Two New Polyoxygenated Triterpenoids from *Actinidia valvata*

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Two new polyoxygenated triterpenoids, (2β,3α,6α)-2,3,6,20,23,30-hexahydroxyurs-12-en-28-oic acid (**1**) and (2β,3α)-2,3,20,23,24,30-hexahydroxyurs-12-en-28-oic acid *O*-β-D-glucopyranosyl ester (**2**) were isolated from the roots of *Actinidia valvata* DUNN. Their structures were elucidated by means of extensive spectroscopic studies. Both compounds showed moderate cytotoxic activity *in vitro* against the BEL-7402 and SMMC-7721 tumor cell lines.

Introduction. – There are nearly sixty species in the genus *Actinidia*, family Actinidiceae, and some of them are well known worldwide for their delicious kiwi fruits [1]. One species affiliated to the genus *Actinidia*, *Actinidia valvata* DUNN., is a shrub mainly growing in eastern China, whose root is known as 'mao ren shen' in traditional Chinese medicine (TCM) exhibiting antitumor and anti-inflammatory activity, and has been used for the treatment of hepatoma, lung carcinoma and myeloma for a long time [2][3]. However, ignorance of chemical constituents of many Chinese herbal medicines, although their effect on cancer has been shown by long clinical experience, hampered its further study and more widespread agreement [4]. For 'mao ren shen' as Chinese herbal medicine with a long history for curing cancer, it is also the case. To settle this problem, we carried out continuing studies over the past five years, mainly on its antitumor pharmacology. In the course of these studies, two new polyoxygenated triterpenoids (2β,3α,6α)-2,3,6,20,23,30-hexahydroxyurs-12-en-28-oic acid (**1**) and (2β,3α)-2,3,20,23,24,30-hexahydroxyurs-12-en-28-oic acid *O*-β-D-glucopyranosyl ester (**2**) (see *Fig. 1*), together with seven known triterpenoids were obtained. Specially, it was striking that these two new compounds were both oxygenated at C(20) among so many reported triterpenoids with a pentacyclic carbon skeleton [5]. Here, we report the structure elucidation and isolation of these two new compounds.

Results and Discussion. – The roots of *Actinidia valvata* DUNN. were extracted with 80% EtOH. The concentrated extract was suspended in H_2O and successively extracted with petroleum ether (60–90°), AcOEt, and BuOH. The AcOEt-soluble extract was repeatedly subjected to column chromatography to yield compound **1**. The BuOH-soluble extract also was repeatedly subjected to column chromatography to yield

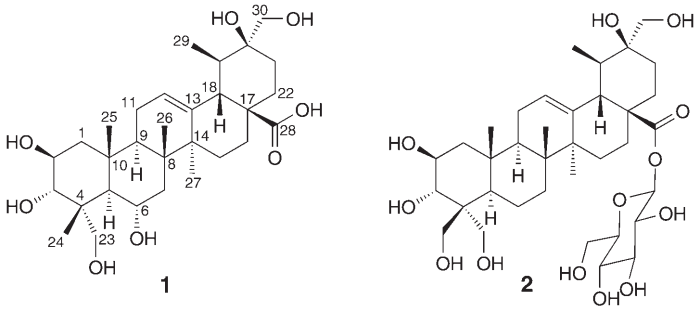

Fig. 1. *Structures of compounds* **1** *and* **2**

compound **2**. Both compounds were highly oxygenated triterpenoids with an urs-12-ene skeleton. Their structures were elucidated by detailed spectroscopic analysis.

Compound **1** was obtained as a white amorphous powder. The compound was optically active, with $[\alpha]_D^{25} = 56.28$ ($c = 0.1$, MeOH), and had the molecular formula $C_{30}H_{48}O_8$, with seven degrees of unsaturation, as determined according to a *pseudo*-molecular-ion peak at 559.3247 ($[M + Na]^+$; calc. 559.3247) in the HR-ESI-MS.

Compound **1** displayed a positive *Liebermann–Burchard* test. The IR spectrum featured absorptions of OH (3410 cm^{-1}), C=O (1643 cm^{-1}), and olefinic (1626 cm^{-1}) groups. Analysis of the ^{13}C-NMR (DEPT) spectrum revealed 30 C-atom signals, including five Me groups, nine CH_2 groups (two of them oxygenated), seven CH groups (three of them oxygenated), one trisubstituted C=C bond, and seven quaternary C-atoms, including one C=O group.

In the ^{1}H-NMR spectrum of compound **1** (see the *Table*), signals at 1.07–1.38 ppm consisting of four *singlets* and one *doublet* (1.31 ppm, *d*, $J = 6.6$ Hz) were assigned to five Me groups. The H-atom *doublet* at 3.47 ppm correlated with the C-atom at 47.77 ppm (*d*) in the HMQC spectrum, and correlated with the C-atoms at 35.89 (*d*), 47.78 (*s*), 12.72 (*q*), and 179.50 ppm (*s*), and a trisubstituted C=C group in the HMBC spectrum (*Fig. 2*). Thus, the signals for the H-atom at 3.47 ppm and the C-atom at 47.77 ppm (*d*) both were assigned to H–C(18); the signal at 47.78 ppm (*s*) to C(17), and the signal at 35.89 ppm (*d*) to H–C(19), and the signal at 179.50 ppm was assigned to CO(28).

In the HMBC spectrum, a clear correlation of the Me group at 1.31 ppm (*d*, $J =$ 6.6 Hz), and 12.72 ppm (*q*), respectively, with H–C(18) and another C-atom at 73.07 ppm (*s*) was observed (*Fig. 2*). From this correlation data, the Me group (Me(29)) was deduced to be attached to C(19), and the C-atom at 73.07 ppm (*s*) was assigned to the oxigenated C(20). The two H-atoms at 3.87 (*d*, $J = 10.2$ Hz) and 4.16 ppm (*d*, $J = 10.2$ Hz) showed HMQC correlations to the C-atom at 69.70 ppm (*t*), and both correlated to H–C(19) and C(20) in the HMBC spectrum. Therefore, the C-atom at 69.70 ppm (*t*) was assigned to C(30).

In the HMBC spectrum, not only correlations of Me(25) with the C-atoms with signals at 47.75 (*t*), 39.96 (*s*), and 55.40 ppm (*d*) were observed, but also a correlation of Me(24) and CH_2(23) with the C-atom at 55.40 ppm (*d*), which was assigned to C(5).

Table. *^{1}H- and ^{13}C-NMR Data of* **1** (in C_5D_5N) *and* **2** (in CD_3OD). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(C)[a])	δ(H)[b])	δ(C)[a])	δ(H)[b])
1	47.75 (*t*)	2.19 (*dd*, *J* = 12, 4.2), 1.31 (*dd*, *J* = 12, 4.2)	42.62 (*t*)	1.28 (*dd*, *J* = 12, 4.2), 1.60 (*dd*, *J* = 12, 4.2)
2	68.48 (*d*)	4.19–4.24 (*m*)	67.14 (*d*)	3.84–3.88 (*m*)
3	79.14 (*d*)	3.87 (*d*, *J* = 9)	73.85 (*d*)	4.00 (*d*, *J* = 2.4)
4	44.77 (*s*)	–	48.06 (*s*)	–
5	55.40 (*d*)	1.79 (*d*, *J* = 10.2)	44.92 (*d*)	1.53–1.56 (*m*)
6	66.75 (*d*)	4.27–4.41 (*m*)	19.46 (*t*)	1.33–1.35 (*m*)
7	45.16 (*t*)	1.92 (br.)	34.71 (*t*)	1.28–1.30 (*m*), 1.54–1.56 (*m*)
8	40.83 (*s*)	–	41.03 (*s*)	–
9	47.47 (*d*)	1.83–1.85 (*m*)	48.72 (*d*)	1.71–1.74 (*m*)
10	39.96 (*s*)	–	38.91 (*s*)	–
11	23.81 (*t*)	1.99–2.01 (*m*)	24.63 (*t*)	1.93–1.95 (*m*), 1.74–1.76 (*m*)
12	125.54 (*d*)	5.53 (br.)	127.25 (*d*)	5.27 (br.)
13	139.02 (*s*)	–	138.83 (*s*)	–
14	42.74 (*s*)	–	43.33 (*s*)	–
15	28.68 (*t*)	1.23–1.25 (*m*)	29.14 (*t*)	1.09–1.10 (*m*), 1.88–1.89 (*m*)
16	24.32 (*t*)	2.22–2.25 (*m*)	24.63 (*t*)	1.98–2.00 (*m*), 1.74–1.76 (*m*)
17	47.78 (*s*)	–	47.80 (*s*)	–
18	47.77 (*d*)	3.47 (*d*, *J* = 12.6)	47.86 (*d*)	2.67 (*d*, *J* = 12)
19	35.89 (*d*)	2.41 (*d*, *J* = 12.6)	35.80 (*d*)	2.00–2.03 (*m*)
20	73.07 (*s*)	–	73.67 (*s*)	–
21	31.26 (*t*)	1.97–2.01 (*m*)	30.92 (*t*)	1.44–1.46 (*m*)
22	32.30 (*t*)	2.42–2.45 (*m*), 2.76–2.79 (*m*)	32.00 (*t*)	1.28–1.30 (*m*), 1.53–1.55 (*m*)
23	69.72 (*t*)	1.38 (*s*)	68.91 (*t*)	3.55 (*d*, *J* = 4.8), 3.65 (*d*, *J* = 4.8)
24	14.73 (*q*)	4.41 (*d*, *J* = 10.2), 4.43 (*d*, *J* = 10.2)	64.45 (*t*)	3.68 (*dd*, *J* = 10.2, 1.8), 3.77 (*dd*, *J* = 10.2, 1.8)
25	18.14 (*q*)	1.07 (*s*)	17.32 (*q*)	0.99 (*s*)
26	18.59 (*q*)	1.15 (*s*)	17.88 (*q*)	0.81 (*s*)
27	23.71 (*q*)	1.23 (*s*)	23.89 (*q*)	1.10 (*s*)
28	179.50 (*s*)	–	177.83 (*s*)	–
29	12.72 (*q*)	1.31 (*d*, *J* = 6.6)	12.29 (*q*)	0.85 (*d*, *J* = 6.6)
30	69.70 (*t*)	3.87 (*d*, *J* = 10.2), 4.16 (*d*, *J* = 10.2)	69.43 (*t*)	3.25 (*d*, *J* = 10.8), 3.53 (*d*, *J* = 10.8)
1′			95.86 (*d*)	5.30 (*d*, *J* = 7.8)
2′			74.00 (*d*)	3.31–3.34 (*m*)
3′			78.56 (*d*)	3.33–3.36 (*m*)
4′			71.30 (*d*)	3.33–3.35 (*m*)
5′			78.06 (*d*)	3.38–3.90 (*m*)
6′			62.64 (*t*)	3.77 (*dd*, *J* = 12, 1.8), 3.64 (*dd*, *J* = 12, 1.8)

[a]) Recorded at 150 MHz, multiplicity determined by DEPT. [b]) Recorded at 600 MHz.

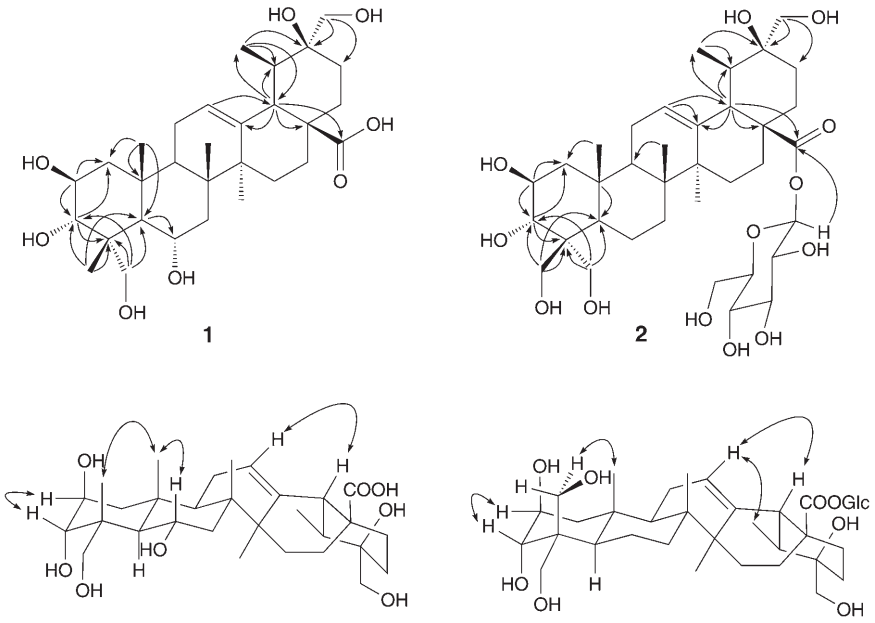

Fig. 2. *Key HMBC* (→) *and NOESY* (↔) *correlations of compound* **1** *and* **2**

Besides, H–C(5) showed a correlation with the C-atom at 66.75 ppm, which was assigned to C(6), and is oxygenated.

The HMBC spectrum further showed correlations of H–C(2) with C(1) and C(3), H–C(3) with C(1) and C(4), and CH_2(23) with C(3), which strongly supported the assignment of C(2) and C(3).

In the NOSEY spectrum (*Fig. 2*), correlations of H–C(2) and H–C(3), and H–C(6) and Me(25) were observed. Thus, the configurations of HO–C(2), HO–C(3), and HO–C(6), were determined as 2β, 3α, and 6α, respectively. The assignment of HO–CH_2(23) was supported by the correlation of one H-atom of Me(24) with Me(25) in the NOSEY spectra.

As no correlation of CH_2(30) and H–C(18) was observed in the NOSEY spectrum, the configuration of HO–C(20) was assigned as 20β, though the HO–CH_2(30) group as 20α.

The ^{1}H- and ^{13}C-NMR spectra (*Table*), in combination with HMQC, HMBC and NOSEY data (*Fig. 2*), established the structure of compound **1** as (2β,3α,6α)-2,3,6,20,23,30-hexahydroxyurs-12-en-28-oic acid.

The compound **2** was obtained as slightly yellow, amorphous powder. The compound was optically active, with $[\alpha]_D^{25} = 32.34$ ($c = 0.1$, MeOH), and had the molecular formula $C_{36}H_{58}O_{13}$, with eight degrees of unsaturation, as determined according to the ion peak at 721.3770 ($[M + Na]^+$; calc. 721.3775) in the HR-ESI-MS.

Compound **2** showed positive *Liebermann–Burchard* and *Molish* tests. The IR spectrum showed absorptions of OH (3455 cm^{-1}) and C=O (1661 cm^{-1}) groups. Analysis of the ^{13}C-NMR (DEPT) spectrum revealed 36 C-atom signals, including four

Me groups, twelve CH_2 (four of them oxygenated), eleven CH (seven of them oxygenated), one trisubstituted C=C bond, and seven quaternary C-atoms including one C=O group (*Table*).

In the ^{1}H-NMR spectrum of compound **2**, signals at 0.81 – 1.10 ppm consisting of three *singlets* and one *doublet* (0.85 ppm, *d*, $J = 6.6$ Hz), were assigned to four Me groups. In the HMQC spectrum, one H-atom *doublet* (2.67 ppm, *d*, $J = 12$ Hz) correlated with a C-atom signal at 47.86 ppm (*d*). At the same time, this H-atom correlated with the C-atoms at 35.80 (*d*), 47.80 (*s*), 12.29 (*q*), 177.83 (*s*), and a trisubstituted C=C group in the HMBC spectrum (*Fig. 2*).

The H-atom at 2.67 ppm and the C-atom at 47.86 (*d*) were assigned to H–C(18), and the C-atom at 47.80 (*s*) to C(17). The C-atom at 35.80 ppm (*d*) was assigned to H–C(19), and the signal at 177.83 ppm was assigned to C(28) (ester group). It is a key step for the structure elucidation of compound **2** that the H–C(18) was correctly assigned.

In the HMBC spectrum, a clear correlation of the Me group at 0.85 ppm (*d*, $J =$ 6.6 Hz) and 12.29 ppm (*q*) with H–C(18) and another C-atom at 73.67 ppm (*s*) was observed, thus the Me group was assigned to Me(29), and the C-atom at 73.67 ppm (*s*) was assigned to C(20), which was oxygenated.

In the HMQC spectrum, the H-atoms at 3.25 and 3.53 ppm (2*d*, $J = 10.8$ Hz) were both correlated to a secondary C-atom at 69.43 ppm; in the HMBC spectrum, they showed a correlation with H–C(19) and C(20). The above mentioned secondary C-atom at 69.43 ppm was assigned to CH_2(30).

In the HMBC spectrum, correlations of H–C(2) with CH_2(1) and H–C(3), of H–C(3) with CH_2(1) and C(4), and of CH_2(23) and CH_2(24) with H–C(3) were observed and led to the assignment of H–C(2), H–C(3), CH_2(23), and CH_2(24). In the NOSEY spectrum, correlations of H–C(2) with H–C(3), CH_2(24) with Me(25), and H–C(12) with H–C(18) were observed, and no correlation between CH_2(30) and H–C(18) was observed. Thus, the configurations of HO–C(2), HO–C(3), and HO–C(20) were determined as 2β, 3α, and 20β, respectively. The ^{1}H-NMR and ^{13}C-NMR data (*Table*) of the glycon moiety of compound **2** indicated the precence of glucose. The C-atom at 95.86 ppm (*d*) and the H-atom at 5.30 ppm (*d*, $J = 7.8$ Hz) were assigned to the anomeric H–C(1′) group. In the HMBC spectrum, a correlation of the anomeric H-atom with the C=O group indicated that glucose was connected to the 28-position. The coupling constant of 7.8 Hz implied the β-configuration of the anomeric H-atom.

The ^{1}H- and ^{13}C-NMR spectra (*Table*), in combination with HMQC, HMBC, and NOSEY data (*Fig. 2*), established the structure of compound **2** as (2β,3α)-2,3,20,23,24,30-hexahydroxyurs-12-en-28-oic acid *O*-β-D-glucopyranosyl ester.

Compounds **1** and **2** showed moderate *in vitro* cytotoxic activity against BEL-7402 (IC_{50} values of 15.5 mg and 17.2 mg, resp.) and SMMC-7721 (IC_{50} values of 16.4 mg and 9.8 mg, resp.), as determined by a classical MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay.

This work was supported by the *National Natural Science Foundation of China* (Grant No. 30572360) and *Special Project of TCM Modernization of Science and Technology Commission of Shanghai Municipality* (Grant No. 04DZ19808).

Experimental Part

General. Silica-gel plates (*Sinopharm Chemical Reagent Co., Ltd*) were used for TLC analysis. 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-600* 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 roots of *Actinidia valvata* DUNN. were collected in Changshan County, Zhejiang Province, P. R. China, in October 2006, and identified by Prof. *Han-Chen Zheng*, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University. A voucher specimen (No. 20061005) was deposited at the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The powdered plant material of the roots of *Actinidia valvata* DUNN. (30 kg) was refluxed in 80% EtOH soln. for 3 times, 1.5 h each time. The extract was concentrated under reduced pressure and resulted in a brown syrup, which was partitioned between H_2O and petroleum ether (PE), AcOEt, and BuOH successively. The AcOEt-soluble fraction (124.6 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 to afford 19 fractions (*Fr. 1–19*). *Fr. 2* was repeatedly subjected to CC (*Pharmadex LH-20* and *RP C-18*) to yield compound **1** (8.9 mg). The BuOH soluble fraction (280.6 g) was subjected to CC on SiO_2, eluting with $CHCl_3$/MeOH/H_2O 10 : 1 : 0.1, 7 : 1 : 0.1, 5 : 1 : 0.1, 3 : 1 : 0.1, 2 : 1 : 0.1 to afford 9 fractions (*Fr. 1–9*). *Fr. 3* was repeatedly subjected to CC (*Pharmadex LH-20* and *RP C-18*) to yield compound **2** (5.7 mg).

(2β,3α,6α)-2,3,6,20,23,30-Hexahydroxyurs-12-en-28-oic Acid (**1**). White amorphous powder. M.p. 256–257°. $[\alpha]_D^{25} = 56.28$ ($c = 0.1$, MeOH). IR (KBr): 3410, 2922, 2852, 1643, 1626, 1406, 1383, 1096, 1030. ^1H-NMR (600 MHz, C_5D_5N), and ^{13}C-NMR (150 MHz, C_5D_5N): *Table.* ESI-MS: 559.83. HR-ESI-MS: 559.3247 ($[M + Na]^+$, $C_{30}H_{48}NaO_8^+$; calc. 559.3247).

(2β,3α)-2,3,20,23,24,30-Hexahydroxyurs-12-en-28-oic acid O-*β*-D-*Glucopyranosyl Ester* (**2**). Slightly yellow amorphous powder. M.p. 96–98°. $[\alpha]_D^{25} = 32.34$ ($c = 0.1$, MeOH). IR (KBr): 3455, 2982, 2922, 2879, 1724, 1661, 1643, 1567, 1383, 1278, 1227, 1203, 1186, 1077, 1026. ^1H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): *Table.* ESI-MS: 721.87 (MS), 559.11 (MS^2). HR-ESI-MS: 721.3770 ($[M + Na]^+$, $C_{36}H_{58}NaO_{13}^+$; calc. 721.3775).

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Received October 17, 2007