Neuroprotective Xanthone Glycosides from *Swertia punicea*

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Two new dimeric xanthone *O*-glycosides, puniceasides A (**1**) and B (**2**), a new trimeric *O*-glycoside, puniceaside C (**3**), and two new trimeric *C*-glycosides, puniceasides D (**4**) and E (**5**), together with 12 known xanthones were isolated from the entire plant of *Swertia punicea*. The structures of **1**−**5** were determined by HRESIMS and NMR spectroscopic methods. Compounds **2**, **6**, and **7** exhibited potent neuroprotective activity against H_2O_2-induced PC12 cell damage.

The genus *Swertia* (Gentianaceae) includes about 170 species widely distributed throughout the world. Many species of this genus have been used as medicinal herbs for the treatment of choleretic, hepatic, and inflammatory diseases in different countries and regions.[1,2] In mainland China, *Swertia punicea* is called "Ganyancao" and is used as a traditional folk medicine to treat fever, intoxification, jaundice, and cholecystitis.[3] Previous phytochemical studies have led to reports of xanthone derivatives, iridoid glycosides, and triterpenoids from *S. punicea*.[4,5] In a continued effort to search for new bioactive compounds from this species, an investigation of the xanthones from *S. punicea* was undertaken, and this has led to the isolation of 17 xanthones, including five new compounds, puniceasides A−E (**1**−**5**), and 12 known xanthones. The known compounds were identified as swertiabisxanthone-I 8′-*O*-*β*-D-glucopyranoside (**6**),[6] swertiabisxanthone I,[7] 3-*O*-demethylswertipunicoside (**7**),[4] swertipunicoside,[5] norswertianolin,[8] swertianolin,[8] norbellidifodin,[8] bellidifodin,[8] mangiferin,[9] campestroside,[10] gentisin,[11] and swerchirin.[9] In this paper, we report the isolation and structural elucidation of the new xanthone glycosides **1**−**5**, as well as the neuroprotective activity evaluation of xanthones **2**, **6**, and **7** against H_2O_2-induced PC12 cell damage.

Puniceaside A (**1**) was obtained as a pale yellow powder. The HRESIMS gave a $[M + Na]^+$ ion at *m/z* 703.0924 (calcd for $C_{32}H_{24}O_{17}Na$ 703.0911), establishing the molecular formula of **1** as $C_{32}H_{24}O_{17}$, with 21 degrees of unsaturation. The UV spectrum showed absorption bands at 258, 283, 318, and 337 nm. The ^1H NMR spectrum (Table 1) displayed six aromatic proton signals at δ_H 6.19 (brs, H-2′), 6.41 (brs, H-4′), 7.22 (s, H-6′), 6.32 (s, H-2), 7.11 (d, $J = 9.0$ Hz, H-6), and 7.07 (d, $J = 9.0$ Hz, H-7) and a group of signals for a *β*-glucopyranosyloxy unit with the anomeric proton resonating at δ_H 4.75 (d, $J = 7.8$ Hz, H-1″). The ^{13}C NMR spectrum (Table 1) showed 32 carbon resonances, consisting of two carbonyls [(δ_C 183.5, 180.9 (C-9, C-9′)], 24 aromatic carbons, and signals for a *β*-glucopyranosyloxy unit ($\delta_{C\text{-}1''\text{-}C\text{-}6''}$ 103.6, 73.6, 76.0, 69.8, 77.5, and 60.9). The HRESIMS and UV and NMR specotroscopic data of **1** were closely comparable to those of the known dimeric xanthone glycoside swertiabisxanthone-I 8′-*O*-*β*-D-glucopyranoside (**6**),[6] suggesting that compound **1** might be an isomer of **6**. On comparing the ^{13}C NMR spectrum of **1** with that of **6**, the major differences were the chemical shifts of C-2 and C-4. The former signal (C-2) in **1** had an upfield shift from δ_C 113.2 (in **6**) to δ_C 98.2, similar to that of C-2′ (δ_C 98.8), and the latter signal (C-4) in **1** shifted from δ_C 95.0 (in **6**) to δ_C 102.3, implying that the two xanthone units of **1** are connected by a C-4−C-7′ linkage. This was further confirmed by the key HMBC cross-peak of H-6′ with C-4. The D-configuration of the glucopyranosyl unit was confirmed via acid hydrolysis (see Experimental Section).[12] Hence, the structure of **1** was determined as 8-(*β*-D-glucopyranosyloxy)-1,3,5,1′,3′,5′,8′-heptahydroxy-[4,7′-bi-9*H*-xanthene]-9,9′-dione.

Puniceaside B (**2**) was obtained as a yellow powder. The molecular formula of **2** was assigned as $C_{32}H_{28}O_{17}$ on the basis of HRESIMS ($[M + Na]^+$ at *m/z* 707.1219, calcd 707.1224), representing 19 degrees of unsaturation, two degrees less and four mass units more than compound **6**. The UV absorption bands at λ_{max} 255, 281, and 332 nm were comparable to those of **6**. Similarities in the ^1H and ^{13}C NMR spectroscopic data (Table 1) indicated that **2** is an analogue of **6**. The significant differences between **2** and **6** were the resonances of two oxygenated methine [δ_H 4.55, δ_C 65.5; δ_H 4.81, δ_C 68.6] and two methylene [δ_H 1.68, 2.16, δ_C 26.5; 1.98 (2H), δ_C 27.1] groups of **2** instead of two olefinic protons (H-6 and H-7) and four olefinic carbon (C-5, C-6, C-7, and C-8) resonances in **6**, suggesting that the C_5/C_6 and C_7/C_8 bonds of **2** are saturated. This conclusion was supported by observed $^1H-^1H$ COSY correlations (H-5/H-6/H-7/H-8) and HMBC cross-peaks (H_2-6/C-4b, C-5, and C-8 and H_2-7/C-8a, C-8, and C-5) (Figure 1). Furthermore, the chemical shifts of H-5, H_2-6, H_2-7, and H-8 in **2** were similar to those of tetrahydroswertianolin.[13] The *cis* configuration of H-5 and H-8 was confirmed by NOEs observed between H-5 and H-8 in the NOESY experiment. The D-glucopyranosyl unit was also determined via acid hydrolysis.[12] Therefore, the structure of **2** was characterized as 8-(*β*-D-glucopyranosyloxy)-5,6,7,8-tetrahydro-1,3,5,1′,3′,5′,8′-heptahydroxy-[2,7′-bi-9*H*-xanthene]-9,9′-dione. It is interesting to note that the H-6′ resonances appeared as two singlets at δ_H 7.10 and 7.12 (Table 1), as in swertifrancheside, indicating that rotamers exist for **2**.[14] The two singlets of H-6′ were converted to one singlet when the temperature of the ^1H NMR experiment was increased to 120 °C (see Supporting Information).

Puniceaside C (**3**) was obtained as a pale yellow powder. Its molecular formula was determined as $C_{45}H_{34}O_{23}$ by HRESIMS at *m/z* 943.1587 ($[M + H]^+$ calcd 943.1569) and 965.1403 ($[M + Na]^+$ calcd 965.1389), indicating 29 degrees of unsaturation. The ^1H NMR data (Table 2) displayed the presence of six aromatic protons including two *meta*-coupled protons at δ_H 6.41 and 6.20 (each brs, H-4″, H-2″) and four singlets at δ_H 6.54 × 2 (H-4, H-4′), 7.12 (H-6′), and 7.17 (H-6″). The ^{13}C NMR data (Table 2) of **3** exihibited 45 carbons attributable to three carbonyls (δ_C 183.6 × 2, 180.9), two oxygenated methines (δ_C 65.5, 68.6), two methylenes

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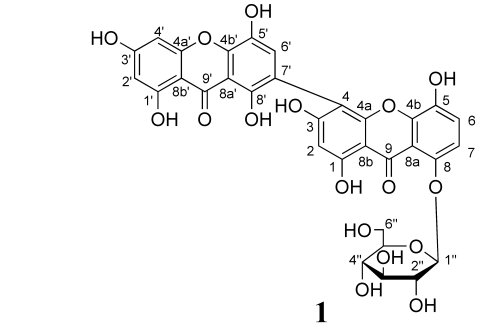
Chart 1



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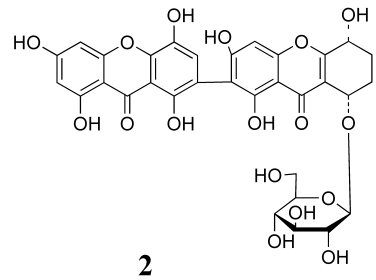

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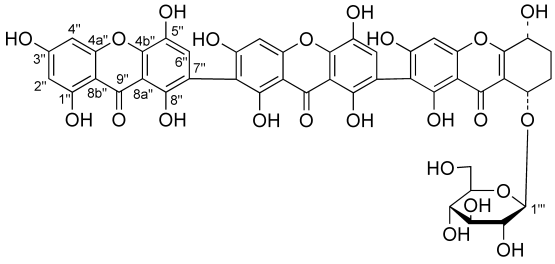

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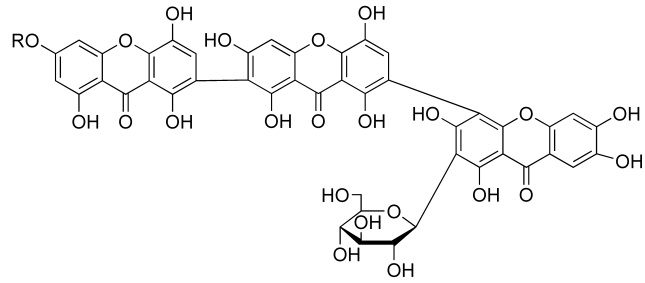
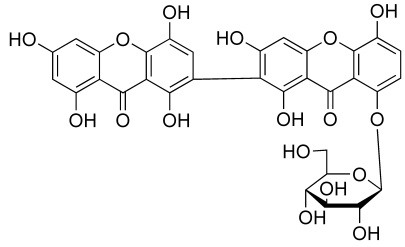
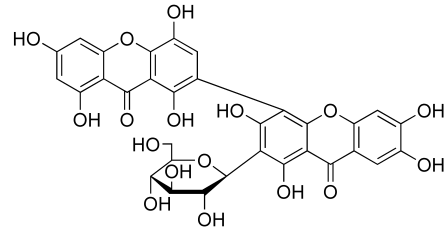


4 R = H
5 R = CH_3



6



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(δ_C 26.5, 27.1), 32 unsaturated carbons, and a β-glucopyranosyloxy unit. The ^{1}H and ^{13}C NMR spectroscopic data of **3** (Table 2) were found to be close to those of **2** except for the additional resonances of a 1,3,5,8-tetrahydroxyxanthone unit [δ_H 6.41, 6.20 (each brs, H-4″, H-2″), 7.17 (s H-6″); δ_C 183.6], which was confirmed by the HMBC experiment (Figure 2). Hence, **3** could be proposed as a trimeric xanthone glycoside, since, to the best of our knowledge, no xanthone trimers have been reported thus far. The C-2−C-7′ and C-2′−C-7″ linkages of **3** were identified by the HMBC cross-peaks of H-6′/C-2 and H-6″/C-2′. Furthermore, on acid hydrolysis of **3**, the glucopyranosyl unit was identified as D-glucose. Consequently, **3** was assigned as 8-(β-D-glucopyranosyloxy)-5,6,7,8-tetrahydro-1,3,5,1′,3′,5′,8′,1″,3″,5″,8″-undecahydroxy-[2,7′:2′,7″-ter-9*H*-xanthene]-9,9′,9″-trione.

Puniceasides D and E (**4** and **5**) were obtained as pale yellow powders. Their molecular formulas were established as $C_{45}H_{30}O_{23}$ and $C_{46}H_{32}O_{23}$ by HRESIMS at *m*/*z* 961.1054 ([M + Na]$^+$ calcd for $C_{45}H_{30}O_{23}Na$ 961.1076) and *m*/*z* 975.1241 ([M + Na]$^+$ calcd for $C_{46}H_{32}O_{23}Na$ 975.1232), respectively. The ^{1}H and ^{13}C NMR spectroscopic data of **4** and **5** showed similarities to those of **3** (Table 2). However, a significant difference was a *C*-β-glucopyranosyl unit [δ_H 4.82 (d, J = 9.6 Hz H-1‴), δ_C 74.1 (C-1‴) for **4**; δ_H 4.83 (d, J = 10.2 Hz H-1‴), δ_C 74.1 (C-1‴) for **5**][5] instead of a *O*-β-glucopyranosyl unit of **3**, indicating that **4** and **5** are two unprecedented trimeric xanthone *C*-glycosides. On comparing the ^{1}H and ^{13}C NMR data (Table 2) of **4** and **5** with those of a known dimeric xanthone *C*-glycoside, 3-*O*-demethylswertipunicoside (**7**),[5] the differences were the additional 1,3,5,8-tetrahydroxyxanthone [δ_H 6.45, 6.24 (each brs, H-4″, H-2″), 7.16 (s, H-6″); δ_C 184.0 (C-9″)] and 1,5,8-trihydroxy-3-methoxyxanthone [δ_H 6.68, 6.43 (each brs, H-4″, H-2″), 7.22 (s, H-6″), 3.93 (3H, s, MeO); δ_C 184.2 (C-9″), 56.3 (MeO)] units, which were confirmed by relevant HMBC correlations. Thus, the C-4−C-7′, and C-2′−C-7″ linkages of **4** and **5** were revealed by the same HMBC cross-peaks of H-6′/C-4 and H-6″/C-2′. Owing to insufficient amounts of sample, the absolute configurations of the *C*-β-glucopyranosyl units of **4** and **5** remain to be determined. Hence, **4** and **5** were assigned as 2-(β-glucopyranosyl)-1,3,6,7,1′,3′,5′,8′,1″,3″,5″,8″-dodecahydroxy-[4,7′:2′,7″-ter-9*H*-xanthene]-9,9′,9″-trione and 2-(β-glucopyranosyl)-1,3,6,7,1′,3′,5′,8′,1″,5″,8″-undecahydroxy-3″-methoxy-[4,7′:2′,7″-ter-9*H*-xanthene]-9,9′,9″-trione, respectively. In the ^{1}H NMR spectra of **4** and **5** (Table 2), H-6′ resonated as two singlets, which were converted to one singlet when the temperature of the ^{1}H NMR experiment was increased to 120 °C (see Supporting Information), as in the case of **2**, suggesting that rotamers exist in **4** and **5**.

The 12 known xanthones were identified by the interpretation of their spectroscopic data and comparison with literature values.[4−11] Compounds **6**, swertiabisxanthone I, and campestroside were isolated for the first time from the title plant.

Compounds **1**−**7**, swertipunicoside, norswertianolin, swertianolin, norbellidifodin, bellidifodin, and mangiferin were evaluated for their potential neuroprotective activities against H_2O_2-induced PC12 cell damage, using a MTT assay.[15] The dimeric xanthone glycosides **2**, **6**, and **7** displayed potent neuroprotective activity (Table 3). It is noteworthy that **6** and **7** potently stimulated the damaged PC12 cells to grow owing to

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1** and **2** in DMSO-d_6 (δ in ppm, J in Hz in parentheses)

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
1		162.0 C		159.1 C
2	6.32 s	98.2 CH		107.1 C
3		164.4 C		162.3 C
4		102.3 C	6.54 s	93.3 CH
4a		153.8 C		156.2 C
4b		145.1 C		167.3 C
5		141.3 C	4.55 m	65.5 CH
6	7.11 d (9)	120.5 CH	1.98 m	26.5 CH_2
			1.98 m	
7	7.07 d (9)	112.5 CH	1.68 m	27.1 CH_2
			2.18 m	
8		149.3 C	4.89 brt (2.1)	68.6 CH
8a		107.1 C		116.4 C
8b		102.8 C		103.4 C
9		183.5 C		181.0 C
1′		162.2 C		162.3 C
2′	6.19 brs	98.8 CH	6.23 brs	98.5 CH
3′		167.4 C		166.5 C
4′	6.41 brs	94.6 CH	6.44 brs	94.4 CH
4a′		157.5 C		157.6 C
4b′		142.8 C		142.9 C
5′		136.3 C		136.5 C
6′	7.22 s	127.0 CH	7.10 s, 7.12 s	126.7 CH
7′		113.5 C		114.6 C
8′		150.7 C		150.1 C
8a′		112.5 C		108.0 C
8b′		100.8 C		101.2 C
9′		180.9 C		183.9 C
glc-1″	4.75 d (7.8)	103.6 CH	4.55 d (7.2)	104.0 CH
2″	3.36 m	73.6 CH	2.92 m	74.0 CH
3″	3.30 m	76.0 CH	3.15 m	76.6 CH
4″	3.18 m	69.8 CH	3.05 m	70.1 CH
5″	3.28 m	77.5 CH	3.16 m	77.0 CH
6″	3.71 brd (11.4)	60.9 CH_2	3.69 brd (12.0)	61.1 CH_2
	3.48 m		3.44 m	

their high cell viabilities, which were up to 123.0% ± 5.6 and 157.8% ± 6.0, at a concentration of 25 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured on an AA10R digital polarimeter in MeOH at 25 °C. UV spectra were recorded on a TU-1901-UV-UIS spectrophotometer. IR spectra were recorded in KBr with an Avatar 360 FT-IR spectrophotometer. NMR spectra were obtained on an INOVA SX-600 spectrometer with solvent (DMSO-d_6) peaks being used as reference. HRESIMS were obtained on a Bruker APEX IV FT mass spectrometer. Preparative HPLC was performed on a Spectra Series HPLC system (Thermo Separation Products) equipped with a P100 pump and a UV 100 detector with a Zorbax-SB-ODS column (20 mm ×150 mm, 5 μm; flow rate at 2.0 mL/min; wavelength detection at 254 nm). All solvents were analytical grade or higher (Beijing Chemical Co. Ltd.).

Plant Material. *Swertia punicea* was purchased from Bozhou herbal market, Anhui Province, People's Republic of China, in August 2007, and identified by D.-A.G. A voucher specimen (SP-001) was deposited in the herbarium of the Department of Pharmacognosy, School of Pharmaceutical Sciences, Peking University, Beijing.

Extraction and Isolation. The air-dried plant material (8.5 kg) was powdered and extracted with MeOH. After evaporation of the solvent, the pooled residues were suspended in water and extracted sequentially

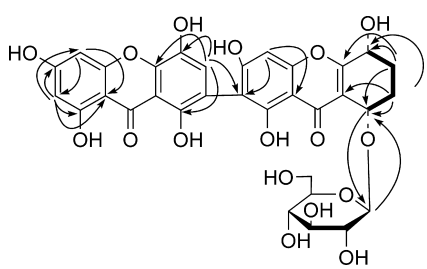

Figure 1. Key HMBC (→) and ^{1}H−^{1}H COSY (—) correlations of **2**.

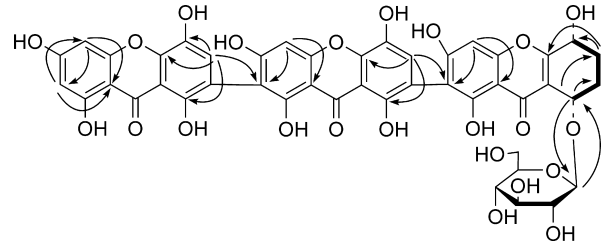

Figure 2. Key HMBC (→) and ^{1}H−^{1}H COSY (—) correlations of **3**.

with petroleum ether (3 × 2.5 L), EtOAc (3 × 2.5 L), and *n*-BuOH (3 × 2.5 L). The petroleum ether fraction (17 g) was subjected to silica gel column chromatography (200−300 mesh), eluted in a step gradient manner with petroleum ether−acetone (100:0 → 0:100), to give three fractions (F1−F3). F2 was purified by preparative HPLC, eluted with 74% MeOH, to afford swertiabisxanthone I (6.5 mg) and gentisin (9.5 mg). F3 was purified by recrystallization with acetone to obtain swerchirin (1.5 mg).

Part of the EtOAc fraction (300 g) was subjected to silica gel column chromatography (200−300 mesh), eluted with $CHCl_3$−MeOH (100:0 → 0:100), to give four fractions (F1−F4). F1 (1.22 g) was subjected to Sephadex LH-20 column chromatography, eluted with MeOH, to yield norbellidifodin (222 mg). F4 (7.47 g) was also chromatographed on Sephadex LH-20 to give three subfractions (F4a−F4c). F4a was purified by preparative HPLC, eluted with 48% MeOH, to give norswertianolin (40.0 mg) and swertianolin (6.0 mg). F4b was separated by preparative HPLC, eluted with 63% MeOH, to yield **6** (15.0 mg) and swertipunicoside (12.8 mg). F4c (127.9 mg) was purified by preparative HPLC, eluted with 81% MeOH, to afford bellidifodin (6.3 mg).

Part of the *n*-BuOH fraction (26 g) was subjected initially to silica gel column chromatography (200−300 mesh), eluted with $CHCl_3$−MeOH (100:0 → 0:100), to give five fractions (F1−F5). F1 was purified by preparative HPLC, eluted with 40% MeOH, to afford campestroside (14.0 mg). F3 was purified by preparative HPLC, eluted with 35% MeOH, to obtain mangiferin (70.0 mg). F4 (2.6 g) was subjected to Sephadex LH-20 column chromatography, eluted with MeOH, and then purified by preparative HPLC (55% MeOH) to give **1** (4.5 mg) and **7** (600 mg).

The remainder of the EtOAc and *n*-BuOH extracts (400 g) were mixed and subjected to silica gel column chromatography (200−300 mesh), eluted with $CHCl_3$−MeOH (100:0 → 0:100), to give seven fractions (Fr.A−G). Fr.C (90 g) was purified by silica gel column chromatography (200−300 mesh), eluted with $CHCl_3$−MeOH (100:0 → 0:100), to yield four subfractions (Fr.C_1−C_4). Fr.C_3 (40 g) was separated on Sephadex LH-20 column chromatography (52 × 3 cm) with MeOH as eluent and then purified by preparative HPLC to yield **4** (9.3 mg), **5** (20 mg), and **3** (13 mg). Fr.C_2 (4 g) was subjected to Sephadex LH-20 column chromatography (52 × 3 cm), eluted with MeOH, to give 40 subfractions. Subfractions 20 and 21 (140 mg) were purified by preparative HPLC (55% MeOH) to yield **2** (18.0 mg). Subfractions 27−30 (100 mg) were purified by preparative HPLC (32% CH_3CN) to afford **1** (4.5 mg).

Puniceaside A (1): $[\alpha]^{25}_D$ −1.2 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 258 (3.88), 283 (3.85), 337 (3.75) nm; IR (KBr) ν_{max} 3378 (OH), 1625 (C=O), 1583 (C=O), 1494 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; positive-ion HRESIMS $[M + Na]^+$ *m*/*z* 703.0924 (calcd for $C_{32}H_{24}O_{17}Na$, 703.0911).

Puniceaside B (2): $[\alpha]^{25}_D$ +18.1 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 255 (4.55), 281 (4.44), 332 (4.26) nm; IR (KBr) ν_{max} 3401 (OH), 1625 (C=O), 1590 (C=O), 1465 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; positive-ion HRESIMS $[M + Na]^+$ *m*/*z* 707.1219 (calcd for $C_{32}H_{28}O_{17}Na$, 707.1224).

Puniceaside C (3): $[\alpha]^{25}_D$ +8.3 (*c* 1.2, MeOH); UV (MeOH) λ_{max} (log ε) 257 (4.19), 285 (4.13), 337 (3.98) nm; IR (KBr) ν_{max} 3398 (OH), 1652 (C=O), 1622.0 (C=O), 1590 (C=O), 1466 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; positive-ion HRESIMS $[M + H]^+$ *m*/*z* 943.1587 (calcd for $C_{45}H_{35}O_{23}$, 943.1564), $[M + Na]^+$ *m*/*z* 965.1403 (calcd for $C_{45}H_{34}O_{23}Na$, 965.1389).

Puniceaside D (4): pale yellow powder; $[\alpha]^{25}_D$ +23.4 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 258 (4.87), 283 (4.78), 339 (4.63) nm; IR

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of **3**−**5** in DMSO-d_6 (δ in ppm, J in Hz in parentheses)

	3		**4**		**5**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		159.1 C		160.7 C		160.0 C
2		107.8 C		106.8 C		106.8 C
3		162.7 C		159.8 C		159.7 C
4	6.54 s	93.3 CH		102.9 C		102.9 C
4a		156.2 C		153.7 C		153.7 C
4b		167.3 C		150.9 C		150.3 C
5	4.56 m	65.5 CH	6.58 s	102.5 CH	6.55 s	102.3 CH
6	1.98 m	26.5 CH_2		154.2 C		154.9 C
	1.98 m					
7	1.70 m	27.1 CH_2		143.9 C		143.0 C
	2.18 m					
8	4.90 brt (2.1)	68.6 CH	7.38 s	107.9 CH	7.36 s	107.5 CH
8a		116.4 C		111.5 C		111.1 C
8b		103.3 C		101.2 C		101.3 C
9		180.9 C		179.5 C		179.3 C
1′		159.6 C		160.2 C		160.3 C
2′		107.8 C		107.3 C		107.3 C
3′		162.7 C		164.6 C		166.0 C
4′	6.54 s	93.3 CH	6.66 s	94.0 CH	6.60 s	94.3 CH
4a′		157.5 C		157.6 C		157.5C
4b′		142.9 C		143.2 C		144.1 C
5′		136.4 C		136.7 C		136.6 C
6′	7.12 s	126.4 CH	7.22 s, 7.21 s	126.9 CH	7.22 s, 7.20 s	126.7 CH
7′		114.2 C		112.9 C		114.2 C
8′		150.2 C		150.2 C		151.1 C
8a′		107.1 C		107.3 C		107.3 C
8b′		103.3 C		101.0 C		100.5 C
9′		183.6 C		183.9 C		183.5 C
1″		162.3 C		162.3 C		162.0 C
2″	6.20 brs	98.6 CH	6.24 brs	98.6 CH	6.43 brs	97.6 CH
3″		167.3 C		166.5 C		167.0 C
4″	6.41 brs	94.4 CH	6.45 brs	94.4 CH	6.68 brs	93.0 CH
4a″		156.6 C		156.6 C		157.5 C
4b″		142.9 C		143.0 C		143.1 C
5″		136.4 C		136.6 C		136.6 C
6″	7.17 s	126.7 CH	7.16 s	126.6 CH	7.22 s	127.0 C
7″		113.7 C		113.5 C		113.0 C
8″		150.3 C		150.4 C		150.5 C
8a″		107.3 C		102.8 C		103.0 C
8b″		101.0 C		101.4 C		102.3 C
9″		183.6 C		184.0 C		184.2 C
glc-1‴	4.56 d (7.2)	104.0 CH	4.82 d (9.6)	74.1 CH	4.83 d (10.2)	74.1 CH
2‴	2.92 m	74.0 CH	3.36 m	71.8 CH	3.36 m	71.8 CH
3‴	3.15 m	76.6 CH	3.31 m	77.9 CH	3.30 m	78.0 CH
4‴	3.05 m	70.1 CH	3.32 m	69.4 CH	3.32 m	69.4 CH
5‴	3.17 m	77.0 CH	3.28 m	81.3 CH	3.28 m	81.2 CH
6‴	3.70 brd (10.8)	61.1 CH_2	3.63 brd (9.0)	60.1 CH_2	3.65 brd (10.8)	60.2 CH_2
	3.47 m		3.56 brd (9.6)		3.56 brd (10.8)	
OCH_3					3.93 s	56.3 CH_3

(KBr) ν_{max} 3409 (OH), 1622 (C═O), 1615 (C═O), 1475 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; positive-ion HRESIMS $[M + Na]^+$ *m/z* 961.1054 (calcd for $C_{45}H_{30}O_{23}Na$, 961.1076).

Puniceaside E (5): $[\alpha]^{25}_D$ −104 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 258 (4.72), 283 (4.64), 337 (4.48) nm; IR (KBr) ν_{max} 3390 (OH), 1621 (C═O), 1588 (C═O), 1473 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; positive-ion HRESIMS $[M + Na]^+$ *m/z* 975.1241 (calcd for $C_{46}H_{32}O_{23}Na$, 975.1232).

Acid Hydrolysis of Compounds 1−3.[12] Compounds **1** (5 mg), **2** (10 mg), and **3** (10 mg) were each refluxed with 2 N CF_3COOH in aqueous MeOH (10 mL) for 3 h. The reaction mixture was then evaporated to dryness with MeOH until neutral and then diluted with H_2O (5 mL). After being extracted with EtOAc (3 × 5 mL), the aqueous layer was concentrated and compared with reference D-glucose (National Institute for the Control of Pharmaceutical and Biological Products, People's Republic of China) by TLC (silica gel; $CHCl_3$−MeOH−H_2O, 6:4:1). The residue was dissolved in pyridine (1.5 mL). Then 500 μL of HMDS-TMCS (hexamethyldisilazane−trimethylchlorosilane, 3:1) was added, and the mixture was stirred at 60 °C for 30 min. The supernatant was subjected to GC analysis (conditions: Shimadzu GC-14C; SGE Analytical Science AC225 (30 m × 0.22 mm × 0.25 μm) column; carrier gas He; injection temperature 250 °C, detection temperature 250 °C, column temperature 220 °C). D-Glucose (13.89 min) was detected from **1**−**3**.

Bioassay. The neuroprotective activity of compounds **1**−**13** against H_2O_2-induced PC12 cells (Cell Bank of Chinese Academy of Sciences, Shanghai, People's Republic of China) was evaluated in accordance with a reported protocol.[15] Samples were added to the

Table 3. Neuroprotective Effects of Compounds **2**, **6**, and **7** against H_2O_2-Induced PC12 Cell Damage

	control	H_2O_2[a]	edaravone[b]		**2**		**6**		**7**	
concentration (μg/mL)			12.5	25	12.5	25	12.5	25	12.5	25
cell viability (%)[c]	100	64.2 ± 8.0	74.3 ± 3.9	86.9 ± 4.9[e]	75.1 ± 9.0[e]	98.1 ± 6.8[d]	89.1 ± 4.8[d]	123.0 ± 5.6[d]	95.5 ± 15.0[d]	157.8 ± 6.0[d]

[a] Negative control. [b] Positive control. [c] Mean ± SEM (n = 3). [d] $p < 0.05$, compared to H_2O_2 group. [e] $p < 0.01$, compared to H_2O_2 group.

cells, which were seeded into 96-well plates and cultivated for 24 h. The cultures were developed for 6 h and then incubated with 250 μM H_2O_2 for 1 h. Cell viability (% of control) was determined by the MTT method.[13] Edaravone (Nanjing Simcere Pharmaceutical Group, Jiangsu Province, People's Republic of China) was used as positive control, and H_2O_2 was treated as negative control. Experiments were carried out in triplicate. Statistical comparison was made by using one-way ANOVA. The data are expressed as means ± SEM of three assays.

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Supporting Information Available: The HRESIMS, UV, IR, and 1D and 2D NMR data of puniceasides A−E (**1**−**5**) are available free of charge via the Internet at http://pubs.acs.org.

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