Glycosides from Paeonia suffruticosa

Hsiou-Yu DING,[a] Yang-Chang WU,[a] Hang-Ching LIN,*[,b] Yu-Yi CHAN,[c] Pei-Lin WU,[c] and Tian-Shung WU*[,c]

Graduate Institute of Natural Products, Kaohsiung Medical College,[a] Kaohsiung, Taiwan, R.O.C., School of Pharmacy, National Defense Medical Center,[b] Taipei, Taiwan, R.O.C., and Department of Chemistry, National Cheng Kung University,[c] Tainan, Taiwan, R.O.C. Received December 15, 1998; accepted January 23, 1999

Four new glycosides, namely mudanpiosides-G, -H, -I and mudanoside-A together with three known compounds, gallic acid, adenosine and *p*-hydroxybenzoic acid were isolated from the root bark of *Paeonia suffruticosa*. Their structures have been determined on the basis of spectral evidence.

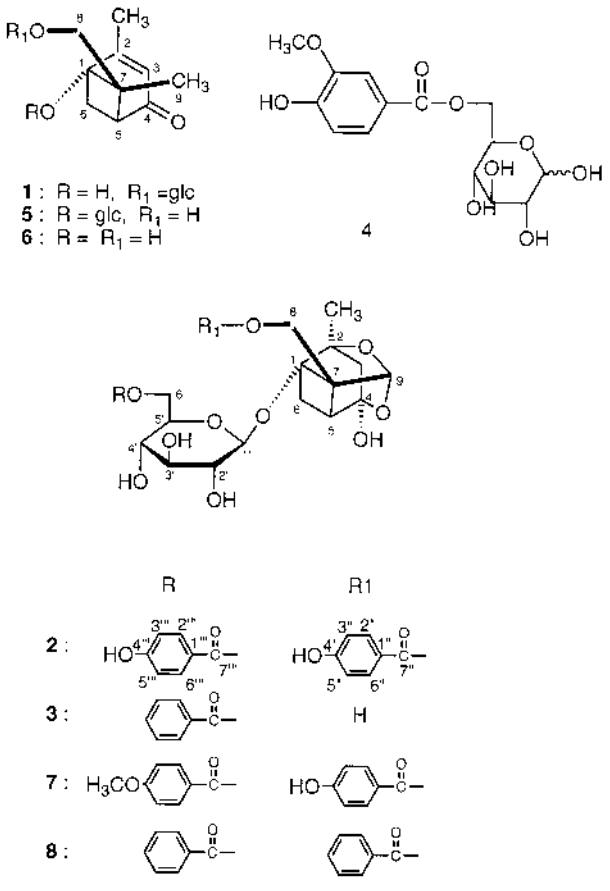
Key words *Paeonia suffruticosa*; Moutan bark; Paeoniaceae; glycoside

The root cortex of *Paeonia suffruticosa* ANDR. (Chinese name: mudanpi) (Paeoniaceae) is a famous traditional Chinese medicine and has been used as an analgesic, sedative and antiinflammatory agent, and as a remedy for cardiovascular, extravasated blood, stagnated blood and female genital diseases.[1—4] In previous papers, we reported on the isolation of acetophenones, monoterpene glycosides, flavonoid and triterpenoids from the root cortex of *Paeonia suffruticosa*.[5—7] In a continuation of our phytochemical studies on the constituents of this plant, we describe here the isolation and structural determination of four new glycosides, mudanpiosides-G (**1**), -H (**2**), -I (**3**) and mudanoside-A (**4**), together with three known compounds, gallic acid (**9**), adenosine (**10**) and *p*-hydroxybenzoic acid (**11**) from the polar fraction of an ethanolic extract.

Results and Discussion

Mudanpioside-G (**1**) was isolated as an optically active colorless syrup. It gave rise to a quasi molecular ion peak at *m/z* 345 $[M+H]^+$ by high resolution fast atom bombardment mass spectrometry (HR-FAB-MS) to establish the molecular formula as $C_{16}H_{24}O_8$, an isomer of mudanpioside-F (**5**).[6] The UV, IR, ^{1}H- and ^{13}C-NMR spectral data were similar to those of **5**, indicating the presence of a monoterpenoid. The ^{1}H-NMR signals at δ 1.63 (s) and 2.19 (s) for the two methyls at C-7 and C-2, respectively; 1.30 (1H, t, J=3.0 Hz) and 1.99 (1H, dd, J=8.4, 3.0 Hz), as well as δ 3.82 and 4.24 (each 1H, d, J=10.2 Hz) for the two methylenes at C-6 and C-8, respectively; δ 2.82 (1H, dd, J=8.4, 3.0 Hz) for a methine at C-5 and δ 5.49 (s) for a vinyl proton at C-3 were assigned by comparison with those of **5**. The remaining signals were attributed to a glucose moiety: at δ 3.93 (1H, ddd, J=8.4, 5.4, 1.6 Hz, H-5′), 4.01 (1H, t, J=8.4 Hz, H-2′), 4.17 (1H, t, J=8.4 Hz, H-4′), 4.21 (1H, t, J=8.4 Hz, H-3′), 4.33 (1H, dd, J=12.0, 5.4 Hz, H-6′), 4.52 (1H, dd, J=12.0, 1.6 Hz, H-6′) and 4.88 (1H, d, J=8.4 Hz, H-1′). An ether linkage between C-1′ of glucose and C-8 of the monoterpenoid was proven by the ^{1}H-^{13}C long range correlations between H-1′ and C-8, along with H-8 and C-1′. The full assignment of ^{1}H- and ^{13}C-NMR signals was conducted according to correaltion spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC) and rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectra (Table 1). Furthermore, hydrolysis of **1** and **5** with β-glucosidase at 37 °C overnight afforded the same aglycon **6** and β-glucose. Therefore, the structure of mudanpioside-G was assigned as **1**.

Mudanpioside-H (**2**) was determined to have the molecular formula $C_{30}H_{32}O_{14}$ by FAB-MS. The spectral data of **2** were similar to those of mudanpioside-B (**7**),[6] which contained a monoterpenoid bearing a glucoside. The monoterpenoid part was found to have ^{1}H-NMR signals at δ 1.67 (3H, s, C-2 Me); 2.28 and 2.45 (each 1H, d, J=12.0 Hz, H-3); 2.24 (1H, d, J=10.4 Hz) and 2.85 (1H, dd, J=10.4, 6.8 Hz) for H-6; 3.06 (1H, d, J=6.8 Hz, H-5); 5.01 and 5.16 (each 1H, d, J=12.0 Hz, H-8) and 5.92 (1H, s, H-9). Moreover, the glucose moiety appeared at δ 4.05 (1H, t, J=8.0 Hz, H-2′), 4.09 (1H, t, J=8.0 Hz, H-4′), 4.10 (1H, dd, J=8.0, 5.6 Hz, H-5′), 4.25 (1H, t, J=8.0 Hz, H-3′), 4.96 (1H, dd, J=11.2, 5.6 Hz, H-6′), 5.19 (1H, d, J=11.2 Hz, H-6′) and 5.14 (1H, d, J=8.0 Hz, H-



* Author to whom correspondence should be addressed.

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data and HMBC, NOESY Correlations of **1** and **5**

C	Compound **1**				Compound **5**	
	δ_C	δ_H (J in Hz)	HMBC	NOESY	δ_C	δ_H (J in Hz)
1	70.2				84.4	
2	177.2		H10		173.4	
3	125.9	5.49, s	H10	H3/H10	121.3	5.78 (quintet, 1.8)
4	205.1		H3		201.1	
5	29.7	2.82 (8.4, 3.0) dd	H3	H5/H6b	48.1	2.95 (dd, 7.2, 2.5)
6	35.7	1.3 (3.0) t		H6a/H6b	43.4	2.63 (d, 9.3)
		1.99 (8.4, 3.0) dd				3.55 (dd, 9.3, 7.2)
7	43.1		H3, 8a, 8b, 9		63.6	
8	76.9	3.82 (10.2) d	H1′	H8a/H8b, 1′	66.3	4.10 (d, 12.1)
		4.24 (10.2) d				4.46 (d, 12.1)
9	24.1	1.63, s	H8a, 8b	H9/H8a, 10	16.1	1.31 (s)
10	18.5	2.19, s			19.9	2.12 (d, 1.8)
1′	105.1	4.88 (8.4) d	H8a, 2′	H-1′/H8a, 3′	99.8	5.15 (d, 8.5)
2′	74.6	4.01 (8.4) t		H2′/H4′	74.7	4.04 (t, 8.5)
3′	78.0	4.21 (8.4, t)	H1′, 4′	H3′/H5′	78.5	4.20 (t, 8.5)
4′	70.9	4.17 (8.4) t	H-3′	H4′/H5′	71.6	4.16 (t, 8.5))
5′	78.0	3.93 (8.4, 5.4, 1.6) ddd	H-4′		78.5	3.90 (m)
6′	62.0	4.33 (12.0, 5.4) dd			62.6	4.29 (dd, 11.7, 5.4)
		4.52 (12.0, 1.6) dd				4.44 (d, 11.7)

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

C	δ_C	δ_H (J in Hz)	HMBC	NOESY
1	88.3		H6a, 10	
2	85.5		H9, 10	
3	44.2	2.28 (12.0) d	H10	H3a/H3b
		2.45 (12.0) d		
4	105.4		H6a, 9	
5	43.2	3.06 (6.8) d	H8a, 8b	
6	22.3	2.24 (10.4) d		H6a/H6b
		2.85 (10.4, 6.8) dd		H6b/H1′
7	71.3		H6a	
8	60.2	5.01 (12.0) d		H8a/H8b
		5.16 (12.0) d		
9	101.2	5.92, s	H8b	
1′	19.3	1.67, s		
1′	99.7	5.14 (8.0) d	H2′	H-1′/H6b, 2′, 3′, 5′
2′	74.3	4.05 (8.0) d	H4′	
3′	77.7	4.25 (8.0) t	H4′	
4′	70.9	4.09 (8.0) t		
5′	74.6	4.10 (8.0, 5.6) dd		
6′	64.1	4.96 (11.2, 5.6) dd		
		5.19 (11.2) d		
1″	120.7		H3″, 5″	
2″	131.8	8.13 (8.0) d	H6″	H2″/H3″
3″	115.5	7.06 (8.0) d	H5″	
4″	131.8		H2″, 6″	
5″	115.5	7.06 (8.0) d		H5″/H6″
6″	131.8	8.13 (8.0) d	H2″	
7″	166.0			
1‴	121.0		H3‴, 5‴	
2‴	131.8	8.27 (8.0) d	H6‴	H2‴/H3‴, 5‴
3‴	115.6	7.19 (8.0) d	H5‴	
4‴	163.1		H2‴, 6‴	
5‴	115.6	7.19 (8.0) d		
6‴	131.8	8.27 (8.0) d	H2‴	
7‴	166.0			

Table 3. ^{1}H- and ^{13}C-NMR Spectral Data and HMBC, NOESY Correlations of **3**

C	δ_C	δ_H (J in Hz)	HMBC	NOESY
1	89.1		H′, 3a, 5, 6a, 6b, 8, 10	
2	86.2		H3a, 6b, 9, 10	
3	45.2	2.26 (12.2) d	H10	H3a/H3b, 10
		2.41 (12.2) d		H3b/H3a, 6, 10
4	106.0		H3a, 3b, 5, 6b, 9	
5	43.8	2.96 (6.8) d	H3a, 6b, 8	H5/H6b, 8
6	23.3	2.12 (10.3) d		H6a/H3b, 6b
		2.83 (10.3, 6.8) dd		
7	73.5		H5, 6a, 8, 9	
8	58.9	4.37 (12.0) d	H5, 9	H8/H5, 6b, 3′
		4.41 (12.0) d		
9	102.0	5.82, s	H8	
10	19.8	1.64, s		
1′	100.0	5.22 (7.8) d	H2′	H1′/H3′, 5′
2′	74.9	4.06 (7.8) t		
3′	78.4	4.24 (7.8) t		H3′/H1′, 2′, 4′, 5′
4′	71.9	4.09 (7.8) t		H4′/H6′a, 6′b
5′	75.1	4.13 (7.8, 6.9) dd		H5′/H6′a, 6′b
6′	65.2	4.69 (10.0, 6.9) dd		
		5.19 (10.0) d		
1″	131.0		H3″, 5″	
2″	129.9	8.22 (7.2) d	H4″, 6″	H2″/H3″
3″	128.8	7.37 (7.2) t	H5″	
4″	133.4	7.45 (7.2) t	H2″, 6″	H4″/H3″, 5″
5″	128.8	7.37 (7.2) t	H3″	H5″/H6″
6″	129.9	8.22 (7.2) d	H2″, 4″	
7″	166.5		H2″, 6″	

1′), which connected to the monoterpenoid by an ether linkage between C-1′ of glucose and C-1 of the monoterpenoid. This was supported by the presence of nuclear Overhauser effect (NOE) between H-1′ and H-6 (Table 2). Two sets of *p*-hydroxybenzoyl group signals were found at δ 7.06 and 8.13 (each 2H, d, J=8.0 Hz) and δ 7.19 and 8.27 (each 2H, d, J=8.0 Hz), respectively. The location of the two *p*-hydroxybenzoyloxy groups on C-8 and C-6′ were inferred by the downfield shift of the signals for H-8 and H-6′ to δ 5.01, 5.16 and 4.96, 5.19, respectively. Similar results were also found for mudanpioside-A—C, benzoylpaeoniflorin and benzoyloxypaeoniflorin.[6] Based on the above spectral analyses, the structure of mudanpioside-H was deduced as **2**, with six

Table 4. ^{1}H- and ^{13}C-NMR Spectral Data and HMBC, NOESY Correlations of **4**

C	δ_C	δ_H (J in Hz)	HMBC	NOESY
1	121.9		H2, 5	
2	113.7	7.84 (1.6) d	H6	H2/H4′, 3-OMe
3	148.4		H2, 5, 3-OMe	
4	153.2		H2, 5, 6	
5	116.2	7.12 (8.0) d		H5/H6
6	124.8	7.88 (8.0, 1.6) dd	H2	H6/H4′
7	166.9		H2, 6, 6′αa, 6′αb, 6′αb, 6′βa, 6′βb	
3-OMe	55.8	3.61, s		
1′α	94.3	5.89 (3.6) d		H1′α/H2′α, 6′αa
1′β	99.1	5.34 (8.0) d	H2′β	H1′β/H2′β, 3′β, 5′β
2′α	75.6	4.23 (8.0, 3.6) dd	H1′α, 3′α, 4′α	
2′β	76.9	4.16 (8.0) t	H3′β	
3′α	75.4	4.78 (8.0) t	H1′α, 2′α, 4′α, 5′α	H3′α/H2′α, 4′α, 5′α
3′β	78.6	4.31 (8.0) t		H3′β/H1′β, 2′β, 6′βa
4′α	72.6	4.22 (8.0) t	H3′α, 5′α	
4′β	72.0	4.22 (8.0) t	H2′β, 3′β, 6′βa, 6′βb	
5′α	71.2	4.99 (8.0, 2.0) dd	H1′α, 3′α, 6′αa, 6′αb	H5′α/H1′α, 3′α
5′β	74.5	4.25 (8.0) d	H6′β	
6′α	65.7	5.07 (11.4) d	H4′α	H6′αa/H2′α, 6′αb
		5.27 (11.4) d		H6′αb/H2′α, 4′α, 6′αa
6′β	65.4	5.09 (11.4) d		H6′βa/H3′β, 4′β, 5′β, 6′βb
		5.29 (11.4) d		H6′βb/H5′β, 6′βa

chiral centers.

Mudanpioside-I (**3**) exhibited the molecular formula $C_{23}H_{28}O_{11}$ by FAB-MS. It consisted of a monoterpene glycoside skeleton, from comparison of spectral data with those of **2**. Compound **3** also showed similar spectral data to those of benzoylpaeoniflorin (**8**). However, only one benzoyloxy group was present in the ^{1}H-NMR spectrum at δ 7.37 (2H, t, J=7.2 Hz, H-3″ and H-5″), 7.45 (1H, t, J=7.2 Hz, H-4″) and 8.22 (2H, d, J=7.2 Hz, H-2″ and H-6″). The downfield shift of H-6′ (δ 4.96, 5.19) indicated that this benzoyloxy substituent was attached to C-6′ of glucose. Based on the above analysis, the structure **3** was assigned to mudanpioside-I.

On the basis of the specific rotational values of compounds **2** and **3**, [α] −10.5° and −9.2°, respectively, similar to those of mudanpioside-A—E, which ranged from [α] −5° to −25°, these new compounds were assumed to have the same absolute configuration as mudanpioside-A—E.[6]

Mudanoside-A (**4**), $C_{14}H_{18}O_9$, showed the presence of a benzoyl substituent and a glucose unit, the hydrolyzed moiety of mudanpioside E.[6] A 3,4-disubstituted benzoyl group was deduced from the signals at δ 7.12 (d, J=8.0 Hz, H-5), 7.84 (d, J=1.6 Hz, H-2) and 7.88 (dd, J=8.0, 1.6 Hz, H-6) in the ^{1}H-NMR spectrum. A methoxy (δ 3.61) and a hydroxy group were located at C-3 and C-4, respectively, from the ^{1}H-^{13}C long range correlations between this methoxy and C-3 (δ 148.4), and the NOE between the methoxy protons and H-2. In addition, two sets of glucose signals, assignable for α and β, were found in the ^{1}H-NMR spectrum which could be grouped by COSY correlations. The downfield shifted H-6′ (δ 5.07, 5.27 for 6′α and 5.09, 5.29 for 6′β) suggested a 3-methoxy -4-hydroxybenzoyloxy group attached to C-6′. Full assignments were determined by COSY, HMQC, HMBC and nuclear Overhauser and exchange spectroscopy (NOESY) spectra. Therefore mudanoside-A was proposed to have the structure **4**.

Three known compounds, gallic acid (**9**),[8] adenosine (**10**)[8] and *p*-hydroxybenzoic acid (**11**)[8] were also isolated and characterized by comparison of their spectral data (UV, IR, NMR, MS) with literature values.

Experimental

UV spectra were recorded in MeOH and IR spectra were determined as KBr discs. ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian Unity Plus NMR spectrometer (400 and 100 MHz), with pyridine-d_5 as solvent and tetramethylsilane (TMS) as internal standard. FAB-MS was measured on a VG70-250AS spectrometer. Optical rotations were recorded on a Jasco DIP-370 digital polarimeter.

Plant Material The root cortex of *P. suffruticosa* was purchased from a local market. A specimen of the plant (NDMC-790701) has been verified by Prof. C. S. Kuoh and deposited at the herbarium of the National Defense Medical Center, Taipei, Taiwan.

Extraction and Separation The root cortex (45 kg) was extracted with 95% followed by 70% EtOH (×4) at room temperature. The combined EtOH extracts were concentrated under reduced pressure to yield a dark-brown syrup which was partitioned between hexane and 90% MeOH. The 90% MeOH layer was concentrated and partitioned with EtOAc and H_2O. The aqueous solution was again partitioned between *n*-BuOH and H_2O. The EtOAc layer was subjected to silica gel column chromatography and eluted with $CHCl_3$ and $CHCl_3$–MeOH (97 : 3) to afford acetophenones. The residue was eluted with MeOH, combined with the *n*-BuOH extract and subjected to column chromatography on silica gel with $CHCl_3$–MeOH, 19 : 1, 9 : 1, 17 : 3 and MeOH, successively. Rechromatography of the first fraction on preparative Lobar RP-8 followed by HPLC separation using phenyl column and 45% MeOH as eluent gave **2** (365.5 mg) and **3** (234.5 mg). In the same manner, the second fraction using 35% MeOH as eluent furnished **11** (720 mg), and the third fraction using 10%, 18% and 25% MeOH as eluent gave **9** (385.0 mg), **1** (43.8 mg), **4** (198.7 mg) and **10** (141.0 mg), successively.

Mudanpioside-G (**1**): Colorless syrup. $[\alpha]_D$ −46.75° (c=0.24, MeOH). UV λ_{max} nm (log ε): 256.8 (3.80). IR ν_{max} cm^{-1}: 3377 (OH), 1682 (C=O). HR-FAB-MS: Calcd for $C_{16}H_{25}O_8$, m/z: 345.1549 [M+1]$^+$, Found 345.1547. FAB-MS m/z (posit., rel. int.): 367 ([M+Na]$^+$, 14), 345 ([M+H]$^+$, 16), 253 (19), 197 (47), 183 (100), 165 (45), 147 (43).

Mudanpioside-H (**2**): Colorless powder. mp 159—161 °C. $[\alpha]_D$ −10.52° (c=0.23, MeOH). UV λ_{max} nm (log ε): 258.0 (4.50). IR ν_{max} cm^{-1}: 3395 (OH), 1705, 1699 (C=O). FAB-MS m/z (posit., rel. int.): 617 ([M+1]$^+$, 1), 599 (6), 461 (3), 391 (1), 317 (8), 283 (26), 242 (3), 220 (5), 179 (28), 154 (91), 121 (100).

Mudanpioside-I (**3**): Colorless powder. mp 123—125 °C. $[\alpha]_D$ −9.2° (c=0.38, MeOH). UV λ_{max} nm (log ε): 229.6 (4.10). IR ν_{max} cm^{-1}: 3500-3250 (OH), 1722(C=O), 1602, 1585. FAB-MS m/z (posit., rel. int.): 481 ([M+1]$^+$, 8), 437 (9), 349 (7), 307 (9), 289 (9), 267 (52), 249 (23), 219

(11), 179 (36), 154 (73), 138 (33), 137 (57), 136 (64), 105 (100).

Mudanoside-A (**4**): Colorless syrup. $[\alpha]_D$ +24.59° (c=0.09, MeOH). UV λ_{max} nm (log ε): 220.2 (4.10), 262.0 (3.90), 292.8 (3.60). IR ν_{max} cm^{-1}: 3377 (OH), 1701 (C=O). HR-FAB-MS: Calcd for $C_{14}H_{19}O_9$, *m*/*z*: 331.1029 $[M+1]^+$, Found 331.1031. FAB-MS *m*/*z* (posit., rel. int.): 331 ($[M+1]^+$, 7), 313 (9), 277 (8), 223 (11), 207 (13), 197 (10), 185 (100), 167 (12), 151 (25), 135 (13), 115 (35), 105 (23).

Hydrolysis of 1 and 5 A solution of **1** (2 mg) and **5** (5 mg) were hydrolyzed with excess β-glucosidase at 37 °C overnight, followed by column chromatography on Sephadex LH-20 and eluted with H_2O and MeOH to afford β-glucose and the same aglycon **6** as a colorless syrup, successively. Compound **6**, $C_{10}H_{14}O_3$, UV λ_{max} nm: 257. IR ν_{max} cm^{-1}: 3386, 2978, 1674, 1381, 1230. EI-MS *m*/*z* (rel. int.): 182 ($[M]^+$, 7), 164 (37), 151 (55), 136 (41), 124 (60), 107 (20), 96 (83), 91 (25), 77 (26), 68 (100), 55 (34). ^{1}H-NMR (CD_3OD) δ: 5.70 (1H, d, J=2.0 Hz, H-3), 4.09 (1H, d, J=12.0 Hz, H-8), 3.78 (1H, d, J=12.0 Hz, H-8), 2.88 (1H, dd, J=9.6, 6.4 Hz, H-6), 2.73 (1H, dd, J=6.4, 2.0 Hz, H-5), 2.42 (1H, d, J=9.6 Hz, H-6), 2.16 (3H, s, H-10), 1.09 (3H, s, H-9).

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