New Triterpenoids Isolated from the Root Bark of *Ulmus pumila* L.

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Three new triterpenoids, 1—3, were isolated from the dried root bark of *Ulmus pumila*. Along with the three new compounds, six known triterpenoids, epifriedelanol (4), friedelin (5), oleanolic acid (6), maslinic acid (7), camaldulenic acid (8), and arjunolic acid (9) were also isolated. The structures of new compounds were established as ulmudiol (bauer-7-ene-1α,3β-diol, 1), dehydroulmudiol [bauer-7,9(11)-diene-1α,3β-diol, 2], and ulmuestone [3α-hydroxy-11α-(4′-hydroxy-3′-methoxy)benzoyloxybauer-1-one, 3], on the basis of extensive 1D and 2D NMR spectroscopic data interpretation. In addition, the cytotoxic activities of these compounds are also reported.

Key words Ulmaceae; *Ulmus pumila*; root bark; triterpenoid; cytotoxicity

Ulmus pumila L. is a deciduous tree, which is widely distributed in East Asia. For centuries, the dried stem and root bark of this species have been used as Traditional Chinese Medicine (TCM) to treat edema, mastitis, gastric cancer and inflammation.[1,2] As a part of our ongoing research to search for novel anticancer agents,[3,4] an EtOAc extract of the root bark of *U. pumila*, was initially selected for testing cytotoxic activity and it fed back a positive result. Therefore, the separation of this extract was carried out to isolate the constituents with cytotoxic activity.

Three new triterpenoids (**1**—**3**) were isolated during this study, and in addition to the six known triterpenoids (**4**—**9**). The structures of the six known compounds were identified as epifriedelanol (**4**), friedelin (**5**),[5] oleanolic acid (**6**),[6] maslinic acid (**7**),[7] camaldulenic acid (**8**), and arjunolic acid (**9**),[8,9] by comparison of their spectral data with values reported in the literature (Fig. 1).

Results and Discussion

Compound **1** was isolated as colorless needles, and the molecular formula was deduced as $C_{30}H_{50}O_2$ by EI-MS, ^{1}H-, ^{13}C-NMR and DEPT spectra. It gave a positive Lieberman–Burchard (LB) test for triterpenoids. The ^{1}H-NMR spectrum of **1** showed the presence of six tertiary methyl groups at δ 0.76, 0.87, 0.97, 1.01, 1.01, and 1.04, two secondary methyl groups at δ 0.90 (3H, d, J=5.8 Hz) and 1.05 (3H, d, J=5.9 Hz), one olefinic proton at δ 5.39 (1H, m). Only two olefinic carbons were observed at δ 115.7 and 145.3 in the ^{13}C-NMR spectrum of **1**. These signals suggested that compound **1** is a pentacyclic triterpene, and two methyl are boned to C-29 and C-30 in E ring separately. It is concluded that compound **1** is a triterpene of bauerene type supported by close compared ^{13}C-NMR spectra data of bauerenyl acetate,[10] and the main difference is in A ring of basic skeleton between these two compounds (Table 1).

The ^{1}H-NMR spectrum also showed the presence of two oxymethine proton at δ 3.77 (1H, br s) and 3.80 (1H, dd, J=4.4, 12.1 Hz), and these two proton correlated with the carbon signals of δ 72.5 and 73.3 respectively in the HMQC spectrum. On the other hand, HMBC spectrum showed that both of the two oxymethine protons correlated with the methlene carbon at δ 34.8, and the correlation of the proton at δ 3.77 with the carbon at δ 73.3 was also shown (Fig. 2). It suggested that compound **1** is bauer-7-ene-1,3-diol.

The proton at δ 3.80 correlated with the methyl of C-23 and C-24, and the proton at δ 3.77 correlated with the methyl of C-25 in HMBC spectrum. Therefore, the chemical shifts of C-1 and C-3 are assigned to δ 72.5 and 73.3 respectively. The coupling patterns of the H-1 and H-3 signals in the ^{1}H-NMR indicated that H-1 was equatorial, and H-3 was axial, and that, accordingly, C-1 and C-3 hydroxy groups were α-axial and β-equatorial, respectively.

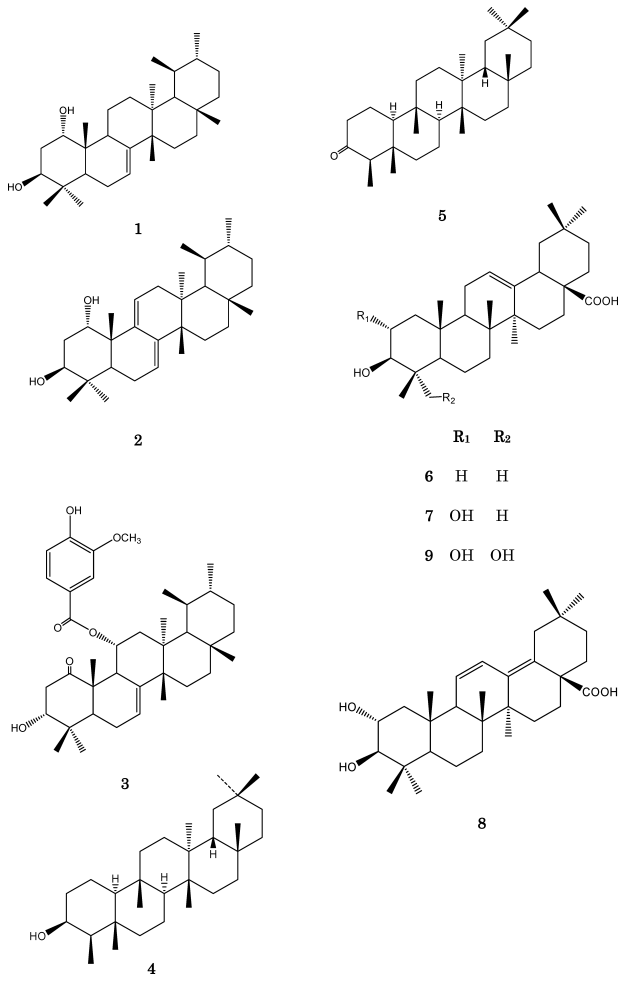

Fig. 1. Chemical Structures of Compounds **1**—**9**

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Table 1. The ^{13}C-NMR Chemical Shift of Compounds **1**, **2**, **3**, and Bauerenyl Acetate ($CDCl_3$)

No.	**1**	**2**	**3**	Bauerenyl acetate
1	72.5	72.1	213.1	36.5
2	34.8	32.6	45.7	24.2
3	73.3	72.7	78.5	81.1
4	39.1	40.1	38.6	37.8
5	43.6	40.9	49.5	50.0
6	24.1	23.1	24.0	24.0
7	115.7	119.8	119.3	116.2
8	145.3	141.2	143.7	145.4
9	40.0	140.8	48.1	48.2
10	39.0	42.7	49.8	35.1
11	16.4	115.0	69.0	16.9
12	32.2	37.4	41.9	32.5
13	37.7	37.7	39.3	37.8
14	41.3	39.1	41.9	41.3
15	28.9	27.2	29.1	28.9
16	31.5	31.3	31.6	31.5
17	32.0	32.0	32.0	32.1
18	54.9	52.6	54.4	55.0
19	35.3	35.5	35.6	35.4
20	32.0	31.9	31.9	38.0
21	29.2	29.2	29.2	29.2
22	37.7	36.7	37.5	37.8
23	27.6	27.8	28.1	27.5
24	14.5	15.2	16.2	15.8
25	13.5	20.8	12.1	13.0
26	23.6	21.0	24.7	23.6
27	22.6	17.0	22.7	22.7
28	38.0	37.7	37.9	32.1
29	25.6	25.1	25.6	25.6
30	22.5	22.4	22.4	22.5
1′				122.7
2′				112.1
3′				146.2
4′				149.8
5′				113.8
6′				123.8
7′				165.3
$-OCH_3$				56.1

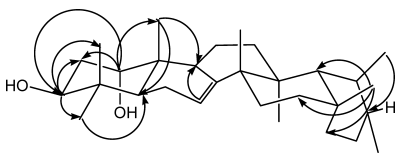

Fig. 2. The Selected HMBC Correlations for Compound **1**

It was reported that the ^{13}C-NMR chemical shifts of C-20 (CH) and C-28 (CH_3) of bauerenyl acetate were assigned as δ 38.0 and 32.1 respectively. However, in the DEPT and HMQC spectrum of compound **1**, the signal of δ 38.0 is shown as methyl carbon signal and δ 32.0 is assigned as a methine carbon signal. The HMBC spectrum of compound **1** also showed that the methyl proton at δ 1.04 (3H, s), which correlated with the carbon at δ 38.0 in HMQC spectrum, correlated with the carbons of C-16, C-18, and C-22; and the proton signal of 29-CH_3 at δ 1.05 correlated with the carbon at δ 32.0. Therefore, the chemical shifts of C-28 and C-20 were assigned to δ 38.0 and 32.0, respectively. The data of ^{13}C-NMR spectrum of bauerenyl acetate reported in literature is incorrect. Thus, the structure of compound **1** was identified as bauer-7-ene-1α,3β-diol. This new compound has been named as ulmudiol.

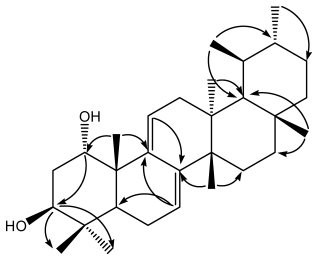

Fig. 3. The Selected HMBC Correlations for Compound **2**

Compound **2** was also obtained as colorless needles, and the LB test for triterpenoids showed a positive result. The molecular formula of **2** was determined as $C_{30}H_{48}O_2$ by EI-MS, ^{1}H-, ^{13}C-NMR and DEPT spectra. The ^{13}C-NMR spectrum of **2** showed chemical shifts similar to that of **1** except that there are four olefinic carbon at δ 115.0, 119.8, 140.8, and 141.2 in compound **2**. The ^{1}H-NMR spectrum of **2** also showed two olefinic proton at δ 5.39 (1H, br d, J=5.0 Hz) and 5.61 (1H, br d, J=4.6 Hz). All those suggested that compound **2** was a dehydrogenated derivative of compound **1**.

The HMQC spectrum of **2** showed correlations between the olefinic proton at δ 5.39 and the carbon at δ 115.0; and the proton at δ 5.61 and the carbon at δ 119.8. The long-range correlations between δ 5.39 and δ 141.2; and δ 5.61 and δ 140.8 were shown in HMBC spectrum of **2**, and these suggested the presence of the following partial structure –CH=C–C=CH–, a conjugated double bond in compound **2** (Fig. 3). This result was further supported by the correlations between δ 5.61 and C-5; H_3-26 and δ 141.2; H_3-25 and δ 140.8 in HMBC spectrum of **2**. Therefore, the olefinic carbon signals of δ 119.8, 141.2, 140.8, and 115.0 were assigned to C-7, C-8, C-9, and C-11 respectively.

It was suggested that A ring of **2** was a structure with 1,3-dihydroxyl by the correlations showed in HMBC spectrum. The chemical shifts of H-1 and H-3 were assigned to δ 4.15 (1H, br s) and 3.89 (1H, dd, J=4.2, 12.0 Hz), respectively, and the coupling patterns of them indicated that H-1 was equatorial, and H-3 was axial, and that, accordingly, C-1 and C-3 hydroxy groups were α-axial and β-equatorial, respectively. Compound **2** is, therefore, bauer-7,9(11)-diene-1α,3β-diol. This new compound has been named as dehydroulmudiol.

Compound **3** was obtained as an amorphous powder, and the LB test for triterpenoids showed a positive result. ESI-MS showed the $[M+NH_4]^+$ ion at m/z 624, and the $[2M+NH_4]^+$ ion at m/z 1230, corresponding to the molecular weight 606. Combined with ^{1}H-, ^{13}C-NMR and DEPT spectra, the molecular formula of **3** was determined as $C_{38}H_{54}O_6$. Its IR spectrum suggested the presence of hydroxyl (3429 cm^{-1}) and carbonyl (1708 cm^{-1}) groups. The signals for a 1,2,4-trisubstituted aromatic moiety [δ 6.90 (1H, d, J=8.2 Hz, H-5′), 7.50 (1H, br d, J=8.2 Hz, H-6′), and 7.54 (1H, br s, H-2′)] and the methoxyl group [δ 3.96 (3H, s)] in the ^{1}H-NMR spectrum together with the fragment ion at m/z 168 consistent with $C_8H_8O_4$ in the EI-MS suggested the presence of a benzoyl ester substituted with a methoxyl and a hydroxyl group in **3**. The placement of substitutions on the benzoyl ester was determined to be a 4-hydroxy-3-methoxybenzoate since correlation of the methoxyl signal at δ 3.96 to C-

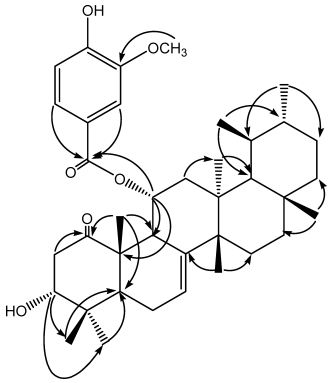

Fig. 4. The Selective HMBC Correlations for Compound **3**

Table 2. The Cytotoxic Effects of Compounds from *U. pumila* on HeLa, A375, and MCF-7 Cells

Compound	IC_{50} (μg/ml)		
	HeLa	A375	MCF-7
1	62.13	31.74	50.66
2	>100	26.27	44.69
3	3.19	13.26	20.17
4	>100	>100	>100
5	>100	>100	>100
6	12.09	>100	>100
7	38.53	26.44	34.34
8	42.67	59.14	54.37
9	7.69	43.21	28.83

3′ (δ 146.2) was observed in the HMBC spectrum (Fig. 4).

The ^{13}C-NMR spectrums of **3** due to the triterpene carbon were nearly identical to those of **1**, except for the downfield shift of the carbon signal at δ 72.5 (C-1) to δ 213.1, and δ 16.4 (C-11) to δ 69.0, suggesting that **3** is a bauerene-skeleton triterpene bearing one ester linkages and one ketonic group. The proton signal at δ 5.34 (H-11) in the ^{1}H-NMR spectrum also supported ester bond in **3**. The 4-hydroxy-3-methoxybenzoate was determined to be attached to C-11 by the long-range correlation seen between δ 165.3 (C-7′) and δ 5.34 (H-11) in the HMBC spectrum. The HMBC spectrum also showed the long-range correlations between δ 213.1 and δ 2.43 (1H, dd, J=5.5, 12.5 Hz, H-2a) and 2.95 (1H, dd, J=7.2, 12.5 Hz, H-2b); and δ 213.1 and δ 1.10 (3H, s, H_3-25), suggesting that the ketonic group is located at C-1. The coupling patterns of H-3 [δ 3.78 (1H, br t, J=5.8 Hz)] and H-11 [δ 5.34 (1H, dt, J=5.0, 9.7 Hz)] showed that H-3 was equatorial, and H-11 was axial, and those indicated that C-3 hydroxy groups was α-axial and the 4-hydroxy-3-methoxybenzoate was connected as α-equatorial. Therefore, **3** was determined to be 3α-hydroxy-11α-(4′-hydroxy-3′-methoxy)benzoyloxybauer-1-one. This new compound has been named as ulmuestone.

The *in vitro* cytotoxicities on three human tumor cell lines (HeLa, A375, and MCF-7) of the isolated compounds were examined also. The results shown in Table 2 indicate that compound **3** showed the stronger cytotoxic effects than others, and the IC_{50} values for HeLa, A375, and MCF-7 are 3.19, 13.26, and 20.17 μg/ml separately.

Experimental

General Procedures The NMR spectra were recorded on Bruker ARX-300 NMR spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz) and Bruker ARX-600 NMR spectrometer (^{1}H, 600 MHz; ^{13}C, 150 MHz), using TMS as an internal standard. EI-MS were recorded on a GC-MS QP5050 spectrometer (Shimadzu, Kyoto, Japan), and ESI-MS were recorded on a LCQ LC-MS (Finnigan, U.S.A.). Column chromatography were performed on silica gel (100—140 and 200—300 mesh, Qingdao Haiyang Chemical, Shandong, China) and Sephadex LH-20 (bead size 25—100 μm, Sigma-Aldrich Chemical, St. Louis, MO, U.S.A.). Analytical TLC was performed using silica gel 60 F_{254} plates (Kieselgel 60 F_{254} precoated plates, Merck, Darmstadt, Germany). Semipreparative HPLC was performed on an ODS C-18 column (9.8 mm×250 mm, 5 μm, Phenomenex, Torrance, CA, U.S.A.) using a water–methanol system as a mobile phase, monitored with an RID detector. All solvents were of analytical reagent or chromatographic reagent grade.

Plant Material The root barks of *U. pumila* were collected from Fuxin, Liaoning Province, China, in May 2000 and identified by Prof. Zheng Cui of Shenyang Pharmaceutical University, China. Fresh root barks were dried in a dark, well-ventilated place, and a voucher specimen has been deposited in the Department of Traditional China Materia Medica, Shenyang Pharmaceutical University.

Extraction and Isolation The air-dried root barks of *U. pumila* (10 kg) were milled and extracted three times with 80% ethanol under reflux. The ethanol extract was filtered and concentrated under reduced pressure. The crude product (1195 g) was successively partitioned with EtOAc and *n*-butanol. The EtOAc layer was concentrated under reduced pressure, and the residue (111.8 g) was subjected to silica gel column chromatography eluted with *c*-hexane and a *c*-hexane–acetone mixture with increasing proportions of acetone. The fractions were collected and combined by monitoring with analytical TLC to afford seven fractions (fractions 1—7) in order of increasing polarity. The fraction 2 was recrystaled by $CHCl_3$ and afforded **4** (980 mg). The fraction 3 was subjected to further coloum chromatography over silica gel using *c*-hexane–EtOAc (5 : 1) and afforded **5** (3.39 mg). The fraction 6 was subjected to coloum chromatography over silica gel using $CHCl_3$, $CHCl_3$–MeOH mixture with increasing proportions of MeOH, then further to coloum chromatography over Sephadex LH-20 using $CHCl_3$–MeOH (1 : 1) to furnish seven fractions (7-1—7-7). Fraction 7-1 on semipreparative HPLC over RP C-18 using 60% MeOH afforded **2** (28.2 mg). Fracton 7-2 was subjected to Sephadex LH-20 coloum chromatography using *c*-hexane–$CHCl_3$–MeOH (5 : 5 : 1) and afforded **6** (90.4 mg). Fraction 7-5 was recrystaled by acetone and yielded **7** (690 mg). Fraction 7-6 was separated on Sephadex LH-20 using *c*-hexane–$CHCl_3$–MeOH (5 : 5 : 1) and purified by semipreparative HPLC by 92% MeOH to give **1** (64.5 mg) and **3** (22.9 mg). **8** and **9** were purified from fraction 7-7 by Sephadex LH-20 (*c*-hexane–$CHCl_3$–MeOH=5 : 5 : 1) and semipreparative HPLC (85% MeOH).

Compound **1**: Colorless needles (MeOH–H_2O=5 : 1); mp 187—189 °C; IR (KBr) cm^{-1}: 3402, 2928, 1046; UV λ_{max} (CH_3CN) nm (log ε): 202 (3.4); EI-MS *m*/*z*: 442 (M^+), 424 ($M-H_2O^+$), 337, 245, 227; ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, 25-CH_3), 0.87 (3H, s, 24-CH_3), 0.97 (3H, s, 27-CH_3), 1.01 (3H, s, 23-CH_3), 1.01 (3H, s, 26-CH_3), 1.04 (3H, s, 28-CH_3), 0.90 (3H, d, J=5.8 Hz, 30-CH_3), 1.05 (3H, d, J=5.9 Hz, 29-CH_3), 1.74 (1H, dd, J=6.2, 12.0 Hz, 5-H), 1.83 (1H, td, J=3.9, 9.8 Hz, 2α-H), 1.90 (1H, dt, J=2.2, 12.9 Hz, 2β-H), 2.02 (1H, m, 6β-H), 2.22 (1H, m, 6α-H), 2.74 (1H, br d, 9-H), 3.77 (1H, br s, 1-H), 3.80 (1H, dd, J=4.4, 12.1 Hz, 3-H), 5.39 (1H, m, 7-H); ^{13}C-NMR data see Table 1.

Compound **2**: Colorless needles (MeOH–H_2O=5 : 1); mp 146—148 °C; IR (KBr) cm^{-1}: 3428, 2927, 1047, 757; UV λ_{max} (CH_3CN) nm (log ε): 245 (4.0); EI-MS *m*/*z*: 440 (M^+), 422 ($M-H_2O^+$), 335, 251, 225; ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, 27-CH_3), 0.88 (3H, s, 26-CH_3), 0.90 (3H, s, 24-CH_3), 0.90 (3H, d, J=6.4 Hz, 30-CH_3), 0.98 (3H, s, 25-CH_3), 1.03 (3H, s, 23-CH_3), 1.04 (3H, d, J=6.6 Hz, 29-CH_3), 1.07 (3H, s, 28-CH_3), 3.89 (1H, dd, J=4.2, 12.0 Hz, 3-H), 4.15 (1H, br s, $W_{1/2}$=4.8 Hz, 1-H), 5.39 (1H, br d, J=5.0 Hz, 11-H), 5.61 (1H, br d, J=4.6 Hz, 7-H); ^{13}C-NMR data see Table 1.

Compound **3**: Amorphous powder; mp 266—268 °C; IR (KBr) cm^{-1}: 3429, 2952, 1707, 1286, 764; UV λ_{max} (CH_3CN) nm (log ε): 288 (3.7); ESI-MS *m*/*z*: 624 ($M+NH_4^+$), 1230 ($2M+NH_4^+$); ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, s, 24-CH_3), 0.87 (3H, s, J=5.6 Hz, 30-CH_3), 1.01 (3H, s, 23-CH_3), 1.01 (3H, d, J=6.0 Hz, 29-CH_3), 1.05 (3H, s, 26-CH_3), 1.05 (3H, s, 28-CH_3), 1.10 (3H, s, 25-CH_3), 1.14 (3H, s, 27-CH_3), 1.30 (1H, br s, 18-H), 1.81 (1H, dd, J=4.6, 12.4 Hz, 5-H), 2.43 (1H, dd, J=5.5, 12.5 Hz, 2α-H), 2.95 (1H, dd, J=7.2, 12.5 Hz, 2β-H), 3.47 (1H, br d, J=10.1 Hz, 9-H), 3.78 (1H, br t,

J=5.8 Hz, 3-H), 3.96 (3H, s, 3′-OCH_3), 5.34 (1H, dt, J=5.0, 10.9 Hz, 11-H), 5.65 (1H, br s, 7-H), 6.90 (1H, d, J=8.2 Hz, 5′-H), 7.50 (1H, d, J=8.2 Hz, 6′-H), 7.54 (1H, s, 2′-H); ^{13}C-NMR data see Table 1.

Cytotoxic Activity The *in vitro* cytotoxic activities test on three human tumor cell lines were performed using MTT methods in a 96-multiwell microtiter integrated system.[3)]

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