Triterpenoids from the Flowers of *Salvia miltiorrhiza*

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A phytochemical investigation of the flowers of *Salvia miltiorrhiza* BUNGE led to the isolation of two new oleanane- and ursane-type triterpenoids, (3α)-olean-12-ene-3,23-diol (**1**) and (3α)-urs-12-ene-3,23-diol (**2**), as well as of four known triterpenoids. Their structures were elucidated on the basis of spectroscopic evidence, including 1D- and 2D-NMR, HR-MS, and the X-ray analysis, and by comparison with literature data.

Introduction. – *Salvia miltiorrhiza* BUNGE, one of the plants of the genus *Salvia* (Labiatae), is a Chinese herb used in the treatment of cardiovascular disease [1]. Dried roots of *Salvia miltiorrhiza* BUNGE (also known as Dan-Shen in Chinese, and Dansham in Korea) are an ancient Chinese drug for the treatment of hemorrhage, menstrual disorders, and swelling [2][3]. They have also been commonly used in traditional Chinese medicine for promoting blood circulation to remove blood stasis, clearing away heat, relieving vexation, nourishing blood, tranquillizing the mind, cooling the blood to relieve carbuncles, and treating hemorrhages, menstrual disorders, and miscarriages [3–5]. Dan-Shen is well known to contain abietane-type diterpenes (tanshinones), such as cryptotanshinone, tanshinone I, and tanshinone IIA [6–8]. However, only a few phytochemical investigations have been performed on the flowers of *Salvia miltiorrhiza* BUNGE. Therefore, a more systematic chemical investigation was carryied out.

Herein, we report two new oleanane- and ursane-type triterpenoids, (3α)-olean-12-ene-3,23-diol (**1**), (3α)-urs-12-ene-3,23-diol (**2**; *Fig. 1*), as well as four known com-

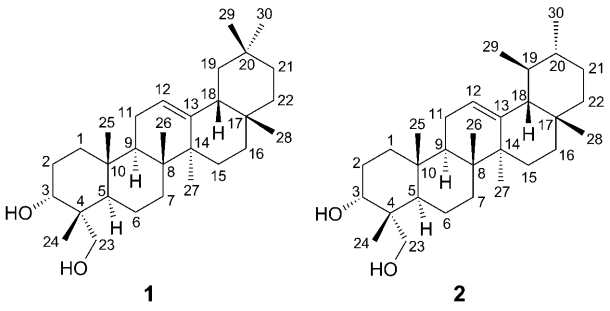

Fig. 1. *Compounds* **1** *and* **2**, *isolated from the flowers of* Salvia miltiorrhiza

pounds, oleanolic acid [9][10], ursolic acid [9][10], corosolic acid [9][10], and maslinic acid [9][10]. Their structures were elucidated on the basis of various 2D-NMR techniques, including HSQC, HMBC, ^{1}H,^{1}H-COSY, and NOESY experiments.

Results and Discussion. – Compound **1** was obtained as an optically active powder. It gave rise to a positive *Liebermann–Burchard* coloration test, indicating a triterpenoid structure. Its HR-ESI-MS exhibited a pseudo-molecular ion $[M+Na]^+$ at m/z 465.3700, which was compatible with the molecular formula $C_{30}H_{50}O_2$. The IR spectrum of **1** showed the presence of OH (3405 cm^{-1}) and an olefinic absorption (1630 cm^{-1}). From the six degrees of unsaturation, it was, thus, concluded that **1** contained five rings. The ^{1}H- and ^{13}C-NMR data of **1** indicated a pentacyclic triterpenoid, assignments being confirmed with the help of 2D-NMR (HSQC, HMBC, NOESY) experiments (*Table 1*).

Table 1. *^{13}C- and ^{1}H-NMR* (C_5D_5N; 500 and 125 MHz, resp.), *^{1}H,^{1}H-COSY, and HMBC Data of Compound* **1**. δ in ppm, J in Hz.

	δ(C)	δ(H)	^{1}H,^{1}H-COSY	HMBC
CH_2(1)	32.8 (*t*)	1.20–1.28, 1.62–1.70 (2*m*)		
CH_2(2)	26.3 (*t*)	1.43–1.50, 1.90–2.00 (2*m*)	H–C(3)	C(1), C(4), C(10), C(25)
H–C(3)	76.6 (*d*)	3.72 (br. *s*)	CH_2(2)	C(1), C(5), C(23), C(24)
C(4)	40.2 (*s*)			
H–C(5)	42.7 (*d*)	1.65 (*m*)	CH_2(6)	
CH_2(6)	18.0 (*t*)	1.35–1.43 (*m*)	H–C(5)	
CH_2(7)	32.1 (*t*)	1.50–1.67 (*m*)		
C(8)	40.0 (*s*)			
H–C(9)	47.4 (*d*)	1.72 (*m*)		
C(10)	36.7 (*s*)			
CH_2(11)	23.4 (*t*)	1.90 (*m*)	H–C(12)	C(8), C(10), C(12), C(13)
H–C(12)	121.6 (*d*)	5.21 (*t*, $J=3.5$)	CH_2(11)	C(9), C(11), C(13), C(14), C(18)
C(13)	145.3 (*s*)			
C(14)	41.8 (*s*)			
CH_2(15)	26.9 (*t*)	0.80–0.90, 1.96–2.03 (2*m*)		
CH_2(16)	26.1 (*t*)	1.00–1.05, 1.70–1.80 (2*m*)		
C(17)	32.5 (*s*)			
H–C(18)	47.2 (*d*)	1.95 (*m*)		C(12), C(13), C(17), C(19), C(21)
CH_2(19)	46.8 (*t*)	1.56 (*m*)		
C(20)	31.1 (*s*)			
CH_2(21)	34.7 (*t*)	1.20–1.44 (*m*)		
CH_2(22)	37.1 (*t*)	1.26–1.40 (*m*)		
CH_2(23)	71.4 (*t*)	3.58, 3.44 (2*d*, $J=11.3$)		C(3), C(4), C(5), C(24)
Me(24)	17.9 (*q*)	0.72 (*s*)		C(3), C(4), C(5), C(23)
Me(25)	16.8 (*q*)	0.99 (*s*)		C(1), C(5), C(9), C(10)
Me(26)	15.6 (*q*)	0.98 (*s*)		C(7), C(8), C(9), C(13)
Me(27)	26.2 (*q*)	1.17 (*s*)		C(8), C(13), C(14), C(15)
Me(28)	28.4 (*q*)	0.84 (*s*)		C(16), C(17), C(18), C(22)
Me(29)	33.3 (*q*)	0.87 (*s*)		C(19), C(20), C(21), C(30)
Me(30)	23.7 (*q*)	0.88 (*s*)		C(19), C(20), C(21), C(29)

The ^{13}C-NMR and DEPT spectra (125 MHz, $CDCl_3$) of **1** allowed the assignment of the 30 signals to seven Me, eleven CH_2, and five CH groups, as well as to seven quaternary C-atoms. The ^{1}H-NMR spectrum of **1** (*Table 1*) revealed the presence of seven Me *s* at δ(H) 0.72, 0.99, 0.98, 1.17, 0.84, 0.87, and 0.88, an O-bearing CH group signal for H–C(3) at δ(H) 3.72 (br. *s*), an O-bearing CH_2 group signal for CH_2(23) at δ(H) 3.44 and 3.58 (2 *d*, $J = 11.3$ Hz), and a *t* at δ(H) 5.21 ($J = 3.5$ Hz, H–C(12)) corresponding to the olefinic H-atom, which was correlated with the signal at δ(C) 121.6 (C(12)). The ^{13}C-NMR spectrum indicated a C=C bond (δ(H) 121.6 and 145.3), and two O-bearing C-atoms (δ(H) 76.6 (OCH), 71.4 (OCH_2)). The above mentioned data and the molecular formular pointed to a triterpenoid with an olean-12-ene skeleton [11].

The signals at δ(H) 3.44 and 3.58 (2 *d*, $J = 11.3$ Hz), which were coupled with δ(C) 71.4 (*t*, C(23)), were assigned to the CH_2(23) moiety bearing an OH function. From the ^{1}H-NMR multiplicity, this OH group could be located at one of the C-atoms C(23) to C(30). The ^{13}C-NMR signals ascribable to C(3) to C(6), C(23), and C(24) were different from those in (3β)-olean-12-ene-3,23-diol [12]. One OH group was considered to be at C(23) or C(24), based on the HMBCs of δ(H) 3.44 and 3.58 (CH_2(23) or CH_2(24)) with δ(C) 17.9 (C(24) or C(23)), 76.6 (C(3)), 40.2 (C(4)), and 42.7 (C(5)). The second OH group of compound **1** could be located at C(3) according to the HMBC cross-peaks of δ(H) 3.72 (H–C(3)) with δ(C) 17.9 (Me), 71.4 (CH_2O), 26.3 (CH_2), 40.2 (C), and 42.7 (CH). Its β-configuration was derived from its coupling pattern (br. *s*). The C=C bond in compound **1** was established to be between C(12) and C(13), as confirmed by HMBC cross-peaks of H–C(9), CH_2(11), and H–C(18) with C(12), and of H–C(9), CH_2(11), and Me(27) with C(13). The relative configuration of **1** was confirmed by a NOESY experiment (*Fig. 2*). The β-position of OH–C(3) was in accord with the NOESY correlation δ(H) 3.72 (H–C(3))/0.99 (Me(25)). The NOESY correlations Me(24)/Me(25) and Me(24)/H–C(3) indicated that the OH group was located at C(23). The structure of **1** was confirmed by the X-ray analysis[1]), as shown in *Fig. 3*. From the above evidence, the structure of compound **1** was unequivocally established as (3α)-olean-12-ene-3,23-diol (**1**; *Fig. 1*).

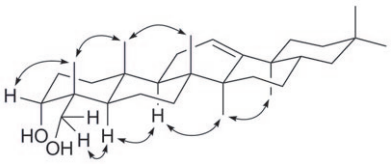

Fig. 2. *Key NOESY correlations of* **1**

Compound **2**, isolated as optically active colorless needles, gave a positive *Liebermann–Burchard* coloration test. Its molecular formula was $C_{30}H_{50}O_2$ according to a HR-ESI-MS (*m*/*z* 465.3698 ($[M + Na]^+$)), corresponding to six degrees of unsaturation. The IR spectrum of compound **2** showed the presence of OH groups (3406 cm^{-1}). The ^{1}H-NMR (*Table 2*) revealed five *s* for Me groups at δ(H) 0.72, 0.98, 0.98, 1.04, and 0.83, two Me *d* at δ(H) 0.82 (*d*, $J = 6.5$ Hz) and 0.94 (*d*, $J = 6.3$ Hz), an

[1]) CCDC-771805 contains the supplementary crystallographic data of **1**. These data can be obtained, free of charge, *via* http://www.ccdc.cam.ac.uk/data_request/cif.

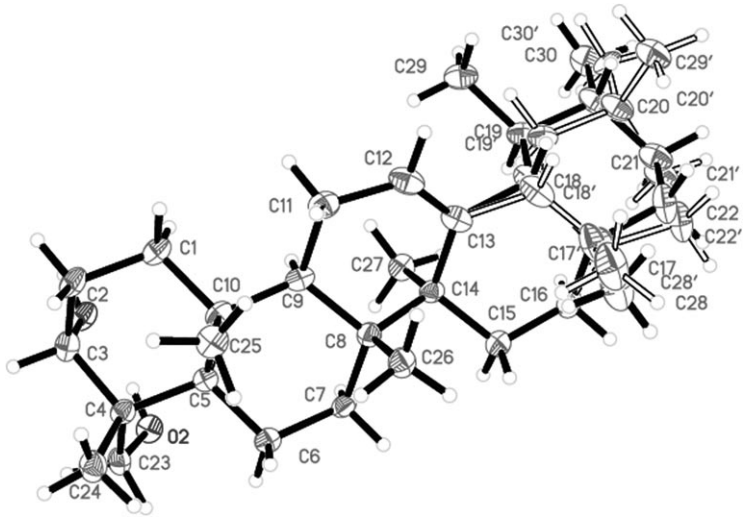

Fig. 3. *Single-crystal X-ray structure of compounds* **1** *and* **2**

O-bearing CH group signal for H–C(3) at δ(H) 3.72 (br. *s*), an O-bearing CH_2 group signal for CH_2(23) at δ(H) 3.44 and 3.58 (2 *d*, $J = 11.3$ Hz), and an olefinic H-atom signal for H–C(12) at δ(H) 5.16 (*t*, $J = 3.5$ Hz). The ^{13}C-NMR spectrum indicated a C=C bond (δ(C) 124.3 and 139.7), and two O-bearing C-atoms (δ(C) 76.6 (CH–O), 71.4 (CH_2O)). The above evidence suggested that compound **2** is a triterpenoid with an urs-12-ene skeleton, an isomer of compound **1**. Careful comparison of the ^{13}C-NMR data of **2** with those of **1** showed that the signals for C(1) to C(11) and C(23) to C(26) were basically identical in both compounds, which indicated identical rings *A*, *B*, and *C*. To confirm the structure of compound **2** in ring *E*, the known triterpenoid (3β)-urs-12-ene-3,24-diol [13] was used as a reference compound. The signals of C(12) to C(22) and of C(27) to C(30) of **2** matched well those of this known triterpenoid, which suggested that their rings *C*, *D*, and *E* were similar. The NMR-data assigments were corroborated by HSQC, HMBC, and NOESY data (*Table 2*). Thus, based on the above evidence, the structure of compound **2** was elucidated as (*3*α)-urs-12-ene-3,23-diol (**2**; *Fig. 1*).

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Co., Ltd.*) and *Toyopearl HW-40* (*Tosoh*). TLC: silica gel GF_{254} plates; visualization under UV light and by spraying with Ce_2SO_4 or phosphomolybdic acid hydrate, followed by heating. HPLC: *Waters 600* with *Waters TP* pump and *UV-2487* detector (*Waters*, USA); *ODS* column (*YMC-Pack ODS-A*, *SH-343-5*; *YMC*, Tokyo, Japan), mobile phase MeOH/H_2O. Optical rotations: *Perkin-Elmer-241-MC* digital polarimeter. IR Spectra: *Perkin-Elmer-577* spectrometer; in cm^{-1}. NMR Spectra: *Bruker-AV-300* instrument; at 500 (^{1}H) and 125 MHz (^{13}C)); δ in ppm rel. to Me_4Si, *J* in Hz. HR-ESI-MS: *Waters-LCT-Premier* instrument; in *m*/*z*.

Table 2. *^{13}C- and ^{1}H-NMR* (C_5D_5N; 500 and 125 MHz, resp.), *$^{1}H,^{1}H$-COSY, and HMBC Data of Compound* **2**. δ in ppm, J in Hz.

	δ(C)	δ(H)	^{1}H,^{1}H-COSY	HMBC
CH_2(1)	32.4 (*t*)	1.20–1.28 1.62–1.70 (2*m*)		
CH_2(2)	26.4 (*t*)	1.45–1.50, 1.90–2.00 (2*m*)	H–C(3)	C(1), C(4), C(10), C(25)
H–C(3)	76.6 (*d*)	3.72 (br. *s*)	CH_2(2)	C(1), C(5), C(23), C(24)
C(4)	40.2 (*s*)			
H–C(5)	42.6 (*d*)	1.65 (*m*)	CH_2(6)	
CH_2(6)	18.1 (*t*)	1.35–1.43 (*m*)	H–C(5)	
CH_2(7)	33.0 (*t*)	1.50–1.67 (*m*)		
C(8)	40.1 (*s*)			
H–C(9)	47.5 (*d*)	1.72 (*m*)		
C(10)	36.7 (*s*)			
CH_2(11)	23.3 (*t*)	1.95 (*m*)	H–C(12)	C(8), C(10), C(12), C(13)
H–C(12)	124.3 (*d*)	5.16 (*t*, J = 3.5)	CH_2(11)	C(9), C(11), C(13), C(14), C(18)
C(13)	139.7 (*s*)			
C(14)	42.2 (*s*)			
CH_2(15)	28.1 (*t*)	0.78–0.85, 0.96–2.03 (2*m*)		
CH_2(16)	26.5 (*t*)	0.90–1.00, 1.70–1.75 (2*m*)		
C(17)	33.7 (*s*)			
H–C(18)	59.0 (*d*)	1.32 (*m*)		C(12), C(13), C(17), C(19),C(21)
H–C(19)	39.6 (*d*)	1.28 (*m*)		
H–C(20)	39.6 (*d*)	1.41 (*m*)		
CH_2(21)	31.2 (*t*)	1.25–1.30 (*m*)		
CH_2(22)	41.6 (*t*)	1.27–1.41 (*m*)		
CH_2(23)	71.4 (*t*)	3.58, 3.44 (2*d*, J = 11.3)		C(3), C(4), C(5), C(24)
Me(24)	18.0 (*q*)	0.72 (*s*)		C(3), C(4), C(5), C(23)
Me(25)	16.9 (*q*)	0.98 (*s*)		C(1), C(5), C(9), C(10)
Me(26)	15.8 (*q*)	1.04 (*s*)		C(7), C(8), C(9), C(13)
Me(27)	23.4 (*q*)	1.13 (*s*)		C(8), C(13), C(14), C(15)
Me(28)	28.7 (*q*)	0.83 (*s*)		C(16), C(17), C(18), C(22)
Me(29)	17.5 (*q*)	0.82 (*d*, J = 6.5)		C(18), C(19), C(20), C(30)
Me(30)	21.3 (*q*)	0.94 (*d*, J = 6.3)		C(19), C(20), C(21), C(29)

Plant Material. Flowers of *Salvia miltiorrhiza* BUNGE were collected in Songxian County, Henan Province, P. R. China, in April 2007. The plant was identified by Prof. *Zhongdong Wang*, Chemical Engineering & Pharmaceutical College, Henan University of Science and Technology, P. R. China. A voucher specimen (No. N20071008) was deposited at the Chemical Engineering & Pharmaceutical College, Henan University of Science and Technology, Luoyang, P. R. China.

Extraction and Isolation. The dried flowers (3.2 kg) of *P. umbrosa* were crushed and then extracted with 95% aq. EtOH (12 l) for 4 h under reflux (3 ×). The pooled EtOH solns. were concentrated, and the resulting residue (180 g) was suspended in H_2O and then successively extracted with petroleum ether, AcOEt, and BuOH.

The petroleum ether fraction afforded, upon evaporation, a residue (50 g), which was further separated by CC (SiO_2 (1 kg), petroleum ether/AcOEt 10 : 1, 8 : 1, 6 : 1, 4 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3, and 0 : 1, then AcOEt/MeOH 19 : 1, 10 : 1, 5 : 1, and 0 : 1): *Fractions 1–20* (by TLC). *Fr. 6* (2300 mg) was subjected to CC (*Toyopearl HW-40*, $CHCl_3$/MeOH 2 : 1), and then purified by HPLC (*ODS-A*, MeOH/H_2O 2 : 8, 3.0 ml/min): **1** (25.6 mg) and **2** (17.8 mg). *Fr. 10* (1.5 g) was subjected to HPLC (*ODS-A*; MeOH/H_2O 2 : 8, 3.0 ml/min): oleanolic acid and ursolic acid.

The AcOEt fraction, after evaporation, was further separated by CC (SiO_2 (2 kg), $CHCl_3$/MeOH 9:1, 8:1, 7:1, 4:1, 2:1, and 0:1): *Fractions 1–18* (by TLC). *Fr. 5* (3510 mg) was subjected to CC (*Toyopearl HW-40*; $CHCl_3$/MeOH 2:1): *Frs. 6.1–6.6*. *Fr. 6.4* (710 mg) was purified by HPLC (*ODS-A*, MeOH/H_2O 3:7, 3.0 ml/min): corosolic acid (12.3 mg) and maslinic acid.

(3α)-Olean-12-ene-3,23-diol (**1**): Amorphous powder. $[\alpha]_D^{25} = +8.61$ ($c = 0.74$, $CHCl_3$). IR (KBr): 3405, 2933, 1630, 1451, 1375, 1262, 1040. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 465.3700 ($[M+Na]^+$, $C_{30}H_{50}NaO_2^+$; calc. 465.3705).

(3α)-Urs-12-ene-3,23-diol (**2**): Colorless needles. $[\alpha]_D^{25} = +10.1$ ($c = 0.67$, $CHCl_3$). IR (KBr): 3404, 2933, 1632, 1451, 1375, 1262, 1043. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 465.3698 ($[M+Na]^+$, $C_{30}H_{50}NaO_2^+$; calc. 465.3705).

X-Ray Crystallographic Analysis of Compound **1**. Formula, $C_{30}H_{50}O_2$, M_r 442.70; monoclinic, space group $P2_12_12_1$, $a = 9.2554(7)$ Å, $b = 12.8342(9)$ Å, $c = 23.016(2)$ Å, $V = 2733.9(4)$ Å^3, $Z = 4$, $d = 1.076$ g/cm^3; crystal dimensions, $0.26 \times 0.20 \times 0.18$ mm. The measurements were performed on a *SMART* (*Bruker*, 1997). The total number of independent and observed reflections was 2740. The crystal structure was resolved and refined by direct methods with SHELXS-97 [14][15]. Final indices: $R = 0.0616$, $R_w = 0.134$. H-Atoms were fixed at their calculated positions.

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