The Components of *Cacalia tangutica*

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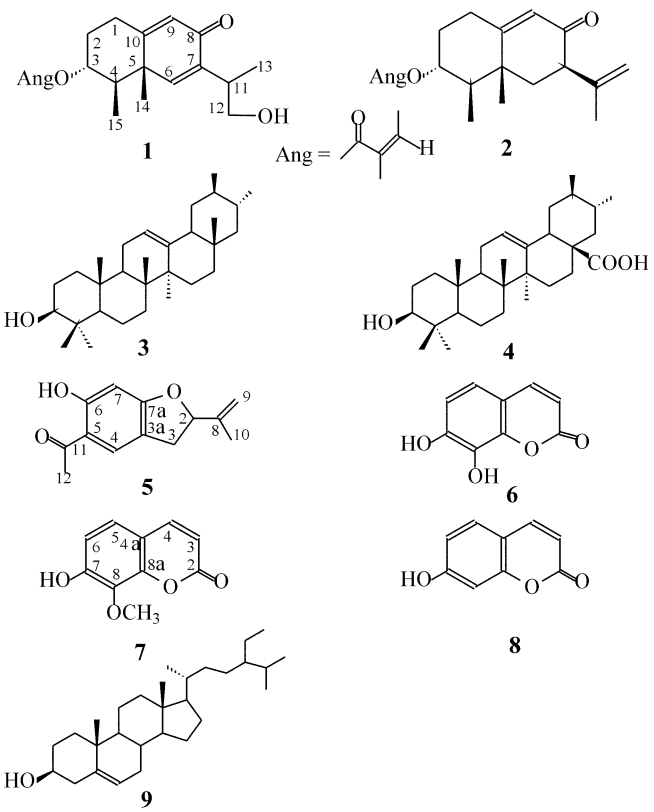
Received January 12, 2004

Key Words : Sesquiterpene, Eremophilane, *Cacalia tangutica*, Antitumor activity

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)$^\alpha$
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

$^\alpha$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-yollow 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).

Acknowledgment. We would like to thank Prof. Ji-zhou Sun of Department of Biology, Lanzhou University for the authentication of the plant.

References

1. Institute of Botany *Iconographia Cormophytorum Sinicorum*; Science Press: Beijing, 1973; p 795.
2. The China Medicinal Materials Co.; *Zhongguo Zhongyao Ziyuan Zhiyao*; Science Press: Beijing, 1994; p 1271.
3. Krasovskaya, N. P.; Kulesh, N. I.; Denisnko, V. A. *Khim. Prir. Soedin* **1989**, 643.
4. Sugama, K.; Hayashi, K.; Mitsuhashi, H. *Phytochemistry* **1985**, *24*, 1531.
5. Gonzales, U. J.; Bassabe, B. P.; Sanchez, M. I.; Fernandez, M. R.; Lopez, E. M.; Fernandez, R. A. *Phytochemistry* **1988**, *27*, 1507.
6. Pieters, L. A.; Vlietnick, A. J. *Planta Med.* **1988**, *54*, 178.
7. Khetwal, K. S.; Maural, K. *Planta Med.* **1988**, *54*, 188.
8. Bolmann, F.; Zdero, C.; Jakupovic, J.; Misra, L. N.; Benerjee, S.; Singh, P.; Baruah, R. N.; Metwally, M. A.; Schmeda-Hirschmann, G.; Vicent, L. P. D.; King, R. M.; Robinson, H. *Phytochemistry* **1985**, *24*, 1249.
9. Aclinou, P.; Benkouider, A.; Massiot, G.; Lemen-Olivier, L. *Phytochemistry* **1991**, *30*, 2083.
10. Zhang, S. M.; Zhao, G. L.; Li, R.; Lin, G. Q. *Phytochemistry* **1998**, *48*, 519.
11. Zhao, Y.; Peng, H.; Jia, Z. J. *J. Nat. Products* **1994**, *57*, 1626.
12. Zhao, Y.; Jia, Z. J.; Yang, L. *Planta Med.* **1994**, *60*, 91.
13. Takagi, I.; Gakuin, Y.; Naya, K. *Chem. Pharm. Bull.* **1977**, *50*, 3320.
14. Dupre, S.; Grenz, M.; Jakupovic, J.; Bohlmann, F.; Niemeyer, H. M. *Phytochemistry* **1991**, *30*, 1211.
15. Bohlmann, F.; Suwita, A.; Zdero, C. *Phytochemistry* **1978**, *17*, 1763.
16. Zdero, C.; Bohlmann, F.; Liddell, J. R. *Phytochemistry* **1989**, *28*, 3532.
17. Bohlmann, F.; Ates, N.; King, R. M.; Robinson, H. *Phytochemistry* **1983**, *22*, 1675.
18. Bohlmann, F.; Ehlers, D.; Zdero, C. *Phytochemistry* **1978**, *17*, 467.
19. Bohlmann, F.; Zdero, C. *Phytochemistry* **1978**, *17*, 1337.
20. Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517.
21. Bohlmann, F.; Grenz, M. *Chem. Ber.* **1970**, *103*, 90.
22. Gray, A. I.; Meegan, C. J.; O'Callaghan, N. B. *Phytochemistry* **1987**, *26*, 257.
23. Chatterjee, A.; Sarkar, S.; Shoolery, J. N. *Phytochemistry* **1980**, *19*, 2219.
24. Lee, S. K.; Nam, K. A.; Heo, Y. H. *Planta Med.* **2003**, *69*, 21.