Two Novel Triterpenoids from *Portulaca oleracea* L.

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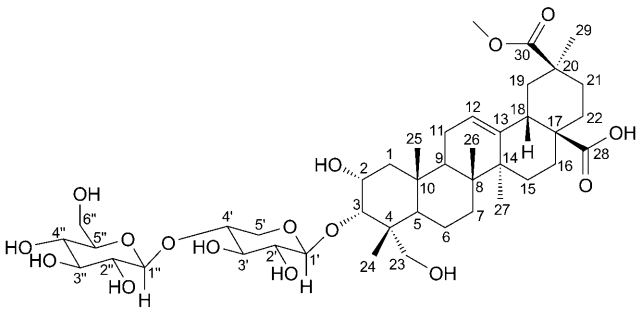
Two novel triterpenoids, (2α,3α)-3-{[4-*O*-(β-D-glucopyranosyl)-β-D-xylopyranosyl]oxy}-2,23-dihydroxy-30-methoxy-30-oxoolean-12-en-28-oic acid (**1**) and (2α,3α)-2,23,30-trihydroxy-3-[(β-D-xylopyranosyl)oxy]olean-12-en-28-oic acid (**2**) were isolated from *Portulaca oleracea* L., and they both showed weak cytotoxic activity assayed with the MTT method.

Introduction. – *Portulaca oleracea* L. (Portulacaceae) is widely distributed all over the world, and is consumed as a nutritious and delicious vegetable abundant in α-linolenic acid and β-carotene in many countries. As a folk medicine with a long history and included in the Chinese pharmacopeia (Chinese name 'Ma-Chi-Xian'), it has also been used as a diuretic, febrifuge, antiseptic, antispasmodic, and vermifuge. In modern pharmacological research, it exhibits a wide range of pharmacological effects, including antibacterial [1], analgesic, anti-inflammatory [2], skeletal muscle relaxant [3][4], and wound-healing [5] activities. Up to now, many constituents from *Portulaca oleracea* L. have been reported, including monoterpenes (portuloside A [6], portuloside B), triterpenoids (α- and β-amyrane type, friedelane), alkanoids (noradrenaline [7], dopamine, oleraceins A – E [8], adenosine), flavonoids (genistin, genistein), coumadin (scopoletin, bergapten, isoimpinellin) [9], *etc.*

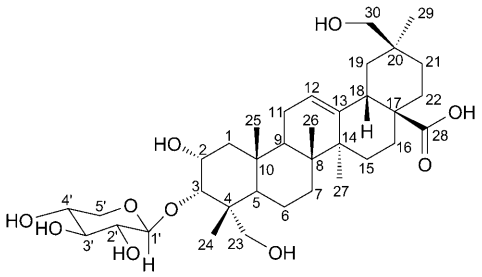
Over the past several years, we carried out a continuous study on its antihypoxia, cytotoxic activity, and active chemical constituents, and a much better effect of antihypoxia has been testified [10][11]. In the present study, two novel β-amyrin type triterpenoids were obtained, whose cytotoxic activity were also assayed with MTT method consequently. Here, we report the structure elucidation and isolation of these two novel compounds based on detailed spectroscopic analysis.

Results and Discussion. – Compound **1** was obtained as a white amorphous powder and it displayed a positive *Liebermann – Burchard* test. It was optically active with $[\alpha]_D^{25} = 34.60$ ($c = 0.1$, MeOH), and had the molecular formula $C_{42}H_{66}O_{16}$, with ten degrees of unsaturation, as determined according to the *pseudo*-molecular ion peak at 849.4249 ($[M+Na]^+$; calc. 849.4249) in the HR-ESI-MS spectrum. Analysis of the ^{13}C-NMR and DEPT spectra revealed 42 C-atom signals, including six Me groups, twelve CH_2 groups (three of them oxygenated), 14 CH groups (eleven of them oxygenated), one trisubstituted C=C bond, and eight quaternary C-atoms including

two C=O groups. In the ^{1}H-NMR spectrum, signals at δ(H) 1.15 (*s*), 1.29 (*s*), 1.36 (*s*), 1.41 (*s*), and 1.64 (*s*) were assigned to five Me groups, and another signal at δ(H) 3.74 (*s*) was assigned to an oxygenated Me group. Based on the analysis of ^{1}H- and ^{13}C-NMR data, especially referring to the chemical shift of the C=C bond (δ(C) 122.50 (*d*), 145.04 (*s*)), the number of Me groups, and positive results of *Liebermann–Burchard* test, compound **1** may be preliminarily deduced as a β-amyrin type triterpenoid.



1



2

In the HMQC spectrum, the Me group at δ(H) 1.41 (*s*) was correlated with the signal at δ(C) 15.09 (*q*), which was correlated with signals at δ(C) 65.38 (*t*) and 83.02 (*d*) in the HMBC spectrum. Thus, the three signals at δ(C) 15.09 (*q*), 65.38 (*t*) and 83.02 (*d*) were assigned to C(24), C(23), and C(3), respectively. The signal at δ(H) 4.31 (*d*, $J = 2.5$) was correlated with the signal of C(3) in the HMQC spectrum and correlated with the signals at δ(C) 71.05 (*d*) and 44.30 (*t*) in the HMBC spectrum. Therefore, the signals at δ(C) 71.05 (*d*) and 44.30 (*t*) were assigned to C(2) and C(1), respectively. In the HMBC spectrum, Me(29) at δ(H) 1.29 (*s*) was correlated with the signal at δ(C) 177.27 (*s*), and the Me group (δ(C) 51.80 (*q*), δ(H) 3.74 (*s*)) was also correlated with the same signal at δ(C) 177.27 (*s*), supporting that C(30) was part of a methyl ester moiety. Besides, the signal for H–C(18) at δ(H) 3.36 (*dd*, $J = 13.6, 2.0$) was correlated with the signal at δ(C) 179.91 (*s*) in the HMBC spectrum, indicating C(28) to be part of a COOH group. Based on the above analysis and in combination with ^{1}H,^{1}H-COSY, HMQC, HMBC spectra, and referring to literature values [12], the remaining C-atoms of compound **1** could be unambiguously assigned (*Table*).

Table. *^{1}H- and ^{13}C-NMR Data of* **1** (in C_5D_5N) *and* **2** (in C_5D_5N). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(C)[a])	δ(H)[b])	δ(C)[a])	δ(H)[b])
1	44.30 (*t*)	1.31–1.35 (*m*), 1.95–2.01 (*m*)	43.86 (*t*)	1.29 (*dd*, *J* = 12.0, 4.2), 2.14 (*dd*, *J* = 12.0, 4.2)
2	71.05 (*d*)	4.76–4.81 (*m*)	70.56 (*d*)	4.77–4.83 (*m*)
3	83.02 (*d*)	4.31 (*d*, *J* = 2.5)	82.64 (*d*)	4.30 (*d*, *J* = 3.0)
4	42.80 (*s*)	–	42.09 (*s*)	–
5	47.86 (*d*)	1.84–1.91 (*m*)	47.40 (*d*)	1.83–1.88 (*m*)
6	18.06 (*t*)	1.73–1.82 (*m*)	17.64 (*t*)	1.68–1.77 (*m*)
7	33.14 (*t*)	1.39–1.33 (*m*), 2.12–2.15 (*m*)	32.67 (*t*)	1.31–1.34 (*m*), 2.14–2.18 (*m*)
8	40.00 (*s*)	–	39.57 (*s*)	–
9	48.69 (*d*)	1.84–1.89 (*m*)	48.21 (*d*)	1.77 (*m*)
10	37.11 (*s*)	–	36.64 (*s*)	–
11	24.03 (*t*)	2.33–2.36 (*m*), 2.42–2.45 (*m*)	23.71 (*t*)	2.01–2.05 (*m*), 2.19–2.23 (*m*)
12	122.50 (*d*)	5.71 (br.)	122.52 (*d*)	5.50 (*s*)
13	145.04 (*s*)	–	144.45 (*s*)	–
14	42.43 (*s*)	–	42.48 (*s*)	–
15	28.45 (*t*)	1.23–1.26 (*m*), 1.32–1.34 (*m*)	27.98 (*t*)	1.20–1.24 (*m*), 2.16–2.19 (*m*)
16	23.98 (*t*)	2.33–2.36 (*m*), 2.42–2.45 (*m*)	23.64 (*t*)	2.15–2.19 (*m*)
17	46.30 (*s*)	–	46.34 (*s*)	–
18	43.49 (*d*)	3.36 (*dd*, *J* = 13.6, 2.0)	41.28 (*d*)	3.65–3.71 (*m*)
19	42.61 (*t*)	2.41–2.47 (*m*)	41.64 (*t*)	1.83–1.86 (*m*)
20	44.29 (*s*)	–	35.53 (*s*)	–
21	30.96 (*t*)	1.37–1.41 (*m*), 1.93–1.97 (*m*)	29.28 (*t*)	1.35–1.56 (*m*), 1.77–1.86 (*m*)
22	34.72 (*t*)	1.95–2.10 (*m*)	32.64 (*t*)	1.56–1.77 (*m*), 2.14–2.16 (*m*)
23	65.38 (*t*)	3.80 (*d*, *J* = 12.0), 4.37 (*d*, *J* = 12.0)	64.96 (*t*)	3.71 (*d*, *J* = 11.0), 4.36 (*d*, *J* = 11.0)
24	15.09 (*q*)	1.41 (*s*)	14.63 (*q*)	1.35 (*s*)
25	17.39 (*q*)	1.64 (*s*)	16.91 (*q*)	1.56 (*s*)
26	17.59 (*q*)	1.15 (*s*)	17.20 (*q*)	1.07 (*s*)
27	26.33 (*q*)	1.36 (*s*)	25.94 (*q*)	1.30 (*s*)
28	179.91 (*s*)	–	178.20 (*s*)	–
29	28.56 (*q*)	1.29 (*s*)	28.04 (*q*)	1.20 (*s*)
30	177.27 (*s*)	–	65.25 (*t*)	3.90 (*d*, *J* = 11.0), 4.00 (*d*, *J* = 11.0)
MeO	51.80 (*q*)	3.74 (*s*)		
1′	106.55 (*d*)	5.05 (*d*, *J* = 8.0)	106.39 (*d*)	5.06 (*d*, *J* = 8.0)
2′	75.19 (*d*)	3.99–4.02 (*m*)	75.11 (*d*)	3.98–4.00 (*m*)
3′	76.42 (*d*)	4.16–4.19 (*m*)	78.13 (*d*)	4.05–4.07 (*m*)
4′	77.15 (*d*)	4.28–4.32 (*m*)	70.71 (*d*)	4.08–4.12 (*m*)
5′	64.96 (*t*)	3.64 (*dd*, *J* = 12.0, 1.8), 4.24 (*dd*, *J* = 12.0, 1.8)	66.84 (*t*)	3.64 (*dd*, *J* = 12.0, 1.8), 4.30 (*dd*, *J* = 12.0, 1.8)
1″	103.70 (*d*)	5.11 (*d*, *J* = 8.0)		
2″	74.33 (*d*)	4.11–4.13 (*m*)		
3″	78.25 (*d*)	4.25–4.28 (*m*)		
4″	71.80 (*d*)	4.22–4.25 (*m*)		
5″	78.93 (*d*)	4.07–4.09 (*m*)		
6″	62.64 (*t*)	4.40 (*dd*, *J* = 12.0, 1.8), 4.62 (*dd*, *J* = 12.0, 1.8)		

[a]) Recorded at 125 MHz, multiplicity by DEPT. [b]) Recorded at 500 MHz.

For the identification of the sugar moiety, compound **1** was hydrolyzed with hydrochloric acid at 80° for 2 h, then the hydrolyzed sample solution was analyzed by comparison with standard monosaccharides by means of the TLC method (BuOH/AcOH/H_2O 4:1:5 (upper layer) as mobile phase, detected by spraying with aniline *O*-phthalic acid). In the HMBC spectrum, the correlation of the anomeric H-atom of xylose at δ(H) 5.05 (*d*, $J = 8.0$) with C(3) was evident. Therefore, the xylose moiety was determined to be connected to C(3). By analyzing the down-field shifted signal of C(4′) (4.28–4.32 ppm) of the xylose moiety and the correlation of the signal of the anomeric H-atom of glucose at δ(H) 5.11 (*d*, $J = 8.0$) with C(4′) of the xylose moiety in the HMBC spectrum, the glucose moiety could be deduced to be connected to C(4′) of the xylose moiety. Due to the coupling constant of 8 Hz of the anomeric H-atoms, both the xylose and the glucose moiety were assigned to have β-configuration.

The ^{1}H- and ^{13}C-NMR spectra (*Table*), in combination with ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY data (*Fig. 1*), established the structure of compound **1** as (2α,3α)-3-{[4-*O*-(β-D-glucopyranosyl)-β-D-xylopyranosyl]oxy}-2,23-dihydroxy-30-methoxy-30-oxoolean-12-en-28-oic acid.

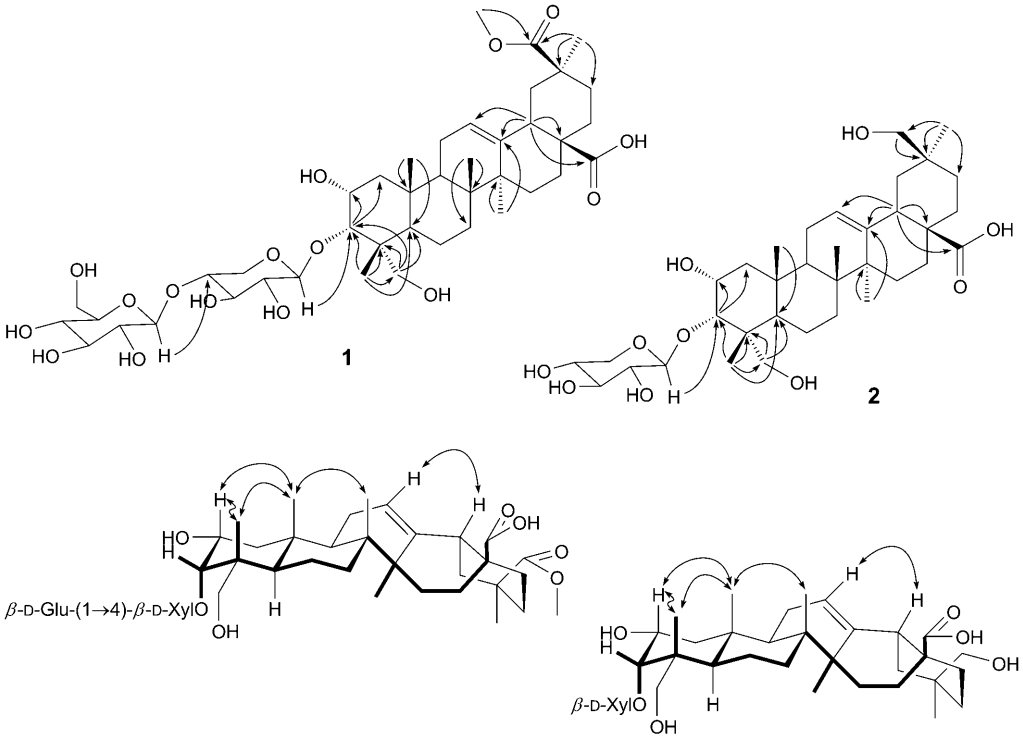


Fig. 1. *Key HMBC* (H→C) *and NOESY* (↔) *correlations of compounds* **1** *and* **2**

Compound **2** displayed a positive *Liebermann–Burchard* test. It was optically active with $[\alpha]_D^{25} = 28.54$ ($c = 0.1$, MeOH), and had the molecular formula $C_{35}H_{56}O_{10}$, with eight degrees of unsaturation, as determined according to the *pseudo*-molecular

ion peak at 659.3773 ($[M+Na]^+$; calc. 659.3771) in the HR-ESI-MS spectrum. Analysis of the ^{13}C-NMR and DEPT spectra revealed 35 C-atom signals, including five Me groups, twelve CH_2 groups (three of them oxygenated), nine CH (six of them oxygenated), one trisubstituted C=C (δ(C) 122.52, 144.45) bond, and seven quaternary C-atoms including one C=O group. In the ^{1}H-NMR spectrum, signals at δ(H) 1.07 (*s*), 1.20 (*s*), 1.30 (*s*), 1.35 (*s*), 1.56 (*s*) were assigned to five Me groups. Based on the above analysis, compound **2** could also be preliminarily deduced as β-amyrin type triterpenoid.

The Me group at δ(H) 1.35 (*s*) was correlated with the signal at δ(C) 14.63 (*q*) in the HMQC spectrum, and correlated with the signals at δ(C) 64.96 (*t*) and 82.64 (*d*) in the HMBC spectrum. Therefore, the three signals at δ(C) 14.63 (*q*), 64.96 (*t*), and 82.64 (*d*) were assigned to C(24), C(23), and C(3), respectively. Due to the correlation of the signal of H–C(3) at δ(H) 4.30 (*d*, $J = 3.0$) with the signals at δ(C) 70.56 (*d*) and 43.86 (*t*) in the HMBC spectrum, the signals at δ(C) 70.56 (*d*) and 43.86 (*t*) were assigned to C(2) and C(1), respectively. In the HMBC spectrum, a correlation of Me(29) with the signal at δ(C) 65.25 (*t*) indicated that C(30) was oxygenated. In the HMBC spectrum, the signal for H–C(18) at δ(H) 3.65–3.71 (*m*) was correlated with the signal at δ(C) 178.20 (*s*), which suggested that C(28) was present as a COOH group. Based on the above analysis of ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra, as well as referring to literature data [12], the remaining C-atoms of compound **2** could be unambiguously assigned (*Table*).

Hydrolysis of compound **2** with HCl furnished xylose as the single sugar unit. In the HMBC spectrum, a correlation of the anomeric H-atom with C(3) indicated that the xylose unit is connected to C(3) in β-configuration ($^3J = 8.0$ for the anomeric H-atom). The ^{1}H- and ^{13}C-NMR spectra (*Table*), in combination with ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY data (*Fig. 1*), established the structure of compound **2** as (2α,3β)-2,23,30-Trihydroxy-3-[(β-D-xylopyranosyl)oxy]olean-12-en-28-oic acid.

Compounds **1** and **2** showed weak *in vitro* cytotoxic activity against the HepG2 tumor cell line as determined by classical MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 *H*-tetrazolium bromide) colorimetric assay (*Fig. 2*).

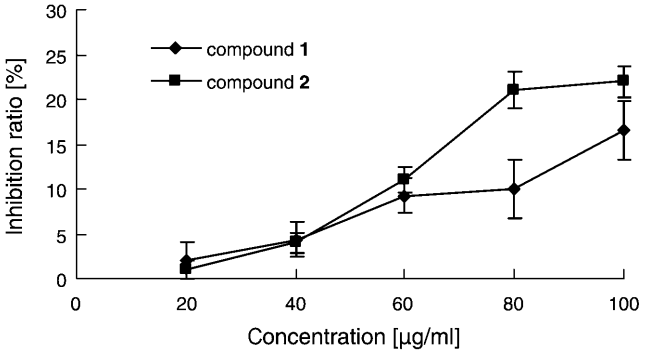


Fig. 2. *Cytotoxic activity results of compounds* **1** *and* **2**

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Experimental Part

General. TLC: Silica-gel plates (*Sinopharm Chemical Reagent Co., Ltd.*). M.p.: *WRS-1A* micro-melting-point apparatus; uncorrected. Optical rotations: *Jasco P-1300* spectropolarimeter. IR Spectra: *Bruker VECTOR-22* spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR, as well as 2D-NMR spectra: *Bruker AVANCE 500* spectrometer; chemical shifts δ in ppm rel. to Me_4Si, coupling constant J in Hz. ESI-MS: *Finnigan LCQ* mass spectrometer; in m/z. HR-ESI-MS: *Q-Tof micro YA019* mass spectrometer.

Plant Material. The aerial parts of *Portulaca oleracea* L. were collected in Henan province, P. R. China in October 2006 and authenticated by Prof. *Han-Chen Zheng*. A voucher specimen has been deposited with the Department of Traditional Chinese Medicine (TCM), Second Military Medical University (20060721).

Extraction and Isolation. The powdered aerial parts of *Portulaca oleracea* L. (40 kg) were refluxed three times in 400 l 80% EtOH soln. for 2.0 h each. The extract was concentrated under reduced pressure to 80 l, then centrifuged at 5000 rpm for 4 min. The precipitation part (680.8 g) was subjected to column chromatography (CC) on silica gel (SiO_2), eluting with $CHCl_3$/MeOH 20:1, 10:1, 7:1, 5:1, 3:1, 1:1, to afford 12 fractions (*Fr. 1–12*). *Fr. 7* and *Fr. 11* were repeatedly subjected to CC (*Pharmadex LH-20* and *RP C-18*) to yield compounds **1** (12.4 mg) and **2** (9.0 mg), resp.

*(2α,3α)-3-{[4-*O*-(β-*D*-Glucopyranosyl)-β-*D*-xylopyranosyl]oxy}-2,23-dihydroxy-30-methoxy-30-oxoolean-12-en-28-oic Acid* (**1**). White amorphous powder. M.p. 185–187°. $[\alpha]_D^{25} = 34.60$ ($c = 0.1$, MeOH). ^1H-NMR (500 MHz, C_5D_5N) and ^{13}C-NMR (125 MHz, C_5D_5N): *Table*. ESI-MS (pos.): 849.48 ($[M + Na]^+$). HR-ESI-MS: 849.4249 ($[M + Na]^+$, $C_{42}H_{66}NaO_{16}^+$; calc. 849.4249).

*(2α,3α)-2,23,30-Trihydroxy-3-[(β-*D*-xylopyranosyl)oxy]olean-12-en-28-oic Acid* (**2**). Light yellow amorphous powder. M.p. 222–224°. $[\alpha]_D^{25} = 28.54$ ($c = 0.1$, MeOH). ^1H-NMR (500 MHz, C_5D_5N) and ^{13}C-NMR (125 MHz, C_5D_5N): *Table*. ESI-MS (pos.): 659.39 ($[M + Na]^+$). HR-ESI-MS: 659.3773 ($[M + Na]^+$, $C_{35}H_{56}NaO_{10}^+$; calc. 659.3771).

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