New Eremophilanolides from *Ligularia hodgsonii*

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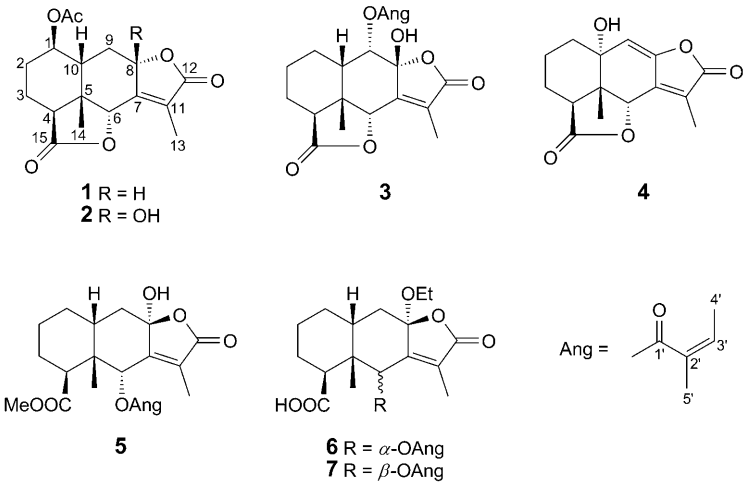
A phytochemical study on the roots and rhizomes of *Ligularia hodgsonii* led to the isolation of seven new eremophilanolides (**1**–**7**), their structures were established as (1*R*,4*S*,5*S*,6*R*,8*S*,10*R*)-1-acetoxyeremophil-7(11)-en-6,15;8,12-diolide (**1**), (1*R*,4*S*,5*S*,6*R*,8*S*,10*R*)-1-acetoxy-8β-hydroxyeremophil-7(11)-en-6,15;8,12-diolide (**2**), (4*S*,5*S*,6*R*,8*R*,9*S*,10*S*)-8-hydroxy-9-(angeloyloxy)eremophil-7(11)-en-6,15;8,12-diolide (**3**), (4*S*,5*S*,6*R*,10*R*)-10-hydroxyeremophil-7(11),8(9)-diene-6,15;8,12-diolide (**4**), (4*S*,5*S*,6*R*,8*R*,10*R*)-6-(angeloyloxy)-8-hydroxyeremophil-7(11)-en-8,12-olide-15-carboxylic acid methyl ester (**5**), (4*S*,5*S*,6*R*,8*R*,10*R*)-6-(angeloyloxy)-8-ethoxyeremophil-7(11)-en-8,12-olid-15-oic acid (**6**), (4*S*,5*S*,6*S*,8*R*,10*R*)-6-(angeloyloxy)-8-ethoxyeremophil-7(11)-en-8,12-olid-15-oic acid (**7**) by means of spectroscopic analyses. The compounds were also evaluated for antibacterial activity, only compound **6** exhibited antibacterial activity against *Bacillus subtilis*.

Introduction. – The genus *Ligularia* (Compositae) contains more than 110 species occurring in China. Approximately 40 species have been used as traditional herbal medicines. A number of sesquiterpenoids, including a few unusual ones from *Ligularia* plants, have been reported in recent years [1]. Due to the continued interest in the genus *Ligularia* [2], we investigated *Ligularia hodgsonii*, a traditional herb used as folk medicine for their antibiotic, antiphlogistic, and antitumor activities [3]. As a result, seven new eremophilanolide-type sesquiterpenes **1**–**7** were isolated from the EtOH extract of the roots and rhizomes of this species. In this article, we report the isolation, structural elucidation, and antibacterial activity of these new sesquiterpenes.

Results and Discussion. – Compound **1**, obtained as a yellow amorphous powder, has the molecular formula $C_{17}H_{20}O_6$, on the basis of the HR-ESI-MS ($[M+Na]^+$, m/z 343.1151; calc. 343.1158). The IR absorptions at 1787, 1765, and 1720 cm^{-1} implied the presence of ester C=O functionalities. The ^{1}H-NMR spectrum (*Table 1*) displays a characteristic Me signal at δ(H) 2.08 (*s*), in conjunction with ^{13}C-NMR data (δ(C) 170.1 (C) and 21.6 (Me)) (*Table 2*), an AcO group was inferred to be present in **1**. In addition, there were 15 C-atom signals in the ^{13}C-NMR spectrum, which include two lactone C=O groups (δ(C) 174.1 and 173.2), a C=C bond (δ(C) 153.9 and 126.5), three O–CH groups (δ(C) 82.4, 77.0, and 71.5), and two Me groups (δ(C) 21.1 and 9.5), which are characteristic signals of eremophilanolide-type sesquiterpene [4][5]. The ^{13}C-NMR data of **1** were nearly superimposable with those of eremophil-7(11)-en-6α,15β;8α,12-diolide [5], indicating that they have the same eremophilane sesquiterpene skeleton, except for the substitution pattern at C(1). Due to the down-field shifted



signal of H–C(1) (δ(H) 4.86) and HMBC correlations of H–C(1) with C(1′), the AcO group was located at C(1). The coupling pattern of H–C(1) (br. *s*) indicated that it occupied an equatorial position. On the basis of biogenetic precedents and in analogy with the known compounds, Me(14) and C(15)=O should be *cis*-configured, and the absolute configurations at C(4) and C(5) were presumed to be (*S*,*S*), respectively [6]. In the NOE difference spectrum, the resonances of H–C(8), H–C(10), H–C(14), and H–C(2′) were enhanced by irradiation of H–C(6), and H–C(6) and H–C(10) were enhanced by irradiation of H–C(8), indicating that H–C(6), H–C(8), H–C(10), H–C(14), and the AcO group were on the same side of the molecular plane, and a *cis*-fused *A*/*B* ring system (*Fig. 1*). Therefore, the structure of **1** was identified as (1*R*,4*S*,5*S*,6*R*,8*S*,10*R*)-1-acetoxyeremophil-7(11)-ene-6,15;8,12-diolide[1]).

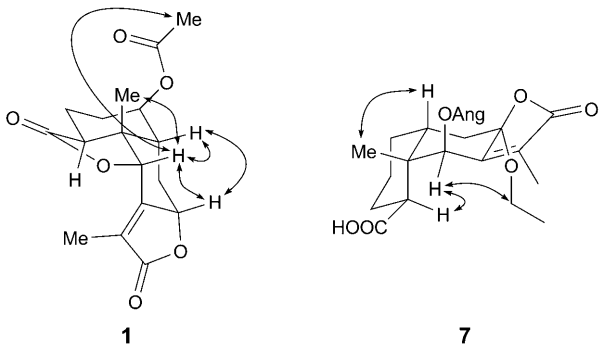


Fig. 1. *Key NOE correlations of compounds* **1** *and* **7**

[1]) For systematic names, see *Exper. Part.*

Table 1. *^{1}H-NMR Data of Compounds* **1**, **2**, *and* **3**. δ in ppm, J in Hz.

	1[a])[b])	**2**[c])[d])	**4**[d])[e])
CH_2(1) or H–C(1)	4.86 (br. *s*)	4.82 (br. *s*)	1.24–1.28 (*m*), 1.56–1.59 (*m*)
CH_2(2)	1.60–1.66 (*m*), 1.90–1.96 (*m*)	1.78–1.81 (*m*), 1.84–1.87 (*m*)	1.56–1.60 (*m*), 1.74–1.77 (*m*)
CH_2(3)	1.73–1.80 (*m*), 1.92–1.98 (*m*)	1.66–1.72 (*m*), 1.76–1.83 (*m*)	1.28–1.34 (*m*), 1.54–1.57 (*m*)
H–C(4)	2.27–2.31 (*m*)	2.70 (*dd*, J = 11.6, 2.4)	2.65 (*dd*, J = 10.8, 2.4)
H–C(6)	4.97 (*d*, J = 1.5)	5.11 (*d*, J = 1.6)	5.38 (*s*)
H–C(8)	4.69 (*dd*, J = 11.7, 4.5)	–	–
CH_2(9) or H–C(9)	1.27–1.38 (*m*), 2.49–2.57 (*m*)	1.89 (*t*, J = 12.8), 2.36 (*dd*, J = 12.8, 5.2)	5.57 (*s*)
H–C(10)	2.23–2.26 (*m*)	2.38–2.42 (*m*)	–
Me(13)	1.99 (*d*, J = 1.5)	1.82 (*d*, J = 1.6)	1.90 (*s*)
Me(14)	1.39 (*s*)	1.37 (*s*)	1.16 (*s*)
AcO	2.08 (*s*)	2.05 (*s*)	–

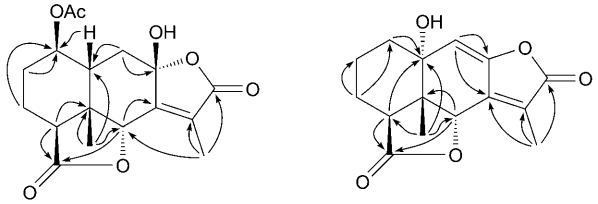
[a]) In $CDCl_3$. [b]) Recorded at 300 MHz. [c]) In (D_6)acetone. [d]) Recorded at 400 MHz. [e]) In (D_6)DMSO.

Table 2. *^{13}C-NMR Data of Compounds* **1**–**7**. δ in ppm.

	1[a])[b])	**2**[c])[d])	**3**[a])[d])	**4**[d])[e])	**5**[a])[d])	**6**[a])[d])	**7**[a])[d])
C(1)	71.5	72.1	22.2	29.8	24.8	25.1	27.8
C(2)	27.2	27.4	22.2	22.5	24.6	24.7	24.6
C(3)	16.0	16.6	18.5	18.4	18.5	18.6	20.9
C(4)	41.0	41.0	42.6	44.9	41.3	41.7	44.6
C(5)	43.9	44.3	44.1	45.5	41.4	41.8	42.9
C(6)	82.4	83.4	82.8	78.8	71.1	71.8	70.4
C(7)	153.9	153.0	150.4	151.1	151.4	150.1	154.6
C(8)	77.0	103.5	103.0	142.5	104.5	106.5	106.6
C(9)	32.4	36.2	73.6	105.5	38.3	38.4	38.7
C(10)	40.2	41.4	38.0	83.8	35.3	35.4	36.3
C(11)	126.5	127.3	129.0	127.3	129.4	132.1	126.5
C(12)	173.2	171.3	171.5	169.6	171.7	171.3	170.9
C(13)	9.5	8.9	8.9	9.1	8.9	9.4	8.1
C(14)	21.1	21.0	20.6	13.9	18.2	18.7	19.2
C(15)	174.1	175.2	176.0	174.4	173.8	179.7	178.1
EtO/AcO	170.1	170.2	–	–	–	58.7	58.9
Me–CO or MeO	21.6	21.3	–	–	51.5	15.0	15.3
AngO							
C(1′)	–	–	166.0	–	166.7	166.9	166.5
C(2′)	–	–	125.9	–	127.1	127.1	126.4
C(3′)	–	–	142.5	–	140.0	140.9	141.9
C(4′)	–	–	16.0	–	15.7	16.2	16.1
C(5′)	–	–	20.6	–	20.4	20.8	20.6

[a]) In $CDCl_3$. [b]) Recorded at 75 MHz. [c]) In (D_6)acetone. [d]) Recorded at 100 MHz. [e]) In (D_6)DMSO.

Compound **2** was obtained as colorless needles. The molecular formula $C_{17}H_{20}O_7$ was deduced from the HR-ESI-MS ($[M+NH_4]^+$, *m/z* 354.1546; calc. 354.1553). The IR spectrum of **2** showed strong absorption peaks of an OH group (3334 cm^{-1}) and C=O groups (1765 and 1718 cm^{-1}). Detailed analysis of the NMR spectra of **2** (*Tables 1* and *2*) indicated the presence of an AcO group (δ(H) 2.05 (*s*); δ(C) 170.2 (C=O) and 21.3 (Me)). ^{1}H-NMR Spectrum (*Table 1*) exhibited resonances of a Me *singlet* at δ(H) 1.37, a Me *doublet* at δ(H) 1.82, two O−CH groups at δ(H) 4.82 and 5.11. Furthermore, the ^{13}C-NMR and DEPT spectra of **2** showed 15 C-atoms including two Me groups, three CH_2 and four CH groups, and six quaternary C-atoms, which revealed that compound **2** was an eremophilanolide-type sesquiterpene. The ^{1}H- and ^{13}C-NMR signals of **2** were fully assigned by means of ^{1}H,^{1}H-COSY, HSQC, and HMBC (*Fig. 2*) experiments. A comparison of the spectroscopic data of **2** with those of **1** showed that the two compounds were very similar; the only difference was that **2** has an additional OH group, located at C(8), which could be confirmed by the correlations of 1.89 (*t*, *J* = 12.8, H_a−C(9)), 2.36 (*dd*, *J* = 12.8, 5.2, H_b−C(9)) to the C-atom signal at δ(C) 103.5 (C(8)) in the HMBC spectrum. The (*S*)-configuration at C(8) was deduced from the observation of a homoallylic coupling between H−C(6) and Me(13) in this lactone [6]. Similar to **1**, the only AcO group was attached at C(1) and was on the same side as Me(14). As a result, **2** was firmly established as (1*R*,4*S*,5*S*,6*R*,8*S*,10*R*)-1-acetoxy-8β-hydroxyeremophil-7(11)-ene-6,15;8,12-diolide[1]).



2 **4**

Fig. 2. *Key HMBC data of compounds* **2** *and* **4**

To the best of our knowledge, compounds **1** and **2** are the first eremophilandiolide-type sesquiterpene derivatives that have substituents at C(1).

Compound **3** was obtained as a colorless oil. The molecular formula $C_{20}H_{24}O_7$ for **3** was determined by the HR-ESI-MS (*m/z* 394.1858, $[M+NH_4]^+$). Analysis of the NMR data of **3** (*Tables 2* and *3*) indicated that it has the typical eremophilanolide-type sesquiterpene skeleton and an angeloyloxy group (δ(H) 6.12 (*qq*, *J* = 7.2, 1.2, H−C(3′)), 1.91 (*dq*, *J* = 7.2, 1.2, Me(4′)), and 1.62 (*dq*, *J* = 1.2, 1.2, Me(5′)); δ(C) 166.0 (C(1′)), 125.9 (C(2′)), 142.5 (CH(3′)), 16.0 (Me(4′)), and 20.6 (Me(5′))). Except for the signals for the angeloyloxy group, the ^{1}H- and ^{13}C-NMR data of **3** were similar to those of 8β-hydroxyeremophil-7(11)-ene-6α,15β;8α,12-diolide [5], which led to the conclusion that **3** was an angeloyloxy derivative of this compound. The position of the angeloyloxy group was determined to be at C(9) by the chemical shift value of H−C(9) ((δ(H) 5.65 (*d*, *J* = 4.4)) and HMBC correlations of H−C(9) with C(1′). In the NOE difference spectrum, H−C(6), H−C(9), and H−C(14) were enhanced by irradiation of H−C(10), which demonstrated that H−C(14) was *cis*-oriented with respect to

H–C(6), H–C(9), and H–C(10). A long-range coupling between H–C(6) and H–C(13) indicated that OH–C(8) was also *cis*-oriented in respect to H–C(14). Thus, compound **3** was established as (4*S*,5*S*,6*R*,8*R*,9*S*,10*S*)-8-hydroxy-9-(angeloyloxy)-eremophil-7(11)-ene-6,15;8,12-diolide[1]).

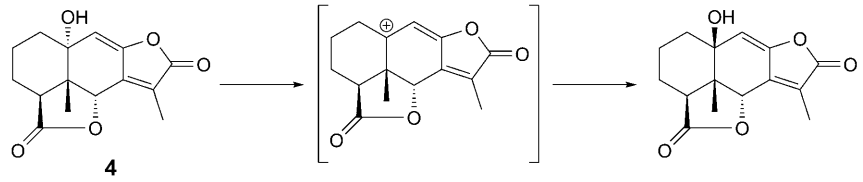
Table 3. *^{1}H-NMR Data* (400 MHz) *of Compounds* **3** *and* **5**–**7**. In $CDCl_3$, δ in ppm, J in Hz.

	3	**5**	**6**	**7**
CH_2(1)	1.64–1.68 (*m*), 1.85–1.90 (*m*)	1.34–1.38 (*m*), 1.73–1.78 (*m*)	1.36–1.41 (*m*), 1.79–1.83 (*m*)	1.25–1.28 (*m*), 1.93–1.98 (*m*)
CH_2(2)	1.20–1.24 (*m*), 1.86–1.90 (*m*)	1.64–1.58 (*m*), 1.78–1.83 (*m*)	1.36–1.41 (*m*), 1.71–1.75 (*m*)	1.57–1.62 (*m*), 1.87–1.92 (*m*)
CH_2(3)	1.41–1.49 (*m*), 1.82–1.87 (*m*)	1.51–1.56 (*m*), 1.85–1.88 (*m*)	1.53–1.56 (*m*), 1.79–1.81 (*m*)	1.54–1.59 (*m*), 1.73–1.77 (*m*)
H–C(4)	2.65 (*dd*, *J* = 11.6, 2.0)	2.32–2.36 (*m*)	2.37 (*dd*, *J* = 12.4, 3.2)	2.81 (br. *s*)
H–C(6)	5.17 (*d*, *J* = 2.0)	5.51 (*s*)	5.69 (*s*)	5.92 (*s*)
CH_2(9) or H–C(9)	5.65 (*d*, *J* = 4.4)	1.94–1.99 (*m*), 2.06–2.11 (*m*)	2.09–2.13 (*m*), 1.99–2.03 (*m*)	1.92–1.95 (*m*), 2.21–2.24 (*m*)
H–C(10)	2.44 (*dd*, *J* = 5.4, 5.1)	2.26–2.32 (*m*)	2.27–2.30 (*m*)	2.41–2.44 (*m*)
Me(13)	1.92 (*d*, *J* = 2.0)	1.91 (*s*)	2.02 (*s*)	1.81 (*d*, *J* = 1.2)
Me(14)	1.22 (*s*)	1.17 (*s*)	1.19 (*s*)	1.07 (*s*)
MeO or EtO	–	3.63 (*s*)	2.85–2.93 (*m*), 3.26–3.33 (*m*), 1.03 (*t*, *J* = 6.8)	3.39–3.46 (*m*), 3.49–3.56 (*m*), 1.24 (*t*, *J* = 7.2)
AngO				
H–C(3′)	6.12 (*qq*, *J* = 7.2, 1.2)	6.08 (*dq*, *J* = 7.2, 1.2)	6.14 (*q*, *J* = 7.2)	6.28 (*dq*, *J* = 7.2, 1.6)
Me(4′)	1.91 (*dq*, *J* = 7.2, 1.2)	1.95 (*dq*, *J* = 7.2, 1.2)	1.98 (*d*, *J* = 7.2)	2.08 (*dq*, *J* = 7.2, 1.6)
Me(5′)	1.62 (*dq*, *J* = 1.2, 1.2)	1.83 (*dq*, *J* = 1.2, 1.2)	1.88 (*s*)	1.99 (*dd*, *J* = 1.6, 1.6)

Compound **4** was obtained as a colorless amorphous powder. The molecular formula $C_{15}H_{16}O_5$ was derived from EI-MS (M^+, m/z 276) which was in agreement with the NMR data. Fifteen C-atom signals and two Me signals at δ(H) 1.90 and 1.16 were found in the ^{13}C- and ^{1}H-NMR spectra, respectively, suggesting that **4** is an eremophilane sesquiterpene. Furthermore, one olefinic H-atom *singlet* at δ(H) 5.57 in conjunction with ^{13}C-NMR resonances at δ(C) 105.5, 127.3, 142.5, 151.1, 169.6, and 174.4 strongly suggested the structure of an eremophil-7(11);8(9)-dien-6,15;8,12-diolide. Due to the long-range correlations observed from the H-atom signals at δ(H) 1.16 (*s*, Me(14)), 1.24–1.28 (*m*, H_a–C(1)), 1.56–1.59 (*m*, H_b–C(1)), and 2.65 (*dd*, *J* = 10.8, 2.4, H–C(4)) to the C-atom signal at δ(C) 83.8 (C(10)) in the HMBC spectrum (*Fig. 2*), C(10) was inferred to be oxygenated. By comparison of the NMR data of **4** with those of the known sesquiterpene 10β-hydroxyeremophil-7(11),8(9)-dien-6α,15β;8α,12-diolide [7], it was found that the ^{1}H and ^{13}C spectral data of both compounds were nearly the same, except that the C-atom shifts of the signals for C(1), C(9), and C(10) indicated that **4** is epimeric at C(10). In addition, 10β-hydroxyeremophil-7(11),8(9)-ene-6α,15β;8α,12-diolide was obtained by letting **4** stand in DMSO for prolonged periods

of time. This can be rationalized by means of a stepwise mechanism, first, the loss of the OH group at C(10) leads to an allylic carbocation, subsequent nucleophilic attack by a OH group from the other face of the tertiary carbocation yields the epimer of **4** (*Scheme*). On this basis, **4** was deduced to be (4*S*,5*S*,6*R*,10*R*)-10-hydroxyeremophil-7(11),8(9)-dien-6,15;8,12-diolide[1]).

Scheme. *Proposed Epimerization Pathway of* **4**



Compound **5** was obtained as colorless crystals, and had the molecular formula $C_{21}H_{28}O_7$, as determined by HR-ESI-MS (*m*/*z* 410.2175, $[M+NH_4]^+$). Comparing the data and features of NMR with those of known compounds, the structure of **5** was similar to that of 6α-(angeloyloxy)-8α-hydroxyeremophil-7(11)-en-8β,12-olid-15-oic acid [5], except for the presence of a MeO group (δ(H) 3.63 (*s*) and δ(C) 51.5 (Me)). The location of the MeO group at C(15) was confirmed by HMBC correlations of the MeO H-atoms at δ(H) 3.63 with the C-atom at δ(C) 173.8 (C(15)). In the NOE difference spectrum of **5**, irradiation of Me(14) caused an NOE enhancement of the signals of H–C(6) and H–C(10). Thus, H–C(6), H–C(10), and Me(14) were *cis*-oriented. The OH–C(8) on the other side of the molecular plane was deduced from the absence of a long-range coupling between H–C(6) and Me(13). Therefore, **5** was determined as (4*S*,5*S*,6*R*,8*R*,10*R*)-6-(angeloyloxy)-8-hydroxyeremophil-7(11)-en-8,12-olide-15-carboxylic acid methyl ester[1]).

Compound **6** was isolated as colorless crystals, and its molecular formula was assigned as $C_{22}H_{30}O_7$ from HR-ESI-MS (*m*/*z* 424.2333, $[M+NH_4]^+$). Comparison of the NMR data of **6** with 6β-angeloyloxy-8α-methoxyeremophil-7(11)-en-8β,12-olid-15-oic acid [8] showed that these two compounds differed only by the presence of a EtO group (δ(H) 2.85–2.93, 3.26–3.33 (*m*, each 1 H) and 1.03 (*t*, $J=6.8$, 3 H); δ(C) 58.7 (CH_2), and 15.0 (Me)) in **6** rather than the MeO group in 6β-(angeloyloxy)-8α-methoxyeremophil-7(11)-en-8β,12-olid-15-oic acid, which suggested that **6** was 6-angeloyloxy-8-ethoxyeremophil-7(11)-en-8β,12-olid-15-oic acid. This structure was further confirmed by the HMBC experiment. NOE Difference spectra of **6** showed that irradiation of Me(14) enhances H–C(6) and H–C(10). Thus, the configurations of H–C(6), H–C(10), and Me(14) favor to be *cis*-orientation. The assumption that EtO–C(8) was *trans*-oriented deduced from the absence of a homoallylic coupling between H–C(6) and Me(13) [6]. Consequently, **6** was characterized as (4*S*,5*S*,6*R*,8*R*,10*R*)-6-(angeloyloxy)-8-ethoxyeremophil-7(11)-en-8,12-olid-15-oic acid[1]).

Compound **7** was obtained as a colorless oil and had the molecular formula $C_{22}H_{30}O_7$, as deduced from HR-ESI-MS (*m*/*z* 424.2331, $[M+NH_4]^+$). Comparison of the data and features of NMR with those of **6** suggested that **7** was the C(6)-epimer of **6**. The structure was determined as (4*S*,5*S*,6*S*,8*R*,10*R*)-6-(angeloyloxy)-8-ethoxy-

eremophil-7(11)-en-8,12-olid-15-oic acid by ^{1}H,^{1}H-COSY, HMBC, and NOE spectroscopic analysis (*Fig. 1*).

To confirm whether compounds **6** and **7** are artifacts from the use of 95% EtOH as solvent for extraction, a further supply of the plant was obtained and extracted with MeOH. However, compounds **6** and **7** were not detected in the MeOH extract by LC-MS. Thus, compounds **6** and **7** were established as artifacts of extraction.

Antibacterial assays of all compounds, **1**–**7**, against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* were carried out by the doubling dilution method [9]. Chloramphenicol was used as a positive control. The results indicated that **6** showed weak antibacterial activity against *Bacillus subtilis* (minimum inhibitory concentration (*MIC*)): **6** (128 μg/ml); chloramphenicol (4 μg/ml).

Financial support by the *National Basic Research Program Projects of China* (2007CB108903) and the *National Natural Science Foundation of China* (No. 20621091 QT Program) is gratefully acknowledged.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Factory*), *Sephadex LH-20* (*Amersham Pharmacia Biotech*), *RP-18* SiO_2 (150–200 mesh, *Merck*). Thin-layer chromatography (TLC): silica gel GF_{254} (SiO_2; 10–40 mm; *Qingdao Marine Chemical Factory*); detection under UV light and visualized by spraying with 5% H_2SO_4 in EtOH (*v*/*v*), followed by heating. M.p.: *X-4* melting-point apparatus (*Beijing TECH Instrument Co. Ltd.*, P. R. China); uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet Avatar 360* FT-IR spectrometer; in cm^{-1}. NMR Spectra: *Varian Mercury-300*/*400BB* spectrometer; δ in ppm, *J* in Hz, with Me_4Si as standards or residual solvent peak used for referencing. EI-MS: *HP-5988A GC*/*MS* instrument; in *m*/*z* (rel. %). HR-ESI-MS: *Bruker APEX-II* mass spectrometer.

Plant Material. The roots and rhizomes of *L. hodgsonii* were collected from Jixi county, Anhui Province, P. R. China, in October 2005, and authenticated by Prof. *Guo-Liang Zhang* from the College of Life Science, Lanzhou University. A voucher specimen (No. 200610LH) was deposited with the Institute of Organic Chemistry, Lanzhou University.

Extraction and Isolation. The air-dried and powdered materials (3.0 kg) were extracted with 95% EtOH (8 l) at r.t. three times. The extract was evaporated under vacuum to give a residue (243 g), which was subjected to CC (SiO_2; petroleum ether (PE)/acetone 30:1 to 1:1) to afford eleven fractions (*Fr. 1–11*). *Fr. 6* was re-subjected to CC (SiO_2; PE/$CHCl_3$/MeOH 100:100:1 to 1:1:1) to give seven fractions (*Fr. 6-1–6-7*). *Fr. 6-2* was separated by CC (SiO_2; PE/$CHCl_3$/MeOH 100:100:1 to 1:1:1) to afford **4** (4 mg). *Fr. 7* was purified by CC (SiO_2; PE/$CHCl_3$/MeOH 100:100:1 to 1:1:1) to yield seven fractions (*Fr. 7-1–7-7*). *Fr. 7-4* was chromatographed by CC (SiO_2; PE/acetone 50:1 to 1:1) to give six fractions (*Fr. 7-4-1–7-4-6*). *Fr. 7-4-3* was separated by CC (*RP-18*; 25 to 75% aq. MeOH) to afford **6** (80 mg). *Fr. 7-5* was subjected to CC (SiO_2; PE/acetone 30:1 to 1:1) to give seven fractions (*Fr. 7-5-1–7-5-7*). *Fr. 7-5-3* was separated by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 2:1) to provide **5** (18 mg). **7** (2 mg) was isolated from *Fr. 7-5-4* by prep. TLC (SiO_2; PE/$CHCl_3$/MeOH 15:15:4). *Fr. 8* was subjected to CC (SiO_2; PE/$CHCl_3$/MeOH 100:100:1 to 1:1:1) to give seven fractions (*Fr. 8-1–8-7*). *Fr. 8-5* was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 2:1), followed by prep. TLC (SiO_2; PE/$CHCl_3$/AcOEt 1:1:1) to afford **3** (35 mg). *Fr. 9* was subjected to CC (SiO_2; PE/$CHCl_3$/MeOH 100:100:1 to 1:1:1) to give seven fractions (*Fr. 9-1–9-7*). *Fr. 9-3* was separated by CC (SiO_2; PE/acetone 50:1 to 1:1) to give **1** (4 mg). **2** (15 mg) was obtained from *Fr. 9-4* by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 2:1).

1β-Acetoxyeremophil-7(11)-ene-6α,15β;8α,12-diolide (=*(2a*S*,5*R*,5a*R*,6a*S*,9b*R*,9c*S*)-2a,3,4,5,5a,6,6a,8,9b,9c-Decahydro-9,9c-dimethyl-2,8-dioxo-2*H*-naphtho[2,3*-b:*4,5*-b′c′*]difuran-5-yl Acetate*; **1**). Yellow amorphous powder. M.p. 220–221°. $[\alpha]_D^{20} = +46$ ($c = 0.40$, $CHCl_3$). IR (KBr): 2940, 1787, 1765, 1720. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 343.1151 ($[M+Na]^+$, $C_{17}H_{20}NaO_6^+$; calc. 343.1158).

1β-Acetoxy-8β-hydroxyeremophil-7(11)-ene-6α,15β;8α,12-diolide (= *(2aS,5R,5aR,6aS,9bR,9cS)-2a,3,4,5,5a,6,6a,8,9b,9c-Decahydro-6a-hydroxy-9,9c-dimethyl-2,8-dioxo-2H-naphtho[2,3-b:4,5-b′c′]difuran-5-yl Acetate*; **2**). Colorless needles. M.p. 210–212°. $[\alpha]_D^{20} = +66$ ($c = 0.15$, MeOH). IR (KBr): 3334, 2965, 1765, 1718. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 354.1546 ($[M+NH_4]^+$, $C_{17}H_{24}NO_7^+$; calc. 354.1553).

9α-(Angeloyloxy)-8β-hydroxyeremophil-7(11)-ene-6α,15β;8α,12-diolide (= *(2aS,5aS,6S,6aR,9bR,9cS)-2a,3,4,5,5a,6,6a,8,9b,9c-Decahydro-6a-hydroxy-9,9c-dimethyl-2,8-dioxo-2H-naphtho[2,3-b:4,5-b′c′]difuran-6-yl (2Z)-2-Methylbut-2-enoate*; **3**). Colorless oil. $[\alpha]_D^{20} = +100$ ($c = 0.70$, $CHCl_3$). IR (KBr): 3403, 2954, 1773. ^{1}H- and ^{13}C-NMR: *Tables 3* and *2*. HR-ESI-MS: 394.1858 ($[M+NH_4]^+$, $C_{20}H_{28}NO_7^+$; calc. 394.1866).

10α-Hydroxyeremophil-7(11),8(9)-diene-6α,15β;8α,12-diolide (= *(2aS,5aR,9bR,9cS)-3,4,5,5a,9b,9c-Hexahydro-5a-hydroxy-9,9c-dimethyl-2H-naphtho[2,3-b:4,5-b′c′]difuran-2,8(2aH)-dione*; **4**). Colorless amorphous powder. M.p. 79–80°. $[\alpha]_D^{20} = +317$ ($c = 0.05$, MeOH). IR (KBr): 3433, 2956, 1777. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 276 (5, M^+), 259 (14, $[M-OH]^+$), 43 (100, $C_3H_7^+$).

6α-(Angeloyloxy)-8α-hydroxyeremophil-7(11)-en-8β,12-olide-4β-carboxylic Methyl Acid Ester (= *Methyl (4R,4aS,5S,8aR,9aR)-2,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-hydroxy-3,4a-dimethyl-4-{[(2Z)-2-methylbut-2-enoyl]oxy}-2-oxonaphtho[2,3-b]furan-5-carboxylate*; **5**). Colorless crystals. M.p. 135–137°. $[\alpha]_D^{20} = +162$ ($c = 0.22$, $CHCl_3$). IR (KBr): 3432, 2927, 1762, 1712. ^{1}H- and ^{13}C-NMR: *Table 3* and *2*. HR-ESI-MS: 410.2175 ($[M+NH_4]^+$, $C_{21}H_{32}NO_7^+$; calc. 410.2179).

6α-(Angeloyloxy)-8α-ethoxyeremophil-7(11)-en-8β,12-olide-15β-oic Acid (= *(4R,4aS,5S,8aR,9aR)-9a-Ethoxy-2,4,4a,5,6,7,8,8a,9,9a-decahydro-3,4a-dimethyl-4-{[(2Z)-2-methylbut-2-enoyl]oxy}-2-oxonaphtho[2,3-b]furan-5-carboxylic Acid*; **6**). Colorless crystals. M.p. 200–203°. $[\alpha]_D^{20} = +152$ ($c = 0.41$, $CHCl_3$). IR (KBr): 3434, 2930, 1762, 1733. ^{1}H- and ^{13}C-NMR: *Tables 3* and *2*. HR-ESI-MS: 424.2333 ($[M+NH_4]^+$, $C_{22}H_{34}NO_7^+$; calc. 424.2335).

6β-(Angeloyloxy)-8α-ethoxyeremophil-7(11)-en-8β,12-olide-15β-oic Acid (= *(4S,4aS,5S,8aR,9aR)-9a-Ethoxy-2,4,4a,5,6,7,8,8a,9,9a-decahydro-3,4a-dimethyl-4-{[(2Z)-2-methylbut-2-enoyl]oxy}-2-oxonaphtho[2,3-b]furan-5-carboxylic Acid*; **7**). Colorless oil. $[\alpha]_D^{20} = -77$ ($c = 0.30$, $CHCl_3$). IR (KBr): 3425, 2934, 1767, 1722. ^{1}H- and ^{13}C-NMR: *Tables 3* and *2*. HR-ESI-MS: 424.2331 ($[M+NH_4]^+$, $C_{22}H_{34}NO_7^+$; calc. 424.2335).

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Received June 26, 2008