A New Triterpene and New Sesquiterpenes from the Roots of *Ligularia sagitta*

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One new triterpene and seven new eremophilane-type sesquiterpenes were isolated from the roots of *Ligularia sagitta*, among them **1a**, an *O*-acetylated oleanane type triterpene, **2a** and **3a**, two 11-nor eremophilane-type sesquiterpenes, **4a**, **5**, and **6**, three standard eremophilane-type sesquiterpenes, and **7** and **8**, two tri-nor eremophilane-type sesquiterpenes. Their structures were established by extensive spectral analyses (1D, 2D-NMR, IR, and MS). Compound **1a** showed moderate cytotoxicity against two tumor cell lines.

Introduction. – The genus *Ligularia* (Compositae) is widely distributed in China with about 100 species, of which more than 20 have long been used in traditional folk medicine. This genus possesses efficacies of antipyretics, relieving phlegm and cough, invigorating circulation of blood and soothing pain [1]. A previous study showed that the main components of *Ligularia* are eremophilane sesquiterpenes and pyrrolizidine alkloids with strong physiological activities [2]. In our long-standing interest in the study of biodiversity and searching for bioactive compounds from several *Ligularia* species [3], the results showed that the components from the same genus, even from the same species displayed remarkable taxonomic differences because of the variability in the ecological environments and collection seasons [3c][4]. As a part of our ongoing investigation on the chemical constitutents of the roots of *Ligularia sagitta* collected from Gannan Tibet Autonomous Region (2000–3800 m above sea level) in Gansu Province of P. R. China, one new triterpene and seven new eremophilane-type sesquiterpenes (*Fig. 1*) were obtained. In this report, we describe the isolation and the structure elucidation (by ^{1}H- and ^{13}C-NMR, COSY, HMBC, and NOESY experiments) of these new compounds.

Results and Discussion. – 1. *Structure Elucidation*. The petroleum ether (b.p. 60–90°)/Et_2O/MeOH (1:1:1)-extracts of roots of *L. sagitta* were submitted to column chromatography on silica gel, yielding fractions that were further purified to give compounds **5**–**8**; compounds **1a**–**4a** were obtained after acetylation of the corresponding mixture, otherwise, they were difficult to separate.

Compound **1a** was obtained as a white amorphous powder. The HR-ESI-MS experiment gave an ion peak of $[M+NH_4]^+$ at *m/z* 616.4212, consistent with the molecular formula of $C_{36}H_{54}O_7$ ($C_{36}H_{58}NO_7^+$; calc. 616.4213), which accounted for ten degrees of unsaturation. The IR spectrum showed the absorption bands of C=O (1737 and 1706 cm^{-1}) and C=C (1634 cm^{-1}).

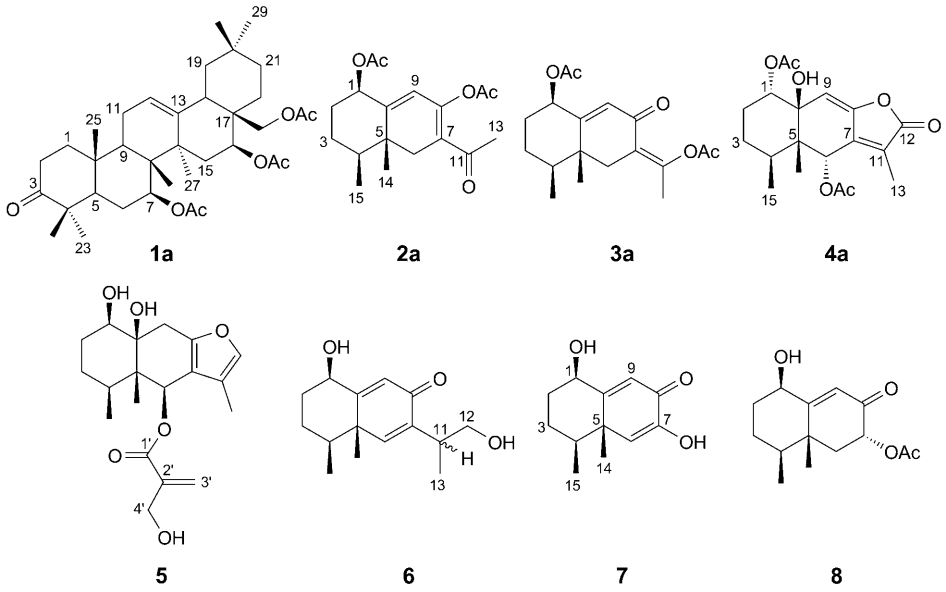

Fig. 1. *Structures of compounds* **1a**–**4a** *and* **5**–**8**

The ^{1}H- and ^{13}C-NMR (*Table 1*) spectra of compound **1a** showed the typical signals of three AcO groups at δ(H) 1.94 (*s*, Me), 2.01 (*s*, Me), 2.09 (*s*, Me), and δ(C) 170.3 (C), 170.3 (C), 171.5(C), 21.8 (Me), 21.6 (Me), 21.3 (Me). In particular, with the help of an HMBC experiment, the ^{1}H-NMR spectrum displayed seven Me signals at δ(H) 0.89 (*s*, Me(29), Me(30)); 1.02 (*s*, Me(23)); 1.05 (*s*, Me(25)); 1.09 (*s*, Me(24)); 1.16 (*s*, Me(26)); 1.33 (*s*, Me(27)), an olefinic signal at δ(H) 5.34 (br. *s*, H–C(12)), two oxygenated CH groups at δ(H) 5.47 (*dd*, J = 11.4, 5.4, CH), 5.12 (br. *d*, J = 11.4, CH), and an oxygenated CH_2 group at δ(H) 4.07 (*d*, J = 10.4, H_a–C(28)); 4.01 (*d*, J = 10.4, H_b–C(28)); the ^{13}C-NMR spectrum showed 30 C-atom signals (7 × Me, 9 × CH_2, 6 × CH, 8 × C[1])) for the skeleton of **1a**, and additionally three AcO groups, which indicated that compound **1a** was a pentacyclic triterpene with a trisubstituted C=C bond.

With the aid of HMBC experiments, an oleane-type triterpene skeleton was suggested by the key HMBC correlations of the seven Me groups (*Fig. 2*), and the correlations of Me(23) and Me(24) with C(3) at δ(C) 216.9 (C) indicated that C(3) is a C=O group; correlation of Me(26) with C(7) at δ(C) 75.2 (CH) indicated that an AcO group was connected with C(7). With the consideration of a chair-like conformation of the five rings of the skeleton of olean-type triterpenes, the coupling pattern of H–C(7) at δ(H) 5.12 (br. *d*, J = 11.4, CH) indicated that H–C(7) should be axial and α-orientated. The HMBC correlation of the H-atom at δ(H) 5.47 (*dd*, J = 11.4, 5.4, CH) with C(28) at δ(C) 66.2 (CH_2) indicated that another AcO group was connected to C(16) or C(22). In an NOE difference experiment (*Fig. 3*), irradiation of the H-atom at

[1]) Assigned through a DEPT spectrum.

Table 1. *^{13}C-NMR Data for Compound* **1a**[a]). δ in ppm.

	δ(C)		δ(C)
CH_2(1)	38.9 (*t*)	H−C(16)	68.5 (*d*)
CH_2(2)	33.8 (*t*)	C(17)	39.6 (*s*)
C(3)	216.9 (*s*)	H−C(18)	43.4 (*d*)
C(4)	47.2 (*s*)	CH_2(19)	46.0 (*t*)
H−C(5)	51.9 (*d*)	C(20)	30.9 (*s*)
CH_2(6)	26.3 (*t*)	CH_2(21)	34.1 (*t*)
H−C(7)	75.2 (*d*)	CH_2(22)	23.6 (*t*)
C(8)	44.5 (*s*)	Me(23)	27.1 (*q*)
H−C(9)	46.3 (*d*)	Me(24)	21.8 (*q*)
C(10)	36.8 (*s*)	Me(25)	15.5 (*q*)
CH_2(11)	23.6 (*t*)	Me(26)	11.3 (*q*)
H−C(12)	124.0 (*d*)	Me(27)	27.0 (*q*)
C(13)	141.3 (*s*)	CH_2(28)	66.2 (*t*)
C(14)	44.5 (*s*)	Me(29)	33.1 (*q*)
CH_2(15)	34.4 (*t*)	Me(30)	23.6 (*q*)

[a]) Recorded in $CDCl_3$ with Me_4Si as an internal standard. AcO: δ(C) 170.3, 170.3, 171.5, 21.8 (Me), 21.6 (Me), 21.3 (Me).

δ(H) 5.47 (*dd*, J = 11.4, 5.4, CH) produced an NOE enhancement (9.24%) of Me(27), which revealed that the AcO group in question is located at C(16) with β-configuration.

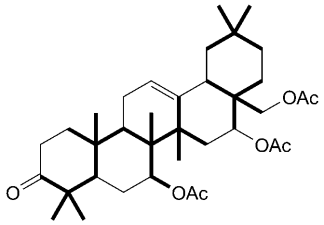

Fig. 2. *Key HMBC correlations of compound* **1a**

The above described analysis of the 1D- and 2D-NMR data, together with a comparative study of NMR data of compound **1a** with those of 16,28-dihydroxyolean-12-en-3-one [5], enabled us to elucidate the structure of compound **1a** as 7β,16β,28-triacetoxyolean-12-en-3-one.

The molecular formula of compound **2a**, an optically active oil, could be deduced as $C_{18}H_{24}O_5$ from a HR-ESI-MS experiment with a signal at m/z $[M+Na]^+$ 343.1512 (calc. 343.1521), which accounted for seven degrees of unsaturation. The IR absorptions at 1655, 1737, and 1765 cm^{-1} pointed to the presence of an α,β-unsaturated ketone moiety.

The ^{1}H-NMR spectrum (*Table 2*) of **2a** showed three Me signals at δ(H) 2.34 (*s*), 0.96 (*s*), and 0.97 (*d*, J = 5.7), the ^{13}C-NMR spectrum showed 14 C-atom signals (3 × Me, 3 × CH_2, 3 × CH, 5 × C^1)) for the skeleton of **2a**, and additionally two AcO groups at δ(H) 2.26 (*s*, Me), 2.02 (*s*, Me), and δ(C) 170.1 (C), 168.5 (C), 21.7 (Me), 21.4 (Me).

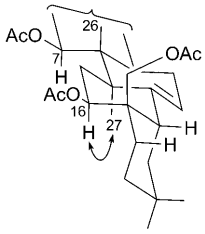

Fig. 3. *Key NOE correlation observed for H–C(16) of compound* **1a**

Table 2. *^{1}H- and ^{13}C-NMR Data for Compounds* **2a** *and* **3a**. δ in ppm.

	2a[a])		**3a**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	5.48 (br. *s*)	72.4 (*d*)	5.44 (br. *s*)	73.7 (*d*)
H_a–C(2)	1.80–1.91 (*m*)	29.9 (*t*)	1.95–2.00 (*m*)	31.1 (*t*)
H_b–C(2)	1.62–1.65 (*m*)		1.65–1.71 (*m*)	
H_a–C(3)	1.62–1.65 (*m*)	25.6 (*t*)	1.65–1.71 (*m*)	25.5 (*t*)
H_b–C(3)	1.40–1.45 (*m*)		1.40–1.50 (*m*)	
H–C(4)	1.48–1.50 (*m*)	43.1 (*d*)	1.40–1.50 (*m*)	42.1 (*d*)
C(5)	–	37.5 (*s*)	–	40.2 (*s*)
H_a–C(6)	2.92 (*d*, J = 12.2)	25.6 (*t*)	2.81 (*d*, J = 14.0)	39.6 (*t*)
H_b–C(6)	2.18 (*d*, J = 12.2)		2.10 (*m*)	
C(7)	–	121.4 (*s*)	–	121.6 (*s*)
C(8)	–	150.3 (*s*)	–	190.0 (*s*)
H–C(9)	5.88 (*s*)	125.1 (*d*)	6.02 (*s*)	131.1 (*d*)
C(10)	–	151.4 (*s*)	–	155.4 (*s*)
C(11)	–	196.7 (*s*)	–	161.4 (*s*)
Me(13)	2.34 (*s*)	31.0 (*q*)	2.28 (*d*, J = 2.4)	19.0 (*q*)
Me(14)	0.96 (*s*)	17.1 (*q*)	1.07 (*s*)	18.6 (*q*)
Me(15)	0.97 (*d*, J = 5.7)	16.1 (*q*)	0.96 (*d*, J = 6.4)	15.6 (*q*)
AcO	2.26 (*s*)	21.7 (*q*), 170.1 (*s*)	2.21 (*s*)	21.5 (*q*), 170.1 (*s*)
AcO	2.02 (*s*)	21.4 (*q*), 168.5 (*s*)	2.01 (*s*)	21.0 (*q*), 171.1 (*s*)

[a]) Recorded in $CDCl_3$ with Me_4Si as an internal standard.

These findings indicated that compound **2a** is an eremophilane derivative containing an α,β-unsaturated ketone moiety [3c].

The spin coupled systems of H–C(1), CH_2(2), CH_2(3), and H–C(4), revealed by a ^{1}H,^{1}H-COSY spectrum, and the key HMBC correlations (*Fig. 4*) of the three Me groups of compound **2a** confirmed the eremophilane sesquiterpene. The HMBC correlations of Me(13) with C(11) and C(7), and CH_2(6) with C(7), C(11), C(5), C(14), and C(8) showed C(11) as C=O group, correlations of H–C(9) with C(7), C(1), C(5), and C(8), and Me(13) with C(7) pointed to the location of an AcO group at C(1). It also indicated the presence of a tetrasubstituted $\Delta^{7,8}$-C=C bond and a trisubstituted $\Delta^{9,10}$-C=C bond. Thus, C(8) must bear an AcO group.

For biogenetic reasons, the Me groups at C(4) and C(5) were both assigned as the β-orientation [6]. With the consideration of a chair-like conformation of the *A* ring of the skeleton of eremophilane sesquiterpenes, the broad *singlet* of H–C(1) at δ(H) 5.48 (br.

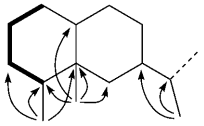

Fig. 4. *Key* $^1H,^1H$*-COSY and HMBC correlations of compounds* **2a**–**4a**, **5**, *and* **6** (only substructure shown)

s, CH) with a possible small coupling constant pointed to H–C(1) as being α-orientated [3c][7].

From the above described analysis of the 1D- and 2D-NMR data, the structure of compound **2a** was elucidated as (1*R*,4*S*,4a*R*)-6-acetyl-1,2,3,4,4a,5-hexahydro-4,4a-dimethylnaphthalene-1,7-diyl diacetate.

Compound **3a** has the same molecular formula as **2a** according to its HR-ESI-MS at *m*/*z* $[M+H]^+$ 321.1701 (calc. 321.1702). The IR absorptions at 1633, 1681, and 1739 cm^{-1} showed the presence of an α,β-unsaturated ketone moiety. The ^1H- and ^{13}C-NMR spectral data of compound **3a** (*Table 2*) closely resembled those of compound **2a**, indicating that **3a** has a close structural relationship to **2a**.

The HMBC correlations of Me(13) with C(7) and C(11), and CH_2(6) with C(5), Me(14), C(7), C(8), and C(11), showed C(8) as C=O group, and also indicated the presence of a tetrasubstituted $\Delta^{7,11}$-C=C bond, which is supported by the evidence that Me(13) at δ(H) 2.28 (*d*, $J = 2.4$, Me) showed a homoallylic coupling with H_α–C(6) [7]. HMBC correlations of Me(15) with C(10), and H–C(9) with C(1), C(5), C(7), and C(8) showed the presence of a trisubstituted $\Delta^{9,10}$-C=C bond; thus, C(11) must bear an AcO group. As in the case of **2a**, **3a** also contained a β-orientated AcO group at C(1), accounting for the broad *singlet* of H–C(1) at δ(H) 5.44 (br. *s*, H–C(1)).

The above described analysis of 1D- and 2D-NMR data further determined the structure of **3a** as (1*Z*)-1-[(5*R*,8*S*,8a*R*)-5-acetoxy-6,7,8,8a-tetrahydro-8,8a-dimethyl-3-oxonaphthalen-2(1*H*)-ylidene]ethyl acetate.

Compound **4a** was obtained as an optically active oil, the molecular formula was determined as $C_{19}H_{24}O_7$ by the signal of a HR-ESI-MS experiment at *m*/*z* $[M+Na]^+$ 387.1413 (calc. 387.1420). The IR spectrum showed absorption bands of OH (3422 cm^{-1}), C=C (1675 cm^{-1}), and C=O (1780 and 1740 cm^{-1}).

The ^1H-NMR spectrum (*Table 3*) of **4a** showed three Me group signals at δ(H) 2.03 (*s*, Me), 1.18 (*s*, Me), and 0.87 (*d*, $J = 5.6$, Me), the ^{13}C-NMR spectrum (*Table 3*) showed 15 C-atom signals ($3\times$ Me, $2\times CH_2$, $4\times$ CH, $6\times C^1$)) for the skeleton of **4a**, and additionally two AcO groups at δ(H) 2.17 (*s*, Me), 2.13 (*s*, Me), and δ(C) 170.1 (C), 169.4 (C), 21.4 (Me), 21.1 (Me). This evidence indicated that compound **4a** is an eremophilane derivative with an α,β-unsaturated lactone γ-moiety [8].

In the HMBC spectrum, correlations of Me(14) with C(4), C(5), C(6), and C(10), and of H–C(6) with C(5), C=O of AcO, and C(10) indicated that a OH group is located at C(10) and an AcO group at C(6); correlations of H–C(1) with C(10), C(9), and C=O of AcO indicated an AcO group at C(1); correlations of Me(13) with the tetrasubstituted $\Delta^{7,11}$-C=C bond, and C(12)=O, H–C(6) with C(7), C(11), and C(8), together with the correlations of H–C(9) with C(5), C(7), and C(8), confirmed the presence of an α,β-unsaturated γ-lactone.

The relative configuration of compound **4a** was obtained by a combination of coupling patterns and an NOE difference spectrum, the coupling pattern of H–C(1) at

Table 3. *^{1}H- and ^{13}C-NMR Data for Compound* **4a**, **5**, *and* **6**. δ in ppm.

	4a[a])		**5**[b])[c])		**6**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	5.12 (*dd*, J = 12.0, 4.0)	73.7 (*d*)	4.28 (br. *s*)	70.0 (*d*)	4.50 (br. *s*)	73.7 (*d*)
H_a–C(2)	1.80–1.91 (*m*)	28.6 (*t*)	1.95–2.00 (*m*)	28.5 (*t*)	1.95–2.00 (*m*)	35.1 (*t*)
H_b–C(2)	1.62–1.65 (*m*)		1.65–1.71 (*m*)		1.65–1.71 (*m*)	
H_a–C(3)	1.62–1.65 (*m*)	28.5 (*t*)	1.65–1.71 (*m*)	28.2 (*t*)	1.65–1.71 (*m*)	25.3 (*t*)
H_b–C(3)	1.40–1.45 (*m*)		1.40–1.50 (*m*)		1.40–1.50 (*m*)	
H–C(4)	1.48–1.50 (*m*)	34.3 (*d*)	2.30–2.40 (*m*)	36.1 (*d*)	1.50–1.60 (*m*)	41.9 (*d*)
C(5)	–	46.4 (*s*)	–	51.8 (*s*)	–	44.0 (*s*)
H–C(6)	5.97 (*s*)	69.9 (*d*)	6.67 (*d*, J = 2.4)	73.7 (*d*)	6.80 (*s*)	153.4 (*d*)
C(7)	–	142.1 (*s*)	–	147.0 (*s*)	–	138.8 (*s*)
C(8)	–	149.1 (*s*)	–	136.1 (*s*)	–	187.9 (*s*)
H_a–C(9)	6.04 (*s*)	107.1 (*d*)	2.00–2.10 (*m*)	36.1 (*t*)	6.14 (*s*)	126.1 (*d*)
H_b–C(9)	–		1.80–1.90 (*m*)		–	
C(10)	–	76.1 (*s*)	–	80.3 (*s*)	–	166.4 (*s*)
C(11)	–	128.5 (*s*)	–	122.0 (*s*)	3.00 (*m*)	34.7 (*d*)
H_a–C(12)	–	170.3 (*s*)	7.65 (*s*)	147.0 (*d*)	3.57 (*dd*, J = 10.8, 5.4)	67.6 (*t*)
H_b–C(12)	–		–		3.51 (*dd*, J = 12.0, 4.0)	
Me(13)	2.03 (*s*)	9.5 (*q*)	1.84 (*s*)	8.7 (*q*)	1.09 (*d*, J = 6.6)	16.0 (*q*)
Me(14)	1.18 (*s*)	16.1 (*q*)	1.21 (*s*)	10.0 (*q*)	1.31 (*s*)	19.1 (*q*)
Me(15)	0.87 (*d*, J = 5.6)	11.0 (*q*)	0.85 (*d*, J = 6.3)	18.0 (*q*)	1.09 (*d*, J = 6.6)	16.4 (*q*)
AcO	2.17 (*s*)	21.4 (*q*), 170.1 (*s*)	–	–	–	–
AcO	2.13 (*s*)	21.1 (*q*), 169.4 (*s*)	–	–	–	–

[a]) Recorded in $CDCl_3$ with Me_4Si as an internal standard. [b]) Recorded in acetone with Me_4Si as an internal standard. [c]) hydroxymethylacryloyl substituent: δ(H) 6.35 (*d*, J = 1.8, H_a–C(3)); 6.06 (*d*, J = 2.4, H_b–C(3)); 4.36 (*s*, CH_2(4)); δ(C) 166.6 (C(1)); 142.1 (C (2)); 125.5 (CH_2(3)); 60.9 (CH_2(4)).

δ(H) 5.12 (*dd*, J = 12.0, 4.0, CH) revealed the β-orientation of H–C(1), which was further confirmed by the evidence that irradiation of H–C(1) produced an NOE enhancement of Me(15) (2.81%); irridiation of Me(14) produced an NOE enhancement of H–C(6) (1.82%), indicating that H–C(6) is β-orientated, OH–C(10) was determined to be β-orientated with the consideration of the relative low-field chemical shift of Me(14) in relation to Me(15) [9]. A comparative study of the NMR data of compound **4a** (*Table 3*) with those of 10β-hydroxyeremophila-8(9),7(11)-diene-6α,15;8,12-diolide [10] confirmed the above result.

The above described analysis of the 1D- and 2D-NMR data further determined the structure of **4a** as (4*R*,4a*S*,5*S*,8*S*,8a*S*)-2,4,4a,5,6,7,8,8a-octahydro-8a-hydroxy-3,4a,5-trimethyl-2-oxonaphtho[2,3-*b*]furan-4,8-diyl diacetate.

Compound **5** was a white amorphous powder, the EI-MS gave a peak of M^+ at m/z 350. Together with the analysis of ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra, the molecular formula could be deduced as $C_{19}H_{26}O_6$. The IR spectrum showed the

absorption bands of OH (3410 cm^{-1}), an α,β-unsaturated ester (1629, 1699, 1740, and 1780 cm^{-1}), and a furane moiety (1161 and 1010 cm^{-1}).

The ^{1}H-NMR spectrum (*Table 3*) of **5** showed three Me groups for an eremophilane sesquiterpene at δ(H) 1.84 (*s*), 1.21 (*s*), and 0.85 (*d*, $J = 6.3$), a hydroxymethylacryloyl group could be distinguished by a typical set of signals at δ(H) 6.35 (*d*, $J = 1.8$, CH), 6.06 (*d*, $J = 2.4$, CH), 4.36 (*s*, CH_2), and δ(C) 166.6 (C), 142.1 (C), 125.5 (CH_2), 60.9 (CH_2), which is supported by the fragment ion peak at *m/z* 85.0 ($[C_4H_5O_2]^+$). Another set of signals at δ(H) 7.65 (*s*, CH), and δ(C) 147.0 (CH), 147.0 (C), 136.1 (C), 122.0 (C) revealed the presence of a trisubstituted furan moiety.

The HMBC correlations of Me(14) with C(5), C(6), and C(10), and of H–C(6) with C(1′) revealed the position of the OH group at C(10), and the hydroxymethylacryloyl group at C(6); correlations of Me(13) with C(12), C(11), and C(7), of H–C(6) with C(5), C(8), and C(11), and of H–C(12) with C(8), C(11), and C(7), confirmed the presence of a furan moiety. The broad *singlet* of H–C(1) at δ(H) 4.28 (br. *s*, CH) showed the relative configuration of H–C(1) as α-orientated. Irradiation of H–C(6) produced an NOE enhancement of H–C(4) (1.82%), indicating that H–C(6) was α-orientated. OH–C(10) was determined to be β-orientated with the consideration of the relatively low-field chemical shift of Me(14) in relation to Me(15) [9][11a], and a comparative study of NMR data of compound **5** (*Table 3*) with those of known compounds [11].

The above described analysis of the 1D- and 2D-NMR data further determined the structure of **5** as (4*S*,4a*S*,5*S*,8*R*,8a*S*)-4,4a,5,6,7,8,8a,9-octahydro-8,8a-dihydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-4-yl (2-(hydroxymethyl)prop-2-enoate.

Compound **6** was obtained as a white amorphous powder. The molecular-ion peak was observed at *m/z* 250 in the EI-MS, the formula corresponding to this ion peak was determined as $C_{15}H_{22}O_3$ by combined spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, and DEPT). The IR spectrum of **6** indicated the presence of OH (3433 cm^{-1}), and an $\alpha,\beta,\alpha',\beta'$-unsaturated ketone moiety (1629, 1664, and 1699 cm^{-1}). A comparative study revealed that the NMR data of compound **6** (*Table 3*) was very similar to those of related known compounds [12].

The ^{1}H-NMR spectrum of **6** displayed three Me groups for an eremophilane sesquiterpene at δ(H) 1.31 (*s*, Me), 1.09 (*d*, $J = 6.6$, 2 × Me), two olefinic signals at δ(H) 6.80 (*s*, CH), and 6.14 (*s*, CH) for two pairs of trisubstituted C=C bonds, it also showed one oxygenated CH group at δ(H) 4.50 (br. *s*, CH) and one oxygenated CH_2 group at δ(H) 3.57 (*dd*, $J = 10.8$, 5.4, CH), 3.51 (*dd*, $J = 12.0$, 4.0, CH). The ^{13}C-NMR spectrum showed 15 C-atom signals (3 × Me, 3 × CH_2 (one oxygenated), 5 × CH (one oxygenated), 4 × C) with the aid of DEPT experiments.

The ^{1}H,^{1}H-COSY spectrum showed two spin coupling systems *a* (H–C(1), CH_2(2), CH_2(3), and H–C(4)) and *b* (CH_2(12), H–C(11), and Me(13)). In the HMBC spectrum, the correlations of Me(14) with C(5), C(6), and C(10), and Me(15) with C(4) and C(5) indicated the presence of two pairs of C=C bonds at C(6) and C(10), correlations of Me(13) with C(11), C(7), and C(12), and of H–C(12) with C(7), together with the correlations of H–C(6) with C(8), and C(10), and of H–C(9) with C(7) further confirmed the C=C bonds as C(6)=C(7) and C(9)=C(10), and also revealed the presence of a C=O group at C(8). The position of the OH group at C(1) was deduced from the HMBC correlations of H–C(9) with C(1), and of H–C(1) with

C(9) and C(5). The relative configuration of H–C(1) was determined to be α by the broad *singlet* at δ(H) 4.50 (br. *s*, H–C(1)).

From the above discussed data, the structure of **6** was finally determined as (4a*S*,5*S*,8*R*)-5,6,7,8-tetrahydro-8-hydroxy-3-(1-hydroxypropan-2-yl)-4a,5-dimethylnaphthalen-2(4a*H*)-one by analysis of 1D- and 2D-NMR data.

Compound **7** was obtained as a white amorphous powder. The molecular formula was determined as $C_{12}H_{16}O_3$ by a HR-ESI-MS experiment at *m/z* $[M+H]^+$ 209.1170 (calc. 209.1178). The IR spectrum of **7** indicated the presence of OH (3443 cm^{-1}), and an $\alpha,\beta,\alpha',\beta'$-unsaturated ketone moiety (1629, 1686 cm^{-1}). A comparative study revealed that the NMR data of compound **7** (*Table 4*) was similar to those of a known related compound [3c].

Table 4. *^{1}H- and ^{13}C-NMR Data for Compound* **7** *and* **8**. δ in ppm.

	7[a])		**8**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	4.59 (br. *s*)	74.2 (*d*)	4.36 (br. *s*)	73.0 (*d*)
H_a–C(2)	2.05–2.32 (*m*)	35.0 (*t*)	2.05–2.32 (*m*)	33.1 (*t*)
H_b–C(2)	1.60–1.70 (*m*)		1.60–1.70 (*m*)	
H_a–C(3)	1.80–1.90 (*m*)	25.0 (*t*)	1.80–1.90 (*m*)	30.0 (*t*)
H_b–C(3)	1.40–1.50 (*m*)		1.40–1.50 (*m*)	
H–C(4)	1.50–1.60 (*m*)	43.0 (*d*)	1.50–1.60 (*m*)	43.8 (*d*)
C(5)	–	44.7 (*s*)	–	40.8 (*s*)
H_a–C(6)	6.33 (*s*)	126.7 (*d*)	2.21 (*dd*, J = 12.3, 5.4)	43.1 (*t*)
H_b–C(6)			2.00 (*dd*, J = 12.3, 3.0)	
H–C(7)	–	146.0 (*s*)	5.56 (*dd*, J = 14.1, 5.4)	71.7 (*s*)
C(8)	–	182.4 (*s*)	–	194.9 (*s*)
H–C(9)	6.28 (*s*)	123.7 (*d*)	5.86 (*s*)	124.8 (*d*)
C(10)	–	169.4 (*s*)	–	170.5 (*s*)
Me(14)	1.38 (*s*)	19.6 (*q*)	1.44 (*s*)	15.2 (*q*)
Me(15)	1.10 (*d*, J = 6.3)	16.7 (*q*)	0.94 (*d*, J = 6.9)	19.0 (*q*)
OH	6.20 (*s*)	–	–	–
AcO	–	–	2.17 (*s*)	21.1 (*q*), 170.5 (*s*)

[a]) Recorded in $CDCl_3$ with Me_4Si as an internal standard.

The ^{1}H-NMR spectrum of **7** displayed two Me groups at δ(H) 1.38 (*s*) and 1.10 (*d*, J = 6.3), two olefinic signals at δ(H) 6.33 (*s*, CH) and 6.28 (*s*, CH) for two pairs of trisubstituted C=C bonds, and an oxygenated CH at δ(H) 4.59 (br. *s*, CH). The ^{13}C-NMR spectrum showed 12 C-atom signals (2 × Me, 2 × CH_2, 4 × CH (one oxygenated), 4 × C[1])) for the skeleton of **7**.

The key ^{1}H,^{1}H-COSY and HMBC correlations (*Fig. 5*) showed that compound **7** was a tri-nor eremophilane sesquiterpene. In the HMBC spectrum, correlations of H–C(6) with C(5), C(7), C(8), C(10), and Me(14), and of H–C(9) with C(7), C(1), and C(5), confirmed the presence of an $\alpha,\beta,\alpha',\beta'$-unsaturated ketone moiety. Correlations of the H-atom of a OH group at δ(H) 6.20 (*s*) with C(6), C(7), and

C(8) confirmed the location of OH at C(7), correlations of H–C(9) with C(1), and H–C(1) with C(9) and C(5) indicated that another OH group was located at C(1).

The above discussed analysis of the 1D- and 2D-NMR data of compound **7** further showed the structure of **7** as (4a*S*,5*S*,8*R*)-5,6,7,8-tetrahydro-3,8-dihydroxy-4a,5-dimethylnaphthalen-2(4a*H*)-one.

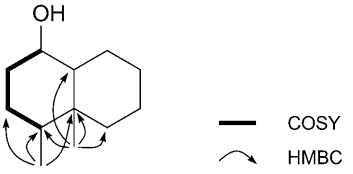

Fig. 5. *Key ^{1}H,^{1}H-COSY and HMBC correlations of compounds* **7** *and* **8** (only substructure shown)

Compound **8** was obtained as a white amorphous powder. The HR-ESI-MS experiment showed the molecular formula as $C_{14}H_{20}O_4$ at *m/z* $[M+Na]^+$ 275.1259 (calc. 275.1259). The IR spectrum of **8** indicated the presence of OH (3410 cm^{-1}), and an α,β-unsaturated ketone moiety (1736 and 1656 cm^{-1}). The NMR data of compound **8** (*Table 4*) was similar to those of **7**.

The ^{1}H-NMR spectrum of **8** displayed two Me groups at δ(H) 1.44 (*s*) and 0.94 (*d*, $J = 6.9$), one olefinic signal at δ(H) 5.86 (*s*, CH), and two oxygenated CH groups at δ(H) 5.56 (*dd*, $J = 14.1, 5.4$, CH) and 4.36 (br. *s*, CH). The ^{13}C-NMR showed 12 C-atom signals ($2 \times$ Me, $3 \times CH_2$, $4 \times$ CH (two oxygenated), $3 \times C^1$)) for the skeleton of **8**, and additionally two signals for an AcO group (δ(H) 2.17 (*s*, Me), δ(C) 170.5 (C) and 21.1 (Me)).

Compound **8** was also determined as a tri-nor eremophilane sesquiterpene with the aid of the key ^{1}H,^{1}H-COSY and HMBC correlations (*Fig. 5*). The HMBC correlations of CH_2(6) with C(8), C(7), C(5), and Me(14), together with the crosspeak of H–C(7)/CH_2(6) in the ^{1}H,^{1}H-COSY spectrum, indicated the presence of an AcO group at C(7). H–C(1) was deduced to be α-orientated due to the coupling pattern of a broad *singlet* at δ(H) 4.36 (br. *s*, CH), irradiation of H–C(7) produced an NOE enhancement of Me(14) (2.01%), indicating that H–C(7) must be β-orientated.

The above described analysis of the 1D- and 2D-NMR data further determined the structure of **8** as (2*R*,5*R*,8*S*,8a*R*)-1,2,3,5,6,7,8,8a-octahydro-5-hydroxy-8,8a-dimethyl-3-oxonaphthalen-2-yl acetate.

2. *Biological Studies.* Compound **1a** was tested for its cytotoxic activity against HL-60 (human promyelocytic leukemia), SMMC-7721 (human hepatoma), and HeLa (human cervical carcinoma) cells according to the sulforhodamine B (SRB) method [13] (use of vincristine sulfate as a positive control with IC_{50} values 11.2 μg/ml against HL-60, 26.7 μg/ml against SMMC-7721, and 8.3 μg/ml against HeLa) as reported previously. Compound **1a** showed moderate cytotoxicity against HL-60 and SMMC-7721 cells with IC_{50} values of 11.2 and 42.6 μg/ml, respectively, while it was not bioactive against HeLa cells with an IC_{50} value of more than 100 μg/ml.

Experimental Part

General. TLC: GF_{254} (10–40 μ); spots were detected under UV or by heating after spraying with 5% H_2SO_4 in EtOH (*v*/*v*). Column chromatography (CC): silica gel (SiO_2; 200–300 mesh) was supplied by the *Qingdao Marine Chemical Factory* in P. R. China. The reversed phase pre-coated TLC plates: *RP-18* F_{254} (size 20 × 20 cm, Schichtdicke 0.25 mm); supplied by *E. Merck*, Germany. M.p.: *Kofler* melting-point apparatus, uncorrected. Optical rotations: *Perkin Elmer 341* polarimeter. IR: *Nicolet NEXUS 670* FT-IR; in cm^{-1}. NMR: *Varian Mercury plus-300* NMR spectrometer; with Me_4Si as an internal standard. EI-MS: *HP-5988A* GC/MS instrument; *m*/*z* (rel. int.). HR-ESI-MS: *Bruker Daltonics APEX II* 47e spectrometer.

Plant Material. The roots of *L. sagitta* MAXIM were collected from Gannan Tibet Autonomous Region (S. A. 2000–3800 m), Gansu Province of P. R. China in August 2005. It was identified by Prof. *Guo-Liang Zhang*, School of Life Sciences, Lanzhou University. A voucher specimen (No. 20050920) was deposited with the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The air-dried roots of *L. sagitta* (10 kg) were pulverized, and extracted with mixed solvent (petroleum ether (PE) (b.p. 60–90°)/Et_2O/MeOH 1:1:1) three times (7 d each time) at r.t. The extract was concentrated under reduced pressure, the residue (400 g) was subjected to SiO_2 CC, and eluted with a step gradient of PE/acetone (20:1, 10:1, 5:1, 3:1, and 1:1). Five fractions (*Fr. 1–5*) were collected according to TLC. *Fr. 3* (PE/acetone 5:1; 50 g) was purified by repeated chromatography over a SiO_2 column with PE/acetone (5:1) to afford **7** (5 mg), **8** (3 mg), and a mixture. After acetylation of the mixture, **2a** (5 mg) and **3a** (3 mg) were obtained. *Fr. 4* (PE/acetone 3:1; 40 g) after SiO_2 CC (200–300 mesh; 400 g) with PE/acetone (3:1) as eluent, three mixtures were obtained. Two of them were then rechromatographed by *RP-18* PTLC (H_2O/MeOH 1:3, two times each) to afford **5** (2 mg) and **6** (13 mg), resp., and the third one was acetylated to afford **1a** (2 mg) and **4a** (3 mg).

(7β,16β)-3-Oxoolean-12-ene-7,16,28-triyl Triacetate (**1a**). White amorphous powder. M.p. 190–192°. $[\alpha]_D^{20} = +4.0$ ($c = 0.2$, $CHCl_3$). IR (film): 2927, 2855, 1737, 1706, 1634, 1459, 1372, 1242, 1021. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.89 (*s*, Me(29), Me(30)); 1.02 (*s*, Me(23)); 1.05 (*s*, Me(25)); 1.09 (*s*, Me(24)); 1.16 (*s*, Me(26)); 1.33 (*s*, Me(27)); 1.94 (*s*, AcO); 2.01 (*s*, AcO); 2.09 (*s*, AcO); 4.01 (*d*, $J = 10.4$, H_b–C(28)); 4.07 (*d*, $J = 10.4$, H_a–C(28)); 5.12 (br. *d*, $J = 11.4$, H–C(7)); 5.34 (br. *s*, H–C(12)); 5.47 (*dd*, $J = 11.4$, 5.4, H–C(16)). ^{13}C-NMR: *Table 1*. HR-ESI-MS: 616.4212 ($[M + NH_4]^+$, $C_{36}H_{58}NO_7^+$; calc. 616.4213).

*(1*R*,4*S*,4a*R*)-6-Acetyl-1,2,3,4,4a,5-hexahydro-4,4a-dimethylnaphthalene-1,7-diyl Diacetate* (**2a**). Optically active oil. $[\alpha]_D^{20} = +26$ ($c = 0.5$, $CHCl_3$). IR (film): 2959, 2931, 2865, 1765, 1737, 1655, 1595, 1370, 1238, 1189, 1016. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 343.1512 ($[M + Na]^+$, $C_{18}H_{24}NaO_5^+$; calc. 343.1521).

*(1*Z*)-1-[(5*R*,8*S*,8a*R*)-5-(Acetyloxy)-3,5,6,7,8,8a-hexahydro-8,8a-dimethyl-3-oxonaphthalen-2(1*H*)-ylidene]ethyl Acetate* (**3a**). Optically active oil. $[\alpha]_D^{20} = +14$ ($c = 0.3$, $CHCl_3$). IR (film): 2928, 2856, 1758, 1739, 1681, 1633, 1371, 1255, 1180, 1015. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 321.1701 ($[M + H]^+$, $C_{18}H_{25}O_5^+$; calc. 321.1702).

*(4*R*,4a*S*,5*S*,8*S*,8a*S*)-2,4,4a,5,6,7,8,8a-Octahydro-8a-hydroxy-3,4a,5-trimethyl-2-oxonaphtho[2,3-*b*]furan-4,8-diyl Diacetate* (**4a**). Optically active oil. $[\alpha]_D^{20} = -3$ ($c = 0.3$, $CHCl_3$). IR (film): 3422, 2922, 2853, 1780, 1740, 1675, 1376, 1235, 1039, 1021. ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-MS: 387.1413 ($[M + Na]^+$, $C_{19}H_{24}NaO_7^+$; calc. 387.1420).

*(4*S*,4a*S*,5*S*,8*R*,8a*S*)-4,4a,5,6,7,8,8a,9-Octahydro-8,8a-dihydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-4-yl 2-(Hydroxymethyl)prop-2-enoate* (**5**). White amorphous powder. M.p. 185–186°. $[\alpha]_D^{20} = -10$ ($c = 0.2$, $CHCl_3$). IR (film): 3410, 2927, 2856, 1780, 1740, 1699, 1663, 1629, 1367, 1215, 1161, 1099, 1061, 1010. ^{1}H- and ^{13}C-NMR: *Table 3*. EI-MS: 350 (5, M^+), 289 (100), 280 (65), 262 (98), 244 (28), 215 (30), 177 (85), 109 (20), 85 (80).

*(4a*S*,5*S*,8*R*)-5,6,7,8-Tetrahydro-8-hydroxy-3-(1-hydroxypropan-2-yl)-4a,5-dimethylnaphthalen-2(4a*H*)-one* (**6**). White amorphous powder. M.p. 190–192°. $[\alpha]_D^{20} = -1.7$ ($c = 1.3$, $CHCl_3$). IR (film): 3433, 2928, 2853, 1739, 1699, 1664, 1629, 1467, 1234, 1208, 1022. ^{1}H- and ^{13}C-NMR: *Table 3*. EI-MS: 250 (10, M^+), 232 (10), 221 (100), 175 (60), 147(60), 135 (70), 91 (60).

*(4a*S*,5*S*,8*R*)-5,6,7,8-Tetrahydro-3,8-dihydroxy-4a,5-dimethylnaphthalen-2(4a*H*)-one* (**7**). White amorphous powder. M.p. 187–189°. $[\alpha]_D^{20} = -1.4$ ($c = 0.5$, $CHCl_3$). IR (film): 3443, 2934, 2857, 1759,

1738, 1686, 1629, 1367, 1239, 1181, 1016. ^{1}H- and ^{13}C-NMR: *Table 4*. HR-ESI-MS: 209.1170 ($[M+H]^+$, $C_{12}H_{17}O_3^+$; calc. 209.1178).

*(2*R*,5*R*,8*S*,8a*R*)-1,2,3,5,6,7,8,8a-Octahydro-5-hydroxy-8,8a-dimethyl-3-oxonaphthalen-2-yl Acetate* (**8**). White amorphous powder. M.p. 188–191°. $[\alpha]_D^{20} = +3.0$ ($c = 0.3$, $CHCl_3$). IR (film): 3410, 2926, 2856, 1756, 1736, 1656, 1373, 1236, 1161, 1015. ^{1}H- and ^{13}C-NMR: *Table 4*. HR-ESI-MS: 275.1259 ($[M+Na]^+$, $C_{14}H_{20}NaO_4^+$; calc. 275.1259).

Cytotoxicity Assay. Testing for *in vitro* cytotoxic activities against SMMC-7721 (human hepatoma), HL-60 (human promyelocytic leukemia), and HeLa (human cervical carcinoma) cells of compound **1a** was carried out according to the sulforhodamine B (SRB) method [11]. Vincristine sulfate was used as a positive control. The absorbency of extracted sulforhodamine B at 515 nm was measured on a microplate reader. The experiments were carried out in triplicate. Each run entailed 5–6 concentrations of the compounds being tested.

The percentage survival rate of cells exposed to the compound was calculated by assuming the survival rate of untreated cells to be 100%. Plotting the compound concentrations *vs.* the growth rates of cells provided the half inhibitory concentration IC_{50} values of the compound.

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Received March 14, 2008