A New Triterpene and Phenolic Compounds from the Roots of *Pteroxygonum giraldii*

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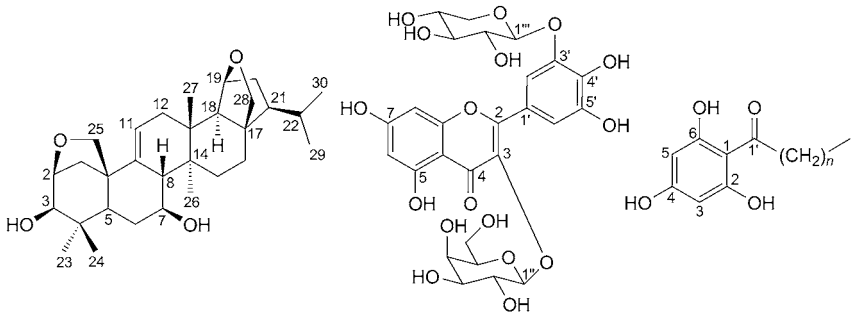
A chemical investigation of the roots of *Pteroxygonum giraldii* led to the isolation of a new arborane-type triterpene, pteroxygonumnol A (**1**), a new myricetin glycoside, myricetin 3-*O*-*β*-D-galactopyranoside 3′-*O*-*β*-D-xylopyranoside (**2**), and a group of phenolic lipids, **3**–**6**, along with four known phenolic compounds, (−)-epigallocatechin, (−)-epigallocatechin gallate, gallic acid, and 2-(4-hydroxyphenyl)-acetic acid. Their structures were elucidated on the basis of extensive spectroscopic analyses.

Introduction. – The genus *Pteroxygonum* belongs to the Polygonaceae family and comprises only one species, *Pteroxygonum giraldii* DAMMER et DIELS, which is an indigenous plant of China [1]. In traditional Chinese medicine, the roots of *P. giraldii*, locally known as '*QiaoMaiQi*', are used for the treatment of gastroenteritis, dysentery, tonsillitis, haematemesis, burns, and lumbago [2]. A series of hexaoxygenated flavonoids from this plant has been reported [3][4]. In our subsequent investigation, a new arborane-type triterpene, pteroxygonumnol A (**1**), a new myricetin glycoside, myricetin 3-*O*-*β*-D-galactopyranoside 3′-*O*-*β*-D-xylopyranoside (**2**), and a group of phenolic lipids, **3**–**6** (*Fig. 1*), along with four known phenolic compounds, (−)-epigallocatechin (EGC) [5], (−)-epigallocatechin gallate (EGCG) [6], gallic acid [7], and 2-(4-hydroxyphenyl)acetic acid [8], were isolated from the title plant.



1 **2** **3 – 6** *n* = 18, 20, 22, 24

Fig. 1. *The structures of compounds* **1**–**6**

Here, we describe the isolation and structure elucidation of the new compounds, and the isolation of the known compounds. Their structures were elucidated based on the combination of various NMR (^{1}H- and ^{13}C-NMR, COSY, HSQC, HMBC, and NOESY) spectroscopic and mass spectrometric (HR-ESI-MS) techniques.

Results and Discussion. – Compound **1** was isolated as white needles. The HR-ESI-MS (positive-ion mode) exhibited a *quasi*-molecular-ion peak at *m/z* 493.3281 ([*M* + Na]$^+$; calc. 493.3288), corresponding to the molecular formula $C_{30}H_{46}O_4$. The IR spectrum showed absorption at 3442 cm^{-1} ascribed to OH groups. The ^{1}H-, ^{13}C-NMR, DEPT, and HSQC spectra exhibited signals assigned to six Me groups (δ(H) 0.86 (*d*, *J* = 6.5), 1.20 (*s*), 1.21 (*s*), 1.22 (*s*), 1.25 (*s*); δ(C) 15.5, 16.3, 22.8, 23.3, 26.1, and 28.4), two CH_2 groups (δ(H) 3.71, 3.84 (2*d*, *J* = 7.5, 1 H each), 3.36, 4.32 (2*d*, *J* = 8.0, 1 H each); δ(C) 68.9 and 73.4), four O-bearing CH groups (δ(H) 3.85 (*d*, *J* = 4.5), 3.92 (*ddd*, *J* = 10.5, 10.5, 3.5), 4.23 (*d*, *J* = 1.0), 4.72 (*dd*, *J* = 5.5, 5.5); δ(C) 76.8, 72.7, 77.7, and 80.8), and a trisubstituted olefinic moiety (δ(H) 5.74 (br. *d*, *J* = 6.5); δ(C) 119.0 and 140.4). The ^{1}H- and ^{13}C- NMR spectroscopic data of **1** (*Table 1*) were similar to those of rubianoside I and rubianol-f [9] except for the signals due to the 2,25-epoxy ring, which was confirmed by the long-range correlations between the signals of H–C(2) (δ(H) 4.72) and C(25) (δ(C) 73.4), and between those of CH_2(25) (δ(H) 4.32) and C(2) (δ(C) 80.8) in the HMBC spectrum (*Fig. 2*). The relative configuration of **1** was elucidated by a NOESY experiment (*Fig. 3*), which showed NOE correlations between the following H-atom pairs: H–C(3), and H–C(5) and Me(23); H–C(5), and H–C(7) and Me(23); Me(26), and H–C(7) and H–C(18); CH_2(25), and Me(24) and H–C(8); Me(27), and H–C(8) and CH_2(28a); and CH_2(28b), and H–C(22), Me(29) and Me(30). Based on these data, the structure of **1** was identified, and named pteroxygonumnol A.

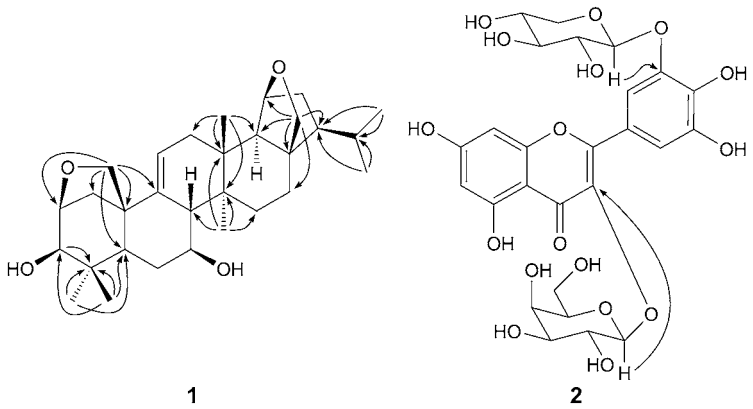


Fig. 2. *Selected HMBCs* (H → C) *of* **1** *and* **2**

Compound **2** was isolated as a yellow powder. The HR-ESI-MS (positive-ion mode) exhibited a *quasi*-molecular-ion peak at *m/z* 635.1210 ([*M* + Na]$^+$; calc. 635.1219), corresponding to the molecular formula $C_{26}H_{28}O_{17}$. The IR, UV, and

Table 1. *^1H-* (500 MHz, C_5D_5N) *and ^{13}C-NMR Data* (125 MHz, C_5D_5N) *of* **1**. δ in ppm, J in Hz[a]).

	δ(H)	δ(C)
CH_2(1)	2.34 (*d*, $J = 11.0$), 2.28	35.2
H–C(2)	4.72 (*dd*, $J = 5.5, 5.5$)	80.8
H–C(3)	3.85 (*d*, $J = 4.5$)	76.8
C(4)		37.7
H–C(5)	1.78	47.3
CH_2(6)	2.26, 2.04	37.2
H–C(7)	3.92 (*ddd*, $J = 10.5, 10.5, 3.5$)	72.7
H–C(8)	1.95	52.2
C(9)		140.4
C(10)		50.3
H–C(11)	5.74 (br. *d*, $J = 6.5$)	119.0
CH_2(12)	1.97, 1.82	38.4
C(13)		36.3
C(14)		39.6
CH_2(15)	2.98 (br. *d*, $J = 11.0$), 1.73 (*dd*, $J = 13.5, 3.5$)	30.4
CH_2(16)	1.80, 1.66	26.2
C(17)		48.9
H–C(18)	1.52 (br. *s*)	58.2
H–C(19)	4.23 (*d*, $J = 1.0$)	77.7
CH_2(20)	1.88, 1.24	41.7
H–C(21)	1.22	54.7
H–C(22)	1.65	31.4
Me(23)	1.25 (*s*)	28.4
Me(24)	1.22 (*s*)	26.1
CH_2(25)	4.32 (*d*, $J = 8.0$), 3.36 (*d*, $J = 8.0$)	73.4
Me(26)	1.20 (*s*)	15.5
Me(27)	1.21 (*s*)	16.3
CH_2(28)	3.84, 3.71 (*d*, $J = 7.5$)	68.9
Me(29)	0.86 (*d*, $J = 6.5$)	22.8
Me(30)	0.86 (*d*, $J = 6.5$)	23.3

[a]) The assignments were based on DEPT, COSY, HSQC, HMBC, and NOESY experiments, with multiplicities and coupling constants in parentheses. Overlapped signals were reported without multiplicities.

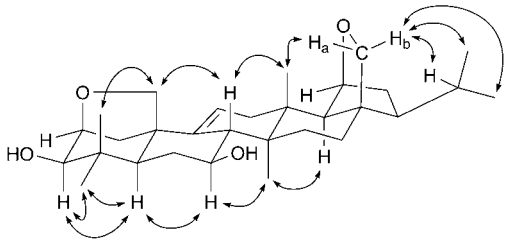


Fig. 3. *Key NOESY* (H ↔ H) *correlations of* **1**

NMR spectra indicated that **2** was a derivative of myricetin with a sugar unit. In the ^{1}H-NMR spectrum (*Table 2*), two *meta*-coupled *doublet*s ($J = 2.0$) in the aromatic

Table 2. *^1H-* (500 MHz, (D_6)DMSO) *and ^{13}C-NMR Data* (125 MHz, (D_6)DMSO) *of* **2**. δ in ppm, J in Hz[a]).

	δ(H)	δ(C)
Aglycone		
C(2)		156.3
C(3)		134.5
C(4)		178.2
C(5)		161.9
H–C(6)	6.19 (d, J = 2.0)	99.4
C(7)		164.9
H–C(8)	6.40 (d, J = 2.0)	94.2
C(9)		156.9
C(10)		104.6
C(1′)		120.8
H–C(2′)	7.45 (d, J = 2.0)	110.5
C(3′)		145.8
C(4′)		139.5
C(5′)		146.2
H–C(6′)	7.36 (d, J = 2.0)	112.3
3-*O*-Gal		
H–C(1″)	5.36 (d, J = 8.0)	102.4
H–C(2″)	3.54–3.58 (m)	71.3
H–C(3″)	3.37	73.2
H–C(4″)	3.63 (br. s)	68.0
H–C(5″)	3.33	76.0
CH_2(6″)	3.44–3.48 (m), 3.28	60.1
3′-*O*-Xyl		
H–C(1‴)	4.80 (d, J = 7.5)	103.7
H–C(2‴)	3.38	73.3
H–C(3‴)	3.32	75.6
H–C(4‴)	3.40	69.5
CH_2(5‴)	3.79 (dd, J = 11.5, 5.0), 3.27	65.7

[a]) The assignments were based on COSY, HSQC, and HMBC experiments, with multiplicities and coupling constants in parentheses. Overlapped signals were reported without multiplicities.

region (δ(H) 6.40 and 6.19, 1 H each) indicated a 5,7-dihydroxy *A*-ring, and the signal at δ(H) 12.61 was assigned to HO–C(5) due to its high chemical shift caused by intermolecular H-bonding. Another two *meta*-coupled *doublet*s (J = 2.0) in the aromatic region (δ(H) 7.45 and 7.36, 1 H each) suggested a 3′,4′,5′-tri-*O*-substituted asymmetric *B*-ring. In the upfield region of the spectrum, there were signals due to two anomeric H-atoms of a sugar moiety (δ(H) 5.36 (d, J = 8.0) and 4.80 (d, J = 7.5)) and eleven sugar C-atoms, evidencing the presence of a pentosyl and a hexosyl group. The ^{1}H- and ^{13}C-NMR data of the sugar moiety were very similar to those of 3-methylmyricetin 3′-*O*-β-D-xylopyranoside [3] and myricetin 3-*O*-β-D-galactopyranoside [10], which indicated the presence of a terminal xylose (δ(H) 4.80 (d, J = 7.5); δ(C) 103.7, 73.3, 75.6, 69.5, and 65.7) and a terminal galactose (δ(H) 5.36 (d, J = 8.0); δ(C) 102.4, 71.3, 73.2, 68.0, 76.0, and 60.1) residue. Acid hydrolysis afforded galactose

and xylose in agreement with the ^{1}H- and ^{13}C-NMR data. The positions of attachment of the glycosyl moieties to the aglycone were deduced by the HMBC correlations (*Fig. 2*) observed for H–C(1″) of Gal (δ(H) 5.36)/C(3) of aglycone (δ(C) 134.5), and H–C(1‴) of Xyl (δ(H) 4.80)/C(3′) of aglycone (δ(C) 145.8). Based on the above results, the structure of compound **2** was established as myricetin 3-*O*-β-D-galactopyranoside 3′-*O*-β-D-xylopyranoside.

A mixture of compounds **3**–**6** was isolated as white powder. The IR spectrum suggested the presence of H-bonded CO (1642 cm^{-1}) and OH (3395 cm^{-1}) groups. In the HR-ESI-MS (negative-ion mode) spectrum, four *quasi*-molecular ion peaks were found at *m*/*z* 419.3170 ([*M* – H]$^-$; calc. 419.3167), 447.3480 ([*M* – H]$^-$; calc. 447.3480), 475.3794 ([*M* – H]$^-$; calc. 475.3493), and 503.4109 ([*M* – H]$^-$; calc. 503.4106), corresponding to the molecular formulae $C_{26}H_{44}O_4$, $C_{28}H_{48}O_4$, $C_{30}H_{52}O_4$, and $C_{32}H_{56}O_4$, respectively, which indicated a mixture of four compounds. The ^{1}H-NMR spectrum displayed three OH signals at δ(H) 13.91 (2 H, H-bonded OH) and 12.67 (1 H, a free OH group), a signal for two aromatic H-atoms at δ(H) 6.47 (*s*), and a series of long-chain aliphatic alkane H-atom signals at δ(H) 3.42 (*t*, *J* = 7.5, 2 H), 1.89 (*quint.*, *J* = 7.5, 2 H), 1.42 (*m*, 2 H), 1.27 (br. *s*, –CH_2–), and 0.85 (*t*, *J* = 7.0, Me). The ^{13}C-NMR spectrum exhibited a group of Ph signals at δ(C) 165.3, 165.0 (2 C), 104.3, 94.8 (2 C), which evidenced a symmetric benzene ring; a CO group at δ(C) 205.3, and a series of long-chain alkane C-atom signals at δ(C) 43.1 (–CH_2–X), 31.0, 28.8–28.7, 28.5, 24.2, 21.8 (–CH_2–), and 13.1 (Me). The H-atom signals were similar to those of the known compound 1-(2,4,6-trihydroxyphenyl)icosan-1-one [11]. Thus, the difference between the four compounds was the length of the alkane chain, which could be deduced from the molecular formulae. Finally, concluded that this mixture was comprised of a known compound, 1-(2,4,6-trihydroxyphenyl)icosan-1-one (**3**), and three new ones, 1-(2,4,6-trihydroxyphenyl)docosan-1-one (**4**), 1-(2,4,6-trihydroxyphenyl)tetracosan-1-one (**5**), and 1-(2,4,6-trihydroxyphenyl)hexacosan-1-one (**6**).

The four known compounds, (–)-epigallocatechin, (–)-epigallocatechin gallate, gallic acid, and 2-(4-hydroxyphenyl)acetic acid, were identified by comparison of their spectroscopic data with literature values. This is the first report of these compounds from the genus *Pteroxygonum*, except gallic acid.

This work was supported by the *Program for New Century Excellent Talents in University* (NCET-09–0589) and the *China Postdoctoral Science Foundation* (No. 20090450775).

Experimental Part

General. All solvents used were of anal. grade (*Tianjin Jiangtian Chemical Technology Co. Ltd.*, P. R. China). TLC: Silica gel GF_{254} plates (*Qingdao Haiyang Chemical Co. Ltd.*, P. R. China); spots visualized by UV light (254/365 nm), and by spraying with 5% H_2SO_4 and 1% $AlCl_3$ reagents, followed by heating. Column chromatography (CC): silica gel (SiO_2; 100–200 and 200–300 mesh; *Qingdao Haiyang Chemical Co. Ltd.*, P. R. China), *LiChroprep RP-18* (40–63 μm; *Merck*, Germany), and *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, Sweden). M.p.: *XT4A microscope* apparatus; uncorrected. Optical rotations: *Rudolph Research Analytical Autopol II automatic* polarimeter. UV Spectra: *SHIMADZU UV-2450* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker Tensor 27* spectrometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AV-500* and *Varian INOVA 500 FT* instruments; δ in ppm rel. to Me_4Si as internal standard; *J* in Hz. HR-ESI-MS: *Varian 7.0T FT-ICR* mass spectrometer; in *m*/*z*.

Plant Material. The roots of *P. giraldii* were collected in September and October 2007, in Mei County, Shaanxi Province, P. R. China, and authenticated by Prof. *Zhen-Hai Wu*, Northwest A&F University. A voucher specimen (S20060811) was deposited with the School of Pharmaceutical Science and Technology, Tianjin University, P. R. China.

Extraction and Isolation. Fresh roots of *P. giraldii* (18 kg) were refluxed with 90% and then with 60% EtOH twice, resp. The extracts were combined and concentrated to give a residue (3 kg), which was suspended in H_2O to a final volume of 10 l, and partitioned with petroleum ether (PE), $CHCl_3$, AcOEt, and BuOH. The AcOEt extract (130 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH 98:2 → 65:35) to give 83 fractions. *Frs. 22–29* (63.5 g) were combined and further submitted to CC (SiO_2; PE/Me_2CO 9:1 → 5:5) to give the mixture of **3**–**6** (20 mg) and 2-(4-hydroxyphenyl)acetic acid (100 mg). *Frs. 36–45* (24 g) were combined and further purified by CC (SiO_2; CH_2Cl_2/MeOH 95:5 → 85:15; and *Sephadex LH-20*; MeOH) to afford gallic acid (500 mg). *Frs. 46–64* (42 g) were combined and further purified by repeated CC (SiO_2; CH_2Cl_2/MeOH 9:1 → 75:25; *RP-18* SiO_2; MeOH/H_2O 5:5; and *Sephadex LH-20*; MeOH) to afford compound **1** (30 mg), (−)-epigallocatechin (20 mg), and (−)-epigallocatechin gallate (100 mg).

The BuOH extract (1200 g) was subjected to *D101* macroporous resin CC and eluted with H_2O, followed by increasing concentrations of EtOH in H_2O (30%, 50%, and 95% EtOH) to yield five fractions. The 30% EtOH eluate (330 g) was then exposed to CC (SiO_2; AcOEt/MeOH/H_2O 96:4:2 → 7:3:1) to give 71 fractions. *Frs. 25–37* (46 g) were combined and further purified by CC (SiO_2; $CHCl_3$/MeOH/H_2O 75:25:2 → 4:6:1) to afford compound **2** (100 mg).

Acid Hydrolysis. Compound **2** (10.1 mg) was dissolved in 4 ml of 1M HCl (H_2O/dioxane 1:1) and heated in a H_2O bath at 80° for 2 h, then the dioxane was evaporated, and the aglycone was removed by extracting with AcOEt (4 × 4 ml). The aq. layer was neutralized with Ag_2CO_3, centrifuged, and evaporated to dryness. The monosaccharides were identified as Xyl and Gal by TLC (SiO_2; AcOEt/MeOH/H_2O/AcOH 13:3:4:2) comparision with authentic sugars.

Pteroxygonumnol A (= rel-*(3S,4R,7S,7aS,7bS,9aR,10S,12S,12aR,12bR,14bR)-3,4,5,5a,6,7,7a,7b,8,9,10,11,12,12a,12b,13-Hexadecahydro-5,5,7b,12b-tetramethyl-10-(propan-2-yl)-12,9a-(epoxymethano)-3,14b-methanocyclopenta[7,8]phenanthro[1,2-c]oxepine-4,7(1H)-diol*; **1**). White powder. M.p. 296–297°. $[\alpha]_D^{25} = +28.2$ ($c = 0.78$, C_5H_5N). UV (MeOH): 222 (3.12), 268 (2.72). IR (KBr): 3442, 2924, 2347, 1641, 1059. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 493.3281 ($[M + Na]^+$, $C_{30}H_{46}NaO_4^+$; calc. 493.3288).

Myricetin 3-O-β-D-Galactopyranoside 3′-O-β-D-Xylopyranoside (=*2-[3,4-Dihydroxy-5-(β-D-xylopyranosyloxy)phenyl]-5,7-dihydroxy-4-oxo-4H-chromen-3-yl β-D-Galactopyranoside*; **2**). Yellow powder. M.p. 194–196°. $[\alpha]_D^{25} = -42.9$ ($c = 0.63$, C_5H_5N). UV (MeOH): 225 (4.34), 357 (4.33). IR (KBr): 3421, 2913, 1657, 1607, 1505, 1351, 1202, 1046. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 635.1210 ($[M + Na]^+$, $C_{26}H_{28}NaO_{17}^+$; calc. 635.1219).

Mixture of Phenolic Lipids 1-(2,4,6-Trihydroxyphenyl)henicosan-1-one, 1-(2,4,6-Trihydroxyphenyl)-tricosan-1-one, 1-(2,4,6-Trihydroxyphenyl)pentacosan-1-one, and 1-(2,4,6-Trihydroxyphenyl)heptacosan-1-one (**3**–**6**, resp.). White powder. UV (MeOH): 228 (4.10), 286 (4.20). IR (KBr): 3395, 2918, 2849, 1642, 1467, 1222, 1072. ^{1}H-NMR (500 MHz, C_5D_5N): 13.91 (br. *s*, HO–C(2/6)); 12.67 (br. *s*, HO–C(4)); 6.47 (*s*, H–C(3/5)); 3.42 (*t*, $J = 7.5$, CH_2(2′)); 1.89 (*quint.*, $J = 7.5$, CH_2(3′)); 1.27 (br. *s*, $–CH_2–$); 0.85 (*t*, $J = 7.0$, Me). ^{13}C-NMR (125 MHz, C_5D_5N): 205.3 (C(1′)); 165.3 (C(4)); 165.0 (C(2/6)); 104.3 (C(1)); 94.8 (C(3/5)); 43.1 (C(2′)); 31.0, 28.8–28.7, 28.5, 24.2, 21.8 ($–CH_2–$); 13.1 (Me). HR-ESI-MS: 419.3170 ($[M - H]^-$, $C_{26}H_{43}O_4^-$; calc. 419.3167), 447.3480 ($[M - H]^-$, $C_{28}H_{47}O_4^-$; calc. 447.3480), 475.3794 ($[M - H]^-$, $C_{30}H_{51}O_4^-$; calc. 475.3493), and 503.4109 ($[M - H]^-$, $C_{32}H_{55}O_4^-$; calc. 503.4106), resp.

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Received June 24, 2011