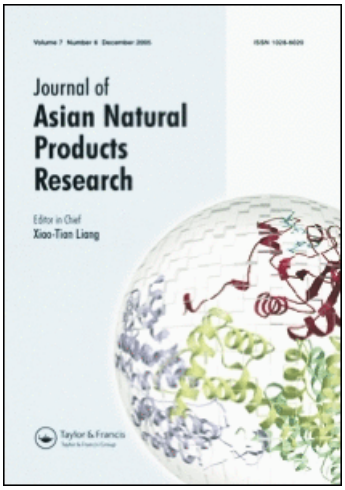

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

ent-Kaurenoids from the roots of *Cacalia pilgeriana*

E.-W. Li[a]; K. Gao[a]; Z.-J. Jia[a]
[a] State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, China

To cite this Article Li, E.-W. , Gao, K. and Jia, Z.-J.(2007) '*ent*-Kaurenoids from the roots of *Cacalia pilgeriana*', Journal of Asian Natural Products Research, 9: 3, 191 — 195
To link to this Article: DOI: 10.1080/10286020500530656
URL: http://dx.doi.org/10.1080/10286020500530656

Journal of Asian Natural Products Research, Vol. 9, No. 3, April–May 2007, 191–195

ent-Kaurenoids from the roots of *Cacalia pilgeriana*

E.-W. LI, K. GAO* and Z.-J. JIA*

State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China

(Received 11 August 2005; revised 25 November 2005; in final form 29 November 2005)

Two new *ent*-kaurenoids,19-acetoxyl-*ent*-3β,17-dihydroxykaur-15-ene (**1**), 19-acetoxyl-*ent*-3β-hydroxykaur-15-en-17-al (**2**), together with seven known *ent*-kaurenoids: *ent*-kaur-16-en-19-al (**3**), *ent*-kaur-16-en-19-oic acid (**4**), *ent*-kauran-16β,17-diol (**5**), *ent*-15β, 16β-epoxy-17-hydroxykauran-19-oic acid (**6**),19-acetyl-*ent*-3β-hydroxyl-kaur-16-ene (**7**), *ent*-3β,19-dihydroxykaur-16-ene (**8**), *ent*-17-hydroxy-kaur-15-ene (**9**), were isolated from *Cacalia pilgeriana*. Their structures were elucidated by spectroscopic methods including 2D NMR spectral analysis.

Keywords: *Cacalia pilgeriana*; Compositae; *ent*-Kaurenoid; 19-acetoxyl-*ent*-3β,17-dihydroxykaur-15-ene,19-acetoxyl-*ent*-3β-hydroxykaur-15-en-17-al

1. Introduction

The genus *Cacalia* (Compositae) is widespread in North-eastern areas of Asia and in America. There are about 50 species distributed in the North-western and South-western regions of China [1], of which about 26 species have long been used as traditional Chinese medicinal herbs for the treatment of mobilisation of blood circulation, and relieving coughs and phlegm [2,3]. *Cacalia pilgeriana* (Diels) Ling is a perennial herbage with antifungal activity [4]. The genus *Cacalia* was characterised by containing sesquiterpenes [5–7]; only Nasr reported a series of kaurenoid diterpenes from *Cacalia bulbifera* [8]. Wang *et al.* [9] previously reported some known compounds: a chromene derivative, an eudesmane sesquiterpene, several triterpenes and sterols from the aerial parts of *Cacalia pilgeriana*. In continuation of our studies on the Compositae to seek new structural and active compounds, we re-collected the roots of *Cacalia pilgeriana* in August 2002 and investigated the chemical constituents of the roots. Here we report the isolation and structure elucidation of two new *ent*-kaurenoids (**1**–**2**) and seven known *ent*-kaurenoids (**3**–**9**) from the roots of *Cacalia pilgeriana*.

2. Results and discussion

The MeOH extract of the roots of *Cacalia pilgeriana* was subjected to column chromatography over silica gel and fractioned further by repeated chromatography and

*Corresponding author. Email: npchem@lzu.edu.cn; jiazj@lzu.edu.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500530656

preparative TLC to yield two new *ent*-kaurenoids (**1**–**2**) and seven known *ent*-kaurenoids (**3**–**9**). The known compounds *ent*-kaur-16-en-19-al (**3**) [8], *ent*-kaur-16-en-19-oic acid (**4**) [8], *ent*-kauran-16β,17-diol (**5**) [10,11], *ent*-15β,16β-epoxy-17-hydroxykauran-19-oic acid (**6**) [12], 19-acetyl-*ent*-3β-hydroxykaur-16-ene (**7**) [13], *ent*-3β,19-dihydroxykaur-16-ene (**8**) [14], and *ent*-17-hydroxykaur-15-ene (**9**) [11] were identified by direct comparison of their spectral data (MS, ^{1}H NMR, ^{13}C NMR) with those reported in the literature.

Compound **1** was obtained as colourless crystals from acetone. The molecular formula $C_{22}H_{34}O_4$ was obtained based on the EI-MS at *m/z* 362 $[M]^+$, which was also confirmed by its HRESI-MS spectrum at *m/z* 345.2425 $[M-H_2O+H]^+$ and 327.2299 $[M-2H_2O+H]^+$. The ^{1}H NMR spectrum of **1** showed the signals for three tertiary methyls (δ 1.03, 1.13, s and 2.07, s, each 3H), two oxygenated methylenes (δ 4.12 and 4.33, d, each 1H, 11.2 Hz; δ 4.55, s, 2H), an oxygenated methine (δ 3.28, dd, 1H, 11.2, 5.2 Hz) and an olefinic methine (δ 5.48, s, 1H). Furthermore, the ^{13}C NMR and DEPT spectra showed 22 signals for $3\times CH_3$, $9\times CH_2$ (two of which were oxygenated), $5\times CH$ (one was oxygenated) and $5\times C$ (one was carbonyl). The information mentioned above suggested that **1** was a kaurane diterpene and the negative optical rotation of **1** indicated an enantiomer of kaurane, namely *ent*-kaurane [8,10–14]. The NMR data of **1** were similar to those of the known *ent*-3β,19-dihydroxykaur-16-ene and its diacetated derivative [14], the differences were only that the olefinic bond in **1** was located at C-15 (16), and a hydroxylmethyl group at C-17. This was confirmed by the correlations of H-15 with C-16 and C-17, H-17 with C-15 in the HMBC spectrum. The acetoxyl group located at C-19 which was deduced by the presence of the HMBC cross peak of H-19 with C═O. Furthermore, the correlations in the HMBC spectrum between H-18 with C-3 and C-19, and H-3 with C-19 were also observed. The chemical shift of H-3 (δ3.28, dd, 11.2, 5.2 Hz) showed the hydroxyl group was α-orientated [15]. Thus, compound **1** was determined as 19-acetoxyl-*ent*-3β,17-dihydroxykaur-15-ene.

Compound **2**, colourless gum, has the molecular formula $C_{22}H_{32}O_4$ deduced by its HRESI-MS spectrum at *m/z*361.2369 $[M+H]^+$. The ^{1}H NMR and ^{13}C NMR were very similar to those of **1** except for the presence of a —CHO in **2** (C-17: δ_H9.72, s, 1H; δ_C 189.4) instead of the $-CH_2OH$ in **1** (C-17: δ_H4.55, s, 2H; δ_C 75.0). The chemical shifts of H-13 and H-15 were downfield from δ_H 2.64, 5.48 to δ_H 3.04, 6.55 respectively due to the presence of aldehyde group. Therefore, compound **2** was 19-acetoxyl-*ent*-3β-hydroxykaur-15-en-17-al.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Kofler melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer 341 polarimeter. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), DEPT and 2D NMR spectra were recorded on a Bruker AM 400FT-NMR spectrometer using $CDCl_3$ as the solvent, TMS as internal standard. HRESI-MS and EI-MS data were obtained on a Bruker Daltonics APEX II 47e spectrometers and HP5988 instrument, respectively. Silica gel (200–300 mesh) used for CC and silica gel GF_{254} (10–40 μ) used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, P.R. China. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

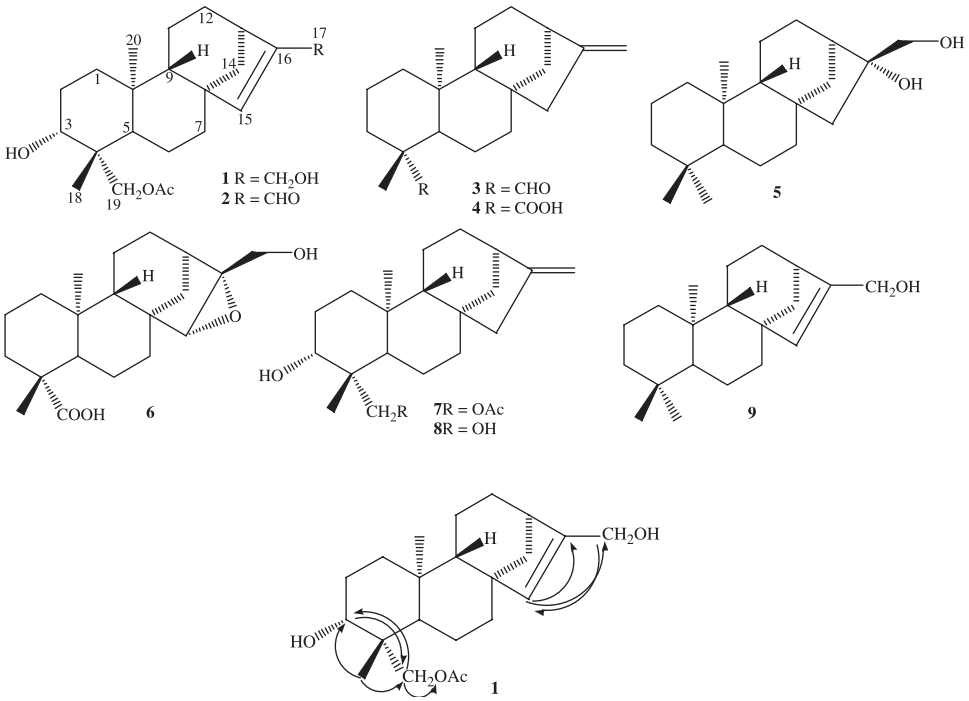

Figure 1. Key HMBC correlations for **1**.

3.2 Plant material

The roots of *Cacalia pilgeriana* were collected in Qinling Mountain, Shaanxi Province, China, in August 2002 and were identified by Professor Yao-Jia Zhang, School of Life Sciences, Lanzhou University. A voucher specimen (No. 20020801) is deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

3.3 Extraction and isolation

The air-dried roots of *Cacalia pilgeriana* (2.6 kg) were powdered and extracted with methanol (each time 8 L for 7 days) three times successively at room temperature. The combined methanol extracts were evaporated under reduced pressure to yield a residue (140 g), which was subjected to silica gel (200–300 mesh, 1200 g) CC eluting with petroleum ether (60–90°C)/EtOAc (10:1 10 L; 5:1 10 L; 1:1 10 L; 0:1 4 L). Four total fractions (A–D) were obtained from 85 fractions indicated by TLC. Fraction A (10:1) gave compounds **3** (300 ml, 45 mg) and **4** (200 ml, 30 mg) by repeated recrystallisation in EtOAc. Fraction B (2.0 g) was re-subjected to CC on silica gel eluting with petroleum ether/EtOAc (10:1 1000 ml; 5:1 1000 ml; 3:1 500 ml) to give 3 sub-fractions (Fr. B_1–Fr. B_3) combined on TLC analysis. Fraction B_1 (0.4 g) was further subjected to CC on silica gel eluting with petroleum ether/EtOAc (9:1, 800 ml) to give **1** (20 mg) and **7** (20 mg). Fraction B_2 (1.0 g) was further subjected to CC on silica gel eluting with petroleum ether/EtOAc (5:1, 1000 ml) to give **8** (50 mg), **5** (8 mg) and the crude **2** and **9**, the latter compounds were purified by PTLC (the same solution petroleum ether/EtOAc, 1:1, $R_f = 0.35$, 0.65, respectively) to

Table 1. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT data of **1** and **2**.

No.	*1*			*2*		
	δ_H	δ_C	*DEPT*	δ_H	δ_C	*DEPT*
1		39.1	CH_2		38.4	CH_2
2		25.3	CH_2		25.1	CH_2
3	3.28 (dd, 11.2, 5.2)	79.2	CH	3.27 (dd, 11.2, 6.0)	79.0	CH
4		42.3	C		42.2	C
5		55.5	CH		55.4	CH
6		19.4	CH_2		19.2	CH_2
7		43.4	CH_2		42.8	CH_2
8		48.9	C		46.8	C
9		48.1	CH		46.6	CH
10		39.0	C		38.8	C
11		18.8	CH_2		18.6	CH_2
12		27.2	CH_2		27.2	CH_2
13	2.64 (m)	41.5	CH	3.04 (m)	40.8	CH
14		38.8	CH_2		37.9	CH_2
15	5.48 (s)	140.4	CH	6.55 (s)	140.0	CH
16		141.3	C		148.8	C
17	4.55 (s)	75.0	CH_2	9.72 (s)	189.4	CH
18	1.13 (s)	22.4	CH_3	1.14 (s)	22.5	CH_3
19	4.12 (d, 11.2)	65.3	CH_2	4.11 (d, 11.7)	65.2	CH_2
	4.33 (d, 11.2)			4.32 (d, 11.7)		
20	1.03 (s)	17.8	CH_3	1.06 (s)	17.8	CH_3
		171.2	C		171.1	C
	2.07 (s)	21.1	CH_3	2.07 (s)	21.1	CH_3

Measured in $CDCl_3$; all values are in ppm, coupling constant in Hz.

afford **2** (1.5 mg) and **9** (30 mg). Fraction B_3 (0.2 g) was further subjected to CC on silica gel eluting with petroleum ether/EtOAc (3:1, 150 ml) to give **6** (4 mg).

3.3.1 19-Acetoxyl-*ent*-3β,17-dihydroxykaur-15-ene (1). Colourless crystals (acetone), mp 157–158°C; $[\alpha]_D^{23}$ − 55 (*c* 1.0, CH_3OH); EI-MS *m/z* (rel. int.): 362 $[M]^+$(1.3), 344 $[M-H_2O]^+$(1.0), 300 (5.3), 159 (6.3), 105 (25), 91 (42), 43 (100); HRESI-MS *m/z*: 345.2425 $[M-H_2O + H]$ (calcd for $[C_{22}H_{34}O_4-H_2O + H]^+$345.2424) and 327.2299 $[M-2H_2O + H]^+$ (calcd for $[C_{22}H_{34}O_4-2H_2O + H]^+$327.2319); ^{1}H NMR and ^{13}C NMR spectral data, see table 1.

3.3.2 19-Acetoxyl-*ent*-3β-hydroxykaur-15-en-17-al (2). Colourless gum, $[\alpha]_D^{23}$ − 82 (*c* 0.15, CH_3OH); EI-MS *m/z* (rel. int.): 360 $[M]^+$(1.0), 300 (7.6), 282 (3.8), 119 (41), 105 (19), 91 (36), 43 (100); HRESI-MS *m/z*: 361.2369 $[M + H]^+$ (calcd for $[C_{22}H_{32}O_4 + H]^+$361.2373); ^{1}H NMR and ^{13}C NMR spectral data, see table 1.

Acknowledgements

This work was supported by the NSFC (No.20372029 and No.20021001) and by the Key Project of Chinese Ministry of Education (No. 104178).

References

[1] K.C. How. *A Dictionary of the Families and Genera of Chinese Seed Plants*, 2nd ed., p. 75, Science Press, Beijing (1998).
[2] Chinese Crude Drugs Co., *Zhongguo Zhongyao Ziyuan Zhiyao*, p. 1271, Science Press, Beijing (1994).
[3] Z.W. Xue, Y.Q. Yu. *Index Flora of Chinese Medicinal Herbs*, p. 754, People's Health Press, Beijing (1996).
[4] H.J. Shao, J.T. Feng, J. Han, G.Z. Li, X. Zhang. *J. Northwest Sci. Tech. Univ. Agric. Forest.*, **31**, 59 (2003).
[5] S.M. Zhang, G.L. Zhao, R. Li, G.Q. Lin. *Phytochenistry*, **48**, 519 (1998).
[6] M.J. Mao, Z.J. Jia. *Planta Med.*, **68**, 55 (2002).
[7] M.J. Mao, Z.D. Yang, Z.J. Jia. *Planta Med.*, **69**, 745 (2003).
[8] A.E.E. Nasr, K. Genjiro, T. Tsunematsu. *Phytochenistry*, **14**, 1660 (1975).
[9] P.F. Wang, C. Yang, Z.J. Jia. *J. Lanzhou Univ.*, (Natural Sciences) **39**, 61 (2003).
[10] F. Bohlmann, J. Jakupovic, H. Robinson, R.M. King. *Phytochemistry*, **19**, 2769 (1980).
[11] J. Kitajima, N. Noda, Y. Ida, T. Komori, T. Kawasaki. *Chem. Pharm. Bull.*, **30**, 3922 (1982).
[12] W. Herz, P. Kulanthaivel, K. Watanabe. *Phytochemistry*, **22**, 2021 (1983).
[13] M. Toyota, E. Nakaishi, Y. Asakawa. *Phytochemistry*, **43**, 1057 (1996).
[14] F. Piozzi, G. Savona, J.R. Hanson. *Phytochenistry*, **19**, 1237 (1980).
[15] S.J. Croker, P. Gaskin, M.H. Beale, J.R. Lenton. *Phytochemistry*, **39**, 11 (1995).