Notes

The Sesquiterpenes from *Cacalia tangutica*

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Cacalia tangutica belonged to the tribe Compositae have long been used as Chinese traditional folk herbs to treat headache, dizziness, hemiplegia, rheumatism, tussis and phlegm.[1] Resently, our continuing studies on this plant revealed the presence of diversiform sesquiterpenes from a petrol extract of the aerial parts.[2] The seven sesquiterpenes isolated were three eremophilane sesquiterpenes (**1-3**)[3-5] including novel one (**1**), one known guaianetype sesquiterpeoid (**4**),[6] one alloromadendrane sesquiterpene (**5**)[7,8] and two eudesmane sesquiterpenes (**6**, **7**)[9-11] (Figure 1).

Compound **1**, a pink gum, $[\alpha]_D^{20}$ +10 (*c* 1.30, $CHCl_3$), has the molecular formula $C_{15}H_{22}O_4$ (HR-ESIMS: *m/z* 267.1597 $[M+1]^+$, calcd. for $C_{15}H_{23}O_4$ 267.1591). Its IR and UV spectra showed the presence of a hydroxyl (3323 cm^{-1}) and α,β-unsaturated carbonyl systems - a ketone (1660, 1613 cm^{-1} and λ_{max} 244 nm, 203 nm). Analysis of the ^1H NMR and ^{13}C NMR (DEPT) spectrum of **1** along with HMQC experiment, the fifteen signals in ^{13}C NMR and the signals of four methyl groups (δ_H: 1.17 s, δ_C: 18.5; δ_H: 1.27 d, J = 6.6 Hz, δ_C: 11.8; δ_H: 1.51 s, δ_C: 24.4; δ_H: 1.55 s, δ_C: 24.7) identified **1** as eremophlane sesquiterpene. The two olefinic signals (δ_H: 6.06 s, δ_C: 125.3 (CH); δ_H: 7.21 s, δ_C: 150.9 (CH)) combined with HMBC correlations (δ_H: 6.06 s/δ_C: 138.7 (C), 42.9 (C), and 30.2 (CH_2); δ_H: 7.21 s/δ_C: 47.3 (CH), 42.9 (C), 138.7 (C), 165.7 (C), 185.7 (C), and 83.4 (C)) indicated the presence of characteristics of an 8-one-eremophila-6,9-diene derivative. An additional hydroxy and a peroxyl groups were required for the molecular formula $C_{15}H_{22}O_4$. The signals appeared at δ_H 3.69 (ddd, 1H, J = 11.4, 11.1, 4.2 Hz) and δ_C 71.1 (CH) suggested the hydroxy group was equatorial stereochemistry at C-3,[11] while the signals at δ_H 1.51 s, 1.55 s, 8.78 brs (H-peroxyl, D_2O exchanged) and δ_C 24.4 (CH_3), 24.7 (CH_3), 83.4 (C) suggested the peroxyl group was at C-11.[14] This was supported by the long range coupling of C-3 (71.1, CH) with the methyl proton (1.27 d, J = 6.6 Hz, H-15) and the long range coupling of C-11 (83.4, C) with the methyl protons (1.51 s, H-12; and 1.55 s, H-13) in the HMBC spectrum. In the ^1H-^1H COSY spectrum, H-4 (δ 1.42 d, J = 11.4 Hz) and H-2 (δ 2.27 m) were also correlated with H-3.

To allow the assignments of structure **1** rigorously, a simple reductive reaction has been taken place as followed (see Figure 2). Compound **1** has been selectively reduced to compound **1-1** by potassium iodide in the solution of dilute acetic acid.

The produce **1-1**, a pale yellow oil, has the molecular

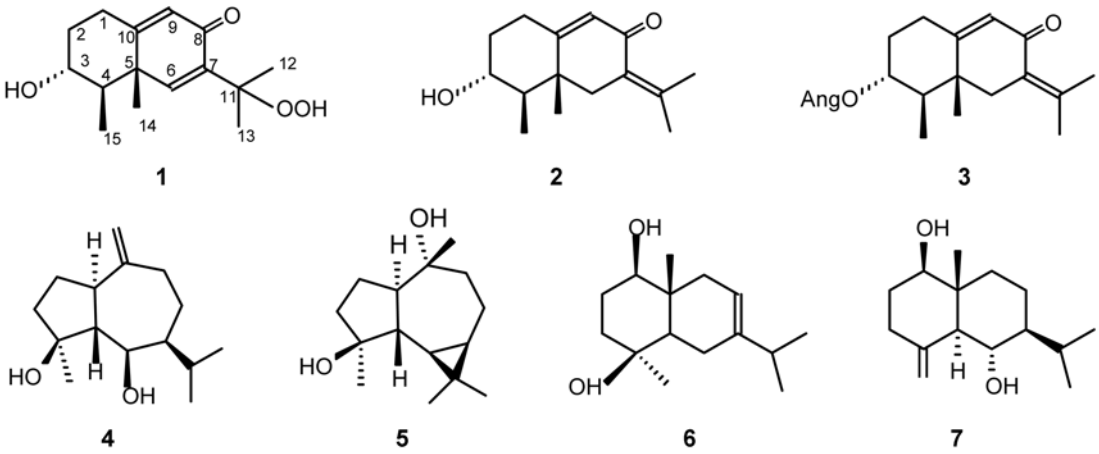

Figure 1. The Sesquiterpenes from *Cacalia tangutica.*

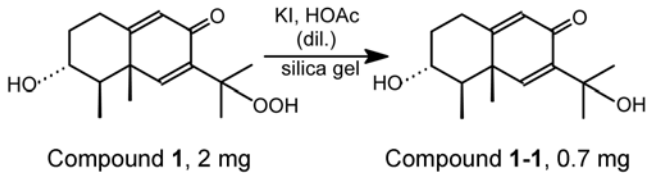

Figure 2. The selectively reductive reaction of compound **1**.

formula $C_{15}H_{22}O_3$ (HR-ESIMS: *m/z* 273.1464 $[M+Na]^+$, calcd. for $C_{15}H_{22}O_3Na$ 273.1461; EI-MS: *m/z* (% ÷ 100) = 250 $[M]^+$ (27), 235 $[M-CH_3]^+$ (521), 217 $[235-H_2O]^+$ (778), 199 $[217-H_2O]^+$ (411), 175 (675), 43 (10000)). In the ^1H NMR of **1-1**, the olefinic signal (6.91 s, H-6) and the methyl signal (1.47s, H-12) shifted to upfield compared with the olefinic signal (7.21 s, H-6) and the methyl signal (1.51 s, H-12) of **1**, at the same time the methyl signal (1.56 s, H-13) of **1-1** shifted to downfield compared with the methyl signal (1.55 s, H-13) of **1**. It was identical with petasitin.[12,13] These indicate that compound **1** has been deoxidized to petasitin and it further demonstrated that a peroxyl group was in structure **1**.[14]

In the NOE spectrum of **1**, the NOEs [H-3 with H-14 (3.3%) and H-15 (1.8%)] were appeared. It was concluded that compound **1** was 3α-hydroxy-11-peroxyl-eremophila-6, 9-dien-8-one.

Six known compounds **2-7** were the results after repeated column chromatography of the petrol extract of the aerial parts of *Cacalia tangutica* and were deduced by spectral data as two eremophilane sesquiterpenes: isopetasol (**2**)[3,4] and isopetasin (**3**),[3,5] one guaianetype sesquiterpeoid: Teucladiol (**4**),[6] one alloromadendrane sesquiterpene: armadendrane-4β, 10α-diol (**5**),[7,8] and two eudesmane sesquiterpenes: oplodiol (**6**)[9,10] and 1β,6α-dihydroxyedues-4(15)-ene (**7**).[11]

Compound **1** was tested for *in vitro* antitumor activity against BEL-7402 (human liver carcinoma) and A-549 (human lung cancer) by the method of the cells stained with sulforhodamine B (SRB).[15] Test plates were incubated for 3 days. The inhibiting activity with IC_{50} values (23.9 μg/mL, 21.8 μg/mL) were determined as compared with Etoposide[16] (IC_{50} values: 7.00 μg/mL, 7.14 μg/mL). The result showed that compound **1** was able to inhibit the growth of BEL-7402 and A-549 within measure.

Experimental Section

General Methods. IR spectra were measured on a Nicolet AVATAR 360 FT-IR instrument (KBr pellet). UV spectra was measured on a Shimadzu UV-260 spectrometer. 1D and 2D NMR spectrometer were measured on a Bruker AM-400FT-NMR spectrometer and a Varian Mercury-300BB NMR spectrometer with TMS as inernal standard. HRESI-MS were recorded on a Bruker APEX II, EI-MS on a HP 5988A GC/MS instrument. Optical rotations were measured using Perkin Elmer Model 341. Silica gel (200-300 mesh) was used for CC, silica GF_{254} (10-40 μ) for TLC were supplied by the Qingdao Marine Chemical factory, Qingdao, P. R. China. Spots were detected on TLC under UV lamp or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

Plant Material. The aerial parts of *Cacalia tangutica.* were collected in Minhe county, Qinhai province of China in October 1997, and identified by Prof. JiZhou Sun of Department of Biology, Lanzhou University. A voucher specimen (NO. 0108298) is deposited in Department of Biology, Lanzhou Univerisity.

Extraction and Isolation. Dried, powdered aerial parts (5750 g) of *Cacalia tangutica* were extracted with methanol by percolation at room temperature to give a residue (796 g) after evaporation. This residue was partitioned between petroleum ether (60-90º) and H_2O. The petroleum ether (60-90º)-soluble portion (118 g) was separated on CC over 1000 g silica gel with a gradient of petroleum ether (60-90º)-acetone (40 : 1; 20 : 1; 18 : 1; 15 : 1; 12 : 1; 10 : 1; 7 : 1; 5 : 1; 3 : 1; 1 : 1 and 0 : 1) as eluent. Compound **1** (8 mg) was isolated during elution with petroleum ether (60-90º)-acetone (10 : 1) and afforded after prep. tlc of the eluates 5-7 with C_6H_6-EtOAc (15 : 1).

Compounds **2**, **4** and **7** were obtained from the fractions of petroleum ether (60-90 ºC)/acetone (18 : 1; 15 : 1; 15 : 1) and chromatographed on silica gel prep. plate using petroleum ether (60-90º)-EtOAc (15 : 1).

The fractions of petroleum ether (60-90 ºC)/acetone (12 : 1; 12 : 1; 10 : 1) was purified by a silica gel column and eluting with a gradient of petrol-EtOAc (20 : 1; 18 : 1; 15 : 1; 12 : 1; 10 : 1; 7 : 1; 5 : 1; 3 : 1; 1 : 1 and 0 : 1) to yield pure compounds **3**, **5** and **6**.

3α-Hydroxy-11-peroxyl-eremophila-6,9-dien-8-one (1): $C_{15}H_{22}O_4$, a pink gum. $[\alpha]_D^{20}$: +10 (*c* 1.30, $CHCl_3$); HR-ESIMS: *m/z* 267.1597 $[M+1]^+$, calcd. for $C_{15}H_{23}O_4$ 267.1591; EI-MS: *m/z* (% ÷ 100) = 266 $[M]^+$ (18), 248 $[M-H_2O]^+$ (204),

Table 1. The NMR spectral data of compound **1** (300 MHz, $CDCl_3$ TMS as internal standard)

No.	δ_H (ppm)	δ_C (DEPT) (ppm)	HMBC[a]
1	2.00 m, 2.47 m	30.2 (CH_2)	C-1 / H-(2), 9
2	1.38 m, 2.27 m	36.3 (CH_2)	C-2 / H-(1)
3	3.69 ddd (11.4, 11.1, 4.2 Hz)	71.1 (CH)	C-3 / H-15
4	1.42 m	47.3 (CH)	C-4 / H-6, 14, (15)
5		42.9 (C)	C-5 / H-1, (6), 9, (14), 15
6	7.21 s	150.9 (CH)	C-6 / H-14
7		138.7 (C)	C-7 / H-(6), 9, 12, 13
8		185.7 (C)	C-8 / H-6
9	6.06 s	125.3 (CH)	C-9 / H-1
10		165.7 (C)	C-10 / H-(1), 6, 14
11		83.4 (C)	C-11 / H-6, (12), (13)
12	1.51 s	24.4 (CH_3)	C-12 / H-(13)
13	1.55 s	24.7 (CH_3)	C-13 / H-(12)
14	1.17 s	18.5 (CH_3)	C-14 / H-4, 6
15	1.27 d (6.6 Hz)	11.8 (CH_3)	C-15 / H-(4)

[a]Two-bond correlations are indicated in parentheses.

235 (1172), 233 (615), 230 (815), 43 (10000); UV (MeOH): λ_{max} = 203, 244 nm; IR (KBr): ν_{max} = 1029, 1265, 1374, 1451, 1613, 1660, 2867, 2928, 2978, 3323 cm^{-1}; ^1H and $^{13}CNMR$ ($CDCl_3$, 300MHz) see Table 1.

Petasitin (1-1): $C_{15}H_{22}O_3$, pale yellow oil. HR-ESIMS: *m/z* 273.1464 $[M+Na]^+$, calcd. for $C_{15}H_{22}O_3Na$ 273.1461; EI-MS: *m/z* (% ÷ 100) = 250 $[M]^+$ (27), 235 $[M-CH_3]^+$ (521), 217 $[235-H_2O]^+$ (778), 199 $[217-H_2O]^+$ (411), 175 (675), 43 (10000); ^1H NMR ($CDCl_3$, TMS): δ3.69 m (H-3), 6.91 s (H-6), 6.10 s (H-9), 1.47 s (H-12), 1.56 s (H-13), 1.16 s (H-14), 1.25 d (J = 6.0 Hz, H-15).

Antitumor Testing. *In vitro* antitumor activities against BEL-7402 (human liver carcinoma) and A-549 (human lung cancer) of compound 1 by the method of the cells stained with sulforhodamine B (SRB) carried out according to:[15] Test plates were incubated for 3 days at 37 °C in a 5% CO_2 incubator. After the incubation periods, cells were fixed by the addition of aqueous TCA solution (4 °C for 30 min) and the fixed cells were stained with SRB (0.4% w/v in 1% aqueous acetic acid) for 30 min, the bound dye was solubilized with 200 μL of 10 mM tris-base (pH 10.0), and absorbance was determined at 515 nm in Vis region.

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