New Oplopane-Type Sesquiterpenes from *Ligularia narynensis*

by **Xue Gao**[a]), **Chang-Jun Lin**[b]), **Wei-Dong Xie**[a]), **Tong Shen**[a]), and **Zhong-Jian Jia***[a])

[a]) State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, 730000 P. R. China
(phone: +(86)0931-8912408; fax: +(86)0931-8912582; e-mail: jiazj@lzu.edu.cn)
[b]) School of Life Sciences, Lanzhou University, Lanzhou, 730000 P. R. China

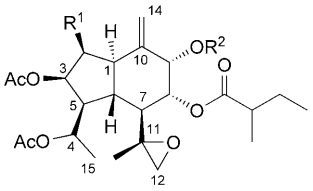
Four new, highly oxygenated oplopane-type sesquiterpenes (**3**–**6**) were isolated from the roots of *Ligularia narynensis*, together with four known compounds, and their structures were elucidated by spectroscopic methods and comparison with literature data. Selected congeners were evaluated for their *in vitro* cytotoxic activities against cultured SMMC-7721 (human hepatoma), L02 (human hepatocyte), and HL-60 (human promyelocytic leukemia) cells, but were found to be inactive.

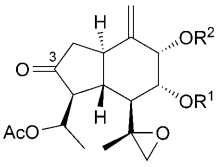
Introduction. – The genus *Ligularia* has been placed taxonomically in the tribe Senecioneae of the family Compositae, with *ca*. 110 species being distributed within mainland China [1]. More than 27 *Ligularia* species have been used as traditional Chinese medicinal herbs against fever, pain, inflammation, and intoxication, and to invigorate blood circulation [2]. In our previous chemical and biological investigation of this genus [3], we found that eremophilanolides and benzofuranolides are the most-widespread secondary metabolites, oplopane sesquiterpenes being rare within *Ligularia*.

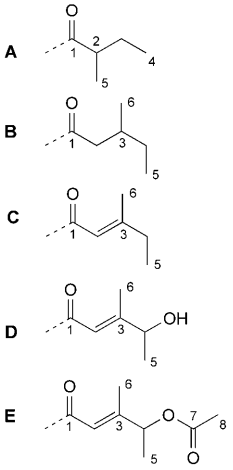
Recently, we reported the structure determination of the two oplopane-type sesquiterpenes **1** and **2** from *Ligularia narynensis* [4]. In a continuation of our phytochemical studies, we herein report the isolation and structure elucidation of four new, highly oxygenated oplopane-type sesquiterpenes, compounds **3**–**6**, from the roots of *L. narynensis*, together with two known eremophilanolides. Selected isolates were evaluated against a small panel of human-cancer cell lines for potential cytotoxic effects.

Results and Discussion. – The pulverized, air-dried roots of *L. narynensis* were extracted with petroleum ether/Et_2O/MeOH 1:1:1. Extensive purification by column chromatography on silica and *RP-18* gel afforded compounds **1**–**6**, and two eremophilanolides (see below).

For compound **3**, the molecular formula $C_{30}H_{42}O_{10}$ was determined by HR-ESI-MS (*m*/*z* 580.3111 ($[M+NH_4]^+$, $C_{30}H_{46}NO_{10}^+$; calc. 580.3122)). Its IR spectrum showed absorption bands for C=O (1734) and C=C (1655 cm^{-1}) groups. In the ^{1}H- and ^{13}C-NMR spectra of **3** (*Tables 1* and *2*), one AcO group, one (4-acetoxy-3-methylpent-2-enoyl)oxy group, and one (2-methylbutanoyl)oxy group were identified, as supported by the EI mass spectrum. Analysis of 1D- and 2D-NMR (*Tables 1–3*) and circular-dichroism (CD) data enabled us to elucidate the structure of **3** as (1*S*,3a*R*,5*S*,6*R*,-7*S*,7a*R*)-1-(1-acetoxyethyl)octahydro-6-[(2-methylbutanoyl)oxy]-4-methylidene-7-[(2*S*)-2-methyloxiran-2-yl]-2-oxo-1*H*-inden-5-yl (2*E*)-4-acetoxy-3-methylpent-2-enoate (sys-







1 R^1 = **A** R^2 = **E**
2 R^1 = H R^2 = **C**
5 R^1 = **A** R^2 = **D**
6 R^1 = **A** R^2 = **C**

3 R^1 = **A** R^2 = **E**
4 R^1 = **B** R^2 = **E**

tematic name), which corresponds to (1*R*,5*S*,6*R*,7*S*,8*R*,9*S*,11*S*)-4-acetoxy-9-[(4-acetoxy-4-methylsenecioyl)oxy]-8-[(2-methylbutanoyl)oxy]-11,12-epoxyoplop-10(14)-en-3-one[1]).

Besides the above ester groups, the NMR spectra of **3** showed resonances for a C=O group (δ(C) 212.5), a methylidene [δ(H) 4.93, 5.29 (2 br. *s*, 1 H each); δ(C) 113.4 (CH_2), 141.9 (C)], and an epoxide [δ(H) 2.70, 2.82 (2*d*, J = 4.2 Hz, 1 H each); δ(C) 52.9 (CH_2), 55.0 (C)]. Moreover, the NMR signals indicated one CH_2, seven CH (including three oxygenated ones), and two Me groups, in which one was attached to a tertiary C-atom, the other being connected to a secondary C-atom. To accommodate ten degrees of unsaturation, compound **3** was proposed to have a bicyclic sesquiterpene skeleton, with an epoxy group, a keto group, and an exocyclic C=C bond, in agreement with an oplopane sesquiterpene skeleton [5].

Further 2D-NMR experiments (^{1}H,^{1}H-COSY) showed two partial structures for the skeleton of **3**: Me–$(CH)_4$–CH_2 and $(CH)_4$. The C–C connectivities of both fragments were established by HMBC analysis (*Table 3*). The following correlations were found: H–C(14) with C(9), C(10), and C(1); H–C(13) with C(7), C(11), and C(12); H–C(12) with C(7), C(11), and C(13); H–C(15) with C(5) and C(4); H–C(1) with C(3); and H–C(4) with C(3). This further confirmed that **3** was an oplopanol derivative. The positions of the three ester groups were inferred from the HMBC correlations between H–C(4), H–C(8), and H–C(9) (δ(H) 5.12, 5.15, 5.80, resp.) with the ester C=O resonances at δ(C) 170.8, 176.1, and 165.0 of the AcO group and fragments **A** and **E**, respectively.

The relative configuration of **3** was deduced by analysis of the NMR coupling constants and of NOE effects. The coupling constants J(6,5), J(6,7), and J(6,1), all *ca.* 11

---

[1]) As the *IUPAC Recommendations on Nomenclature of Organic Compounds* provide changes in the numbering of corresponding positions due to changes in the substituents apart from the backbone, the numbering based on the oplopane backbone (as depicted) is used throughout to ensure easier reading and data comparison. For systematic names, see *Exper. Part*.

Table 1. *^{1}H-NMR Data of* **1**–**6**. Solvent: $CDCl_3$; δ in ppm, J in Hz.

Position	**1**[a])	**2**[a])	**3**[b])	**4**[b])	**5**[a])	**6**[a])
1	2.51 (*dd*, *J*=11.6, 3.2)	2.34 (*ddd*, *J*=11.4, 11.0, 3.0)	2.65 (*ddd*, *J*=14.0, 11.0, 6.8)	2.65 (*ddd*, *J*=14.0, 11.0, 6.8)	2.52 (*dd*, *J*=11.6, 3.2)	2.50 (*dd*, *J*=11.6, 3.0)
2	5.67 (*dd*, *J*=4.4, 3.2)	1.71 (*ddd*, *J*=11.0, 1.10, 11.0)	2.15 (*dd*, *J*=16.5, 14.0)	2.15 (*dd*, *J*=16.5, 14.0)	5.68 (*dd*, *J*=4.2, 3.2)	5.67 (*dd*, *J*=4.4, 3.0)
	–	2.12 (*ddd*, *J*=11.0, 6.8, 3.0)	2.45 (*dd*, *J*=16.5, 6.8)	2.45 (*dd*, *J*=16.5, 6.8)	–	–
3	5.55 (*dd*, *J*=10.8, 4.4)	5.52 (*ddd*, *J*=11.0, 11.0, 6.8)	–	–	5.55 (*dd*, *J*=10.8, 4.2)	5.55 (*dd*, *J*=10.8, 4.4)
4	5.17 (*m*)	5.12 (*dq*, *J*=6.6, 4.0)	5.12 (*dq*, *J*=6.8, 3.6)	5.12 (*dq*, *J*=6.8, 3.6)	5.18 (*m*)	5.18 (*m*)
5	2.73 (*m*)[c])	2.64 (*ddd*, *J*=11.0, 11.0, 4.0)	2.65 (*dd*, *J*=11.0, 3.6)	2.65 (*dd*, *J*=11.0, 3.6)	2.72 (*m*)[c])	2.74[c]) (*m*)[c])
6	2.00 (*m*)[c])	1.42 (*ddd*, *J*=11.4, 11.0, 11.0)	1.54 (*ddd*, *J*=11.0, 11.0, 11.0)	1.54 (*ddd*, *J*=11.0, 11.0, 11.0)	2.01 (*m*) [c])	2.00 (*m*)[c])
7	1.78 (*m*)[c])	1.82 (*dd*, *J*=11.0, 10.6)	1.99 (*dd*, *J*=11.0, 10.4)	1.99 (*dd*, *J*=11.0, 10.4)	1.76 (*m*)[c])	1.79 (*m*)[c])
8	5.07 (*dd*, *J*=10.6, 3.2)	5.03 (*dd*, *J*=10.6, 3.2)	5.15 (*dd*, *J*=10.4, 3.1)	5.15 (*dd*, *J*=10.4, 3.1)	5.08 (*dd*, *J*=10.6, 3.2)	5.07 (*dd*, *J*=10.8, 3.1)
9	5.67 (br. *d*, *J*=3.2)	5.72 (br. *d*, *J*=3.2)	5.80 (br. *d*, *J*=3.1)	5.77 (br. *d*, *J*=3.1)	5.67 (br. *d*, *J*=3.2)	5.66 (br. *d*, *J*=3.1)
12	2.76 (*d*, *J*=4.0)	2.74 (*d*, *J*=4.0)	2.70 (*d*, *J*=4.2)	2.70 (*d*, *J*=4.2)	2.75 (*d*, *J*=4.0)	2.73 (*d*, *J*=4.0)
	2.84 (*d*, *J*=4.0)	2.81 (*d*, *J*=4.0)	2.82 (*d*, *J*=4.2)	2.82 (*d*, *J*=4.2)	2.82 (*d*, *J*=4.0)	2.84 (*d*, *J*=4.0)
13	1.21 (*s*)	1.19 (*s*)	1.23 (*s*)	1.23 (*s*)	1.21 (*s*)	1.22 (*s*)
14	4.84 (br. *s*)	4.93 (br. *s*)	4.93 (br. *s*)	4.93 (br. *s*)	4.82 (br. *s*)	4.84 (br. *s*)
	5.24 (br. *s*)	5.21 (br. *s*)	5.29 (br. *s*)	5.29 (br. *s*)	5.25 (br. *s*)	5.25 (br. *s*)
15	1.44 (*d*, *J*=6.8)	1.46 (*d*, *J*=6.6)	1.24 (*d*, *J*=6.8)	1.24 (*d*, *J*=6.8)	1.45 (*d*, *J*=6.6)	1.45 (*d*, *J*=6.6)
AcO	2.04 (*s*)	2.12 (*s*)	–	–	1.98 (*s*)	1.96 (*s*)
AcO	1.96 (*s*)	2.00 (*s*)	2.11 (*s*)	2.11 (*s*)	2.04 (*s*)	2.05 (*s*)
A[d])						
2	2.40 (*m*)	–	–	–	2.39 (*m*)	2.37 (*m*)
3	1.48 (*m*), 1.75 (*m*)	–	–	–	1.48 (*m*), 1.75 (*m*)	1.48 (*m*), 1.75 (*m*)
4	0.89 (*t*, *J*=7.4)	–	–	–	0.89 (*t*, *J*=7.6)	0.89 (*t*, *J*=7.6)
5	1.14 (*d*, *J*=7.2)	–	–	–	1.15 (*d*, *J*=7.2)	1.15 (*d*, *J*=7.2)
A or **B**[e])						
2	2.40 (*m*)	2.40 (*m*)	2.40 (*m*)	2.40 (*m*)	2.40 (*m*)	2.37 (*m*)
3	1.48 (*m*), 1.75 (*m*)	1.48 (*m*), 1.79 (*m*)	1.48 (*m*), 1.75 (*m*)	2.08 (*m*)	1.48 (*m*), 1.75 (*m*)	1.48 (*m*), 1.75 (*m*)

Table 1 (cont.)

Position	**1**[a])	**2**[a])	**3**[b])	**4**[b])	**5**[a])	**6**[a])
4	0.97 (*t*, *J*=7.2)	0.90 (*t*, *J*=7.0)	0.90 (*t*, *J*=7.2)	1.22 (*m*), 1.32 (*m*)	0.97 (*t*, *J*=7.2)	0.97 (*t*, *J*=7.0)
5	1.19 (*d*, *J*=7.0)	1.17 (*d*, *J*=7.0)	1.16 (*d*, *J*=7.2)	0.86 (*t*, *J*=7.2)	1.20 (*d*, *J*=7.2)	1.20 (*d*, *J*=7.2)
6	–	–	–	0.93 (d, *J*=6.6)	–	–
C–**E**[f])						
2	5.82 (br. *s*)	5.63 (br. *s*)	5.86 (br. *s*)	5.86 (br. *s*)	5.93 (br. *s*)	5.61 (br. *s*)
4	5.23 (*q*, *J*=7.0)	2.16 (*q*, *J*=7.0)	5.24 (*q*, *J*=7.0)	5.24 (*q*, *J*=7.0)	4.26 (*q*, *J*=7.0)	2.16 (*q*, *J*=7.2)
5	1.33 (*d*, *J*=6.8)	1.07 (*t*, *J*=7.0)	1.34 (*d*, *J*=7.0)	1.34 (d, *J*=7.0)	1.31 (d, *J*=6.8)	1.07 (*t*, *J*=7.0)
6	2.08 (br. *s*)	2.13 (br. *s*)	2.10 (br. *s*)	2.10 (br. *s*)	2.08 (br. *s*)	2.13 (br. *s*)
8	2.11 (*s*)		2.10 (*s*)	2.10 (*s*)		

[a]) Recorded at 400 MHz. [b]) Recorded at 300 MHz. [c]) Overlapping. [d]) 2-Methylbutanoyl substituent (**A**). [e]) 3-Methylpentanoyl substituent (**B**) for **4**. [f]) 3-Methylpent-2-enoyl (**C**) for **2** and **6**; 4-hydroxy-3-methylpent-2-enoyl (**D**) for **5**; 4-acetoxy-3-methylpent-2-enoyl (**E**) for **1**, **3**, and **4**.

Hz, indicated that H–C(6) was *trans*-oriented with respect to H–C(5), H–C(7), and H–C(1). Furthermore *J*(8,7) and *J*(8,9), 10.4 and 3.1 Hz, respectively, indicated that H–C(8) and H–C(9) were on the same side of the molecular plane as H–C(6). Irradiation of H–C(6) produced an NOE enhancement of H–C(13) (3.7%), and irradiation of H–C(7) gave rise to an enhancement of H_b–C(12) (2.9%). Thus, H–C(13) was located on the same side as H–C(6). The absolute configuration of **3**, *i.e.*, (1*R*,5*S*,6*R*,7*S*,8*R*,9*S*,11*S*), was deduced by circular dichroism (CD), in which a negative *Cotton* effect was observed at 305 nm ($\Delta\varepsilon = -2.85$) [6].

The HR-ESI mass spectrum of **4** displayed the $[M+Na]^+$ signal at *m*/*z* 599.2819 (calc. 599.2832), which, in combination with the NMR data (*Tables 1* and *2*), suggested the molecular formula $C_{31}H_{44}O_{10}$. The IR spectrum indicated the presence of C=O (1734) and C=C (1655 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra of **4** were very similar to those of **3**, except for one more CH_2 group in the former. Thus, compound **4** was identified as the 3-methylpentanonoyl analogue of **3**, with the same absolute configuration, as established by CD analysis.

The HR-ESI mass spectrum of **5** showed the $[M+Na]^+$ signal at *m*/*z* 687.3349 (calc. 687.3356), in accord with the molecular formula $C_{35}H_{52}O_{12}$. Its IR spectrum indicated the presence of ester C=O groups (1733), a C=C bond (1653), and an OH function (3449 cm^{-1}). Comparing the NMR spectra of **5** (*Tables 1* and *2*) with those of **3**, the keto C=O group (δ(C) 212.5) and one CH_2 (δ(H) 2.15, 2.45; δ(C) 41.7) in **3** were replaced by two oxymethines (δ(C) 71.1 and 72.2) in **5**. Two more ester groups, an AcO and a (2-methylbutanoyl)oxy moiety, appeared correspondingly. Furthermore, the 4-acetoxy-3-methylpent-2-enoyl group in **3** was replaced by a 4-hydroxy-3-methylpent-2-enoyl function. So, compound **5** was secured as (1*S*,2*R*,3*S*,3a*R*,5*S*,6*R*,7*S*,7a*S*)-2-acetoxy-1-(1-acetoxyethyl)octahydro-3,6-bis[(2-methylbutanoyl)oxy]-4-methylidene-7-[(2*S*)-2-methyloxiran-2-yl]-1*H*-inden-5-yl (2*E*)-4-hydroxy-3-methylpent-2-enoate.

Table 2. *^{13}C-NMR (DEPT) Data of Compounds* **1**–**6**. In $CHCl_3$; δ in ppm.

Position	**1**[a])	**2**[a])	**3**[b])	**4**[b])	**5**[a])	**6**[a])
1	44.6 (*d*)	41.4 (*d*)	40.9 (*d*)	40.9 (*d*)	44.7 (*d*)	44.6 (*d*)
2	71.0 (*d*)	33.6 (*t*)	41.7 (*t*)	41.7 (*t*)	71.1 (*d*)	71.1 (*d*)
3	72.2 (*d*)	72.9 (*d*)	212.5 (*s*)	212.5 (*s*)	72.2 (*d*)	72.2 (*d*)
4	69.7 (*d*)	70.1 (*d*)	68.5 (*d*)	68.5 (*d*)	69.7 (*d*)	69.7 (*d*)
5	44.9 (*d*)	45.3 (*d*)	56.4 (*d*)	56.4 (*d*)	45.0 (*d*)	44.9 (*d*)
6	43.7 (*d*)	47.4 (*d*)	45.7 (*d*)	45.7 (*d*)	43.7 (*d*)	43.6 (*d*)
7	49.2 (*d*)	49.0 (*d*)	48.9 (*d*)	48.9 (*d*)	49.0 (*d*)	49.0 (*d*)
8	72.3 (*d*)	73.2 (*d*)	72.7 (*d*)	72.7 (*d*)	72.3 (*d*)	72.4 (*d*)
9	73.2 (*d*)	72.2 (*d*)	72.7 (*d*)	73.6 (*d*)	73.0 (*d*)	72.6 (*d*)
10	137.8 (*s*)	142.9 (*s*)	141.9 (*s*)	141.9 (*s*)	137.9 (*s*)	138.0 (*s*)
11	55.1 (*s*)	55.1 (*s*)	55.0 (*s*)	55.0 (*s*)	55.0 (*s*)	55.1 (*s*)
12	53.0 (*t*)	53.0 (*t*)	52.9 (*t*)	52.9 (*t*)	53.0 (*t*)	53.0 (*t*)
13	16.3 (*q*)	16.1 (*q*)	15.1 (*q*)	15.1 (*q*)	16.3 (*q*)	16.2 (*q*)
14	115.2 (*t*)	112.0 (*t*)	113.4 (*t*)	113.4 (*t*)	115.0 (*t*)	114.9 (*t*)
15	16.2 (*q*)	16.1 (*q*)	15.1 (*q*)	15.1 (*q*)	16.3 (*q*)	16.2 (*q*)
Me*C*O	169.9 (*s*)	170.6 (*s*)	–	–	170.2 (*s*)	170.2 (*s*)
*Me*CO	21.0 (*q*)	21.3 (*q*)	–	–	21.0 (*q*)	21.1 (*q*)
Me*C*O	170.2 (*s*)	170.3 (*s*)	170.8 (*s*)	170.8 (*s*)	169.8 (*s*)	169.8 (*s*)
*Me*CO	21.0 (*q*)	21.0 (*q*)	21.3 (*q*)	21.3 (*q*)	21.7 (*q*)	21.0 (*q*)
A[c])						
1	176.0 (*s*)	–	–	–	176.1 (*s*)	176.1 (*s*)
2	41.1 (*d*)	–	–	–	41.1 (*d*)	41.1 (*d*)
3	26.3 (*t*)	–	–	–	26.2 (*t*)	26.2 (*t*)
4	11.5 (*q*)	–	–	–	11.5 (*q*)	11.5 (*q*)
5	16.2 (*q*)	–	–	–	16.2 (*q*)	16.2 (*q*)
A or **B**[c])						
1	176.0 (*s*)	176.1 (*s*)	176.1 (*s*)	172.6 (*s*)	176.0 (*s*)	176.1 (*s*)
2	41.1 (*d*)	41.1 (*d*)	41.1 (*d*)	41.4 (*t*)	41.7 (*d*)	41.6 (*d*)
3	26.5 (*t*)	26.2 (*t*)	26.3 (*t*)	31.5 (*d*)	26.5 (*t*)	26.5 (*t*)
4	11.9 (*q*)	11.5 (*q*)	11.6 (*q*)	29.1 (*t*)	11.9 (*q*)	11.9 (*q*)
5	17.5 (*q*)	16.1 (*q*)	16.2 (*q*)	11.1 (*q*)	17.5 (*q*)	17.5 (*q*)
6	–	–	–	19.2 (*q*)	–	–
C–**E**[c])						
1	164.9 (*s*)	165.4 (*s*)	165.0 (*s*)	165.0 (*s*)	165.1 (*s*)	165.2 (*s*)
2	114.9 (*d*)	114.0 (*d*)	114.9 (*d*)	114.9 (*d*)	113.5 (*d*)	113.9 (*d*)
3	158.0 (*s*)	162.7 (*s*)	158.2 (*s*)	158.2 (*s*)	162.6 (*s*)	163.0 (*s*)
4	73.5 (*d*)	33.7 (*t*)	73.7 (*d*)	73.7 (*d*)	72.4 (*d*)	33.7 (*t*)
5	19.1 (*q*)	11.7 (*q*)	19.2 (*q*)	19.2 (*q*)	21.0 (*q*)	11.8 (*q*)
6	15.2 (*q*)	18.8 (*q*)	15.1 (*q*)	15.1 (*q*)	15.1 (*q*)	18.9 (*q*)
7	169.8 (*s*)	–	170.0 (*s*)	170.0 (*s*)	–	–
8	21.2 (*q*)	–	21.2 (*q*)	21.2 (*q*)	–	–

[a]) Recorded at 100 MHz. [b]) Recorded at 75 MHz. [c]) For substituents **A**–**E**, see *Table 1* and chemical formulae.

Table 3. *Key HMBC Interactions for* **3**–**6**

H-atom	C-Atom			
	3[a])	**4**[a])	**5**[b])	**6**[b])
1	3, 6, 7,10	3, 6, 10	6, 10, 14	6, 10, 14
2	3, 6, 10	3, 6, 10	5, 6; 1 of **A**	1; 1 of **A**
3	–	–	1, 2, 4, 6; C=O of Ac	1, 2, 4, 6; C=O of Ac
4	3, 5, 15; C=O of Ac	3, 5, 15; C=O of Ac	3, 5, 15; C=O of Ac	3, 5, 15; C=O of Ac
5	2, 3, 7, 15	2, 3, 7, 15	2, 3, 4, 7, 15	2, 4, 7, 15
6	2, 4, 5, 8	2, 4, 5	1, 4, 5, 7, 8, 10	1, 4, 5, 8, 10
7	8, 11, 12, 13	8, 11, 12, 13	8, 11, 12, 13	8, 11, 12, 13
8	7, 9, 11; 1 of **A**	7, 9, 11; 1 of **B**	7, 9, 11; 1 of **A**	9, 11; 1 of **A**
9	1, 7, 8, 10, 14; 1 of **E**	1, 8, 10, 14; 1 of **E**	1, 7, 8, 10, 14; 1 of **D**	7, 8, 10, 14; 1 of **C**
12	7, 11, 13	7, 11, 13	7, 11, 13	7, 11
13	7, 11, 12	7, 11, 12	7, 11, 12	7, 11, 12
14	1, 9, 10	1, 9	1, 9, 10	9, 10
15	4, 5	4, 5	4, 5	4, 5

[a]) Recorded at 300 MHz in $CDCl_3$. [b]) Recorded at 400 MHz in $CDCl_3$.

When irradiating H–C(3), H–C(2), H–C(5), and H–C(1) of **5**, NOE enhancements of 4.3, 4.9, and 3.7% were observed, and irradiation of H–C(8), H–C(6), and H–C(9) gave rise to values of 3.14 and 5.49%, respectively. Moreover, considering the $J(2,3)$, $J(3,5)$, $J(7,8)$, and $J(8,9)$ values of 4.2, 10.8, 10.6, and 3.2 Hz, respectively, the relative configuration of **5** was assigned as shown. Since the *absolute* configurations of **3** and **4** had already been established, we assumed that it is retained also in the close congener **5**.

The formula of compound **6** was determined as $C_{35}H_{52}O_{11}$ by HR-ESI-MS (*m*/*z* 671.3407 ($[M+Na]^+$, $C_{35}H_{52}NaO_{11}^+$; calc. 671.3407)). The IR spectrum of **6** showed absorption bands for ester C=O (1734) and C=C (1649 cm^{-1}) groups. Its NMR data were very similar to those of **5**, except for a 3-methylpent-2-enoyl instead of a 4-hydroxy-3-methylpent-2-enoyl group.

Four known compounds were also isolated from *L. narynensis*: 3β,4-diacetoxy-9α-(4-acetoxy-4-methylsenecioyloxy)-2β,8α-di(2-methylbutyryloxy)-11α,12-epoxyoplop-10(14)-ene (**1**) [4], 3β,4-diacetoxy-8α-(2-methylbutyryloxy)-9α-(4-methylsenecioyloxy)-11α,12-epoxyoplop-10(14)-ene (**2**) [4], eremophil-7(11)-en-6α,15;8α,12-diolide [7], and 8β-hydroxyeremophil-7(11)-en-6α,15;8α,12-diolide [7]. These compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Compounds **1**, **2**, and **6** were assayed for their cytotoxic activities toward human hepatoma SMMC-7721, human hepatocytes L02, and human promyelocytic leukemia HL-60 cells, according to the sulforhodamine B (SRB) method [8]. However they were all inactive ($IC_{50} > 100$, 23.5, and 24.6 μg/ml, resp.).

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh) and *RP-18* gel (60–50 μm). TLC: Silica gel GF_{254} plates (10–40 μm); detection under UV light or by heating after spraying with 5% H_2SO_4 in EtOH. M.p.: *Kofler* melting-point apparatus; uncorrected. Optical rotation: *Perkin-Elmer 341* polarimeter. CD spectra: *Olis RSM 1000CD* apparatus; $\Delta\varepsilon$ in l mol^{-1} cm^{-1} (λ in nm). IR Spectra: *Nicolet 170sx NEXUS 670* FT-IR spectrometer; in cm^{-1}. NMR Spectra: *Varian Mercury* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. EI-MS: *HP-5988A GC/MS* instrument; in *m*/*z* (rel. %). HR-ESI-MS: *Bruker APEX II* mass spectrometer.

Plant Material. The roots of *Ligularia narynensis* were collected in the Tianshan Mountains, Xinjiang Uygur Autonomous Region, P. R. China, in August 2003, and identified by Prof. *Guo-Liang Zhang*, Department of Biology, Lanzhou University. A voucher specimen was deposited at the Institute of Organic Chemistry, Lanzhou University.

Extraction and Isolation. The air-dried roots of *L. narynensis* (3.5 kg) were pulverized and extracted three times with petroleum ether (b.p. 60–90°)/Et_2O/MeOH 1:1:1 at r.t. The extract was concentrated under reduced pressure to afford a residue (70 g), which was subjected to CC (700 g SiO_2 (200–300 mesh); petroleum ether/acetone 30:1, 15:1, 8:1, 2:1): four fractions (Fr.). *Fr. 2* (eluted with petroleum ether/acetone 15:1; 9.6 g) was separated by CC (96 g SiO_2 (300–400 mesh); petroleum ether/AcOEt 10:1 and 4:1): *Fr. 2.1* and *Fr. 2.2*. *Fr. 2.1* (eluted with petroleum ether/AcOEt 10:1; 3.8 g) was purified by repeated CC (*RP-18*) to afford **1** (20 mg) and **2** (65 mg) upon elution with acetone/H_2O 5:4, as well as **3** (4 mg) and **4** (3 mg) with acetone/H_2O 5:3. *Fr. 2.2* (eluted with petroleum ether/AcOEt 4:1; 5.2 g) was subjected to CC (52 g SiO_2 (300–400 mesh); petroleum ether/AcOEt 8:1 and 2:1) to provide **6** (16 mg) and, after repeated CC on *RP-18* gel eluting with acetone/H_2O 5:3, eremophil-7(11)-en-6α,15;8α,12-diolide (20 mg). *Fr. 3* (eluted with petroleum ether/acetone 8:1; 9.2 g) afforded 8β-hydroxyeremophil-7(11)-en-6α,15;8α,12-diolide (13 mg) by CC (SiO_2; $CHCl_3$/AcOEt 30:1 → 8:1) followed by recrystallization from acetone; from the corresponding mother liquor, **5** (3 mg) was obtained by CC (1 g SiO_2 (300–400 mesh); $CHCl_3$/AcOEt 15:1).

(1S,3aR,5S,6R,7S,7aR)-1-(1-Acetoxyethyl)octahydro-6-[(2-methylbutanoyl)oxy]-4-methylidene-7-[(2S)-2-methyloxiran-2-yl]-2-oxo-1H-inden-5-yl (2E)-4-Acetoxy-3-methylpent-2-enoate (**3**). Colorless gum. $[\alpha]_D^{20} = +16.7$ ($c = 3.0$, $CHCl_3$). CD ($CHCl_3$): −2.85 (306). IR (KBr): 2923, 1734, 1655, 1372, 1242, 1152, 1075, 1039, 756. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 43 (46, $[COCH_3]^+$), 85 (11, $[COCH(CH_3)CH_2CH_3]^+$), 155 (4, $[COCH{=}C(CH_3)CH(CH_3)OAc]^+$). HR-EI-MS: 580.3111 ($[M+NH_4]^+$, $C_{30}H_{46}NO_{10}^+$; calc. 580.3122).

(1S,3aR,5S,6R,7S,7aR)-1-(1-Acetoxyethyl)octahydro-4-methylidene-7-[(2S)-2-methyloxiran-2-yl]-6-[(3-methylpentanoyl)oxy]-2-oxo-1H-inden-5-yl (2E)-4-Acetoxy-3-methylpent-2-enoate (**4**). Colorless gum. $[\alpha]_D^{20} = +13.3$ ($c = 3.0$, $CHCl_3$). IR (KBr): 2923, 1734, 1655, 1372, 1242, 1152, 1075, 1039, 756. CD ($CHCl_3$): −1.65 (306). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 43 (44, $[COCH_3]^+$), 99 (13, $[COCH_2CH(CH_3)CH_2CH_3]^+$), 155 (4, $[COCH{=}C(CH_3)CH(CH_3)OAc]^+$). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-EI-MS: 599.2819 ($[M+Na]^+$, $C_{31}H_{44}NaO_{10}^+$; calc. 599.2832).

(1S,2R,3S,3aR,5S,6R,7S,7aS)-2-Acetoxy-1-(1-acetoxyethyl)octahydro-3,6-bis[(2-methylbutanoyl)-oxy]-4-methylidene-7-[(2S)-2-methyloxiran-2-yl]-1H-inden-5-yl (2E)-4-Hydroxy-3-methylpent-2-enoate (**5**). Colorless gum. $[\alpha]_D^{20} = +85.2$ ($c = 3.5$, $CHCl_3$). IR (KBr): 3449, 2970, 1733, 1653, 1371, 1244, 1181, 1150, 1072, 1045, 755. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-EI-MS: 687.3349 ($[M+Na]^+$, $C_{35}H_{52}NaO_{12}^+$; calc. 687.3356).

(1S,2R,3S,3aR,5S,6R,7S,7aS)-2-Acetoxy-1-(1-acetoxyethyl)-octahydro-3,6-bis[(2-methylbutanoyl)-oxy]-4-methylidene-7-[(2S)-2-methyloxiran-2-yl]-1H-inden-5-yl (2E)-3-Methylpent-2-enoate (**6**). Colorless gum. $[\alpha]_D^{20} = +105.5$ ($c = 3.7$, $CHCl_3$). IR (KBr): 2969, 1734, 1649, 1243, 1145, 1098, 1073, 1044, 755. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-EI-MS: 671.3407 ($[M+Na]^+$, $C_{35}H_{52}NaO_{11}^+$; calc. 671.3407).

REFERENCES

[1] 'The Geography of Spermatophytic Families and Genera', Ed. A. M. Lu, Science Press, Beijing, 1999, Vol. 1, p. 530.
[2] L.-S. Chen, *Chin. Tradit. Herbal Drugs* **1987**, *18*, 1431.
[3] Z.-J. Jia, H.-M. Chen, *Phytochemistry* **1991**, *30*, 3132; Y. Zhao, Z.-J. Jia, R.-X. Tan, L. Yang, *Phytochemistry* **1992**, *31*, 2785; Y. Zhao, S. Parsons, B. A. Smart, R.-X. Tan, Z.-J. Jia, H. Sun, D. W. H. Rankin, *Phytochemistry* **1997**, *53*, 6195; W.-S. Wang, K. Gao, Z.-J. Jia, *Planta Med.* **2000**, *66*, 189; X.-Q. Li, K. Gao, Z.-J. Jia, *Planta Med.* **2003**, *69*, 350.
[4] X. Gao, T. Shen, W.-D. Xie, Z.-J. Jia, *Chin. Chem. Lett.* **2006**, *17*, 341.
[5] A. Arciniegas, A.-L. Pérez-Castorena, S. Reyes, J. L. Contreras, A. R. de Vivar, *J. Nat. Prod.* **2003**, *66*, 225.
[6] D. N. Kirk, W. J. Klyne, *J. Chem. Soc., Perkin Trans. 1* **1976**, *7*, 762.
[7] Y. Zhao, Z.-J. Jia, H.-R. Peng, *J. Nat. Prod.* **1995**, *58*, 1358.
[8] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107.

Received April 26, 2006