New Eremophilenolactones from *Senecio nemorensis*

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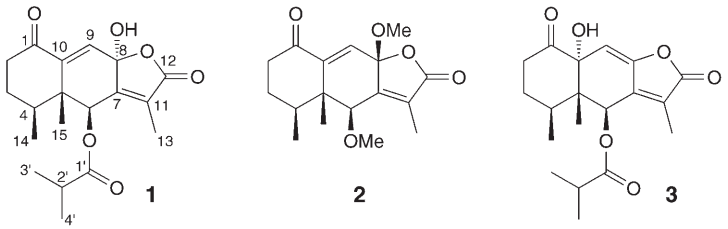
Three new eremophilane sesquiterpenes were isolated from the MeOH extract of the roots of *Senecio nemorensis*. Their structures were identified as 8α-hydroxy-6β-(isobutanoyloxy)-1-oxoeremophila-7(11),9-dieno-12,8β-lactone (**1**), 6β,8β-dimethoxy-1-oxoeremophila-7(11),9-dieno-12,8α-lactone (**2**), and 10α-hydroxy-6β-(isobutanoyloxy)-1-oxoeremophila-7(11),8-dieno-12,8-lactone (**3**), respectively, based on spectroscopic data, including IR, EI-MS, HR-ESI-MS, and 1D- and 2D-NMR data.

Introduction. – The genus *Senecio* belongs to the Senecioneae tribe of the Compositae family with more than 200 species occurring in China [1], of which several species have been used as traditional Chinese medicinal herbs for the treatment of inflammation, malaria, scald, and heatstroke [2]. Many species of *Senecio* have been studied, and the results show that pyrrolizidine alkaloids and eremophilane sesquiterpenes are the characteristic secondary metabolites [3]. *Cheng et al.* [4] have reported the structures of a new and two known franoeremophilane sesquiterpenes isolated from *S. nemorensis* collected in northwest China at about 2000 m altitude. With the aims of finding antibacterial terpenes and discovering the relations between chemical constituents and ecology circumstances, we investigated the chemical constituents of the roots of this plant collected in east China, and isolated three new sesquiterpenes. The results indicate that eremophilenolactones instead of franoeremophilanes are the characteristic sesquiterpenes of the plants collected in the humid and lower-altitude area.

Results and Discussion. – The pulverized, air-dried roots of *S. nemorensis* were extracted with MeOH and partitioned with hexane, $CHCl_3$, and H_2O. The $CHCl_3$-soluble fraction was purified by column chromatography (silica gel) and prep. TLC to yield compounds **1**–**3** and β-sitosterol.



Compound **1** was isolated as a colorless gum. The molecular formula was deduced to be $C_{19}H_{24}O_6$ from the quasimolecular-ion peak at *m/z* 371.1468 ($[M+Na]^+$) in the HR-ESI-MS. Its IR spectrum showed the absorption bands of OH (3328 cm^{-1}), C=C (1634 cm^{-1}), and C=O (1723 and 1706 cm^{-1} moieties). The ^{1}H- and ^{13}C-NMR (DEPT) spectra of **1** (*Table*) were very similar to those of 6β,8α-dihydroxy-1-oxoeremophila-7(11),9-dieno-12,8β-lactone [5], except for the presence of an additional isobutanoyl group (δ(H) 1.23, 1.25, and 2.69 (Me_2CH); δ(C) 176.5, 34.4, 18.2, and 18.9 (Me_2CHCO)). Detailed analysis of 1D- and 2D-NMR (*Fig.*) enabled us to establish the structure of **1** as 8α-hydroxy-6β-(isobutanoyloxy)-1-oxoeremophila-7(11),9-dieno-12,8β-lactone[1]).

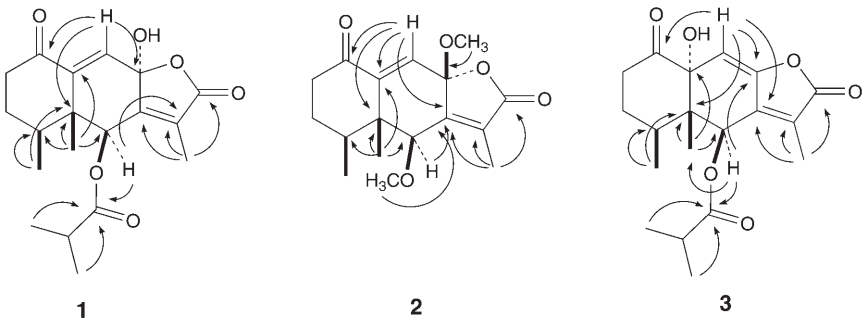


Figure. *Key HMBC correlations* (H → C) *for* **1**–**3**

Apart from the signals of the isobutanoyl group, the ^{1}H- and ^{13}C-NMR (DEPT) spectrum of **1** (*Table*) showed the presence of 15 C-atoms, *i.e.*, three Me, two CH_2, and three CH groups, and five quaternary C-atoms, indicating a sesquiterpene skeleton. The chemical shifts and coupling pattern of three characteristic ^{1}H-NMR signals at δ(H) 1.81 (*d*, *J* = 1.2 Hz, 3 H), 1.02 (*d*, *J* = 6.3 Hz, 3 H), and 0.97 (*s*, 3 H), together with the signals for a quaternary C-atom at δ(C) 100.0, suggested an eremophil-7(11)-eno-12,8-lcatone skeleton [6]. The chemical shift of the signal at δ(C) 201.9 (C) indicated that the carbonyl group was conjugated with a C=C bond. The ^{1}H-NMR signals at δ(H) 6.39 (*s*, H–C(9)) and 5.75 (*d*, *J* = 1.2 Hz, H–C(6)) allowed to position the C=C between C(9) and C(10) and the isobutanoyloxy at C(6) [7]. The HMBC data further confirmed the position of the isobutanoyloxy and α,β-unsaturated ketone groups (*Fig.*). The correlations δ(H) 5.75 (H–C(6))/δ(C) 153.6 (C(7)), 124.1 (C(11)), 40.9 (C(4)), 50.0 (C(5)), and 13.5 (C(15)) indicated that C(6) is oxygenated. The correlation δ(H) 5.75 (H–C(6))/δ(C) 176.5 (C(1′)) together with the lower-field shift of H–C(6) suggested that the isobutanoyloxy group was attached to C(6) [5]. The HMBC correlations δ(H) 6.39 (H–C(9))/δ(C) 201.9 (C(1)), 153.6 (C(7)), and 50.0 (C(5)), and δ(H) 0.97 (Me(15))/δ(C) 148.2 (C(10)) confirmed the position of the C=C bond between C(9) and C(10) and the location of the ketone group at C(1). The relative configurations of **1** were determined on the basis of the analysis of coupling constants. *Naya et al.* [8] reported that the homoallylic spin coupling (*J* = 1.2–1.5 Hz) between H_α–C(6) and Me(13) could be observed in 12,8β-lactons but was absent in 12,8α-lactones. Thus, the coupling constant *J*(6,13) of 1.2 Hz in **1** indicated a β-positioned isobutanoyloxy group at C(6).

The molecular formula of compound **2** was determined as $C_{17}H_{22}O_5$ by HR-ESI-MS (*m/z* 307.1535 ($[M+H]^+$)). The IR spectrum showed the absorptions of an α,β-

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

Table. *^{1}H- and ^{13}C-NMR* (DEPT) *Data of* **1**–**3** *in* $CDCl_3$[1]). δ in ppm, J in Hz.

	1[a])		**2**[b])		**3**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	201.9 (*s*)	–	200.8 (*s*)	–	207.8 (*s*)
H_a–C(2)	2.39–2.52 (*m*)	40.1 (*t*)	2.39–2.48 (*m*)	40.3 (*t*)	2.21–2.29 (*m*)	36.0 (*t*)
H_b–C(2)	2.54–2.66 (*m*)		2.57–2.62 (*m*)		3.15–3.26 (*m*)	
H_a–C(3)	1.68–1.77 (*m*)	28.7 (*t*)	1.66–1.74 (*m*)	27.4 (*t*)	1.53–1.67 (*m*)	30.9 (*d*)
H_b–C(3)	1.76–1.83 (*m*)		1.69–1.78 (*m*)		1.75–1.81 (*m*)	
H–C(4)	2.16–2.24 (*m*)	40.9 (*d*)	1.83–1.91 (*m*)	32.9 (*d*)	2.85–2.96 (*m*)	32.7 (*d*)
C(5)	–	50.0 (*s*)	–	48.9 (*s*)	–	50.8 (*s*)
H–C(6)	5.75 (*d*, J = 1.2)	76.1 (*d*)	4.10 (*s*)	78.8 (*d*)	6.52 (*d*, J = 1.6)	71.8 (*d*)
C(7)	–	153.6 (*s*)	–	153.1 (*s*)	–	144.5 (*s*)
C(8)	–	100.0 (*s*)	–	101.8 (*s*)	–	150.7 (*s*)
H–C(9)	6.39 (*s*)	126.3 (*d*)	6.63 (*s*)	124.8 (*d*)	6.21 (*s*)	104.6 (*d*)
C(10)	–	148.2 (*s*)	–	146.7 (s)	–	78.5 (*s*)
C(11)	–	124.1 (*s*)	–	130.7 (*s*)	–	125.2 (*s*)
C(12)	–	170.5 (*s*)	–	169.8 (*s*)	–	170.1 (s)
Me(13)	1.81 (*d*, J = 1.2)	8.4 (*q*)	1.97 (*s*)	8.8 (*q*)	1.82 (*d*, J = 1.6)	8.6 (*q*)
Me(14)	1.02 (*d*, J = 6.3)	17.5 (*q*)	0.99 (*d*, J = 6.7)	14.3 (*q*)	0.89 (*d*, J = 6.7)	17.0 (*q*)
Me(15)	0.97 (*s*)	13.5 (*q*)	0.76 (*s*)	17.8 (*q*)	0.78 (*s*)	9.2 (*q*)
MeO–C(6)			3.38 (*s*)	51.4 (*s*)		
MeO–C(8)			3.40 (*s*)	58.0 (*s*)		
^{i}PrCOO:						
C(1′)	–	176.5 (*s*)			–	176.4 (*s*)
H–C(2′)	2.69 (*qq*, J = 7.2, 7.2)	34.4 (*d*)			2.67 (*qq*, J = 6.9, 6.9)	34.4 (*d*)
Me(3′)	1.23 (*d*, J = 7.2)	18.2 (*q*)			1.23 (*d*, J = 6.9)	18.5 (*q*)
Me(4′)	1.25 (*d*, J = 7.2)	18.9 (*q*)			1.26 (*d*, J = 6.9)	18.6 (*q*)

[a]) Measured at 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR. [b]) Measured at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR.

unsaturated γ-lactone (1769 cm^{-1}) and an α,β-unsaturated ketone (1705 and 1630 cm^{-1}). The ^{1}H- and ^{13}C-NMR (DEPT) spectrum were similar to those of **1**, except for the presence of two MeO groups instead of the isobutanoyl moiety (*Table*). The structure of **2** was determined as 6β,8β-dimethoxy-1-oxoeremophila-7(11),9-dieno-12,8α-lactone[1]).

The HMBC data of **2**, *i.e.*, the correlations δ(H) 3.40 (MeO–C(8))/δ(C) 101.8 (C(8)) and δ(H) 3.38 (MeO–C(6))/δ(C) 78.8 (C(6)) indicated that the MeO groups were attached to C(8) and C(6), respectively (*Fig.*). The MeO–C(6) was in β orientation as shown by the correlation peak H–C(6)/Me(15) observed in the NOESY [9]. The absence of homoallylic spin coupling between δ(H) 4.10 (*s*, H–C(6)) and 1.97 (*s*, Me(13)) suggested the β orientation of MeO–C(8) [8].

Compound **3** is an isomer of **1**. Its EI-MS showed the molecular-ion peak at m/z 348. The molecular formula $C_{19}H_{24}O_6$ was determined by HR-ESI-MS (m/z 366.1907 ($[M + NH_4]^+$)). Its IR spectrum also showed the absorption bands of OH (3506 cm^{-1}), C=C (1673 and 1644 cm^{-1}), and C=O (1762, 1736, and 1703 cm^{-1}) moieties. From the spectral data (see *Table* and *Fig.*), compound **3** was identified as 10α-hydroxy-6β-(isobutanoyloxy)-1-oxoeremophila-7(11),8-dieno-12,8-lactone[1]).

The quaternary-C-atom signal at δ(C) 78.5 in the ^{13}C-NMR spectrum of **3** revealed the presence of the quaternary OH group. The olefinic proton appeared at δ 6.21 as a *s* disclosing that the neighboring olefinic C-atom is a quaternary one. Thus, the quaternary OH group should be located at C(10) and a C=C bond between C(8) and C(9), thus allowing the olefinic H–C(9) to appear as a *s*. In the HMBC plot, the cross-peak δ(H) 0.78/δ(C) 78.5 further confirmed that the OH was located at C(10). The HMBC cross-peaks δ(H) 6.21 (H–C(9))/δ(C) 207.8 (C(1)) suggested that the ketone carbonyl group was at C(1). The HMBC cross-peaks δ(H) 0.78 (Me(15))/δ(C) 71.8 (C(6)), and δ(H) 6.52 (H–C(6))/δ(C) 9.2 (C(15)), 50.8 (C(5)), 125.2 (C(11)), 144.5 (C(7)), and 176.4 C(1′) indicated that isobutanoyloxy was attached to C(6). In the ^{1}H-NMR spectrum (*Table*), the relatively higher-field chemical shift of Me(15) in relation to that of Me(14) suggested a *trans*-decalin system in **3**, which means α orientation for OH–C(10) [10].

Experimental Part

General. Column chromatography (CC): silica gel (200–300 and 300–400 mesh). TLC: silica gel GF_{254} from *Qingdao Marine Chemical Factory*, China; petroleum ether of b.p. 60–90° in eluent mixtures; detection under UV light or by heating after spraying with 5% H_2SO_4 in EtOH. IR Spectra: *Nicolet NEXUS-670*-FT-IR spectrometer; in cm^{-1}. Optical rotations: *Perkin-Elmer 341* polarimeter. ^{1}H- and ^{13}C-NMR (DEPT) and 2D-NMR Spectra: *Varian Mercury-plus-400* and *-plus-300* spectrometer; δ in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz. MS: *Bruker APEX--II* spectrometer for HR-ESI, and *HP 5988A* GC/MS instrument for EI-MS; in *m*/*z* (rel. %).

Plant Material. The roots of *Senecio nemorensis* were collected from Kunyu Mountains, Weihai, People's Republic of China, in August 2006, and identified by Associate Prof. *Hong Zhao* of the Marine College, Shandong University at Weihai. A voucher specimen was deposited in the Laboratory of Botany, Marine College, Shandong University at Weihai.

Extraction and Isolation. The air-dried rhizome of *Senecio nemorensis* (680 g) were pulverized and extracted with MeOH three times (7 days each time) at r.t. The solvent was evaporated and the residue (56 g) suspended in hot H_2O (60°; 200 ml). This suspension was extracted successively with hexane and $CHCl_3$. The $CHCl_3$-soluble fraction was concentrated to afford a residue (22 g) which was subjected to CC (silica gel (200–300 mesh; 220 g), petroleum ether/acetone 20:1, 10:1, and 5:1): *Fractions 1–3. Fr. 1* (with petroleum ether/acetone 20:1; 2.4 g) was subjected to CC (silica gel, petroleum ether/AcOEt 10:1) and then recrystallized: *β-sitosterol* (380 mg). *Fr. 2* (with petroleum ether/acetone 10:1; 6.7 g) was subjected to CC (silica gel; petroleum ether/acetone 15:1): **1** (18 mg) and a mixture (120 mg). This mixture was further purified by prep. TLC ($CHCl_3$/acetone 150:1): **2** (R_f 0.67; 12 mg) and **3** (R_f 0.42; 8 mg). No interesting spot was found in *Fr. 3* (with petroleum ether/acetone 5:1; 7.8 g).

8α-Hydroxy-6β-(isobutanoyloxy)-1-oxoeremophila-7(11),9-dieno-12,8β-lactone (= rel-*(4*R,*4a*S, *5*R,*9a*S*)-2,4,4a,5,6,7,8,9a-Octahydro-9a-hydroxy-3,4a,5-trimethyl-2,8-dioxonaphtho[2,3-*b*]furan-4-yl 2-Methylpropanoate*; **1**): Colorless gum. $[\alpha]_D^{27} = -106.6$ ($c = 5.3$, $CHCl_3$). IR (KBr): 3328, 2910, 1723, 1706, 1634, 1472, 1456, 1075, 1039. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 278 (1), 260 (19), 231 (4), 217 (8), 204 (6), 189 (3), 177 (3), 149 (3), 115 (4), 91 (11), 83 (31), 71 (38), 57 (18), 55 (26), 43 (100). HR-ESI-MS: 371.1468 ($[M+Na]^+$, $C_{19}H_{24}NaO_6^+$; calc. 371.1465).

6β,8β-Dimethoxy-1-oxoeremophila-7(11),9-dieno-12,8α-lactone (= rel-*(4*R,*4a*S,*5*R,*9a*R*)-4a,6,7,9a-Tetrahydro-4,9a-dimethoxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2,8(4*H*,5*H*)-dione*; **2**): Colorless gum. $[\alpha]_D^{27} = -94.9$ ($c = 0.7$, $CHCl_3$). IR (KBr): 2924, 2844, 1769, 1705, 1630, 1466, 1456, 1075, 1039. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 306 (8), 291 (3), 231 (3), 203 (4), 187 (2), 177 (3), 164 (3), 163 (2), 97 (25), 83 (52), 71 (35), 55 (47), 57 (68), 43 (100). HR-ESI-MS: 307.1535 ($[M+H]^+$, $C_{17}H_{22}O_5^+$; calc. 307.1540).

10α-Hydroxy-6β-(isobutanoyloxy)-1-oxoeremophila-7(11),8-dieno-12,8-lactone (= rel-*(4*R,*4a*R,*5*R,*8a*S*)-2,4,4a,5,6,7,8,8a-Octahydro-8a-hydroxy-3,4a,5-trimethyl-2,8-dioxonaphtho[2,3-*b*]furan-4-yl 2-Methylpropanoate*; **3**): Colorless gum. $[\alpha]_D^{27} = -231.2$ ($c = 0.8$, $CHCl_3$). IR (KBr): 3506, 3262, 2913, 1762, 1736, 1703, 1673, 1644, 1482. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 348 (1), 278 (2), 260 (15), 232 (24), 217 (11), 177 (34), 165 (17), 91 (16), 83 (21), 77 (16), 71 (37), 57 (63), 43 (100). HR-ESI-MS: 366.1907 ($[M+NH_4]^+$, $C_{19}H_{28}NO_6^+$; calc. 366.1911).

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