The Components of *Cacalia tangutica*

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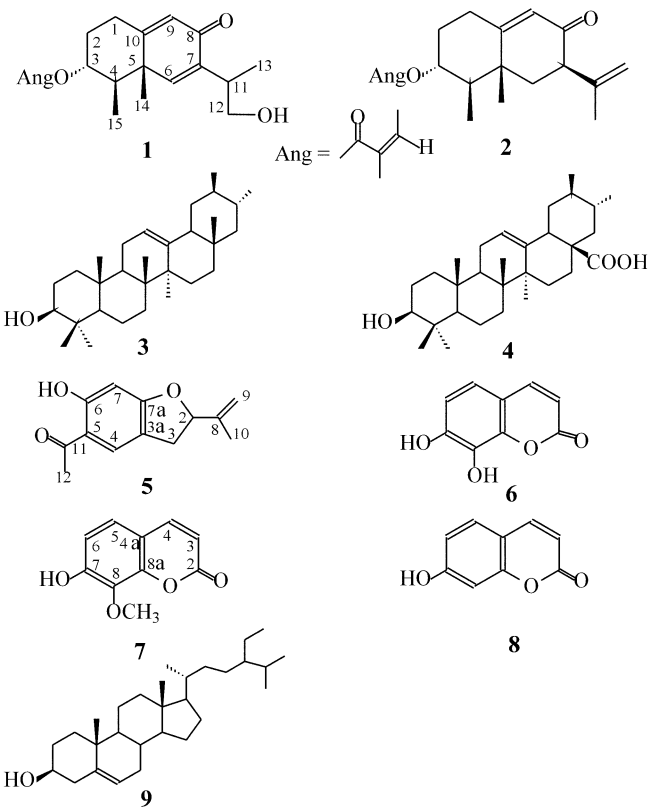
The genus *Cacalia* belongs to the tribe Senecioneae with more than 60 species occurring in China,[1] of which *ca* 26 have long been used as Chinese traditional folk herbs.[2] Several species of genus *Cacalia* have been investigated due to their antioxidant, antiradical and anti-histamine activities.[3] The presence of pyrrolizidine alkaloids and sesquiterpenes in many species of the tribe Senecioneae is well documented.[4-9] Recently, we have investigated the chemical constituents of *Cacalia tangutica* (Senecioneae). From a methanol extract of the root, one new eremophilane sesquiterpene, 12-hydroxy-3α-angeloyloxy-eremophila-6,9-dien-8-one (**1**) combined with eight known compounds: one sesquiterpene, petasine (**2**), two triterpenes, α-amyrin (**3**) and ursolic acid (**4**), one isopentenyl acetophnon derivative, hydroxytremetone (**5**), three cumarins, daphnetol (7,8-dihydroxycumarin) (**6**), hydrangetin (**7**), umbelliferone (**8**), one steroid, β-sitosterol (**9**) were isolated. The structure of the neo-sesquiterpene was elucidated by means of ^{1}H and ^{13}C NMR spectroscopic studies, including long rang correlation spectra with inverse detection (HMBC), ^{1}H-^{1}H COSY, NOE and NOESY.



Compounds 1-9

Compound **1**, a yellow oil, $[\alpha]_D^{20}$ -26° (*c* 1.03, $CHCl_3$), has the molecular formula $C_{20}H_{28}O_4$ (HR-ESIMS: *m/z* 333.2064 $[M-1]^+$, calcd. for $C_{20}H_{28}O_4$ 333.2060). Its IR and UV spectra show the presence of α,β-unsaturated carbonyl system-a ketone (1663 cm^{-1} and λ_{max} 243 nm) and an ester (1715 and 1233 cm^{-1}). The spectral data also indicated that the fourth oxygen atom seemed to be an alcohol (3395 cm^{-1}). The existence of a (*Z*)-2-methyl-2-butenoate (angeloyloate) moiety as well as the ester group in **1**, was inferred from the NMR signals, [δ_H 6.10 m (1H), 1.95 dq (*J* = 7.6 Hz, 1.4 Hz, 3H), 1.86 br s (3H); δ_C 167.5 s, 127.7 s, 138.5 d, 20.5 q, 14.2 q], by analogy with those of the constituents.[10,11]

The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of 8-one-eremophila-6,9-diene derivatives reported in the literature except the primary alcohol group and secondary angeloyloate ester.[10-14] A comparison of the ^{1}H NMR spectral data with those of the corresponding 1β-hydroxy derivative[12] indicated that the C-1 position was not substituted. The downfield shifted signal of H-3 was due to the 3-angeloyloxy while the signal δ 5.01td (*J* = 11.2 Hz, 4.8 Hz, H-3) showed that the angeloyloxy was α-oriented.[14-18] These were confirmed by the correlations between H-2 and H-4 with H-3 in the ^{1}H-^{1}H COSY spectrum and the correlation between H-3 and H-14 in the NOESY spectrum respectively. In the NOE spectrum of **1**, the NOEs [H-15 with H-14 (31.0%) and H-3 (1.8%)] and [H-3 with H-14 (3.0%) and H-15 (1.5%)] were also appeared. According to the methylene signal δ 60.4 in ^{13}C NMR spectra (DEPT), the signal at δ 4.13 dd (*J* = 14.3 Hz, 6.8 Hz, H-12) in ^{1}H NMR was assigned to the two protons attached to the carbon atom (δ 60.4) bearing primary alcohol group. This was then supported by the cross placed between H-11 and H-12 in the ^{1}H-^{1}H COSY.

In the HMBC spectrum, H-9 (δ 6.10) was correlated with C-1 (δ 30.1), C-2 (δ 29.7), C-5 (δ 43.0) and C-7 (δ 141.4). H-6 (δ 6.92) was correlated with C-4 (δ 44.5), C-5 (δ 43.0), C-7 (δ 141.4), C-8 (δ 187.7), C-10 (δ 166.1) and C-14 (δ 18.5). H-14 (δ 1.23) was correlated with C-4 (δ 44.5), C-5 (δ 43.0), C-6 (δ 147.8) and C-10 (δ 166.1). H-15 (δ 1.14) was correlated with C-3 (δ 72.6), C-4 (δ 44.5), and C-5 (δ 43.0),

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H-13 (δ 1.26) was correlated with C-12 (δ 60.4) and C-7 (δ 141.4), H-3 (δ 5.01) was correlated with the carboxylic carbon of angeloyloxy (δ 167.5). It was determined that compound **1** was 12-hydroxy-3α-angeloyloxy-eremophila-6,9-dien-8-one.

Eight known compounds **2-9** were the results after repeated column chromatography of the methanol extract of the root of *Cacalia tangutica* and were deduced by spectral data as petasine (**2**),[19] two triterpenes, α-amyrin (**3**)[20] and ursolic acid (**4**).[20] one isopentenyl acetophnon derivative, hydroxytremetone (**5**),[21] three cumarins, daphnetol (**6**),[22] hydrangetin (**7**),[22] umbelliferone (**8**).[23] one steroid, β-sitosterol (**9**).

The compound **1** was tested for *in vitro* antitumor activity against BEL 7402 (human liver carcinoma) by the method of the cells stained with sulforhodamine B (SRB).[24] Test plates were incubated for 3 days. IC_{50} values were determined for compounds **1** (20.70 μg/mL). This result showed that compound **1** was able to inhibit the growth of BEL 7402 with IC_{50} values below 100 μg/mL.

Experimental Section

General Methods. IR spectra were measured on a Nicolet AVATAR 360 FT-IR instrument KBr. 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 Marino 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 root 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.

Table 1. The NMR data of **1** (400 MHz, $CDCl_3$)

No.	δ_C (DEPT)	δ_H	HMBC (carbon)[a]
1	30.1 (CH_2)	2.33m, 2.60 m	(2), 3, 8, 9, (10)
2	29.7 (CH_2)	1.60m, 2.20 m	(3), 4, 9, 10
3	72.6 (CH)	5.01 td (11.2, 4.8)	(4), OAng (167.5)
4	44.5 (CH)	1.69m	(3), (5), 14
5	43.0 (C)		
6	147.8 (CH)	6.92 s	4, (5), (7), 8, 10, 14
7	141.4 (C)		
8	187.7 (C)		
9	125.3 (CH)	6.10 m	1, 2, 5, 7
10	166.1 (C)		
11	21.0 (CH)	1.26 m	(12)
12	60.4 (CH_2)	4.13 dd (14.3, 6.8)	7, 13
13	28.9 (CH_3)	1.26 m	7, 12
14	18.5 (CH_3)	1.23 s	4, (5), 6, 10
15	15.8 (CH_3)	1.14 d (6.8)	3, (4), 5

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

Extraction and Isolation. Dried, powdered root (1000 g) of *Cacalia tangutica* were extracted with methanol by percolation at room temperature to give a residue (102 g) after evaporation. This residue was separated on CC over 1000 g silica gel with a gradient of petroleum ether (60-90°)-ethyl acetate as eluent. Compound **1** was isolated during elution with petroleum ether (60-90°)-ethyl acetate (8 : 1). Prep. tlc of eluates 5-7 with C_6H_6-EtOAc (12 : 1) afforded 8 mg of **1**.

Compound 1: $C_{20}H_{28}O_4$, yellow oil; $[\alpha]_D^{20}$: -26° ($CHCl_3$, *c* 1.03); IR ν : 3395, 2923, 1715, 1663, 1617, 1457, 1265, 1233 cm^{-1}; UV λ_{max}^{MeOH} : 243 nm; HR-ESIMS: *m/z* 333.2064 $[M+1]^+$, calcd. 333.2060 for $[C_{20}H_{28}O_4+H]^+$; EI-MS: *m/z* (% ÷ 10) 332.05 $[M]^+$ (3), 317 $[M-CH_3]^+$ (203), 249 $[M-Ang]^+$ (3), 217 $[317-HOAng]^+$ (199), 83 $[Ang]^+$ (938), 43 (1000); ^1H and ^{13}C NMR ($CDCl_3$, 400 MHz) see Table 1.

Compound 2: $C_{20}H_{28}O_3$, colorless oil; EI-MS: *m/z* (%) 316.20 $[M]^+$ (3), 233 $[M-Ang]^+$ (1), 216 $[233-H_2O]^+$ (11), 201 $[216-CH_3]^+$ (10), 148 (100), 83 $[Ang]^+$ (13); ^1H NMR ($CDCl_3$, 400 MHz): δ 2.40 (1H, m, H-1), 2.53 (1H, m, H-1), 1.50 (1H, m, H-2), 2.00 (1H, m, H-2), 4.92 (1H, ddd, *J* = 11, 11, 4.5 Hz, H-3), 2.02 (1H, m, H-4), 2.00 (1H, m, H-6), 2.30 (1H, m, H-6), 3.12 (1H, dd, *J* = 14, 5.0 Hz, H-7), 5.79 (1H, d, *J* = 1.6 Hz, H-9), 4.82 (1H, s, H-12), 4.99 (1H, dq, *J* = 1.2 Hz, H-12), 1.74 (3H, s, H-13), 1.24 (3H, s, H-14), 0.97 (3H, d, *J* = 6.8 Hz, H-15), OAng: δ 6.10 (1H, qq, *J* = 7.2, 1.4 Hz), 1.89 (3H, m), 1.96 (3H, m); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 31.6 (C-1), 30.6 (C-2), 73.0 (C-3), 47.3 (C-4), 40.0 (C-5), 41.7 (C-6), 50.3 (C-7), 198.4 (C-8), 124.6 (C-9), 167.6 (C-10), 143.3 (C-11), 114.4 (C-12), 20.6 (C-13), 10.52 (C-14), 15.7 (C-15), OAng: δ 166.7 (C), 127.9 (C), 138.0 (CH), 17.2 (CH_3), 20.0 (CH_3).

Compound 3: $C_{30}H_{50}O$, white needle (MeOH), m.p. 184-186 °C; EI-MS: *m/z* (% ÷ 100) 426.45 $[M]^+$ (533), 411 $[M-CH_3]^+$ (188), 218 (10000), 203 (3958), 189 (2912); ^{13}C NMR (DEPT, $CDCl_3$, 400 MHz): δ 38.8 (C-1), 27.3 (C-2), 79.1 (C-3), 38.8 (C-4), 55.2 (C-5), 18.4 (C-6), 33.0 (C-7), 40.0 (C-8), 47.7 (C-9), 36.9 (C-10), 23.4 (C-11), 124.4 (C-12), 139.6 (C-13), 42.1 (C-14), 28.7 (C-15), 26.6 (C-16), 33.8 (C-17), 59.1 (C-18), 39.7 (C-19), 39.6 (C-20), 31.3 (C-21), 41.5 (C-22), 28.1 (C-23), 15.6 (C-24), 15.7 (C-25), 16.9 (C-26), 23.3 (C-27), 28.1 (C-28), 17.5 (C-29), 21.4 (C-30).

Compound 4: $C_{30}H_{48}O_3$, white powder (MeOH), m.p. 262-264 °C; EI-MS: *m/z* (% ÷ 10) 456.30 $[M]^+$ (26), 438 (320), 423 (461), 410 (57), 300 (18), 248 (1000), 203 (500), 189 (104), 133 (261).

Compound 5: $C_{13}H_{14}O_3$, colorless oil; EI-MS: *m/z* (% ÷ 100) 217.95 $[M]^+$ (3766), 203 $[M-CH_3]^+$ (3682), 175 (2579), 119 (6859), 117 (6747), 43 (10000); ^1H NMR ($CDCl_3$, 400 MHz): δ 5.27 (1H, t, *J* = 8.0 Hz, H-2), 2.95 (1H, dd, *J* = 9.6, 15.0 Hz, H-3), 3.31 (1H, dd, *J* = 8.4, 15.0 Hz, H-3), 7.50 (1H, s, H-4), 6.38 (1H, s, H-7), 4.95 (1H, s, H-9), 5.09 (1H,

s. H-9), 1.76 (3H, s, H-10), 2.55 (3H, s, H-12), OH: 13.0 (1H, s); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 87.6 (C-2), 33.2 (C-3), 113.8 (C-3a), 126.6 (C-4), 143.2 (C-5), 165.8 (C-6), 98.1 (C-7), 166.6 (C-7a), 118.6 (C-8), 112.7 (C-9), 17.0 (C-10), 201.9 (C-11), 26.2 (C-12).

Compound 6: $C_9H_6O_4$, pale-yellow needle (MeOH), m.p. 253-255 ℃; EI-MS: *m/z* (% ÷ 10) 177.90 $[M]^+$ (1000), 150 $[M-H_2O]^+$ (770), 122 (115), 69 (329), 43 (470); ^{1}H NMR (Me_2CO, 400 MHz): δ 6.13 (1H, d, J = 9.5 Hz, H-3), 7.83 (1H, d, J = 9.5 Hz, H-4), 7.01 (1H, d, J = 8.0 Hz, H-5), 6.85 (1H, d, J = 8.0 Hz, H-6); ^{13}C NMR (Me_2CO, 400 MHz): δ 161.3 (C-2), 113.1 (C-3), 145.6 (C-4), 113.4 (C-4a), 119.8 (C-5), 112.4 (C-6), 150.1 (C-7), 132.8 (C-8), 144.4 (C-8a).

Compound 7: $C_{10}H_8O_4$, colorless needle (MeOH), m.p. 154-156 ℃; EI-MS: *m/z* (%)191.95 $[M]^+$ (1000), 177 $[M-CH_3]^-$ (770), 164 (21), 149 (30), 121 (22), 93 (13), 65 (49), 39 (20); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.26 (1H, d, J = 9.4 Hz, H-3), 7.65 (1H, d, J = 9.4 Hz, H-4), 7.13 (1H, d, J = 8.5 Hz, H-5), 6.91 (1H, d, J = 8.5 Hz, H-6), 4.12 (3H, s, H-OCH_3); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 160.4 (C-2), 112.6 (C-3), 144.3 (C-4), 113.2 (C-4a), 123.2 (C-5), 112.2 (C-6), 152.1 (C-7), 133.7 (C-8), 147.2 (C-8a); OCH_3: 61.7.

Compound 8: $C_9H_6O_3$, pale-yellow needle (Me_2CO), m.p. 230-232 ℃; EI-MS: *m/z* (% ÷ 10) 161.95 $[M]^+$ (1000), 134 (997), 105 (332), 78 (351); ^{1}H NMR (Me_2CO, 400 MHz): δ 6.14 (1H, d, J = 9.2 Hz, H-3), 7.85 (1H, d, J = 9.2 Hz, H-4), 7.48 (1H, d, J = 8.4 Hz, H-5), 6.82 (1H, dd, J = 8.4, 2.3 Hz, H-6), 6.73 (1H, d, J = 2.3 Hz, H-8); ^{13}C NMR (Me_2CO, 400 MHz): δ 161.4 (C-2), 112.6 (C-3), 144.8 (C-4), 112.6 (C-4a), 130.4 (C-5), 113.8 (C-6), 162.2 (C-7), 103.1 (C-8), 156.8 (C-8a).

Compound 9: $C_{29}H_{50}O$, colorless needle (Me_2CO), m.p. 138-140 ℃; EI-MS: *m/z* (%) 414.30 $[M]^+$ (26), 396 (10), 381 (5), 329 (9), 303 (7), 273 (6), 255 (11), 213 (10), 199 (5), 159 (11), 145 (18), 95 (28), 81 (40), 43 (100).

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