Eremophilane-Type Sesquiterpene Derivatives from the Roots of *Ligularia lapathifolia*

Dong-Qing Fei,[†] Shi-Gang Li,[‡] Chun-Mei Liu,[†] Gang Wu,[†] 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, and Institute of Basic Medicine, Lanzhou University, Lanzhou 730000, People's Republic of China

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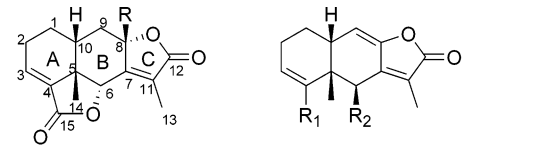
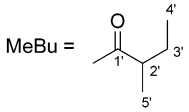
Six new highly oxygenated eremophilane-type sesquiterpene derivatives (**1**−**6**), including a norbisesquiterpene, were isolated from an extract of the roots of *Ligularia lapathifolia*, and their structures were elucidated by spectroscopic methods. The structure of **1** was confirmed by single-crystal X-ray crystallography. In addition, the cytotoxicity of compounds **1**, **2**, **3**, **5**, and **6** was evaluated against selected cancer cell lines, including human stomach carcinoma (MGC-803), human hepatoma (HEP-G2), and murine sarcoma (S-180) cell lines.

The genus *Ligularia* belongs to the Senecioneae tribe of the Compositae family. More than 20 *Ligularia* species are being used in Chinese folk medicine. *L. lapathifolia* (Franch.) Hand-Mazz., named "da du ye cao" in traditional Chinese medicine, is mainly distributed in southwestern China, and its roots have been used by local inhabitants to treat cough and inflammation.[1] Previous work on *L. lapathifolia* led to the isolation of some eremophilane-type sesquiterpene derivatives.[2−4] Due to continued interest in the genus *Ligularia*,[5−9] we reinvestigated this species and obtained six new highly oxygenated eremophilane-type sesquiterpene derivatives (**1**−**6**). Here, we report their isolation and structural elucidation and the results of their cytotoxicity evaluation.



1 R = OH
2 R = OCH_3
3 R = OCH_2CH_3

4 R_1 = $COOCH_3$, R_2 = OMeBu



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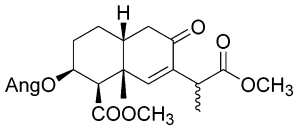

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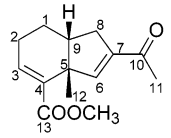

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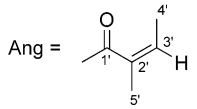

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Results and Discussion

Compound **1** was obtained as colorless crystals and exhibited a molecular ion $[M]^+$ peak at *m*/*z* 276 in the EIMS and a $[2M + Na]^+$ peak at *m*/*z* 575.1893 (calcd for $C_{30}H_{32}O_{10}Na$, 575.1888) in the HRESIMS, corresponding to the molecular formula $C_{15}H_{16}O_5$. The IR spectrum displayed absorption bands for hydroxy (3367 cm^{-1}) and α,β-unsaturated γ-lactone functional groups (1766, 1678 cm^{-1}).[10−12] The ^1H NMR spectrum (Table 1) indicated the presence of a vinylic methyl group at δ 1.95 (d, $J = 2.0$ Hz, H_3-13), a tertiary methyl at δ 1.44 (s, H_3-14), an oxygenated methine at δ 5.24 (d, $J = 2.0$ Hz, H-6), and an olefinic proton at δ 6.87 (dd, $J = 3.2$, 3.6 Hz, H-3), as well as resonances with complex coupling patterns from δ 1.00 to 2.50. The ^{13}C NMR and DEPT spectra (Table 2) showed 15 carbon resonances including two methyl, three methylene, three methine (one oxygenated, one olefinic, and one sp^3 hybridized), and seven quaternary carbons (two lactone carbonyls, three olefinic, one acetal, and one sp^3 hybridized). With eight degrees of unsaturation, **1** apparently contained four rings, apart from two carbonyl and two double-bond functionalities. The ^1H−^1H COSY spectrum of **1** enabled comprehensive systems to be delineated. Vicinal and long-range correlations from the olefinic proton (H-3) to the methylene (H_2-2), with the spin system continuing from H_2-2 through H_2-1 to H-10, and then to H_2-9, indicated the presence of the structural unit ═CH(3)−CH_2(2)−CH_2-(1)−CH(10)−CH_2(9). In the HMBC experiment, long-range correlations between the following protons and carbons were observed: δ 1.44 (H_3-14) and 44.0 (C-5), 129.7 (C-4), 82.1 (C-6), 33.3 (C-10); δ 1.95 (H_3-13) and 126.8 (C-11), 154.1 (C-7), 171.4 (C-12); δ 5.24 (H-6) and 168.8 (C-15), 154.1 (C-7), 126.8 (C-11), 44.0 (C-5), 26.8 (C-14); δ 2.24 (H-9β) and 154.1 (C-7), 103.0 (C-8), 44.0 (C-5), 33.3 (C-10), 21.5 (C-1). These indicated the presence of a sesquiterpene skeleton with two γ-lactone units that is in agreement with an 8-hydroxyeremophil-7(11)-ene-8,12(6,15)-diolide skeleton.[13,14] In addition, the long-range correlations between δ 6.87 (H-3) and 21.5 (C-1), 129.7 (C-4), and 168.8 (C-15) clearly indicated the presence of a 3,4-double bond.

The relative configuration of **1** was elucidated by the NOE difference spectrum, in which the resonances of H-10 and H-6 were enhanced by irradiation of H_3-14, indicating a *cis*-fused A/B ring system and a β-orientated H-6 due to the biogenetic β-orientation of H_3-14.[15] To establish the relative configuration of C-8, a molecular model indicated that in the C(8)-β-OH isomer the dihedral angle between C(6)−β-H and C(11)−CH_3(13) approximated 90°; that is, a homoallylic coupling should be observed. In the C(8)−α-OH isomer, the molecular model showed the dihedral angle to be about 30°, indicating that a homoallylic coupling should be negligible.[13,16] Indeed the ^1H NMR spectrum of **1** showed the presence of homoallylic coupling between H-6 and H_3-13, so the hydroxy group at the C-8 position should be in a β-orientation. The relative configuration of **1** was further confirmed by X-ray crystallographic analysis (Figure 1). Therefore compound **1** was determined as 8β-hydroxyeremophil-3,7(11)-diene-8α,12(6α,15)-diolide.

* To whom correspondence should be addressed. Tel: +86-931-8912592. Fax: +86-931-8912582. E-mail: npchem@lzu.edu.cn.
† State Key Laboratory of Applied Organic Chemistry.
‡ Institute of Basic Medicine.

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Table 1. ^{1}H NMR Data of Compounds **1**−**3**[a] and **4**−**6**[b] in $CDCl_3$ (δ ppm, *J* Hz)

position	1	2	3	4	5	6
1α	1.68−1.72 m	1.68−1.74 m	1.66−1.71 m	1.81−1.85 m	2.14−2.18 m	1.56−1.61 m
1*β*	2.19−2.23 m	2.14−2.20 m	2.17−2.21 m	2.04−2.08 m	1.72−1.78 m	1.69−1.75 m
2α	2.34−2.39 m	2.32−2.37 m	2.32−2.36 m	2.21−2.25 m	1.78−1.84 m	2.18−2.22 m
2*β*	2.40−2.42 m	2.37−2.43 m	2.37−2.41 m	2.32−2.36 m	1.24−1.26 m	2.18−2.22 m
3α	6.87 dd (3.2, 3.6)	6.87 dd (3.2, 3.6)	6.86 dd (3.2, 3.6)	6.92 dd (3.9, 3.6)	4.94 dt (12.3, 5.4)	7.10 dd (4.5, 3.9)
4α					3.13 d (5.4)	
6α				6.64 s	6.44 s	7.09 brs
6*β*	5.24 d (2.0)	5.06 d (2.0)	5.05 d (2.0)			
8α						2.61 brdd (15.7, 6.8)
8*β*						2.22−2.30 m
9α	1.35 t (13.6)	1.30 t (13.2)	1.28 t (13.2)	5.42 brs	2.26 dd (17.5, 4.5)	
9*β*	2.24 dd (13.6, 4.8)	2.23 dd (13.2, 4.8)	2.24 dd (13.2, 4.4)		2.78 dd (17.5, 5.1)	2.22−2.30 m
10*β*	2.02−2.07 m	2.02−2.07 m	2.02−2.06 m	2.98−3.02 m	2.60−2.65 m	
11					3.59 brq (7.5)	2.31 s
12						1.37 s
13	1.95 d (2.0)	2.02 d (2.0)	2.00 d (2.0)	1.97 s	1.32 d (7.5)	
14	1.44 s	1.43 s	1.43 s	1.44 s	1.29 s	
		OCH_3	OCH_2CH_3	OMeBu	OAng	
1′		3.23 s	3.32 dq (7.5, 6.4), 3.53 dq (7.5, 6.4)			
2′			1.24 t (6.4)	2.34−2.37 m		
3′				1.45−1.49 m, 1.63−1.68 m	6.11 qq (7.2, 1.2)	
4′				0.87 t (7.5)	1.95 dq (7.2, 1.2)	
5′				1.12 d (7.2)	1.83 dq (1.2, 1.2)	
				COO*CH*$_3$	COO*CH*$_3$	COO*CH*$_3$
				3.68 s	3.69 s	3.75 s
					3.70 s	

[a] Spectrum was measured at 400 MHz. [b] Spectrum was measured at 300 MHz.

Table 2. ^{13}C NMR Data of Compounds **1**−**3**[a] and **4**−**6**[b] in $CDCl_3$ (δ ppm)

position	1	2	3	4	5	6
1	21.5 t	21.4 t	21.5 t	22.5 t	25.0 t	22.4 t
2	21.8 t	21.7 t	21.7 t	22.8 t	26.3 t	23.2 t
3	137.2 d	137.0 d	137.0 d	142.6 d	70.5 d	141.2 d
4	129.7 s	130.0 s	130.0 s	131.7 s	53.9 d	134.0 s
5	44.0 s	44.0 s	44.0 s	42.2 s	39.6 s	49.9 s
6	82.1 d	82.1 d	82.3 d	67.2 d	150.0 d	151.0 d
7	154.1 s	152.2 s	152.8 s	148.5 s	137.8 s	142.5 s
8	103.0 s	105.2 s	105.1 s	143.8 s	196.3 s	33.2 t
9	36.4 t	35.2 t	35.6 t	108.5 d	39.6 t	45.4 d
10	33.3 d	32.9 d	33.0 d	37.2 d	35.5 d	198.0 s
11	126.8 s	128.6 s	128.3 s	126.7 s	38.0 d	26.4 q
12	171.4 s	170.4 s	170.5 s	171.3 s	174.3 s	25.0 q
13	9.0 q	9.1 q	9.1 q	8.9 q	15.4 q	166.8 s
14	26.8 q	26.8 q	26.9 q	23.4 q	24.7 q	
15	168.8 s	168.5 s	168.5 s	166.5 s	171.8 s	
		OCH_3	OCH_2CH_3	OMeBu	OAng	
1′		50.8 q	59.2 t	175.5 s	166.5 s	
2′			15.1 q	41.4 d	127.2 s	
3′				26.7 t	139.4 d	
4′				11.3 q	15.5 q	
5′				16.4 q	20.1 q	
				COO*C*H$_3$	COO*C*H$_3$	COO*C*H$_3$
				51.7 q	51.2 q	51.4 q
					51.8 q	

[a] Spectrum was measured at 100 MHz. [b] Spectrum was measured at 75 MHz.

Compound **2** was obtained as colorless crystals, and its molecular formula was deduced as $C_{16}H_{18}O_5$ from the HRESIMS data at *m*/*z* 313.1051 $[M + Na]^+$ (calcd for $C_{16}H_{18}O_5Na$, 313.1046). The IR spectrum showed that **2**, like **1**, also contained an α,*β*-unsaturated *γ*-lactone functional group. The ^{1}H NMR and ^{13}C NMR spectra were very similar to those of **1** except for the presence of a methoxy group at δ_H 3.23 (s, H_3-1′) and δ_C 50.8 (C-1′), instead of a hydroxy group at the C-8 position. This could be confirmed by the cross-peak between δ_H 3.23 (H_3-1′) and δ_C 105.2 (C-8) in the HMBC spectrum. Hence **2** is an 8-methoxy derivative of **1**. The relative configuration of **2** was determined to be identical to that of **1** by comparing its NMR data with those of **1** and by the NOE experiment, which showed that the A/B ring system was *cis*-fused, H-6 *β*-orientated and the methoxy group also *β*-orientated. Therefore, compound **2** was assigned as 8*β*-methoxyeremophil-3,7(11)-diene-8α,12(6α,15)-diolide.

Compound **3** was obtained as colorless crystals and gave a $[M + Na]^+$ peak at *m*/*z* 327.1208 (calcd for $C_{17}H_{20}O_5Na$, 327.1203) by HRESIMS, consistent with an elemental formula of $C_{17}H_{20}O_5$. A comparison of the spectroscopic data of **3** with those of **1** and **2** showed that the three compounds were very similar, except that there was at C-8 an ethoxy group in **3**, a hydroxy group in **1**, and a methoxy group in **2**. This could be confirmed by the correlations between δ_H 3.32, 3.53 (H_2-2′) and δ_C 105.1 (C-8) in the HMBC spectrum. The relative configuration of **3** should be the same as that of **1** and **2** by comparison of their NMR data and was confirmed

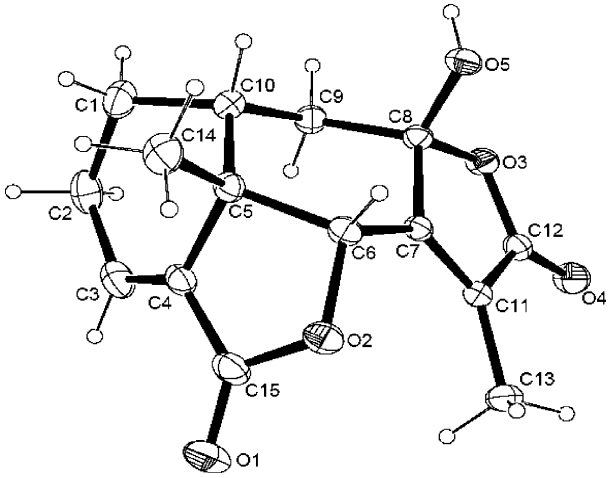

Figure 1. ORTEP diagram of the crystal structure of **1**.

by the NOE difference spectrum of **3**. Thus, compound **3** was assigned as 8β-ethoxyeremophil-3,7(11)-diene-8α,12(6α,15)-diolide.

Compound **4** was obtained as a colorless gum, and its molecular formula was established as $C_{21}H_{26}O_6$ by HRESIMS at *m*/*z* 397.1625 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_6Na$, 397.1622). Its IR spectrum exhibited absorption bands for an α,β-unsaturated γ-lactone functional group (1775, 1649 cm^{-1}).[10−12] The resonances of δ_H 2.36 (m, H-2′), 1.48 and 1.66 (overlapping, H_2-3′), 0.87 (t, J = 7.5 Hz, H_3-4′), and 1.12 (d, J = 7.2 Hz, H_3-5′) in its ^1H NMR spectrum (Table 1) and δ_C 175.5 (C-1′), 41.4 (C-2′), 26.7 (C-3′), 11.3 (C-4′), and 16.4 (C-5′) in its ^{13}C NMR spectrum (Table 2) displayed the presence of a 2-methylbutyryloxy group,[17] which was confirmed by the $C_5H_{10}O_2$ unit due to the fragment ion $[M - 102 (C_5H_{10}O_2)]^+$ peak at *m*/*z* 272 in the EIMS. Additionally, its NMR spectra showed a carbonyl resonance at δ_C 166.5 coupled with the additional methoxy resonances at δ_H 3.68 and δ_C 51.7, strongly indicating the presence of a carboxylic methyl ester group.[14] Besides the five carbons of the 2-methylbutyryloxy group and the methoxy carbon, the ^{13}C NMR spectrum of **4** also showed 15 carbon resonances due to two methyl, two methylene, four methine (one oxygenated, two olefinic, and one sp^3 hybridized), and seven quaternary carbons (two ester carbonyls, four olefinic, and one sp^3 hybridized). Its ^1H NMR spectrum indicated the presence of one tertiary methyl at δ 1.44 (s, H_3-14), one vinylic methyl group at δ 1.97 (s, H_3-13), two olefinic protons at δ 5.42 (brs, H-9) and 6.92 (dd, J = 3.9, 3.6 Hz, H-3), and one oxygenated methine at δ 6.64 (s, H-6), as well as some complex multiplets in the upfield region. In the $^1H-^1H$ COSY spectrum, homonuclear coupling correlations from H-3 through H_2-2 and H_2-1 to H-10 and then to H-9 revealed the presence of the segment ═CH(3)−CH_2(2)−CH_2(1)−CH(10)−CH(9)═. The HMBC experiment showed correlations between δ 1.44 (H_3-14) and 42.2 (C-5), 131.7 (C-4), 67.2 (C-6), 37.2 (C-10); δ 1.97 (H_3-13) and 126.7 (C-11), 148.5 (C-7), 171.3 (C-12); and δ 5.42 (H-9) and 37.2 (C-10), 22.5 (C-1), 42.2 (C-5), 143.8 (C-8), 148.5 (C-7). These observations and biogenetic considerations suggested that **4** had an eremophil-7(11),8-diene-8,12-olide structure.[13,14] The carboxylic methyl ester group was deduced to be at C-4 due to the absence of the H_3-15. One more double bond was determined between C-3 and C-4 by the cross-peaks in the HMBC spectrum: H-3 with C-1, C-4, C-5, and C-15. The 2-methylbutyryloxy group was located at C-6 as a result of the observation of the HMBC correlation between H-6 and C-1′.

The relative configuration of **4** was determined by the NOE difference spectrum, in which the H_3-14 resonance showed an NOE effect to H-10 but not to H-6, indicating a *cis*-fused A/B ring system and an α-orientated H-6. Thus, compound **4** was assigned as 6β-(2ξ-methylbutyryloxy)eremophil-3,7(11),8-trien-8,12-olid-15-oic acid methyl ester.

Compound **5** was obtained as a colorless gum, and its molecular formula was determined as $C_{22}H_{30}O_7$ by HRESIMS at *m*/*z* 407.2058 $[M + H]^+$ (calcd for $C_{22}H_{31}O_7$, 407.2064). Its IR spectrum revealed absorption bands for an α,β-unsaturated ketone (1676 cm^{-1}) and ester carbonyls (1735, 1710 cm^{-1}). The resonances at δ_H 6.11 (qq, J = 7.2, 1.2 Hz, H-3′), 1.95 (dq, J = 7.2, 1.2 Hz, H_3-4′), and 1.83 (dq, J = 1.2, 1.2 Hz, H_3-5′) in its ^1H NMR spectrum (Table 1) and δ_C 166.5 (C-1′), 127.2 (C-2′), 139.4 (C-3′), 15.5 (C-4′), and 20.1 (C-5′) in its ^{13}C NMR spectrum (Table 2), as well as an ion fragment peak at *m*/*z* 306 $[M - 100 (C_5H_8O_2)]^+$ in the EIMS, revealed the presence of an angeloyloxy group.[2] In addition, in its NMR spectra two carboxylic methyl ester groups at δ_H 3.69 (s, OCH_3), δ_C 51.2 (OCH_3), 174.3 (C-12) and δ_H 3.70 (s, OCH_3), δ_C 51.8 (OCH_3), 171.8 (C-15) were observed. Its ^1H NMR spectrum indicated the presence of one tertiary methyl at δ 1.29 (s, H_3-14), one secondary methyl at δ 1.32 (d, J = 7.5 Hz, H_3-13), one olefinic proton at δ 6.44 (s, H-6), one methylene at δ 2.26 (dd, J = 17.5, 4.5 Hz, H-9α) and 2.78 (dd, J = 17.5, 5.1 Hz, H-9β), and several complex multiplets from δ 1.00 to 3.00. Apart from the five carbons of the angeloyloxy group and two methoxy carbons, the ^{13}C NMR spectrum also showed 15 carbon resonances including two methyl, three methylene, five methine (one olefinic, one oxygenated, and three sp^3 hybridized), and five quaternary carbons (two ester carbonyls, one ketone carbonyl, one olefinic, and one sp^3 hybridized).

The structure of **5** was mainly established by 2D NMR experiments. The presence of the two segments CH(4)−CH(3)−CH_2(2)−CH_2(1)-CH(10)−CH_2(9) and CH(11)−CH_3(13) was readily established by the $^1H-^1H$ COSY spectrum, which showed homonuclear correlations from H-4 to H-3, then to H_2-2, the coupling chain continuing from H_2-2 through H_2-1 to H-10 and then to H_2-9, as well as correlations from H_3-13 to H-11. In the HMBC spectrum of **5**, correlations of δ 1.29 (H_3-14) with 39.6 (C-5), 53.9 (C-4), 150.0 (C-6), 35.5 (C-10); δ 7.09 (H-6) with 39.6 (C-5), 137.8 (C-7), 196.3 (C-8), 38.0 (C-11), 35.5 (C-10), 53.9 (C-4); δ 2.26 (H-9α) with 196.3 (C-8), 35.5 (C-10), 25.0 (C-1), 137.8 (C-7), 39.6 (C-5); and δ 1.32 (H_3-13) with 38.0 (C-11), 137.8 (C-7) demonstrated that **5** possessed an eremophilane structure. One carboxylic methyl ester group should be at C-4 due to the absence of the H_3-15. The long-range correlations in the HMBC spectrum from C-12 to H_3-13 and H-11 indicated that the other carboxylic methyl ester group was at C-11. The above spectroscopic data were similar to those of 8-oxoeremophil-6(7)-ene-12,15-dioic acid methyl ester,[14] except for an extra angeloyloxy group in **5**, which was defined as being attached to C-3 by the HMBC cross-peak between H-3 and C-1′. Stereochemically, the $COOCH_3$ function at C-4 should be in a β-orientation due to the fact that H_3-14 and H_3-15 should biogenetically be β-orientated.[15] An NOE difference spectrum of **5** showed that the H_3-14 resonance had an NOE effect on H-10, indicating the existence of a *cis* A/B ring system. The coupling pattern observed for H-3 (dt, J = 12.3, 5.4 Hz) implied that the angeloyloxy group at C-3 was β-equatorial.[18,19] Therefore, compound **5** was determined as 3β-angeloyloxy-8-oxoeremophil-6(7)-ene-12,15-dioic acid methyl ester.

Compound **6** was obtained as a colorless gum and gave a protonated molecular ion $[M + H]^+$ at *m*/*z* 235.1324 (calcd for $C_{14}H_{19}O_3$, 235.1329) by HRESIMS, corresponding to a molecular formula of $C_{14}H_{18}O_3$. The IR spectrum displayed absorption bands for an ester carbonyl (1713 cm^{-1}) and an α,β-unsaturated ketone (1668 cm^{-1}). The ^1H NMR spectrum showed an *O*-methyl resonance at δ 3.75 (s, OCH_3) attributed to a carboxylic methyl ester group, a methyl at δ 2.31 (s, H_3-11) attributed to an acetyl group, a tertiary methyl at δ 1.37 (s, H_3-12), and two olefinic protons at δ 7.09 (brs, H-6) and 7.10 (dd, J = 4.5, 3.9 Hz, H-3), as well as several complex multiplets in the upfield region. The ^{13}C NMR and DEPT spectra showed 14 carbon resonances, including three methyl (one methoxy), three methylene, three

Table 3. IC_{50} Values (μM) for Cytotoxicity of Compounds **1**, **2**, **3**, **5**, and **6**

compound	MGC-803	HEP-G2	S-180
1	>100	>100	>100
2	>100	12.66	73.10
3	53.91	40.53	36.61
5	>100	>100	>100
6	35.38	46.54	19.87

methine (two olefinic and one sp^3 hybridized), and five quaternary carbons (one ester carbonyl, one ketone carbonyl, two olefinic, and one sp^3 hybridized). Apart from an apparent methoxy resonance, the number of skeletal carbons was 13. With an unsaturation degree of six, compound **6** apparently contained two rings, besides two carbonyls and two olefinic groups. Considering that the NMR spectra exhibited one acetyl group, one carboxylic methyl ester group, and one tertiary methyl, a 6,5-fused AB ring system was assumed to be present, since only nine carbons remained to form the ring skeleton. Furthermore, the $^1H-^1H$ COSY spectrum of **6** indicated the presence of the segment $=CH(3)-CH_2(2)-CH_2(1)-CH(9)-CH_2(8)$, and its HMBC spectrum showed the following long-range correlations from δ 1.37 (H_3-12) to 49.9 (C-5), 45.4 (C-9), 151.0 (C-6); from δ 2.31 (H_3-11) to 198.0 (C-10), 142.5 (C-7); from δ 7.09 (H-6) to 49.9 (C-5), 142.5 (C-7), 45.4 (C-9), 198.0 (C-10), 33.2 (C-8); from δ 7.10 (H-3) to 134.0 (C-4), 49.9 (C-5), 166.8 (C-13), 22.4 (C-1); and from δ 3.75 (OCH_3) to 166.8 (C-15), which revealed that **6** was a norbieremophilane sesquiterpene derivative and its structure was similar to the known compound 2-acetyl-3a-methyl-3a,4,5,6,7,7a-hexahydro-1*H*-inden-4-oic acid methyl ester[14] except for the presence of an additional double bond between C-3 and C-4 in **6**. In combination with the NOE difference spectrum, in which the H-9 resonance was enhanced by irradiation of H_3-12, indicating a *cis*-fused A/B ring system, the structure of **6** was determined as 2-acetyl-3aβ-methyl-3a,6,7,7aβ-tetrahydro-1*H*-inden-4-oic acid methyl ester.

The cytotoxic activity of compounds **1**, **2**, **3**, **5**, and **6** was evaluated against human stomach carcinoma (MGC-803), human hepatoma (HEP-G2), and murine sarcoma (S-180) cell lines using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) colorimetric assay as previously reported.[20] The results are shown in Table 3. Compound **2** exhibited moderate activity against HEP-G2 cell lines (IC_{50} 12.66 μM), and compound **6** showed moderate activity against S-180 cell lines (IC_{50} 19.87 μM). Compounds **1**, **3**, and **5** were inactive (IC_{50} > 30 μM). These results indicate that the viabilities of cancer cells after 48 h of incubation with compounds **2** and **6** are specific to the cell-type.

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 HP5988A GCMS spectrometer. HRESIMS data were measured on a Bruker Daltonics APEX II 47e spectrometer. The solvents used for CC were petroleum ether (bp 60−90 °C), acetone, and EtOAc and were distilled prior to use. Silica gel (200−300 mesh) used for CC and silica gel GF_{254} (10−40 μm) 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 roots of *L. lapathifolia* were collected in Muli County, Sichuan Province, People's Republic of China, in August 2003. The plant was identified by Prof. Guo-Liang Zhang, School of Life Sciences, Lanzhou University. A voucher specimen (No. 20030804) was deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The air-dried roots of *L. lapathifolia* (3.6 kg) were powdered and extracted with petroleum ether (bp 60−90 °C)−diethyl ether−acetone (1:1:1) three times (each time 11 L for 7 days) successively at room temperature. The combined extracts were concentrated in vacuum to obtain a residue of 270 g, which was subjected to silica gel (200−300 mesh, 1500 g, 8.0 × 150 cm) CC and eluted with petroleum ether−acetone (50:1, 20:1, 8:1, 5:1, 2:1, 1:1, 0:1). On the basis of differences in composition indicated by TLC, seven pooled fractions (A−G) were obtained. Fraction B (45 g) was further fractionated on a silica gel column (500 g) using petroleum ether−acetone (30:1, 10:1, 5:1, 2:1) to give four crude fractions (B_1-B_4). Fraction B_2 (3.5 g) was further fractionated on a silica gel column (40 g) using petroleum ether−EtOAc (20:1, 10:1, 5:1, 2:1) to obtain compound **5** (109 mg) and a mixture of **4** and **6**, which was purified by preparative TLC using petroleum ether−acetone (15:1, × 3) to give pure **4** (4 mg) and **6** (30 mg). Fraction B_3 (2.3 g) was further fractionated on a silica gel column (30 g) using petroleum ether−acetone (20:1, 8:1, 2:1) to obtain compounds **3** (26 mg) and **2** (31 mg). Fraction D (22 g) was further subjected to column chromatography on silica gel (200 g) and eluted with petroleum ether−acetone (20:1, 10:1, 5:1, 2:1) to give four fractions (D_1-D_4). Fraction D_3 (2.8 g) was further fractionated on a silica gel column (30 g) using petroleum ether−acetone (4:1) to obtain compound **1** (21 mg).

8β-Hydroxyeremophil-3,7(11)-diene-8α,12(6α,15)-diolide (1): colorless crystals (acetone); mp 206−208 °C; $[\alpha]_D^{20}$ +23 (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max} 3367, 2937, 1766, 1678, 1645, 1453, 1386, 1256, 1021, 973 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 276 $[M]^+$ (5), 258 $[M - H_2O]^+$ (5), 231 (5), 217 (8), 187 (5), 175 (7), 91 (57), 43 (100); HRESIMS *m/z* 575.1893 $[2M + Na]^+$ (calcd for $C_{30}H_{32}O_{10}Na$, 575.1888).

8β-Methoxyeremophil-3,7(11)-diene-8α,12(6α,15)-diolide (2): colorless crystals (acetone); mp 203−205 °C; $[\alpha]_D^{20}$ +51 (*c* 0.90, $CHCl_3$); IR (KBr) ν_{max} 2925, 1769, 1686, 1654, 1375, 1256, 1156, 936, 762 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 290 $[M]^+$ (34), 275 (1), 262 (17), 259 (8), 231 (29), 175 (12), 91 (60), 39 (100); HRESIMS *m/z* 313.1051 $[M + Na]^+$ (calcd for $C_{16}H_{18}O_5Na$, 313.1046).

8β-Ethoxyeremophil-3,7(11)-diene-8α,12(6α,15)-diolide (3): colorless crystals (acetone); mp 184−186 °C; $[\alpha]_D^{20}$ +52 (*c* 0.40, $CHCl_3$); IR (KBr) ν_{max} 2928, 1769, 1681, 1649, 1454, 1371, 1256, 1015, 934, 757 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 304 $[M]^+$ (26), 276 (11), 159 (17), 231 (54), 203 (20), 149 (37), 91 (100), 43 (53); HRESIMS *m/z* 327.1208 $[M + Na]^+$ (calcd for $C_{17}H_{20}O_5Na$, 327.1203).

6β-(2ξ-Methylbutyryloxy)eremophil-3,7(11),8-trien-8,12-olide-15-oic acid methyl ester (4): colorless gum; $[\alpha]_D^{20}$ +83 (*c* 0.40, $CHCl_3$); IR (KBr) ν_{max} 2933, 2844, 1775, 1737, 1711, 1649, 1563, 1453, 1379, 1260, 1150, 1020 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 374 $[M]^+$ (5), 290 $[M - MeBuOH]^+$ (4), 272 (24), 257 (28), 240 (100), 212 (14), 185 (15), 91 (43), 57 (99), 43 (97); HRESIMS *m/z* 397.1625 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_6Na$, 397.1622).

3β-Angeloyloxy-8-oxoeremophil-6(7)-ene-12,15-dioic acid methyl ester (5): colorless gum; $[\alpha]_D^{20}$ −15 (*c* 1.90, $CHCl_3$); IR (KBr) ν_{max} 2950, 2874, 1735, 1710, 1676, 1456, 1437, 1382, 1254, 1152, 946, 850 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 406 $[M]^+$ (8), 374 (5), 346 (2), 306 $[M - AngOH]^+$ (6), 83 (100), 55 (41); HRESIMS *m/z* 407.2058 $[M + H]^+$ (calcd for $C_{22}H_{31}O_7$, 407.2064).

2-Acetyl-3aβ-methyl-3a,6,7,7aβ-tetrahydro-1*H*-inden-4-oic acid methyl ester (6): colorless gum; $[\alpha]_D^{20}$ −66 (*c* 0.80, CH_3OH); IR (KBr) ν_{max} 2952, 1713, 1668, 1611, 1436, 1365, 1249, 1230, 1060 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 234 $[M]^+$ (45), 219 (33), 187 (75), 159 (45), 91 (46), 43 (100); HRESIMS *m/z* 235.1324 $[M + H]^+$ (calcd for $C_{14}H_{19}O_3$, 235.1329).

X-ray Crystallography of Compound 1. Crystal data: $C_{15}H_{16}O_5$, formula wt 276.28, crystal dimensions 0.46 × 0.30 × 0.25 mm, monoclinic, space group $P2_1$, $\alpha = 7.340(2)$ Å, $b = 9.715(3)$ Å, $c = 9.555(3)$ Å, $\beta = 99.390(8)°$, $V = 672.3(4)$ Å^3, $Z = 2$, $D_c = 1.365$ g/cm^3, $F(000) = 292$. The reflection data were collected on a Bruker Smart Apex CCD diffractometer, using graphite-monochromated Mo Kα radiation, $\lambda = 0.71073$ Å. A total of 2421 reflections were collected

in the range $2.16° \leq \theta \leq 26.50°$, of which 2253 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. The structure was solved by direct methods using Bruker SHELXS-97 and refined by full-matrix least-squares on F^2 using Bruker SHELXS-97. The final R and R_w factors were 0.0350 and 0.1098, respectively.

Cell Culture. MGC-803 (human stomach carcinoma), HEP-G2 (human hepatoma), and S-180 (murine sarcoma) were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 in RPMI-1640 (Sigma, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco, USA), penicillin (100 U/mL), and streptomycin (100 g/mL). Exponentially growing cells were used throughout the study.

Cytotoxicity Test. Cytotoxicity was determined by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) colorimetric assay.[20] Briefly, aliquots of MGC-803, HEP-G2, and S-180 cells containing 1×10^5 cells/mL were added to each well of 96-well flat-microtiter plates. After 24 h incubation, the cells were treated with compounds **1** and **6** at various concentrations (0.1, 1.0, 10, 100 mg/L) and with **2**, **3**, and **5** at various concentrations (0.15, 1.5, 15, 150 mg/L) and with carboplatin at a concentration of 100 mg/L as positive control. Six replicate wells were used at each point in the experiments. After 48 h of incubation, MTT solution (5 mg/mL in PBS) stored at 4 °C in a dark bottle was added to each well and the plates were incubated for 4 h at 37 °C. An extraction buffer (10% SDS−0.01 M HCl) was added. After an overnight incubation at 37 °C, the optical densities (A) of 570 nm were measured by using a Bio-Rad 550 ELISA microplate reader. The cytotoxicity was calculated as cytotoxicity(%) = [(A_{570} of untreated cells − A_{570} of treated cells)/A_{570} of untreated cells] × 100%.

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

References and Notes

(1) Jiangsu College of New Medicine. *A Dictionary of Traditional Chinese Medicines*; Shanghai Science and Technology Press: Shanghai, 1977; p 154.
(2) Li, Y. S.; Wang, Z. T.; Zhang, M.; Zhou, H.; Chen, J. J.; Luo, S. D. *Planta Med.* **2004**, *70*, 239−243.
(3) Li, Y. S.; Wang, Z. T.; Zhang, M.; Zhou, H.; Chen, J. J.; Luo, S. D. *Nat. Prod. Res.* **2004**, *18*, 99−104.
(4) Fei, D. Q.; Han, Y. F.; Wu, G.; Gao, K. *J. Asian Nat. Prod. Res.* **2006**, *8*, 99−103.
(5) Li, X. Q.; Gao, K.; Jia, Z. J. *Planta Med.* **2003**, *69*, 356−360.
(6) Wu, Q. H.; Wang, C. M.; Cheng, S. G.; Gao, K. *Tetrahedron Lett.* **2004**, *45*, 8855−8858.
(7) Han, Y. F.; Pan, J.; Gao, K.; Jia, Z. J. *Chem. Pharm. Bull.* **2005**, *53*, 1338−1341.
(8) Liu, C. M.; Fei, D. Q.; Wu. Q. H.; Gao, K. *J. Nat. Prod.* **2006**, *69*, 695−699.
(9) Wu, Q. H.; Liu, C. M.; Chen, Y. J.; Gao, K. *Helv. Chim. Acta* **2006**, *89*, 915−922.
(10) Wang, W.; Gao, K.; Jia, Z. *J. Nat. Prod.* **2002**, *65*, 714−717.
(11) Wu, Q. X.; Shi, Y. P.; Yang, L. *Planta Med.* **2004**, *70*, 479−482.
(12) Zhang, S.; Zhao, G.; Li, R.; Lin, G. *Phytochemistry* **1998**, *48*, 519−524.
(13) Moriyama, Y.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3196−3199.
(14) Zhao, Y.; Jia, Z. J.; Peng, H. R. *J. Nat. Prod.* **1995**, *58*, 1358−1364.
(15) Zhao, Y.; Parsons, S.; Smart, B. A.; Tan, R. X.; Jia, Z. J.; Sun, H. D.; Rankin, D. W. H. *Tetrahedron* **1997**, *53*, 6195−6208.
(16) Pérez-Castorena, A. L.; Arciniegas, A.; Guzmán, S. L.; Villaseñor, J. L.; Romo de Vivar, A. *J. Nat. Prod.* **2006**, *69*, 1471−1475.
(17) Gu, J. Q.; Wang, Y. H.; Franzblau, S. G.; Montenegro, G.; Timmermann, B. N. *J. Nat. Prod.* **2004**, *67*, 1483−1487.
(18) Yasunoro, Y.; Masao, K. *Chem. Pharm. Bull.* **1995**, *43*, 1738−1742.
(19) Hanai, R.; Gong, X.; Tori, M.; Kondo, S.; Otose, K.; Okamoto, Y.; Nishihama, T.; Murota, A.; Shen, Y. M.; Wu, S. G.; Kuroda, C. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 1302−1308.
(20) Hussain, R. F.; Nouri, A. M. E.; Oliver, R. T. D. *J. Immunol. Methods* **1993**, *160*, 89−96.

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