of podophyllotoxin were at δ 4.59 (1H, m, H-7), 2.85 (2H, m, H-8 and H-8′), and 4.75 (1H, d, J=8.8 Hz, H-7′),[7)] but those of **1** were at δ 4.60 (1H, d, J=7.6 Hz, H-7), 3.78 (1H, dd, J=7.6, 9.5 Hz, H-8), 5.30 (1H, d, J=10.1 Hz, H-7′), and 3.11 (1H, m, H-8′). They were very similar with those of 4-demethylisopodophyllotoxin which were at δ 3.91 (1H, d, J=7.0 Hz, H-7), 3.38 (1H, dd, J=7.0, 9.4 Hz, H-8), δ 4.48 (1H, d, J=10.5 Hz, H-7′), and δ 2.65 (1H, m, H-8′),[8)] suggesting the configurations of H-7, H-8, H-7′ and H-8′ of **1** were the same as those of 4-demethyl-isopodophyllotoxin. The circular dichroism (CD) [($\Delta\varepsilon$ 290.3 (+1.75), 270 (−2.14), 245.3 (−0.60), 218.3 (−1.08), 202.5 (+11.7)] curve of **1** was similar to that of 4-demethyl-isopodophyllotoxin [($\Delta\varepsilon$ 287 (+2.85), 271.6 (−1.30), 245 (−0.6), 219 (−4.0), 204 (+16.5)],[8)] supporting the above results. Based on these findings, the aglycone of **1** was identified as isopodophyllotoxin.

The ^{1}H-, ^{13}C-NMR spectral data of **1** showed two anomeric signals at δ 5.10 (1H, d, J=8.3 Hz) and 5.02 (1H, d, J=7.4 Hz), and δ 106.0 and 105.1, respectively, indicating **1** should be a diglycoside. Acid hydrolysis of **1** gave a glu-

* To whom correspondence should be addressed. e-mail: ogihara@phar.nagoya-cu.ac.jp



1 2



6: R = Me,
8: R = H,

3: R_1 = H, R_2 = -*O*-glc
4: R_1 = Me, R_2 = -*O*-glc
5: R_1 = Me, R_2 = H
7: R_1 = Me, R_2 = OH
9: R_1 = H, R_2 = OH
10: R_1=H, R_2=H

Chart 1

cose, which was analyzed by HPLC with standard samples. The absolute configuration of the glucose was shown to be D-glucose, according to the method reported by Hara and coworkers.[7] The C-1 of inner glucose was attached to the 7′-OH of the aglycone of **1**, as indicated by the C-7′ chemical shift (δ 77.7), the correlation of H-1 of inner glucose and C-7′ of the aglycone in heteronuclear multiple bond spectroscopy (HMBC), and the correlation of H-1 of inner glucose and H-7′ (δ 5.30, d, J=10.1 Hz) of the aglycone in nuclear Overhauser effect spectroscopy (NOESY). From the HMBC experiment of **1**, the correlation of H-1 of terminal glucose and C-6 (δ 69.5) of inner glucose indicated the C-1 of terminal glucose was linked at 6-OH of inner glucose. Based on these findings, the structure of the new compound of **1** was identified as isopodophyllotoxin 7′-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **2** was obtained as a white powder. A molecular formula of $C_{27}H_{30}O_{13}$ for **2** was determined by HR-FAB-MS. The UV spectrum of **2** showed the absorption bands at λ_{max} (nm) 208, 223, 238 and 290, indicating the existence of benzene rings and a carbonyl group. The IR spectrum of **2** showed the absorption bands at ν_{max} (cm^{-1}) 3550 (–OH), 3350 (–OH) and 1780 (C=O), due to hydroxyl and carbonyl groups, respectively. The ^{1}H-NMR spectrum of **2** exhibited four aromatic proton signals at δ 6.40 (2H, s), 7.37 (1H, s), 6.46 (1H, s), two methoxyl proton signals at δ 3.71 (6H, s), and seven aliphatic proton signals at δ 2.89—5.91 (see Table 1). The relationships of the aliphatic protons was established by ^{1}H–^{1}H shift correlation spectroscopy (COSY). As showed

Table 1. ^{13}C-, ^{1}H-NMR Spectral Data of Compounds **1** and **2**

Positions	**1** (in pyridine-d_5)		**2** (in methanol-d_4)	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	139.2 (C)		135.9 (C)	
2	107.4 (CH)	7.03 (1H, s)	109.7 (CH)	6.40 (1H, s)
3	154.2 (C)		149.0 (C)	
4	137.4 (C)		133.5 (C)	
5	154.2 (C)		149.0 (C)	
6	107.4 (CH)	7.03 (1H, s)	109.7 (CH)	6.40 (1H. s)
7	45.0 (CH)	4.60 (1H, d, J=7.6 Hz)	45.1 (CH)	4.56 (1H, br s)
8	45.1 (CH)	3.78 (1H, dd, J=7.6, 9.5 Hz)	46.5 (CH)	2.98 (1H, m)
9	178.8 (C)		177.1 (C)	
1′	133.7 (C)		132.5 (C)	
2′	107.2 (CH)	8.01 (1H, s)	109.6 (CH)	7.37 (1H, s)
3′	147.0 (C)		148.6 (C)	
4′	147.0 (C)		148.6 (C)	
5′	108.6 (CH)	6.56 (1H, s)	110.2 (CH)	6.46 (1H, s)
6′	135.1 (C)		132.7 (C)	
7′	77.7 (CH)	5.30 (1H, d, J=10.1 Hz)	80.4 (CH)	5.06 (1H, d, J=9.1 Hz)
8′	43.6 (CH)	3.11 (1H, m)	40.6 (CH)	2.96 (1H, m)
9′	69.4 (CH_2)	4.75 (1H, dd, J=10.4, 5.1 Hz) 5.00 (1H, dd, J=10.4, 3.7 Hz)	72.9 (CH_2)	4.21 (1H, dd, J=9.5, 2.3 Hz) 4.69 (1H, dd, J=9.5, 4.1 Hz)
3,5-OMe	56.1 (CH_3)	3.76 (6H, s)	56.9 (CH_3)	3.71 (6H. s)
4-OMe	60.6 (CH_3)	3.89 (3H, s)		
–CH_2–	101.5 (CH_2)	6.00 (1H, d, J=1.2 Hz) 6.12 (1H, d, J=1.2 z)	102.6 (CH_2)	5.91 (1H, d, J=1.4 Hz) 6.00 (1H, d, J=1.4 Hz)
glc (inner)				
1	106.0 (CH)	5.10 (1H, d, J=8.3 Hz)	103.7 (CH)	4.39 (1H, d, J=7.3 Hz)
2	75.3 (CH)	4.16 (1H, dd, J=8.3, 9.1 z)	75.2 (CH)	3.30 (1H, dd, J=7.3, 8.9 Hz)
3	78.4 (CH)	4.08 (1H, t, J=9.1 Hz)	78.2 (CH)	3.83 (1H, t J=8.9 Hz)
4	71.8 (CH)	3.95 (1H, t, J=9.1 Hz)	71.6 (CH)	3.39 (1H, t, J=8.9 Hz)
5	78.7 (CH)	4.12 (1H, ddd, J=9.1, 6.4, 1.9 Hz)	78.2 (CH)	3.31 (1H, ddd, J=8.9, 5.5, 1.7 Hz)
6	69.5 (CH_2)	4.25 (1H, dd, J=12.5, 6.4 Hz) 4.52 (1H, dd, J=12.5, 1.9 Hz)	62.9 (CH_2)	3.74 (1H, dd, J=11.8, 5.5 Hz) 3.90 (1H, dd, J=11.8, 1.7 Hz)
glc (terminal)				
1	105.1 (CH)	5.02 (1H, d, J=7.4 Hz)		
2	75.2 (CH)	3.32 (1H, dd, J=8.9, 7.4 Hz)		
3	78.0 (CH)	3.50 (1H, t, J=8.9 Hz)		
4	71.8 (CH)	4.08 (1H, t, J=8.9 Hz)		
5	78.0 (CH)	3.77 (1H, ddd, J=8.9, 5.5, 1.8 Hz)		
6	62.8 (CH_2)	4.30 (1H, dd, J=11.9, 5.5 Hz) 4.47 (1H, dd, J=11.9, 1.8 Hz)		

in Table 1, the ^{1}H-, ^{13}C-NMR spectral data of the aglycone of **2** were similar to those of 4-demethyl-picropodophyllotoxin,[9] except for the signals due to the sugar moiety. Comparing the spectral and physical data of **2** with those of 4-demethyl-picropodophyllotoxin, the structure of the aglycone of **2** was assigned to 4-demethyl-picropodophyllotoxin. The ^{1}H-, ^{13}C-NMR spectra of **2** showed the aromatic signals at δ 4.39 (1H, d, J=7.3 Hz) and δ 103.7, respectively. Acid hydrolysis of **2** gave a glucose, which was analyzed by the same methods as in **1**. The C-1 of glucose was attached to the 7′-OH of the aglycone, as indicated by the C-7′ chemical shift (δ 80.4) of **2**, the correlation of H-1 of glucose and C-7′ of the aglycone in HMBC, and the correlation of H-1 of glucose

and H-7′ (δ 5.06, 1H, d, J=9.1 Hz) of the aglycone in NOESY. Based on these findings, **2** was identified as 4-demethylpicro-podophyllotoxin 7′-*O*-β-D-glucopyranoside.

The structures of known lignans from this species were characterized by comparison of the physical and spectroscopic data with those in the literature as: 4-demethyl-podophyllotoxin 7′-*O*-β-D-glucopyranoside (**3**), podophyllotoxin 7′-*O*-β-D-glucopyranoside (**4**), deoxy-podophyllotoxin (**5**),[10] picropodophyllotoxin (**6**), podophyllotoxin (**7**),[11] 4-demethyl-picropodophyllotoxin (**8**),[9] 4-demethyl-podophyllotoxin (**9**), and 4-demethyl-deoxy-podophyllotoxin (**10**).[12]

Experimental

General The following instruments were used to obtain physical data: Optical rotations were measured on a JASCO DIP-140 digital polarimeter. NMR spectra were recorded on a JEOL JNM-A 500 spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a JEOL JMS-DX 300 spectrometer.

Silica gel PSQ-100 (Fuji Silysia Chemical Ltd., 100 mesh), Lichroprep. Lobar RP-18 (Merck, 40—63 μm) and Sephadex LH-20 (Pharmacia Biotech) were used for column chromatography. Silica gel 60 F_{254}, RP-18 (Merck) were used for TLC.

Plant Material The roots and rhizomes of *Sinopodophyllum emodi* (Wall.) Ying were collected from the QinLing mountain area, China, in 1999, and were botanically identified by Prof. Li Guangming (Biology Department, Northwest University, China).

Extraction and Isolation Dried roots and rhizomes of *S. emodi* (2.47 kg) were extracted three times with 95% ethanol (each time for 60 min at 50 °C) to give ethanol extract (240 g). The ethanol extract (40 g) was dissolved in water, and then extracted with ether, chloroform, ethyl acetate and *n*-butanol, to give ether extract (6.89 g), chloroform extract (1.33 g), ethyl acetate extract (3.75 g), *n*-butanol extract (18.6 g), and a water layer (12.4 g), respectively.

The ethyl acetate extract (3.75 g) was subjected to a silica gel column chromatography and eluted by the solvent system of $CHCl_3$, $CHCl_3$–MeOH (20 : 1→5 : 1) to give five fractions (fr. A-1—5). Fr. A-1 (0.55 g) was chromatographed on a silica gel column with $CHCl_3$, $CHCl_3$–MeOH (20 : 1→10 : 1) as the eluent to give six fractions (A-1-1—6). Fr. A-1-1 (40 mg) was chromatographed on a silica gel column with $CHCl_3$ as the eluent to give **5** (12 mg). Fr. A-1-2 (72 mg), fr. A-1-4 (82.2 mg) and fr. A-1-6 (90.4 mg) were chromatographed on the silica gel columns with $CHCl_3$–MeOH as the eluent to give **6** (11 mg), **7** (17 mg), **8** (23 mg), and **10** (18 mg), respectively. Fr. A-2 (1.17 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH (16 : 1→8 : 1) as the eluent to give **9** (25 mg) and **2** (42 mg).

The *n*-butanol extract (18.5 g) was subjected to a silica gel column and eluted with the solvent system of $CHCl_3$–MeOH–H_2O (40 : 1 : 0.1→6 : 4 : 1) to give nine fractions (B-1—9) according to their TLC analysis. B-3 (340 mg) and B-5 (344 mg) were subjected to the Sephadex LH-20 columns with MeOH as the eluent to give **4** (214 mg) and **3** (207 mg), respectively. B-7 (997 mg) was chromatographed on a Lobar RP-18 column with 40% MeOH as an eluent to give three fractions (B-7-1—3). B-7-2 (346 mg) was chromatographed on a Sephadex LH-20 column and eluted with MeOH to give **1** (117 mg).

Compound **1** was obtained as colorless needles. $[\alpha]_D^{29}$ −45.67 (c=0.6, MeOH). HR-FAB-MS (positive): m/z 761.2243 $[M+Na]^+$ (Calcd for $C_{34}H_{42}O_{18}Na$: 761.2269). UV λ_{max} (MeOH) nm: 208, 287. IR ν_{max} (KBr) cm^{-1}: 3550, 3350, 1780. CD: $\Delta\varepsilon$ 290.3 (+1.75), 270 (−2.14), 245.3 (−0.60), 218.3 (−1.08), 202.5 (+11.7). ^{1}H-, ^{13}C-NMR (500 MHz, pyridine-d_5) spectral data were given in Table 1.

Compound **2** was obtained as a white powder. $[\alpha]_D^{29}$ −5.18 (c=0.25, MeOH). HR-FAB-MS (positive): m/z 585.1566 $[M+Na]^+$ (Calcd for $C_{27}H_{30}O_{13}Na$: 585.1584). UV λ_{max} (MeOH) nm: 208, 287. IR ν_{max} (KBr) cm^{-1}: 3550, 3350, 1780. CD (MeOH): $\Delta\varepsilon$ 289.9 (+2.94), 275.1 (−2.45), 234.7 (+3.27), 223.2 (−3.31), 214 (+2.04). ^{1}H-, ^{13}C-NMR (500 MHz, CH_3OH-d_4) spectral data were given in Table 1.

Compounds **1** and **2** (1 mg each) were hydrolyzed (2 N HCl, 95 °C, 1 h), and the sugar was detected as a glucose by HPLC, respectively. The absolute configuration of the glucose was determined according to the method reported by Hara and co-workers[7] using GC analysis. GC conditions: column: 3% Silicon SE-30 (2 m×3.2 mm); column temperature: 190 °C; injection temperature: 210 °C; retention time (min): D-glucose 53.0 min.

Acknowledgements This work was supported by the Chinese National Natural Science Foundation and Daiko foundation of Nagoya, Japan. The authors are grateful to Miss S. Kato and Miss K. Takahashi for providing NMR and MS data.

References

1) Stahelin H. F., von Warburg A., *Cancer Res.*, **51**, 5—15 (1991).
2) Podwyssotzki V., *Arch. Exp. Pathol. Pharmakol.*, **13**, 29—52 (1880).
3) Kaplan I. W., *New Orleans Med. Surg. J.*, **94**, 388—390 (1942).
4) Macrae W. D., Towers G. H. N., *Phytochemistry*, **23**, 1207—1201 (1984).
5) Atta-Ur-Rahman, Iqbal Choudhary M. A., Habibu-Ur-Rehman, Kazmi M. H., *Phytochemistry*, **40**, 427—431 (1995).
6) Li G. M., *Xibei Daxue Xueba*, *Ziran Kexueban*, **17**, 100—104 (1987).
7) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **35**, 501—506 (1987).
8) Yu P. Z., Wang L. P., Chen Z. N., *J. Nat. Prod.*, **54**, 1422—1424 (1991).
9) Novelo M., Cruz J. G., Hernanaer L., Pereda-Miranda R., *J. Nat. Prod.*, **56**, 1728—1736 (1993).
10) Broomhead A. J., Dewick P. M., *Phytochemistry*, **29**, 3839—3844 (1990).
11) Stahelin H., *Planta Med.*, **22**, 336—340 (1972).
12) Jackson D. E., Dewick P. M., *Phytochemistry*, **23**, 1147—1152 (1984).