Sesquiterpenoids and Lignans from *Ligularia virgaurea* spp. *oligocephala*

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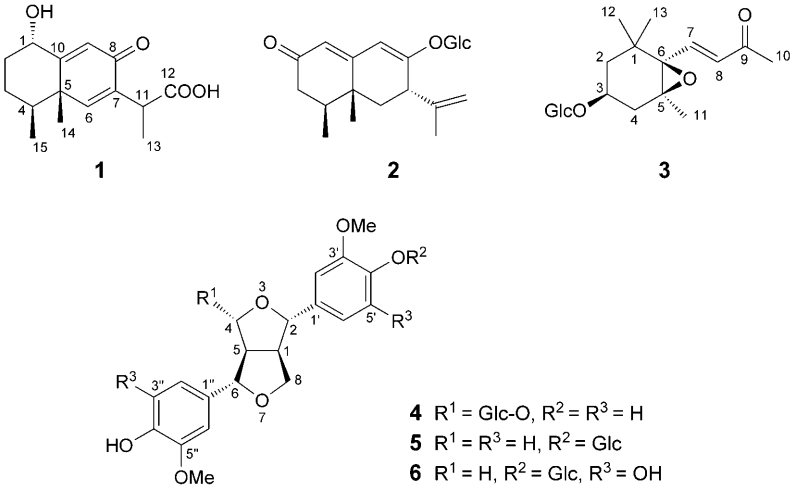
From the BuOH extract of the whole plant of *Ligularia virgaurea* spp. *oligocephala*, a series of sesquiterpenes, sesquiterpene glycosides, and lignan glycosides were isolated, including the three new compounds (1α)-1-hydroxy-8-oxo-eremophila-6,9-dien-12-oic acid (**1**), 8-[(β-D-glucopyranosyl)oxy]eremophila-1(10),8,11-trien-2-one (**2**), and 4-[(β-D-glucopyranosyl)oxy]pinoresinol (**4**). Their structures were elucidated by 1D- and 2D-NMR as well as HR-ESI-MS analyses, and by comparison of the spectroscopic data with those reported for structurally related compounds.

Introduction. – More than 27 *Ligularia* species have long been used as folk remedies due to their antibiotic, antiphlogistic, and antitumor activities [1], sesquiterpenoids being the most-widespread secondary metabolites in this genus [2][3]. Recently, we have isolated some structurally novel sesquiterpenes from the AcOEt extract of *L. virgaurea* spp. *oligocephala* [4–6], which are used in traditional Chinese medicine (TCM) for the treatment of stomachache and nausea [7].

In continuation of our in-depth studies on chemical constituents of *L. virgaurea* spp. *oligocephala*, we now report the isolation and characterization of six compounds from the BuOH extract of this plant, including a new sesquiterpene (**1**), one new and one known sesquiterpene glycoside, **2** and **3** [8], respectively, a new lignan glycoside (**4**), and the two known lignan glycosides **5** [9] and **6** [10].

Results and Discussion. – Compound **1** was obtained as an amorphous, optically active powder, with $[\alpha]_D^{29} = -28.0$ ($c = 0.5$, MeOH). The molecular formula of **1** was deduced as $C_{15}H_{20}O_4$ based on HR-ESI-MS (*m/z* 265.1431 ($[M+H]^+$; calc. 265.1440). The IR spectrum displayed absorption bands for OH (3404), COOH (1709), and α,β-unsaturated C=O (1662 and 1624 cm^{-1}) moieties.

The ^{1}H- and ^{13}C-NMR (DEPT) spectra indicated five quaternary C-atoms, five CH, two CH_2, and three Me groups (*Table 1*). In the ^{1}H-NMR spectrum, there were typical signals for three Me groups at δ(H) 1.27 (*d*, $J = 6.4$ Hz), 1.14 (*s*), and 1.07 (*d*, $J = 6.4$ Hz), as well as signals for C=C–H moieties at δ(H) 6.89 (br. *s*, 1 H) and 6.42 (*d*, $J = 1.6$ Hz, 1 H). In the ^{13}C-NMR spectrum, there were resonances for a keto function at δ(C) 184.6, a COOH function at 177.6, and an oxygenated C-atom at 67.8. Comparing the data and features of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of known compounds [3][11][12], a structure based on an 8-oxo-eremophila-6,9-diene was inferred. The



HMBC cross-peaks between H–C(11)[1]) at δ(H) 3.65–3.67 and C(12) at δ(C) 177.6, and between H–C(1) at δ(H) 4.41 and C(10) at δ(C) 170.7 indicated that the COOH and OH groups were attached at C(11) and C(1), respectively (*Fig. 1*).

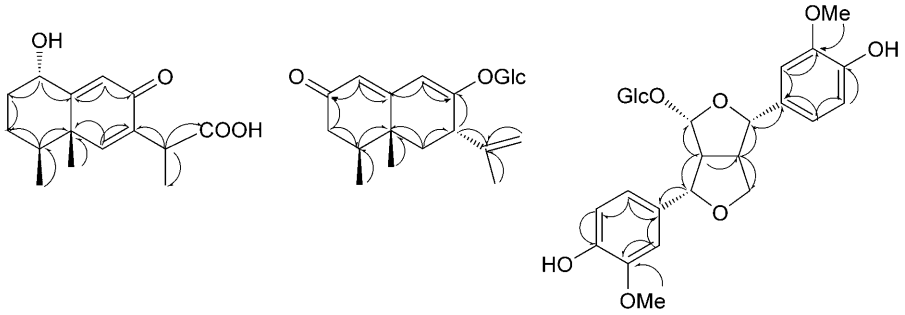


Fig. 1. *Selected HMBC* (H → C) *correlations for* **1**, **2** *and* **4**

The relative configuration of **1** was determined on the basis of a NOESY experiment: correlations between H–C(1) and Me(14), and between Me(14) and Me(15) indicated that the OH group was α-oriented, and that the two Me groups were in β-position. Thus, from the above data, the structure of compound **1** was deduced as (1α)-1-hydroxy-8-oxo-eremophila-6,9-dien-12-oic acid.

Compound **2** was obtained as an amorphous, optically active powder, with $[\alpha]_D^{29} = -119.0$ ($c = 0.8$, MeOH). HR-ESI-MS showed the $[M+H]^+$ signal at m/z 395.2059 (calc. 395.2070), which, together with the NMR data, led to the molecular for-

[1]) Arbitrary atom numbering. For systematic compound names, see the *Exper. Part*.

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. At 400/100 MHz, resp., in CD_3OD; δ in ppm, J in Hz; trivial atom numbering[1]).

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	4.41 (*ddd*, J = 12.0, 5.6, 1.6)	67.8	5.60 (*s*)	121.3
2	2.18 (*m*)	36.6	–	197.9
3	1.60 (*m*)	27.9	2.20 (*m*)	41.7
4	1.63 (*m*)	41.2	1.92 (*m*)	40.0
5	–	43.6	–	37.1
6	6.89 (br. *s*)	151.6	2.20 (*m*)	36.0
7	–	137.3	3.02 (*d*, J = 7.6)	44.0
8	–	184.6	–	163.2
9	6.42 (*d*, J = 1.6)	119.3	5.93 (*s*)	105.1
10	–	170.7	–	166.1
11	3.65–3.67 (*m*)	37.7	–	144.4
12	–	177.6	4.91 (*s*), 4.85 (*s*)	113.3
13	1.27 (*d*, J = 6.4)	15.5	1.87 (*s*)	22.0
14	1.14 (*s*)	16.6	1.06 (*s*)	17.2
15	1.07 (*d*, J = 6.4)	14.8	0.96 (*d*, J = 6.8)	14.5
1′	–		4.99 (*d*, J = 7.6)	99.8
2′	–		3.66 (*m*)	73.4
3′	–		3.70 (*m*)	77.2
4′	–		3.66 (*m*)	70.2
5′	–		3.70 (*m*)	77.0
6′	–		3.88 (*m*)	61.5

mula $C_{21}H_{30}O_7$. The IR spectrum showed typical absorption bands for OH groups (3388 cm^{-1}).

In the NMR spectra of **2** (*Table 1*), the signals at δ(H) 4.99 (*d*, J = 7.6 Hz) and δ(C) 99.8 (*d*), 77.2 (*d*), 77.0 (*d*), 73.4 (*d*), 70.2 (*d*), and 61.5 (*t*) indicated a β-glucopyranosyl (Glc) moiety. The ^{1}H-NMR spectrum showed two C=C–H moieties at δ(H) 5.93 (*s*, 1 H) and 5.60 (*s*, 1 H), two exocyclic methylidene resonances at δ(H) 4.91 (*s*, 1 H) and 4.85 (*s*, 1 H), and three Me groups resonating at δ(H) 1.87 (*s*), 1.06 (*s*), and 0.96 (*d*, J = 6.8 Hz), respectively. The ^{13}C-NMR spectrum of **2** showed a typical signal at δ(C) 197.9 due to an α,β-unsaturated keto function. Careful comparison of the ^{1}H- and ^{13}C-NMR spectroscopic data of **2** with those of **1** and related sesquiterpenes [12][13] led to the conclusion that **2** also had an eremophilane skeleton.

The proposed structure of **2** was further confirmed by 2D-NMR analysis. The HMBC correlations between H–C(1) and C(2) and C(10), between H–C(9) and C(10) and C(8), between H–C(7) and C(6), C(8), and C(11), and between H–C(12) and C(11) suggested a 2-oxo-eremophila-1(10),8,11-triene derivative. Furthermore, HMBC correlations between H–C(8) and C(1′), the anomeric C-atom of the Glc residue, as well as between H–C(1′) and C(8) indicated that the Glc unit was located at C(8) (*Fig. 1*). Thus, from the above data, the structure of **2** was elucidated as 8-[(β-D-glucopyranosyl)oxy]eremophila-1(10),8,11-trien-2-one.

Compound **4** was obtained as an amorphous, optically active powder, with $[\alpha]_D^{29} = -14.0$ (c = 0.7, MeOH). HR-ESI-MS Experiments showed the $[M+NH_4]^+$ sig-

nal at *m/z* 554.2238 (calc. 554.2238), in accord with the molecular formula $C_{26}H_{32}O_{12}$. The IR spectrum of **4** showed a broad absorption at 3361 cm^{-1} (OH), and strong absorptions at 1603, 1517, and 1457 cm^{-1} (Ar).

Inspection of the ^{13}C-NMR (DEPT) spectrum of **4** showed two MeO functions and a Glc residue, together with the resonances for 18 main-skeleton C-atoms (*Table 2*). The ^{1}H-NMR spectrum (*Table 2*) indicated two trisubstituted aromatic rings [δ(H) 7.18 (*d*, *J*=2.0, 1 H); 6.99 (*d*, *J*=2.0, 1 H); 6.96 (*dd*, *J*=8.0, 2.0, 1 H); 6.85 (*dd*, *J*=8.0, 2.0, 1 H); 6.79 (*d*, *J*=8.0, 1 H); and 6.75 (*d*, *J*=8.0 Hz, 1 H)] and two MeO groups at δ(H) 3.85 (*s*) and 3.82 (*s*). A complex *multiplet* assignable to a CH at δ(H) 3.30 (H–C(1)) was found to be coupled with a benzylic resonance at δ(H) 4.97 (*d*, *J*=7.6 Hz, H–C(2)), and with a pair of CH_2 H-atoms at δ(H) 4.17 (*dd*, *J*=9.2, 5.6, H_a–C(8)) and 3.94 (*dd*, *J*=9.2, 2.0 Hz, H_b–C(8)). A second benzylic H-atom at δ(H) 4.84 (*d*, *J*=7.6 Hz, H–C(6)) was found to be coupled with another CH at δ(H) 2.97 (*m*, H–C(5)) through ^{1}H,^{1}H-COSY and HMQC experiments. These signals indicated the presence of a 2,6-diaryl 3,7-dioxabicyclo[3.3.0]octane skeleton similar to syringaresinol [14].

Table 2. *^{1}H- and ^{13}C-NMR Data of* **4**. At 400/100 MHz, resp., in CD_3OD; δ in ppm, *J* in Hz; trivial atom numbering[1]).

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1′	–	133.2	1	3.30 (*m*)	52.7
1″	–	133.0	2	4.97 (*d*, *J*=7.6)	88.7
2′	7.18 (*d*, *J*=2.0)	109.9	4	5.69 (br. *s*)	102.2
2″	6.99 (*d*, *J*=2.0)	109.2	5	2.97 (*m*)	61.4
3′	–	147.3	6	4.84 (*d*, *J*=7.6)	82.4
3″	–	147.2	8	4.17 (*dd*, *J*=9.2, 5.6)	70.7
				3.94 (*dd*, *J*=9.2, 2.0)	
4′	–	145.8	Glc-1	4.94 (*d*, *J*=7.2)	97.9
4″	–	145.6	Glc-2	3.30–3.40[a])	73.3
5′	6.79 (*d*, *J*=8.0)	114.0	Glc-3	3.30–3.40[a])	76.4
5″	6.75 (*d*, *J*=8.0)	114.4	Glc-4	3.30–3.40[a])	70.0
6′	6.96 (*dd*, *J*=8.0, 2.0)	118.4	Glc-5	3.30–3.40[a])	76.3
6″	6.85 (*dd*, *J*=8.0, 2.0)	119.4	Glc-6	3.78 (*m*), 3.60 (*m*)	61.3
MeO	3.85 (*s*)	55.3	MeO	3.82 (*s*)	55.0

[a]) Signal pattern unclear due to overlapping.

By analysis of the HMBC data of **4** (*Fig. 1*), the two MeO groups could be located at C(3′) and C(3″), respectively. The Glc unit was attached at C(4) according to HMBC correlations between the anomeric H-atom of Glc with C(4), and, in turn, between H–C(4) and the anomeric C-atom of Glc. The relative configuration of **4** was determined on the basis of a NOESY experiment, which showed correlations between H–C(6) and both H–C(2) and H–C(4), and based on a correlation between H–C(2) and H–C(8) (*Fig. 2*). From these data, compound **4** was identified as 4-[(β-D-glucopyranosyl)oxy]pinoresinol.

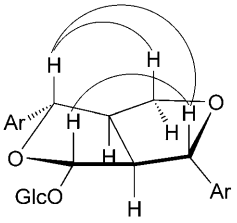


Fig. 2. *Key NOESY correlations for* **4**

Experimental Part

General. TLC: silica gel GF_{254} (10–40 μm; *Qingdao Marine Chemical Factory*). Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Factory*); C_{18} reversed-phase (RP) silica gel (35–75 μm; *Analteck*, Newwark); *HPD-100* resin (*Heibei*, China). Optical rotations: *Perkin Elmer 341* polarimeter. IR Spectra: *Nicolet NEXUS-670* FT-IR spectrophotometer; in cm^{-1}. NMR spectra: *Varian INOVA-400* spectrometer; in CD_3OD soln; δ in ppm rel. to Me_4Si, J in Hz. HR-ESI-MS: *Bruker APEX-II* mass spectrometer; in *m*/*z*.

Plant Material. The air-dried whole plant of *L. virgaurea* spp. *oligocephala* was collected in Huzhu County, Qinghai Province, P. R. China, in August 2002, and was identified by adjunct Prof. *Ji Ma*, Faculty of Pharmacy, First Military Medical University of PLA, Guangzhou, P. R. China. A voucher specimen (No. 2002001) has been deposited at the Key Laboratory for Natural Medicine of Gansu Province.

Extraction and Isolation. The air-dried powder of the whole plant of *L. virgaurea* spp. *oligocephala* (4.0 kg) was extracted at r.t. with 95% EtOH (3×7 d). The crude dry extract (340.0 g) was suspended in H_2O and extracted successively with petroleum ether, AcOEt, and BuOH. The BuOH-soluble part (45 g) was purified by CC (*HPD-100* resin) eluting with H_2O and 95% EtOH successively. The fraction eluted with 95% EtOH was concentrated *in vacuo* to afford a residue (20 g), which was subjected to CC (SiO_2; $CHCl_3$/MeOH 40:1, 20:1, 15:1, 10:1, 8:1, 5:1, and 0:1): seven fractions (*Fr. 1–7*). *Fr. 2* was subjected to CC (SiO_2; $CHCl_3$/MeOH 20:1) to afford **1** (5 mg). *Fr. 3* was subjected to CC (1. SiO_2, $CHCl_3$/MeOH 20:1; 2. RP-C_{18}, MeOH/H_2O 65:35) to provide **3** (4 mg) and **6** (9 mg). *Fr. 4* was subjected to CC (1. SiO_2, $CHCl_3$/MeOH 15:1; 2. RP-C_{18}, MeOH/H_2O 65:35) to furnish **2** (4 mg), **4** (8 mg), and **5** (6 mg).

(1α)-1-Hydroxy-8-oxo-eremophila-6,9-dien-12-oic Acid (=*2-[(5S*,8S*,8aS*)-3,5,6,7,8,8a-Hexahydro-5-hydroxy-8,8a-dimethyl-3-oxonaphthalen-2-yl]propanoic Acid*; **1**). Amorphous powder. $[\alpha]_D^{29} = -28.0$ ($c = 0.5$, MeOH). IR (KBr): 3404, 2934, 1709, 1662, 1624. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 265.1431 ($[M+H]^+$, $C_{15}H_{21}O_4^+$; calc. 265.1440).

8-[(β-D-Glucopyranosyl)oxy]eremophila-1(10),8,11-trien-2-one (=*(4S*,4aR*,6S*)-7-[(β-D-Glucopyranosyl)oxy]-4,4a,5,6-tetrahydro-4,4a-dimethyl-6-(1-methylethenyl)naphthalen-2(3H)-one*; **2**). Amorphous powder. $[\alpha]_D^{29} = -119.0$ ($c = 0.8$, MeOH). IR (KBr): 3388, 2965, 1707, 1604, 1074. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 395.2059 ($[M+H]^+$, $C_{21}H_{31}O_7^+$; calc. 395.2070).

4-[(β-D-Glucopyranosyl)oxy]pinoresinol (=*4,4′-[(1S*,3R*,3aS*,4S*,6aR*)-3-[(β-D-Glucopyranosyl)oxy]tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl]bis(2-methoxyphenol)*; **4**). Amorphous powder. $[\alpha]_D^{29} = -14.0$ ($c = 0.7$, MeOH). IR (KBr): 3361, 1603, 1517, 1457, 1275, 1026. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 554.2238 ($[M+NH_4]^+$, $C_{26}H_{36}NO_{12}^+$; calc. 554.2238).

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