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Cytotoxic Iridoids from the Roots of *Patrinia scabra*

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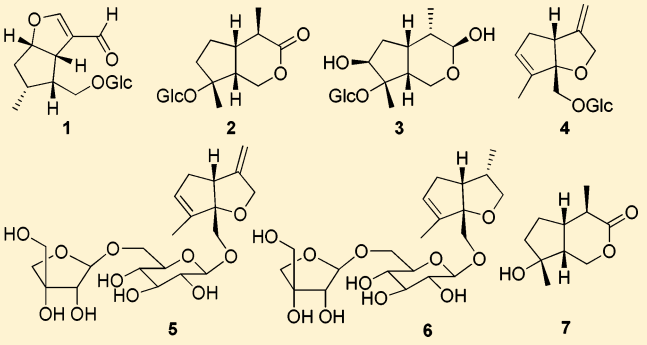
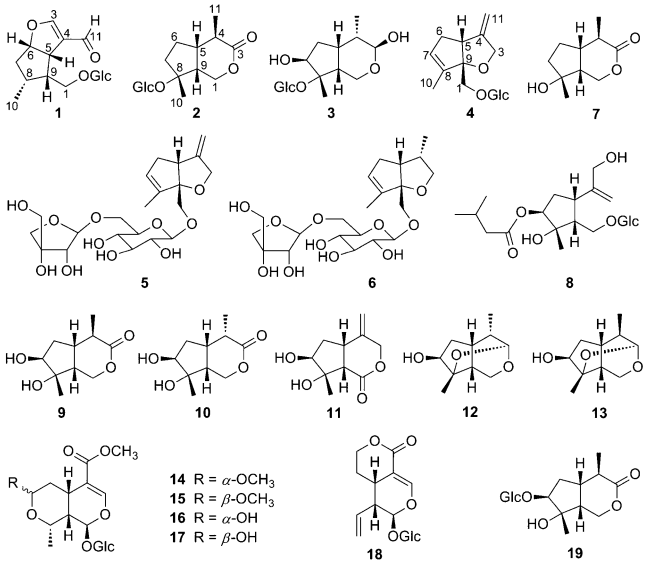
Supporting Information

ABSTRACT: Six new iridoid glucosides, patriridosides D−I (**1**−**6**), and one new iridoid, scabrol A (**7**), along with 12 known non-glycosidic and glycosidic iridoids (**8**−**19**), have been isolated from an ethanolic extract of the roots of *Patrinia scabra*. The cytotoxic activity of the isolated compounds against human cervical carcinoma HeLa cells and gastric carcinoma MNK-45 cells was evaluated using the MTT assay. Compounds **1**, **4**−**6**, **8**, and **18** showed cytotoxic activities against the MNK-45 cell line with respective IC_{50} values of 15.6, 8.7, 9.4, 30.9, 23.8, and 11.2 μM, while only compound **10** showed cytotoxicity against the HeLa cell line, with an IC_{50} value of 24.5 μM.



Patrinia scabra Bunge is a perennial herb belonging to the Valerianaceae family growing mainly in the northeastern part of China. The roots of *P. scabra* are used as a traditional medicine to treat malaria, dysentery, leukemia, gastric cancer, cervical erosion, regulating host immune response, and gynecological diseases.[1−3] Previous investigation of secondary metabolites from the roots of *P. scabra* resulted in the isolation of iridoids,[1−6] flavonoids,[7] terpenoids,[7] and lignans.[8] Phytochemical investigation has revealed that the roots of this plant are rich in iridoids; however, cytotoxicity studies on iridoids have rarely been reported.[9−11] The cytotoxic activity of iridoids against the MNK-45 cancer cell line has not been reported. In our search for biologically active natural products from Chinese medicinal plants, we investigated the iridoid constituents in the ethanolic extract of the roots of *P. scabra*, from which six new iridoid glucosides, patriridosides D−I (**1**−**6**), and one new iridoid, scabrol A (**7**), along with 12 known non-glycosidic and glycosidic iridoids (**8**−**19**) were isolated. Compound **1** represents a new secoiridoid skeleton with an ether bridge between C-3 and C-6. Only a few naturally occurring iridoids with an ether bridge between C-3 and C-6 have been reported in plants of the genus *Patrinia*. This paper describes the isolation and structural elucidation of the above-mentioned compounds and the evaluation of their cytotoxic activities against the HeLa and MNK-45 cancer cell lines.



RESULTS AND DISCUSSION

A 90% aqueous EtOH extract of the roots of *P. scabra* was partitioned between EtOAc and H_2O and between *n*-BuOH and H_2O. The EtOAc and *n*-BuOH layers were subjected repeatedly to column chromatography on CG161M, RP-18, MCI, ODS, Sephadex LH-20, and silica gel to afford six new iridoid glucosides, patriridosides D−I (**1**−**6**), one new iridoid, scabrol A (**7**), and 12 known compounds. The structures of the known compounds were identified as patrinioside (**8**), patriscabrol (**9**), isopatriscabrol (**10**), jatamanin A (**11**), (3*S*,4*S*,5*S*,7*S*,8*S*,9*S*)-3,8-epoxy-7-hydroxy-4,8-dimethylperhydrocyclopenta[*c*]pyran (**12**), (3*S*,4*R*,5*S*,7*S*,8*S*,9*S*)-3,8-epoxy-7-hydroxy-4,8-dimethylperhydrocyclopenta[*c*]pyran (**13**), (7α)-7-*O*-methylmorroniside (**14**), (7β)-7-*O*-methylmorroniside (**15**), (7α-OH)-morroniside (**16**), (7β-OH)-morroniside (**17**), sweroside (**18**), and patriscabroside I (**19**)

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by comparing their spectroscopic data (MS, ^{1}H NMR, ^{13}C NMR) with reported values.[2,3,9,12−14]

Patriridoside D (**1**) was isolated as an amorphous powder and gave a molecular formula of $C_{16}H_{24}O_8$, as determined by HRESIMS ([M + Cl]$^-$ *m*/*z* 379.1154, calcd 379.1159), requiring an index of hydrogen deficiency of five. The IR spectrum showed broad absorption bands for multiple OH groups (3463 cm^{-1}) and a conjugated formyl (1651 cm^{-1}) functionality. Analysis of the ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 3) indicated signals for a double bond [δ_H 7.37 (1H, s, H-3); δ_C 166.8 (C-3) and 125.4 (C-4)], a formyl group [δ_H 9.14 (1H, s, H-11); δ_C 191.2 (C-11)], two methylenes [δ_H 3.95 (1H, dd, *J* = 9.0, 6.7 Hz, H-1a), 3.41 (1H, t, *J* = 8.9 Hz, H-1b), 2.03 (1H, m, H-7a), 1.77 (1H, d, *J* = 13.1 Hz, H-7b); δ_C 71.0 (C-1) and 31.0 (C-7)], four methines [δ_H 3.15 (1H, t, *J* = 4.5 Hz, H-5), 4.50 (1H, m, H-6), 1.93 (1H, m, H-8), 2.04 (1H, m, H-9); δ_C 33.7 (C-5), 88.3 (C-6), 46.0 (C-8), and 57.2 (C-9)], a methyl doublet [δ_H 1.09 (3H, d, *J* = 7.2 Hz, Me-8); δ_C 19.7 (C-10)], and the signals of a glucosyl moiety. The coupling constant of the anomeric proton (δ 4.18, 1H, d, *J* = 7.7 Hz) suggested a β-configured glucose unit. The presence of the β-D-glucopyranosyl moiety was confirmed by acid hydrolysis of **1** with 2 M H_2SO_4. These data led to the preliminary conclusion that **1** was an iridoidal glucoside. The remaining two indices of hydrogen deficiency required by the molecular formula indicated that **1** was a bicyclic iridoid. An HMBC correlation of H-1′/C-1 confirmed the β-D-glucopyranosyl moiety was located at C-1. The COSY spectrum exhibited correlations (Figure 1) between H-9 and H-5, H-5 and H-6, H-6 and H-7, H-7 and H-8, H-8 and H-9, H-8 and H-10, and H-9 and H-1. Further analysis of the 2D NMR HMQC and HMBC data allowed assignment of all proton and carbon signals. Key HMBC correlations (Figure 1) from H-3 to C-6, C-4, C-5, and C-11, from H-11 to C-4, from H-6 to C-3, and from H-5 to C-4, C-3, and C-11 established the gross structure of **1**. The relative configuration of **1** was determined by the 2D NOESY spectrum and biogenetic considerations, prescribing that H-5 and H-9 were β-oriented. NOESY correlations (Figure 1) between H-9 and H-5, H-6, and H-7b and between H-5 and H-7b indicated that these protons were accordingly β-oriented. H-8 showed a correlation with H-6, but no correlations were observed between H-5 and H-10, and H-6 and H-10, suggesting an α-orientation of Me-8. Thus, the structure of patriridoside D (**1**) was defined as (5*S*,6*S*,8*R*,9*R*)-9-(hydroxymethyl)-8-methyl-6,7,8,9-tetrahydro-5*H*-cyclopenta[*b*]furan-4-carbaldehyde 1-*O*-β-D-glucopyranoside.

Compound **1** represents a new secoiridoid skeleton with an ether bridge between C-3 and C-6. It is the first iridoid glycoside from plants of the genus *Patrinia*.

Patriridoside E (**2**) was isolated as an amorphous powder. The molecular formula, $C_{16}H_{26}O_8$, was deduced from the HRESIMS ([M + Na]$^+$ *m*/*z* 369.1500 calcd 369.1519). The IR absorptions of **2** indicated the presence of the hydroxy (3422 cm^{-1}) and lactone carbonyl (1729 cm^{-1}) functional groups. The ^{1}H NMR spectrum (Table 1) exhibited resonances for two methyl groups at δ_H 1.43 (s, H-10) and 1.14 (d, *J* = 6.5 Hz, H-11), three methylene groups at δ_H 4.54 (t, *J* = 11.7 Hz, H-1a), 4.38 (dd, *J* = 11.5, 6.6 Hz, H-1b), 1.94 (m, H-6a), 1.82 (m, H-6b), 2.17 (ddd, *J* = 12.7, 6.5, 3.9 Hz, H-7a), and 1.60 (ddd, *J* = 12.9, 9.9, 6.7 Hz, H-7b), and three methine groups at δ_H 2.65 (dq, *J* = 12.8, 6.4 Hz, H-4), 2.03 (m, H-5), and 2.37 (td, *J* = 11.3, 6.7 Hz, H-9). The ^{13}C NMR and DEPT spectra (Table 3) exhibited 16 carbon signals, consisting of two methyl carbons at δ_C 25.2 (C-10) and 14.1 (C-11), three methylene carbons at δ_C

Table 1. ^{1}H NMR Data for Compounds 1−3 and 7 (400 MHz, δ in ppm, *J* in Hz)

H	1[a]	2[a]	3[a]	7[b]
1	3.95, dd (9.0, 6.7)	4.54, t (11.7)	4.05, dd (12.5, 6.0)	4.33, dd (11.5, 6.8)
	3.41, t (8.9)	4.38, dd (11.5, 6.6)	3.73, dd (12.5, 8.2)	4.41, t (11.5)
3	7.37, s		4.56, d (8.5)	
4		2.65, dq (12.8, 6.4)	1.68, m	2.47, m
5	3.15, t (4.5)	2.03, m	2.41, m	2.03, m
6	4.50, m	1.94, m	2.09, m	1.81, m
		1.82, m	1.47, ddd (14.2, 11.0, 6.8)	1.69, m
7	2.03, m	2.17, ddd (12.7, 6.5, 3.9)	4.39, dd (9.9, 6.6)	2.03, m
	1.77, d (13.1)	1.60, ddd (12.9, 9.9, 6.7)		1.69, m
8	1.93, m			
9	2.04, m	2.37, td (11.3, 6.7)	2.16, m	2.29, m
10	1.09, d (7.2)	1.43, s	1.33, s	1.37, s
11	9.14, s	1.14, d (6.5)	0.99, d (6.8)	1.18, d (6.6)
1′	4.18, d (7.7)	4.43, d (7.8)	4.58, d (7.5)	
2′	3.19, t (8.5)	3.13, t (8.8)	3.21, t (8.2)	
3′	3.37, m	3.36, t (8.6)	3.39, m	
4′	3.27, m	3.28, m	3.28, m	
5′	3.27, m	3.26, m	3.28, m	
6′	3.87, dd (13.2, 3.9)	3.83, dd (11.9, 1.7)	3.87, dd (11.5, 2.3)	
	3.67, dd (12.0, 4.8)	3.64, dd (11.9, 5.2)	3.65, dd (11.5, 5.8)	

[a]Measured in methanol-d_4. [b]Measured in $CDCl_3$.

Table 2. ^{1}H NMR Data for Compounds 4−6[a] (400 MHz, δ in ppm, *J* in Hz)

H	4	5	6
1	3.99, d (10.3)	3.81, d (10.2)	3.73, d (10.4)
	3.80, d (10.3)	3.90, d (10.6)	3.90, m
3	4.46, d (12.6)	4.16, d (12.7)	3.07, dd (10.1, 9.1)
	4.21, dd (12.6, 1.9)	4.39, d (12.7)	3.82, m
4			2.37, m
5	3.32, m	3.23, t (8.5)	2.78, m
6	2.77, m	2.22, d (16.0)	2.21, m (2H)
	2.28, m	2.67, dd (16.0, 6.9)	
7	5.57, brs	5.58, s	5.62, s
10	1.73, brs	1.61, s	1.60, s
11	5.00, dd (5.3, 2.1)	4.99, d (10.6)	0.88, d (6.9)
1′	4.38, d (7.8)	4.41, d (7.9)	4.41, d (7.9)
2′	3.24, t (8.4)	3.23, t (8.5)	3.23, t (8.5)
3′	3.40, m	3.42, m	3.42, m
4′	3.33, m	3.35, m	3.35, m
5′	3.33, m	3.49, m	3.49, m
6′	3.92, dd (12.0, 1.1)	3.67, dd (11.5, 5.7)	3.67, dd (11.5, 5.8)
	3.72, dd (12.0, 5.7)	3.96, d (11.5, 2.5)	3.96, m
1″		5.04, d (2.9)	5.04, d (2.9)
2″		3.93, m	3.93, m
4″		3.83, d (10.0)	3.82, d (10.0)
5″		3.98, d (10.0)	3.98, d (10.0)

[a]Measured in methanol-d_4.

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Table 3. ^{13}C NMR Data for Compounds 1−7 (100 MHz, δ in ppm)

carbon	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[b]
1	71.0, CH_2	67.3, CH_2	63.1, CH_2	71.5, CH_2	71.5, CH_2	72.2, CH_2	66.0, CH_2
3	166.8, CH	179.6, C	98.0, CH	71.6, CH_2	71.3, CH_2	73.1, CH_2	176.8, C
4	125.4, C	39.6, CH	37.9, CH	156.3, C	154.6, C	37.2, CH	39.1, CH
5	33.7, CH	43.5, CH	38.3, CH	48.4, CH	47.6, CH	46.8, CH	42.7, CH
6	88.3, CH	30.3, CH_2	29.7, CH_2	38.7, CH_2	38.2, CH_2	30.3, CH_2	30.2, CH_2
7	31.0, CH_2	38.1, CH_2	78.2, CH	129.7, CH	131.0, CH	132.3, CH	42.6, CH_2
8	46.0, CH	87.4, C	87.8, C	139.8, C	138.6, C	138.3, C	79.7, C
9	57.2, CH	49.7, CH	49.8, CH	100.0, C	99.8, C	99.3, C	46.9, CH
10	19.7, CH_3	25.2, CH_3	18.4, CH_3	12.1, CH_3	12.1, CH_3	12.3, CH_3	27.9, CH_3
11	191.2, CH	14.1, CH_3	14.8, CH_3	104.7, CH_2	106.0, CH_2	11.8, CH_3	13.7, CH_3
1′	104.1, CH	99.1, CH	98.8, CH	104.6, CH	103.7, CH	103.7, CH	
2′	75.3, CH	75.0, CH	75.3, CH	75.0, CH	73.9, CH	73.9, CH	
3′	78.0, CH	78.4, CH	78.2, CH	78.0, CH	76.6, CH	76.6, CH	
4′	71.6, CH	71.7, CH	71.8, CH	71.6, CH	70.5, CH	70.6, CH	
5′	77.9, CH	77.7, CH	77.8, CH	78.0, CH	75.7, CH	75.7, CH	
6′	62.8, CH_2	62.8, CH_2	62.9, CH_2	62.8, CH_2	68.4, CH_2	68.4, CH_2	
1″					109.9, CH	109.9, CH	
2″					77.5, CH	77.4, CH	
3″					80.3, C	80.3, C	
4″					74.5, CH_2	74.4, CH_2	
5″					64.6, CH_2	64.6, CH_2	

[a]Recorded in methanol-d_4. [b]Recorded in $CDCl_3$

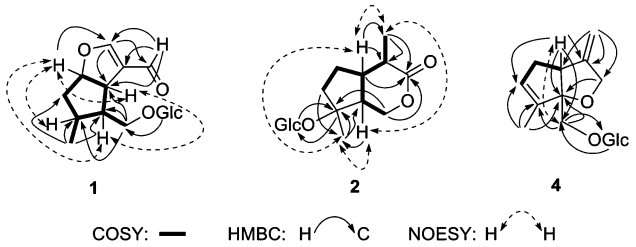

Figure 1. Key COSY, HMBC, and NOESY correlations of 1, 2, and 4.

67.3 (C-1), 30.3 (C-6), and 38.1 (C-7), three methine carbons at δ_C 39.6 (C-4), 43.5 (C-5), and 49.7 (C-9), a quaternary carbon at δ_C 87.4 (C-8), and a lactone carbonyl carbon at δ_C 179.6 (C-3), as well as the six carbon signals of a glucosyl moiety. The $^1H-^1H$ COSY spectrum showed the connectivities (Figure 1) of the proton coupling sequence for the C(1)−C(9)−C(5)−C(6)−C(7) and C(5)−C(4)−C(11) fragments. HMBC correlations (Figure 1) from H-1 and H-5 to C-3, from H-5 to C-4 and C-11, from H-1, H-6, and H-7 to C-8, and from H-7 and H-9 to C-10 were observed. Analysis of the $^1H-^1H$ and $^1H-^{13}C$ correlations, exhibited in the $^1H-^1H$ COSY, HMQC, and HMBC spectra, allowed the establishment of an iridomyrmecin-type iridolactone structure[2] for **2**. The long-range $^1H-^{13}C$ correlation between the anomeric proton and C-8 confirmed that the glucosyl moiety was located at C-8. The coupling constant of the anomeric proton (δ 4.43, 1H, d, J = 7.8 Hz) suggested a β-configured glucose unit. The presence of the β-D-glucopyranosyl moiety was confirmed by acid hydrolysis of **2** with 2 M H_2SO_4.

The relative configuration of **2** was determined by the 2D NOESY and NMR data. Comparison of the coupling constant of **1** ($J_{(1b, 9)}$ = 6.6 Hz) with that of the structurally similar compound patriscabroside I[2] indicated that H-1b [δ 4.38 (dd, J = 11.5, 6.6 Hz)] and H-9 were *trans*-diaxially oriented with H-9 in a β-orientation. According to literature data,[5,15] the chemical shift of C-9 at a relatively high field (δ_C 49.7) indicated that the glucosyl moiety at C-8 was α-oriented. NOESY correlations (Figure 1) between H-9 and H-10, H-9 and H-11, H-5 and H-10, and H-5 and H-11, but the absence of a correlation between H-9 and H-4, indicated that H-5 and the two methyl groups were β-oriented and the glucosyl moiety was accordingly α-oriented. Therefore, the structure of patriridoside E (**2**) was established as (4*R*,5*S*,8*R*,9*R*)-8-hydroxy-4,8-dimethylhexahydrocyclopenta[*c*]pyran-3(1*H*)-one 8-*O*-β-D-glucopyranoside.

Scabrol A (**7**) was isolated as a colorless oil and had the molecular formula $C_{10}H_{16}O_3$ by HRESIMS ($[M + Na]^+$ m/z 207.0995, calcd 207.0997). The NMR data (Tables 1 and 3) were similar to those of **2** except for the absence of the glucosyl moiety signals. The C-8 and C-9 resonances in **7** were shifted upfield by 7.7 and 2.3 ppm compared to those of **2**, while the δ values of C-7 and C-10 in **7** were shifted downfield by 4.5 and 2.8 ppm, due to the absence of a β-D-glucose unit at C-8. The δ values of the remaining carbons in **7** were similar to the corresponding positions of **2** (Table 3). Thus, compound **7** was the aglycone of **2**. On the basis of these results, the structure of scabrol A (**7**) was concluded to be (4*R*,5*S*,8*R*,9*R*)-8-hydroxy-4,8-dimethylhexahydrocyclopenta[*c*]pyran-3(1*H*)-one.

Patriridoside F (**3**) was obtained as an amorphous powder and had a molecular formula of $C_{16}H_{28}O_9$, established by HRESIMS ($[M + Na]^+$ m/z 387.1661, calcd 387.1666). The IR spectrum showed a broad absorption band for hydroxy groups (3406 cm^{-1}). The ^1H NMR spectrum (Table 1) exhibited signals for two methyl groups at δ_H 1.33 (s, H-10) and 0.99 (d, J = 6.8 Hz, H-11), two methylene groups at δ_H 4.05 (dd, J = 12.5, 6.0 Hz, H-1a), 3.73 (dd, J = 12.5, 8.2 Hz, H-1b), 2.09 (m, H-6a), and 1.47 (ddd, J = 14.2, 11.0, 6.8 Hz, H-6b), and five methine groups at δ_H 4.56 (d, J = 8.5 Hz, H-3), 1.68 (m, H-4), 2.41 (m, H-5), 4.39 (dd, J = 9.9, 6.6 Hz, H-7), and 2.16 (m, H-9). The ^{13}C NMR and DEPT spectra (Table 3) exhibited 16 carbon signals, consisting of two methyl carbons at δ_C 18.4 (C-10) and 14.8 (C-11), two methylene carbons at δ_C 63.1 (C-1) and 29.7 (C-6), five methine carbons at δ_C 98.0 (C-3), 37.9 (C-4), 38.3 (C-5), 78.2 (C-7), and 49.8 (C-9), and a quaternary

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carbon at δ_C 87.8 (C-8), as well as the six carbon signals of a glucosyl moiety. The $^1H-^1H$ COSY spectrum showed the connectivities of the proton coupling sequences for the C(1)−C(9)−C(5)−C(6)−C(7), C(5)−C(4)−C(11), and C(4)−C(3) fragments. The correlations of H-7 and H-9 with C-8 in the HMBC spectrum led to the establishment of a cyclopenta[*c*] pyran-type iridoid structure for **3**. The HMBC correlations of H-10 and GlcH-1 with C-8 suggested that the 10-methyl and the glucosyl moieties were located at C-8. The coupling constant of the anomeric proton (δ 4.58, 1H, d, J = 7.5 Hz) suggested a β-configured glucose unit. The presence of the β-D-glucopyranosyl moiety was confirmed by acid hydrolysis of **3** with 2 M H_2SO_4.

The relative configuration of **3** was determined by the 2D NOESY spectrum and on the basis of comparison of NMR data of **3** with reported data.[13] The chemical shift of C-9 at a relatively high field (δ 49.8) indicated that the glucosyl moiety at C-8 was α-oriented and the methyl group at C-8 was β-oriented.[5,15] The NOESY correlations of H-10 with H-9 and H-5, H-9 with H-5 and H-4, H-3 with H-11 and H-1b, and H-4 with H-1a, but the absence of correlations of H-9 with H-3 and H-7, H-5 with H-3 and H-7, and H-10 with H-7, indicated that H-4, H-5, H-9, and Me-10 were β-oriented and H-3, H-7, Me-11, and the glucosyl moiety were α-oriented. Therefore, the structure of patriridoside F (**3**) was defined as (3*S*,4*S*,5*S*,7*S*,-8*S*,9*R*)-4,8-dimethyloctahydrocyclopenta[*c*]pyran-3,7,8-triol 8-*O*-β-D-glucopyranoside.

Patriridoside G (**4**) was obtained as an amorphous powder. The molecular formula was found to be $C_{16}H_{24}O_7$ on the basis of HRESIMS ($[M + Cl]^-$ m/z 363.1217, calcd 363.1210). The IR spectrum indicated the presence of the hydroxy groups (3419 cm^{-1}). The ^1H, ^{13}C, and DEPT NMR spectra for **4** (Tables 2 and 3) revealed the presence of a methyl, a nonoxygenated methine, a nonoxygenated and two oxygenated methylenes, an oxygenated quaternary carbon, a terminal double bond, and a trisubstituted double bond, all of which suggested a molecule of iridoid origin. The remaining two indices of hydrogen deficiency required by the molecular formula indicated that **4** was a bicyclic iridoid. Analysis of the 2D COSY NMR experiment (Figure 1) permitted the establishment of two spin systems, corresponding to the C-3/C-4/C-11 and C-11/C-4/C-5/C-6/C-7/C-8/C-10 portions of **4**. Interpretation of the 2D HMQC and HMBC experiments allowed all of the protons and carbons to be assigned. HMBC correlations (Figure 1) from H-10 to C-9, from H-1 to C-9 and C-5, from H-11 to C-4, C-3, and C-5, and from H-3 to C-9 established connection of the two spin systems and allowed the full structure of compound **4** to be assigned. The HMBC correlations of the anomeric proton with C-1 suggested that the glucosyl moiety was located at C-1. The coupling constant of the anomeric proton (δ 4.38, 1H, d, J = 7.8 Hz) suggested a β-configured glucose unit. The presence of the β-D-glucopyranosyl moiety was confirmed by acid hydrolysis of **4** with 2 M H_2SO_4. The NOESY correlations (Figure 1) between H-5β and H_2-1 required a β-orientation for H_2-1. From the above data and on biogenetic grounds, the structure of patriridoside G (**4**) was established as [(5*R*,9*S*)-8-methyl-4-methylene-4,5,6,9-tetrahydro-3*H*-cyclopenta[*b*]furan-9-yl]methanol 1-*O*-β-D-glucopyranoside.

Patriridoside H (**5**) was obtained as an amorphous powder. The molecular formula of $C_{21}H_{32}O_{11}$ was determined by HRESIMS ($[M + Na]^+$ m/z 483.1829, calcd 483.1835). The ^{13}C NMR spectrum (Table 3) showed 16 signals and was similar to the spectrum of **4**, except for a downfield shift (5.6 ppm) for the Glc-6 resonance and an upfield shift (2.3 ppm) for the Glc-5 resonance. The remaining five signals corresponded to a β-D-apiofuranosyl moiety,[16−19] which had to be attached to C-6′ of the β-D-glucopyranosyl moiety. All the carbons of the apiosyl moiety were assigned through direct $^1H-^{13}C$ correlations in the HMQC spectrum. The anomeric carbon signal at δ 109.9 suggested a β-configured apiose unit. The existence of HMBC between the apiose anomeric proton and C-6 of glucose confirmed that the apiosyl moiety was located at C-6 of glucose. The structure of **5**, therefore, was concluded to be [(5*R*,9*S*)-8-methyl-4-methylene-4,5,6,9-tetrahydro-3*H*-cyclopenta[*b*]furan-9-yl]methanol 1-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside.

Patriridoside I (**6**) was obtained as an amorphous powder and had a molecular formula of $C_{21}H_{34}O_{11}$ on the basis of HRESIMS ($[M + Na]^+$ m/z 485.1991, calcd 485.1993). Compound **6** exhibited NMR spectroscopic features similar to those of **5**. The main difference was the presence of a terminal methyl doublet and a methine multiplet and lack of a terminal double bond in the ^1H NMR spectra of **6**. The NOESY correlations of H_2-1 with H-5β and H-4, but the absence of correlations of H_2-1 with H-11, required a β-orientation for H-4 and H_2-1 and an α-orientation for Me-4. From the above data and on biogenetic grounds, the structure of patriridoside I (**6**) was deduced as [(4*S*,5*R*,9*S*)-4,8-dimethyl-4,5,6,9-tetrahydro-3*H*-cyclopenta[*b*]furan-9-yl]methanol 1-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside.

The cytotoxicity on human cervical carcinoma (HeLa) and gastric carcinoma (MNK-45) cell lines in vitro of compounds **1**−**19** was examined using the microculture tetrazolium (MTT) assay.[20] 5-Fluorouracil was used as a positive control, and the IC_{50} values of these compounds are shown in Table 4. The values represent averages of three independent experiments, each with duplicate samples. Among these isolates, only compound **10** showed cytotoxicity against the HeLa cell line, with an IC_{50} value of 24.5 μM. Compounds **1**, **4**−**6**, **8**, and **18** showed cytotoxic activities against the MNK-45 cell line with respective IC_{50} values of 15.6, 8.7, 9.4, 30.9, 23.8, and 11.2 μM, whereas the other compounds were inactive. As can be seen from the cytotoxicity results in comparison with the inactive compounds (**2**, **3**, **7**, **9**−**17**, and **19**), glycosylation at the C-1 position plays an important role in cytotoxicity against MNK-45 cells. However, introducing an ether bridge between C-7 and C-8 makes secoiridoid glycosides **14**−**17** inactive. Further exploration of related analogues might lead to finding more potent analogues.

Table 4. Cytotoxicity of Compounds 1, 5, 6, 8, 10, and 18 against Tumor Cell Lines[a]

	HeLa	MNK-45
1	>50	15.6
4	>50	8.7
5	>50	9.4
6	>50	30.9
8	>50	23.8
10	24.5	>50
18	>50	11.2
5-fluorouracil	9.5	12.0

[a]Results are expressed as IC_{50} values in μM. Compounds **2**, **3**, **7**, **9**, **11**−**17**, and **19** were inactive for the two cell lines (IC_{50} >50 μM).

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EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 polarimeter. A Shimadzu UV-2401A spectrophotometer was used to obtain the UV spectra. A Bruker Tensor 27 FT-IR spectrometer was used for IR spectra with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. MS and HRMS were performed on an API-QSTAR-Pulsar-1 spectrometer. Column chromatography was carried out on Sephadex LH-20 gel (25−100 μm, Pharmacia Fine Chemical Co. Ltd.), CG161 M (70 μm, Beijing H&E Co. Ltd.), RP-8 MB (100−40/75 μm, Fuji Silysia Chemical Co. Ltd.), MCI gel CHP-20P (75−150 μm, Mitsubishi Chemical Co.), Chromatorex ODS (30−50 μm, Fuji Silysia Chemical Co. Ltd.), and silica gel (200−300 mesh, Qingdao Haiyang Chemical Co. Ltd., P. R. China). Thin-layer chromatography was carried out on silica gel G precoated plates (Qingdao Haiyang Chemical Co. Ltd.), and spots were detected by spraying with 5% H_2SO_4 in EtOH followed by heating.

Plant Material. The dry roots of *Patrinia scabra* Bunge were purchased from Bozhou herbal market in March 2010 and identified by K.-J.W., from the School of Life Sciences, Anhui University, where a voucher specimen (No. 20100303) was deposited.

Extraction and Isolation. The air-dried roots of *P. scabra* (18.0 kg) were powdered and extracted with 90% aqueous EtOH (3 × 60 L) at room temperature. The extracts were combined and concentrated under vacuum to give a residue (1.35 kg). The residue was suspended in H_2O and extracted with petroleum ether, EtOAc, and *n*-BuOH, successively, to give petroleum ether (487 g), EtOAc (243 g), *n*-BuOH (252 g), and aqueous (478 g) fractions. The *n*-BuOH extract was passed through a D101 resin column eluting sequentially with H_2O−MeOH (from 1:0 to 0:1) to generate fractions C_1−C_3. Fraction C_1 was subjected to column chromatography over silica gel (200−300 mesh), eluted with $CHCl_3$−MeOH (from 20:1 to 1:1), to give fractions $C_{1\text{-}1}$−$C_{1\text{-}5}$. Compound **9** (110 mg) was crystallized from $C_{1\text{-}1}$ directly in $CHCl_3$. The surplus $C_{1\text{-}1}$ was chromatographed on CG161 M (30−50% gradient MeOH−H_2O) and then RP-18 (35% MeOH−H_2O) to afford compound **10** (43 mg). Fraction $C_{1\text{-}2}$ was applied to MCI (40% MeOH−H_2O), ODS (10−60% gradient MeOH−H_2O), and then silica gel columns eluted with $CHCl_3$−MeOH (15:1) to yield compounds **1** (31 mg), **4** (20 mg), **14** (28 mg), and **15** (23 mg). Fraction $C_{1\text{-}3}$ was subjected to chromatography over MCI (10−60% gradient MeOH−H_2O), RP-18 (10−50% gradient MeOH−H_2O), Sephadex LH-20 (EtOH), and then a silica gel column eluted with $CHCl_3$−MeOH (15:1) to afford compounds **2** (24 mg) and **19** (38 mg). Fraction $C_{1\text{-}4}$ was applied to MCI, eluting sequentially with H_2O−MeOH (from 1:0 to 0:1), and then repeated chromatography over ODS (10−50% gradient MeOH−H_2O), Sephadex LH-20 (EtOH), and RP-8 eluted with H_2O−MeOH (from 3:7 to 5:5) to yield compounds **16** (24 mg), **17** (28 mg), and **18** (40 mg). Fraction $C_{1\text{-}5}$ was subjected to repeated chromatography over MCI (10−60% gradient MeOH−H_2O) and ODS (45% MeOH−H_2O) to afford compound **8** (39 mg). Compounds **5** (55 mg) and **6** (50 mg) were obtained from fraction C_2 after repeated MCI (10−50% gradient MeOH−-H_2O) and RP-8 (35% MeOH−H_2O). C_3 was subjected to column chromatography over silica gel (200−300 mesh), eluted with $CHCl_3$−MeOH (from 10:1 to 1:1), and then purified over ODS (45% MeOH−H_2O) and MCI (25% MeOH−H_2O) to afford compound **3** (19 mg). The EtOAc extract was subjected to column chromatography over a silica gel column (200−300 mesh), eluted with $CHCl_3$−EtOAc (from 30:1 to 0:1), to generate fractions B_1 and B_2. Fraction B_1 was purified on MCI (60% MeOH−H_2O) and silica gel with a petroleum ether−EtOAc gradient system (from 30:1 to 0:1) to give compounds **7** (10 mg), **12** (17 mg), and **13** (22 mg). B_2 was subjected to chromatography over MCI (60% MeOH−H_2O) and repeated silica gel column chromatography (petroleum ether−EtOAc, 2:1) to yield compound **11** (43 mg).

*Patriridoside D (**1**):* white powder, $[\alpha]_D^{18}$ +6.0 (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.74), 261 (3.98) (nm); IR (KBr) ν_{max}3463, 2960, 2926, 2913, 2870, 1651, 1612, 1458, 1415, 1394, 1238, 1221, 1172, 1099, 1082, 1046, 1016, 996, 964, 751, 630 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; ESIMS *m/z* 379 (100) $[M + Cl]^-$; HRESIMS *m/z* 379.1154 $[M + Cl]^-$ (calcd for $C_{16}H_{24}O_8Cl$, 379.1159).

*Patriridoside E (**2**):* white powder, $[\alpha]_D^{15}$ −25.1 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.11) (nm); IR (KBr) ν_{max}3422, 3394 (OH), 2956, 2926, 2876, 1729, 1636, 1615, 1599, 1457, 1383, 1355, 1332, 1255, 1237, 1188, 1163, 1078, 720, 635 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; ESIMS *m/z* 369 (100) $[M + Na]^+$; HRESIMS *m/z* 369.1500 $[M + Na]^+$ (calcd 369.1519 for $C_{16}H_{26}O_8Na$).

*Patriridoside F (**3**):* white powder, $[\alpha]_D^{14}$ +72.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.39) (nm); IR (KBr) ν_{max}3406, 2923, 2884, 1645, 1458, 1381, 1160, 1076, 992, 637, 603 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; ESIMS *m/z* 387 (100) $[M + Na]^+$; HRESIMS *m/z* 387.1661 $[M + Na]^+$ (calcd 387.1666 for $C_{16}H_{28}O_9Na$).

*Patriridoside G (**4**):* white power, $[\alpha]_D^{25}$ +26.0 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.62) (nm); IR (KBr) ν_{max}3419, 2921, 2875, 2855, 1666, 1635, 1448, 1379, 1328, 1168, 1078, 887, 597 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 363 (100) $[M + Cl]^-$; HRESIMS *m/z* 363.1217 $[M + Cl]^-$ (calcd 363.1210 for $C_{16}H_{24}O_7Cl$).

*Patriridoside H (**5**):* white powder, $[\alpha]_D^{14}$ −3.9 (*c* 0.43, H_2O); UV (MeOH) λ_{max} (log ε) 191 (4.00) (nm); IR (KBr) ν_{max}3419, 2925, 2880, 1665, 1639, 1448, 1366, 1168, 1057, 936, 571 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 483 (100) $[M + Na]^+$; HRESIMS *m/z* 483.1829 $[M + Na]^+$ (calcd 483.1835 for $C_{21}H_{32}O_{11}Na$).

*Patriridoside I (**6**):* white powder, $[\alpha]_D^{15}$ −40.7 (*c* 0.79, H_2O); UV (MeOH) λ_{max} (log ε) 191 (3.80) (nm); IR (KBr) ν_{max} 3420, 2959, 2928, 2878, 1635, 1447, 1379, 1168, 1057, 1021, 824, 753, 569 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; ESIMS *m/z* 485 (100) $[M + Na]^+$; HRESIMS *m/z* 485.1991 $[M + Na]^+$ (calcd 485.1993 for $C_{21}H_{34}O_{11}Na$).

*Scabrol A (**7**):* colorless oil, $[\alpha]_D^{14}$ −39.6 (*c* 0.85, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (2.63) (nm); IR (KBr) ν_{max} 3433, 2962, 2927, 2877, 1727, 1630, 1458, 1381, 1353, 1249, 1176, 1115, 1038, 933 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; ESIMS *m/z* 207 (25) $[M + Na]^+$, 391 (100) $[2M + Na]^+$; HRESIMS *m/z* 207.0995 $[M + Na]^+$ (calcd 207.0997 for $C_{10}H_{16}O_3Na$).

Acid Hydrolysis of 1−6. A 2.0 mg sample was dissolved in a mixture of MeOH (2.0 mL) and 2 mol/L H_2SO_4 (2.0 mL) and refluxed on a boiling water bath for 2 h. The hydrolysate was allowed to cool, diluted 2-fold with distilled H_2O, and partitioned between EtOAc and water. The H_2O-souble fraction was evaporated to dryness. The dried sugar residue was diluted in 1 mL of dry pyridine, treated with 0.5 mL of trimethylchlorosilane, and stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was extracted with ether (1 mL). The ether layer was analyzed by GC.[4] The monosaccharide of compounds **1**−**4** was determined by GC analysis of the derivatives to be D-glucose, of which the retention time was 7.26 min. The monosaccharides of compounds **5** and **6** were determined to be D-glucose and D-apiose, of which the retention times were 7.26 and 8.45 min, respectively.

Cytotoxicity Assay. HeLa and MNK-45 cell lines were cultured in DMEM medium, supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cell cultures were incubated at 95% relative humidity, 5% CO_2, and 37 °C. Cytotoxicity was measured by a microculture tetrazolium (MTT) assay with minor modification to the reference procedure.[20] Briefly, cells were seeded in 96-well microculture plates at 2 × 10^4 cells per well and allowed to adhere for 24 h before drug addition. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with 5-fluorouracil (Sigma) as positive control. After the incubation, 20 μL of MTT (5 mg/mL) was added to each well, and the incubation continued for 4 h at 37 °C. After 4 h, 150 μL of DMSO was added to each well to dissolve the formazan crystals of the viable cells. The plates were read at a wavelength of 570 nm using

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a Wallac 1420 ARVOsx microplate reader (Perkin-Elmer Life and Analytical Sciences, Inc., Boston, MA, USA). IC_{50} values were calculated using SPSS 19.0 statistical analysis software.

■ ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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