Antimicrobial Triterpenoids from *Vladimiria muliensis*

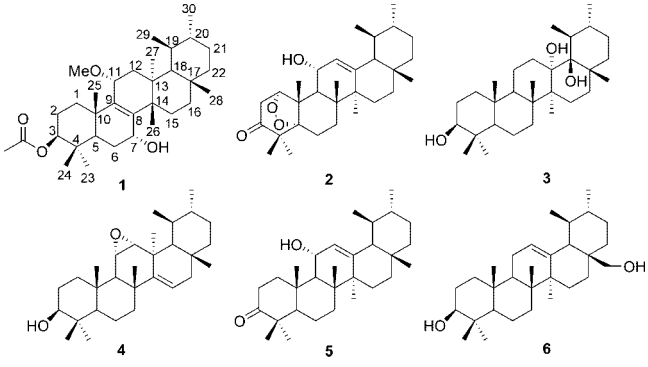
Jian-Jun Chen, Dong-Qing Fei, Sheng-Gao Chen, and Kun Gao*

State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of China

Received September 10, 2007

A new triterpenoid with a rearranged ursane skeleton, 3β-acetoxyl-11α-methoxybauer-8-en-7α-ol (**1**), two new ursane triterpenoids, 1α,5α-dioxy-11α-hydroxyurs-12-en-3-one (**2**) and urs-3β,13α,18β-triol (**3**), together with three known ursane triterpenoids (**4**–**6**) were isolated from the rhizome of *Vladimiria muliensis*. Their structures were elucidated on the basis of spectroscopic methods (IR, EIMS, HRESIMS, 1D and 2D NMR, and X-ray crystallography diffraction). Compounds **2** and **4** exhibited modest antimicrobial activity against *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Bacillus cereus*, and *Staphylococcus aureus*.

The genus *Vladimiria* (Compositae) consists of about 12 species, distributed mainly in southwestern China.[1] The roots of most *Vladimiria* species, especially *V. denticulata* and *V. berardioides*, have been used as traditional Chinese medicine to alleviate pain in abdominal distension, curing indigestion, dysentery, and nausea, and arresting vomiting.[2,3] The characteristic components of these species are sesquiterpenoids,[4,5] triterpenoids, lignans, and phenylpropanoids.[6,7] *Vladimiria muliensis* (H.-M.) Ling, instead of *V. denticulata*, is used by local inhabitants to treat the above symptoms.[2] However, there has been no report about the chemical constituents of *V. muliensis*. In a continuation of our efforts to find new natural antibacterial products from Chinese herb medicine,[8] we studied the dried rhizome of *V. muliensis*, collected in the Muli Autonomic County of Sichuan, China. A new triterpenoid with a rearranged ursane skeleton (**1**), two new (**2** and **3**), and three known ursane triterpenoids (**4**–**6**) were obtained. The antimicrobial activities of **1**–**6** were determined against *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Bacillus cereus*, and *Staphylococcus aureus*. Compounds **2** and **4** showed modest activity, with the former giving an MIC of 14.9 μM/L against *S. aureus*. The structure of compound **1** was confirmed by X-ray crystallography.



Results and Discussion

Compound **1** was obtained as colorless crystals. Its EIMS spectrum revealed a molecular ion peak at *m/z* 514, and the molecular formula, $C_{33}H_{54}O_4$, was determined by HRESIMS (*m/z* 537.3910 $[M + Na]^+$, calc 537.3914). Compound **1** had absorption bands at 3411 cm^{-1} (hydroxy), 1716 cm^{-1} (carbonyl), and 1655 cm^{-1} (olefinic) in the IR spectrum. The ^1H and ^{13}C NMR (DEPT) spectra of **1** displayed signals of six tertiary methyl, two secondary methyl, eight methylene, seven methine (three oxygenated), and seven quarternary (two sp^2 hybridized) carbon atoms, which indicated that compound **1** was a pentacyclic triterpenoid possessing a tetrasubstituted double bond (δ 141.4 and 139.0) and three oxygen-bearing functional groups. These three functional groups were deduced to be one acetoxy (δ_H 2.05, δ_C 21.3 and 170.8), one methoxy (δ_H 3.21, δ_C 55.2), and one hydroxy.

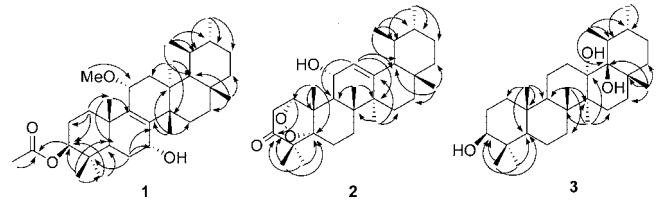


Figure 1. Important HMBC correlations of compounds **1**, **2**, and **3**.

The partial structure of **1** was solved by the HMBC correlations of the protons of the eight methyl groups with the adjacent carbon atoms (Figure 1), especially the correlations of CH_3-27 (δ 0.93) with C-12 (δ 33.8), C-13 (δ 37.0), C-14 (δ 43.9), and C-18 (δ 52.6), which suggested that C-27 had migrated to C-13 from C-14, and C-26 had migrated to C-14 from C-8 of an ursane-type triterpenoid. Thus, **1** was a pentacyclic triterpenoid with a bauerane skeleton.[9,10] Detailed analysis of other correlations in the HMBC spectrum (Figure 1) determined the locations of the following substituents: an acetoxy group at C-3, a methoxy group at C-11, a hydroxy group at C-7, and a double bond between C-8 and C-9.

The relative configuration of **1** was elucidated by the ^1H NMR coupling constants and the NOE experiments. The $^3J_{2,3}$ values (dd, $J = 11.4$, 4.2 Hz) showed that H-3 had an α-axial orientation. The strong NOEs observed between H-11 (δ 3.48) and H-12β (δ 1.88) (4%), H-25 (δ 0.92) (7%), and H-26 (δ 1.02) (4%) and between H-7 (δ 4.20) and H-25 (δ 0.92) (3%), and H-26 (δ 1.02) (4%) indicated that H-7 and H-11 were unambiguously in the β-orientation. Thus, compound **1** was assigned as 11α-methoxy-3β-acetoxybauer-8-en-7α-ol. This new structure was confirmed by a single-crystal X-ray diffraction study (Figure 2).

Compound **2** was obtained as a white powder, and its molecular formula, $C_{30}H_{46}O_4$, was established from HRESIMS (*m/z* 493.3281 $[M + Na]^+$, calc 493.3288), ^{13}C NMR, and DEPT spectra (Table 2). Compound **2** showed absorption bands at 3411 cm^{-1} (hydroxy), 1712 cm^{-1} (carbonyl), and 1660 cm^{-1} (olefinic) in its IR spectrum. Six methyl singlets (δ 0.83, 0.96, 1.04, 1.16, 1.20, and 1.28), two methyl doublets [δ 0.89 (d, $J = 6.0$ Hz), 0.95 (d, $J = 6.0$ Hz)] and the other protons in the ^1H NMR spectrum of **2** (Table 1) were similar to those of the known compound **5**, which indicated that **2** was a derivative of the pentacyclic triterpenoid 11-hydroxyurs-12-

* To whom correspondence should be addressed. Tel: +86-0931-8912592. Fax: +86-931-8912582. E-mail: npchem@lzu.edu.cn.

10.1021/np0704831 CCC: $40.75
Published on Web 02/23/2008

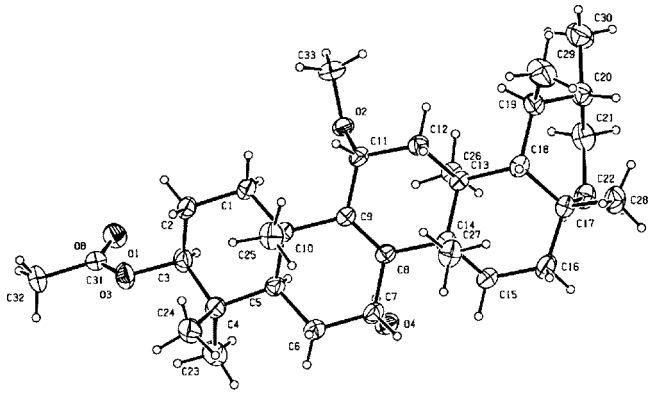


Figure 2. ORTEP drawing of **1**. The solvent molecules are not shown.

Table 1. ^{1}H NMR Data of **1**−**3** (300 MHz (**1**, **3**) or 400 MHz (**2**) in $CDCl_3$; δ in ppm, J in Hz)[a,b]

position	1	2	3
1α	1.48 m	4.27 t (2.8)	0.91 m
1β	1.52 m		1.72 m
2α	1.57 m	2.83 dd (18.8, 2.8)	1.55 m
2β	1.42 m	2.58 br d (18.8)	
3	4.58 dd (11.4,4.2)		3.21 dd (11.1, 5.1)
5	1.70 m		0.79 m
6α	1.97 m	2.06 m	1.54 m
6β	1.93 m	1.86 m	
7	4.20 br s	1.36 m	1.37 m
9		2.55 d (10.4)	1.42 m
11α	3.48 br d (4.8)	4.22 dd (10.4, 2.8)	1.27 m
11β			1.52 m
12α	1.93 m	5.26 d (2.8)	0.90 m
12β	1.88 m		1.64 m
15α	1.36 m	1.68 m	1.58 m
15β	1.24 m	1.11 m	1.08 m
16	1.28 m	1.54 m	1.32 m
18	1.28 m	1.25 m	
19	1.13 m	1.46 m	1.10 m
20	1.24 m	2.06 m	1.61 m
21α	1.38 m	1.28 m	1.16 m
21β	1.48 m	1.52 m	1.58 m
22α	1.42 m	1.50 m	1.64 m
22β	1.53 m	1.61 m	1.86 m
23	0.93 s	1.16 s	0.75 s
24	0.89 s	1.20 s	0.97 s
25	0.92 s	1.04 s	0.82 s
26	1.02 s	0.96 s	1.02 s
27	0.93 s	1.28 s	0.82 s
28	1.06 s	0.83 s	1.04 s
29	1.03 d (6.5)	0.89 d (6.0)	0.94 d (7.2)
30	0.94 d (7.2)	0.95 d (6.0)	1.05 d (6.9)
OAc	2.05 s		
OMe	3.21 s		

[a] Assigned by ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. [b] Some ^{1}H peaks overlapped in the range δ 1.2–2.2; hence the coupling constants could not be measured.

en-3-one.[11] The carbon signals of **2** further confirmed the conclusion due to the presence of an allylic alcohol moiety (C=CH−CHOH) at C-11 (δ 68.2, CH), C-12 (δ 128.0, CH), and C-13 (δ 142.5, C), which was similar to that of **5**. The most significant differences in the ^{13}C NMR spectra of **2** and **5** were that two downfield oxygenated carbons at δ 84.9 (CH) and 90.7 (C) appeared in **2** instead of a methylene carbon at δ 39.4 and a methine carbon at δ 55.5 in **5**. From the molecular formula, $C_{30}H_{46}O_4$, the unsaturation degree of **2** was eight; thus, apart from one carbonyl and one olefinic functionality, the number of rings in **2** should be six, one of which could be attributed to the presence of a peroxide bridge. In the HMBC spectrum of **2**, the correlations (Figure 1) of H-1 (δ 4.27) with C-3 (δ 215.1); H-9 (δ 2.55) with C-1 (δ 84.9), C-7 (δ 41.2), C-10 (δ 44.0), and C-25 (δ 15.9); H-23 (δ 1.16) with C-5 (δ 90.7), C-4 (δ 52.8), C-3 (δ 215.1), and C-24 (δ 20.4); H-24 (δ 1.20) with C-5 (δ 90.7), C-4 (δ 52.8), C-3 (δ 215.1), and C-23 (δ 22.6); and H-25 (δ 1.04) with C-1 (δ 84.9), C-5 (δ 90.7), C-9 (δ 50.1), and C-10 (δ 44.0) indicated a peroxide moiety attached to C-1 and C-5. H-1 and H-11 were assigned β-orientations because of the NOE correlations between H-1 (δ 4.27) and H-25 (δ 1.04) (6%) and between H-11 (δ 4.22) and H-25 (δ 1.04) (8%), and H-26 (δ 0.96) (4%). On the basis of the above evidence, the structure of compound **2** was determined as 1α,5α-dioxy-11α-hydroxyurs-12-en-3-one.

Table 2. ^{13}C NMR Data of **1**−**3** (75 MHz (**1**, **3**) or 100 MHz (**2**) in $CDCl_3$; δ in ppm)[a]

position	1	2	3
1	24.4, CH_2	84.9, CH	38.7, CH_2
2	25.8, CH_2	29.7, CH_2	27.4, CH_2
3	80.7, CH	215.1, C	78.9, CH
4	37.0, C	52.8, C	38.7, C
5	43.7,CH	90.7, C	55.1, CH
6	28.8, CH_2	21.1, CH_2	18.4, CH_2
7	63.9, CH	41.2, CH_2	33.3, CH_2
8	141.4, C	40.3, C	41.1, C
9	139.0,C	50.1, CH	50.5, CH
10	39.4, C	44.0, C	37.1, C
11	73.0, CH	68.2, CH	21.0, CH_2
12	33.8, CH_2	128.0, CH	23.2, CH_2
13	37.0, C	142.5, C	76.6, C
14	43.9, C	42.2, C	41.8, C
15	32.3, CH_2	26.6, CH_2	34.5, CH_2
16	37.9, CH_2	41.3, CH_2	29.2, CH_2
17	31.5, C	33.9, C	43.2, C
18	52.6, CH	59.2, CH	75.8, C
19	36.0, CH	39.5, CH	28.5, CH
20	38.5, CH	40.3, CH	28.4, CH
21	29.6, CH_2	31.1, CH_2	23.2, CH_2
22	33.5, CH_2	28.0, CH_2	38.7, CH_2
23	27.9, CH_3	22.6, CH_3	28.0, CH_3
24	16.7, CH_3	20.4, CH_3	15.4, CH_3
25	20.3, CH_3	15.9, CH_3	15.9, CH_3
26	18.8, CH_3	16.0, CH_3	16.5, CH_3
27	22.9, CH_3	23.1, CH_3	17.9, CH_3
28	32.0, CH_3	28.7, CH_3	18.4, CH_3
29	25.4, CH_3	17.3, CH_3	15.9, CH_3
30	21.3, CH_3	26.1, CH_3	19.1, CH_3
OAc	21.3, CH_3, 170.8, C		
OMe	55.2, CH_3		

[a] Assigned by ^{13}CNMR, DEPT, HMQC, and HMBC spectra.

Compound **3** was obtained as a white powder. Its ^{13}C NMR and DEPT spectra showed 30 carbon signals including six methyl singlets, two methyl doublets, 10 methylene, five methine (one oxygenated), and seven quarternary carbon atoms (two oxygenated) (Table 2). The carbon framework signals of **3** were more similar to those of **2** rather than **1**, especially for C-14 and C-26, which indicated that **3**, like **2**, was an ursane-type pentacyclic triterpenoid with three hydroxy groups. The presence of hydroxy groups was confirmed by its IR spectrum due to a very strong absorption at 3431 cm^{-1}. The ^{1}H NMR spectrum of **3** was similar to those of the known ursan-3β-ol and with six methyl singlets at δ 0.75, 0.82 (× 2), 0.97, 1.02, and 1.04, two methyl doublets at δ 0.94 (d, J = 7.2 Hz) and 1.05 (d, J = 6.9 Hz),[12,13] and a hydroxy-bearing methine proton at δ 3.21 (dd, J = 11.1, 5.1 Hz). Comparison of the ^{13}C NMR data of **3** with those of ursan-3β-ol showed that the chemical shifts of both were similar, except those of the D-ring carbon atoms, and C-27 and C-28: two oxygen-bearing quarternary carbon signals appeared at δ 76.6 and 75.8 in **3**, instead of two methine signals at δ 39.2 (C-13) and 58.1 (C-18) in ursan-3β-ol. The information mentioned above revealed that there was a 3β-hydroxy group in **3**, and the two other hydroxy groups were attached to C-13 and C-18, respectively. In the HMBC spectrum of **3** (Figure 1), the correlations of H-27 (δ 0.82) with C-8 (δ 41.1), C-14 (δ 41.8), C-13 (δ 76.6), and C-15 (δ 34.5); H-28 (δ 1.04) with C-16

Table 3. Antibacterial Activity of Compounds **1**−**6**

	MIC(μM/L)					
compound	*E. coli*	*C. albicans*	*P. aeruginosa*	*E. faecalis*	*B. cereus*	*S. aureus*
1	89.5	89.5	44.7	89.5	178.9	89.5
2	31.9	31.9	31.9	31.9	63.8	14.9
3	140.0	69.6	140.0	50.0	140.0	69.6
4	36.3	36.3	36.3	36.3	72.7	72.7
5	70.4	70.4	70.4	35.2	70.4	35.2
6	142.5	282.8	282.8	142.5	282.8	70.1
ofloxacin	0.43	NT[a]	0.43	0.86	0.86	0.43

[a] NT, not tested.

(δ 29.2), C-17 (δ 43.2), C-18 (δ 75.8), and C-22 (δ 38.7); and H-29 (δ 0.94) with C-18 (δ 75.8), C-19 (δ 28.5), and C-20 (δ 28.4) provided further evidence for the above.

The relative configurations of C-13 and C-18 were determined as follows: the reaction between **3** and anhydrous acetone did not occur at all, in spite of catalysis by pyridinium *p*-toluenesulfonate,[14] which suggested that the two hydroxy groups had opposite orientations. Furthermore, 13-OH was deduced to be in the α-orientation and 18-OH in the β-orientation due to the upfield shift of C-27 and C-28 from δ 23.3 and 28.1 in ursan-3β-ol to δ_C 17.9 and 18.4 in **3** in the ^{13}C NMR spectrum, just as the chemical shift of C-24 (δ 15.4) was more upfield than that of C-23 (δ 28.0) on account of the field effect of 3β-OH. Therefore, the structure of **3** was determined as urs-3β,13α,18β-triol.

Compounds **4**–**6** were identified as 11α,12α-epoxy-D-friedours-14-en-3β-ol,[15] 11α-hydroxyurs-12-en-3-one,[11] and urs-12-ene-3β,28-diol,[16] respectively, by comparing their spectroscopic data with literature values.

The antimicrobial activities of triterpenoids **1**−**6** were tested against *E. coli*, *C. albicans*, *P. aeruginosa*, *E. faecalis*, *B. cereus*, and *S. aureus*. The results (Table 3) indicated that compounds **2** and **4** possessed modest activity against the six kinds of microorganisms. Compound **5** showed modest activity, comparable with that of **2** and **4**, against *E. faecalis* and *S. aureus.* However, compounds **1**, **3**, and **6** exhibited only weak activity against the microorganisms.

It should be emphasized that compound **2** exhibited selective activity against *S. aureus* with an MIC value of 14.9 μM/L and gave the lowest MIC against a wide range of test microorganisms used. Compound **4** gave an inhibition value almost the same as those of **2** against *E. coli*, *C. albicans*, *P. aeruginosa*, and *E. faecalis*. The difference in activities among compounds **1**–**6** may stem from the different substituents. Compound **2** has a peroxide bond, a carbonyl group, and an allylic alcohol group, and compound **4**, an epoxy, a hydroxy group, and double bond, while compound **5** possesses a carbonyl group and an allylic alcohol group, which suggested that the peroxide and epoxy functionalities may be active moieties for antimicrobial activity. At the same time, the result showed that triterpenoids could be antimicrobial-active components of *V. muliensis*.

Experimental Section

General Experimental Procedures. Melting points were determined on an X-4 digital display micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer. NMR spectra were recorded on Varian Mercury-300BB NMR (300 MHz) and Varian Mercury plus-400 (400 MHz) spectrometers with TMS as internal standard. EIMS data were obtained on an HP5988AGCMS spectrometer. HRESIMS data were measured on a Bruker Daltonics APEX II 47e spectrometer. The X-ray crystallographic data were collected on a Bruker Smart CCD diffractometer using graphic-monochromated Mo Kα radiation. Silica gel (200–300 mesh) used for CC and silica gel GF_{254} (10–40 μ) used for TLC were supplied by Qingdao Marine Chemical Factory, Qingdao, P. R. China. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

Plant Material. The rhizome of *V. muliensis* was collected from Muli Autonomic County of Sichuan, China, in August 2004, and authenticated by Prof. Guoliang Zhang from the School of Life Science, Lanzhou University. A specimen (No. 20040814) was deposited at the Natural Product Laboratory of the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The air-dried rhizome of *V. muliensis* (6.25 kg) was pulverized and extracted with MeOH (× 3, 7 days each time) at room temperature. The solvent was evaporated under reduced pressure to give an extract (618 g), which was suspended in hot H_2O (60 °C, 1500 mL). This suspension was extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The petroleum ether soluble fraction was concentrated under reduced pressure to afford a residue (190 g), which was subjected to Si gel column chromatography (200–300 mesh, 1200 g) with a gradient of petroleum ether−EtOAc (100:0, 50:1, 30:1, 10:1, 5:1, 2:1) as eluent, and six fractions (A, B, C, D, E, and F) were collected according to TLC analysis. Fraction D (2.3 g) was separated on a Si gel column (200–300 mesh, 50 g) eluting with $CHCl_3$−EtOAc (20:1) to afford crude crystals of **1**, which were recrystallized from MeOH−acetone to give **1** (20 mg). The EtOAc-soluble fraction was concentrated under reduced pressure to afford a residue (180 g), which was subjected to Si gel column chromatography (200–300 mesh, 1200 g) with a gradient of petroleum ether−acetone (100:0, 50:1, 30:1, 10:1, 5:1, 2:1) as eluent, and six fractions were collected according to TLC analysis. The petroleum ether−acetone fraction of elution (30:1, 20 g) was separated on a Si gel column (200–300 mesh, 100 g) with petroleum ether−EtOAc (10:1, 5:1, 2:1) and a mixture was obtained. The mixture (150 mg) was further separated on a Si gel column (200–300 mesh, 3 g) eluting again with petroleum ether−acetone (15:1) to give compound **3** (14 mg). The petroleum ether−acetone fraction (10:1, 8 g) was separated on a Si gel column (200–300 mesh, 100 g) with petroleum ether−EtOAc (10:1) as eluent to obtain **6** (1.2 g) and a mixture of **2**, **4**, and **5** (60 mg). The mixture was separated by Si gel column chromatography (200–300 mesh, 2 g) with $CHCl_3$−EtOAc (20:1) to obtain **2** (2 mg), **4** (3 mg), and **5** (10 mg).

11α-Methoxy-3β-acetoxybauer-8-en-7α-ol (1): colorless crystals; mp 250–251 °C (MeOH−acetone). $[\alpha]^{20}_D$ −6 (*c* 0.10, $CHCl_3$); IR(KBr) ν_{max} 3411, 1716, 1655 cm^{-1}; ^{1}H NMR (300 Hz) see Table 1; ^{13}C NMR (DEPT) (75 Hz) see Table 2; EIMS *m*/*z* (rel int) 514 $[M]^+$ (4), 496 (3), 483 (1), 397 (3), 289 (2), 278 (2), 173 (2), 149 (3), 135 (5), 123 (11), 109 (9), 84(28), 43 (100). HRESIMS *m*/*z* 537.3910 ($[M + Na]^+$, calcd for $C_{33}H_{54}O_4Na$, 537.3914).

1α,5α-Dioxy-11α-hydroxyurs-12-en-3-one (2): colorless solid; mp 223–224 °C (acetone); $[\alpha]^{20}_D$ +26 (*c* 0.10, $CHCl_3$); IR(KBr) ν_{max} 3410, 1712, 1660 cm^{-1}; ^{1}H NMR (400 Hz) see Table 1; ^{13}C NMR (DEPT) (100 Hz) see Table 2; EIMS *m*/*z* (rel int) 454 (10), 436 (4), 340 (9), 325 (3), 231 (4), 161 (6), 121 (15), 107 (20), 69 (4), 55 (6), 43 (100); HRESIMS *m*/*z* 493.3281 ($[M + Na]^+$, calcd for $C_{33}H_{54}O_4Na$, 493.3288).

Urs-3β,13α,18β-triol (3): colorless solid; mp 213–215 °C (acetone); $[\alpha]^{20}_D$ +10 (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3431 cm^{-1}; ^{1}H NMR (300 Hz) see Table 1; ^{13}C NMR (DEPT) (75 Hz) see Table 2; EIMS *m*/*z* (rel int) 442 (4), 207 (3), 189 (4), 175 (3), 161 (7), 152 (26), 135 (17), 121 (16), 109 (21), 95 (27), 81 (39), 71 (48), 55 (42), 43 (100); HRESIMS *m*/*z* 461.3985 ($[M + H]^+$, calcd for $C_{30}H_{53}O_3$, 461.3989).

X-ray Crystal Structure Analysis of 1.[17] A crystal of **1** with dimensions 0.31 × 0.19 × 0.10 mm was selected for X-ray analysis. Structure analysis was performed using the SHELEXTL-97 program on a PC. Data were collected over a hemisphere of reciprocal space, by a combination of three sets of exposures. The compound crystallized in the monoclinic space group *C*2, with a = 32.341(14) Å, b = 8.429(3) Å, c = 12.051(5) Å, β = 95.82(3)°, V = 3268(2) Å^3, Z = 4, D_{calc} = 1.156 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.079 mm^{-1}, F(000) = 1244, and T = 294(2) K. The SMART program was used to make data corrections. A total of 8787 reflections, collected in the range 1.70° ≤ θ ≤ 25.50°, yielded 5712 unique reflections. The structure was solved using direct methods and was refined by full-matrix least-squares on F^2 values for 3572 $I > 2\sigma(I)$. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R = 0.0608, R_w = 0.0829 with goodness-of-fit = 1.856. Scattering factors were taken from *International Tables for X-ray Crystallography*.[18]

Antimicrobial Assay. The antimicrobial assay was done by the cup-plate method. Sample solutions (0.1–500 μM/L) were prepared by dissolving each compound in DMSO. Ofloxacin (purchased from Beijing Second Pharmaceutical Factory, Beijing, China) was used as a positive control. *S. aureus*, *E. coli*, *P. aeruginosa*, *B. cereus,* and *E. faecalis* were cultured in MH agar plates at 37 °C for 24 h; *C. albicans* was cultured in PDA agar plates at 28 °C for 48 h (all bacteria were purchased from Heilongjiang Province Microorganism Research Institute, Heilongjiang, China). The cultures were then diluted and adjusted to about 1×10^7 CFU/mL in agar medium in dishes. A series of sterile blank cups (8 mm × 10 mm) were placed onto the agar surface, and sample solutions of different concentration were transfused into the cups (each 0.2 mL). The dishes were incubated according to the above conditions. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC). At the lowest concentration of the sample, the microorganisms did not show growth as judged by the presence of turbidity, and MTT was used to measure absorbance at 570 nm. Each test was performed in duplicate and repeated three times.

Acknowledgment. Financial support of the National Basic Research Program of China (No. 2007CB108903) and the National Natural Science Foundation of China (No. 20672052 and No. 20621091) is gratefully acknowledged.

Supporting Information Available: X-ray crystallographic data for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Chen, S. K.; Li, H.; Chen, P. Y. In *Chinese Flora (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, China, 1997; Vol. 78, pp141–153..
(2) Jiangsu New Medical College. *Dictionary of Chinese Herbal Drugs*; Shanghai Science and Technology Press: Shanghai, 1988; pp 353–355..
(3) Zhu, Z. Y.; Feng, Y. X.; Liu, G. S. *Zhong Cao Yao* **1980**, *11*, 507–510.
(4) Sun, C. M.; Syu, W. J.; Don, M. J.; Lu, J. J.; Lee, G. H. *J. Nat. Prod.* **2003**, *66*, 1175–1180.
(5) Tan, R. X.; Jakupovic, J.; Bohlmann, F.; Jia, Z. J.; Schuster, A. *Phytochemistry* **1990**, *29*, 1209–1211.
(6) Yang, H.; Xie, J. L.; Sun, H. D. *Acta Bot. Sinica* **1997**, *39*, 667–669.
(7) Yang, H.; Xie, J. L.; Sun, H. D. *Acta Bot. Yun Nanica* **1997**, *1*, 92–96.
(8) Liu, C. M.; Fei, D. Q.; Wu, Q. H.; Gao, K. *J. Nat. Prod.* **2006**, *69*, 695–699.
(9) Xie, W. D.; Zhang, Q.; Li, P. L.; Jia, Z. J. *Phytochemistry* **2005**, *66*, 2340–2345.
(10) Yaoita, Y.; Kikuchi, M. *J. Nat. Med. (Tokyo)* **1998**, *52*, 273–275.
(11) Luis, J. G.; Andres, L. S. *Nat. Prod. Lett.* **1999**, *13*, 187–194.
(12) Gupta, D. K.; Ali, M.; Bhutani, K. K. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1996**, *35B*, 1079–1084.
(13) Panosyan, A. G.; Mantsakanyan, V. A. *Khim. Prir. Soedin.* **1977**, *1*, 59–69.
(14) Nakata, T.; Fukui, M.; Oishi, T. *Tetrahedron Lett.* **1988**, *29*, 2219–2227.
(15) Tanaka, R.; Matsunago, S. *Phytochemistry* **1988**, *27*, 3579–3584.
(16) Orzalesi, G.; Mezzetti, T.; Rossi, C.; Bellavita, V. *Planta Med.* **1971**, *19*, 30–36.
(17) Crystallographic data for compound **1** have been deposited at the Cambridge Crystallographic Datd Center, CCDC-635451. Copies of the data can be obtained free of charge viawww.ccdc.cam.ac.uk/data_request/cif.
(18) *International Tables for X-Ray Crystallography*; Ibers, J. A., Hamilton, W. C., Eds.; The Kynoch Press: Birmingham, U.K., 1974; Vol. IV.

NP070483L