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Sesquiterpene lactones from *Carpesium abrotanoides*

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ABSTRACT

Phytochemical study on the ethanol extract of the aerial parts of *Carpesium abrotanoides* led to the isolation of two new sesquiterpene lactones, carabrolactone A (1) and carabrolactone B (2). Their structures were elucidated on the basis of extensive spectroscopic analysis.

1. Introduction

Carpesium abrotanoides (Compositae) is a biennial herb and its aerial parts have been used in Chinese and Korean medicines as an insecticide and to treat bruises. The main chemical and bioactive constituents of this medicinal plant are diverse sesquiterpene lactones [1–6], and many of them exhibit antifungal, antibacterial [2,3], and cytotoxic activity [6]. One of our efforts to discover the structurally diverse and biologically significant metabolites from plant resources has led to the isolation of two new sesquiterpene lactones named carabrolactone A (1) and carabrolactone B (2), along with four known sesquiterpene lactones, 2-desoxy-4-*epi*-pulchellin (3), carabrone (4), 11(13)-dehydroivaxillin (5) and 4-*epi*-isoinuviscolide (6), from the aerial parts of *C. abrotanoides*. Herein, details of the isolation and structure elucidation of compounds 1 and 2 are described.

2. Experimental

2.1. General

Melting point was measured on a PHMK 79/2289 micro-melting point apparatus and uncorrected. Optical rotations were obtained on a Horiba SEPA-300 polarimeter. IR spectra were taken on a Bruker Tensor 27 FT–IR spectrometer with KBr pellets. NMR spectra were recorded with a Bruker DRX-500 instrument. EI–MS, ESI–MS and HR–ESI–MS were measured on Finnigan-MAT 90 and API QSTAR Pulsar i mass spectrometers, respectively. Silica gel 200–300 mesh (Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. MPLC was performed on a Büchi Sepacore System equipping pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortechnik AG, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Japan). Fractions were monitored by Agilent 1200 reversed-phase HPLC (Eclipse XDB-C18 column, 5 μm, 4.6×150 mm, 25%–100% MeOH in H_2O over 8 min followed by 100% MeOH to 11 min, 1 ml/min, 30 °C), in combination with TLC (Qingdao Marine Chemical Inc., China).

2.2. Plant material

The aerial parts of *C. abrotanoides* were collected at Tiger Leaping Gorge (alt. 1890 m), Yunnan Province, China and identified by Prof. Dr. Hua Peng. The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The dry aerial parts of *C. abrotanoides* (2.0 kg) were extracted with 95% ethanol at room temperature. The alcohol

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Table 1
NMR spectral data for compounds 1, 7 and 8 in $CDCl_3$

No.	1			7[b]	8[c]
	δ_C	δ_H	HMBC[a]	δ_C	δ_C
1	61.7 (d)	2.71 (dd, 11.1, 1.3, H_α)	C-3, C-9	65.1 (d)	64.9 (d)
2	31.9 (t)	1.56 (ddd, 13.6, 11.5, 11.1, H_β), 2.39 (ddd, 13.6, 5.6, 1.3, H_α)	C-4, C-10	24.0 (t)	24.0 (t)
3	76.1 (d)	3.47 (dd, 11.5, 5.6, H_α)	C-1, C-5, C-15	35.7 (t)	35.7 (t)
4	64.0 (s)	–	–	61.0 (s)	60.8 (s)
5	61.8 (d)	2.79 (br d, 9.4, H_α)	C-3, C-7	64.4 (d)	63.7 (d)
6	25.8 (t)	1.54 (m, H_β), 2.15 (br d, 15.4, H_α)	C-4, C-8, C-11	26.3 (t)	29.4 (t)
7	45.2 (d)	2.40 (m, H_α)	C-5, C-9, C-12, C-13	45.6 (d)	50.8 (d)
8	81.8 (d)	4.29 (ddd, 11.1, 9.0, 1.7, H_β)	C-6, C-10	82.0 (d)	81.8 (d)
9	45.2 (t)	1.43 (dd, 13.7, 11.1, H_α), 2.77 (dd, 13.7, 1.7, H_β)	C-1, C-7, C-14	45.1 (t)	45.0 (t)
10	57.6 (s)	–	–	57.7 (s)	57.0 (s)
11	40.4 (d)	2.84 (m, H_α)	C-8	40.6 (d)	42.6 (d)
12	177.8 (s)	–	–	177.9 (s)	175.9 (s)
13	11.6 (q)	1.25 (d, 7.7)	C-7, C-12	11.5 (q)	13.3 (q)
14	18.1 (q)	1.41 (s)	C-1, C-9	18.2 (q)	18.1 (q)
15	10.7 (q)	1.30 (s)	C-3, C-5	16.3 (q)	16.2 (q)

The assignments were unambiguously achieved by a combination of 1D- and 2D-NMR experiments. [a]Only three-bond correlations were listed. [b,c]The column data were cited from [7,5], respectively.

extract was concentrated to give a residue (80 g), which was subjected to silica gel column chromatography eluted with a solvent system of petroleum ether (PE)/acetone. The fraction (10 g) eluted by PE:acetone=2:1 was repeatedly subjected to silica gel ($CHCl_3$:MeOH=50:1) and Sephadex LH-20 ($CHCl_3$:MeOH=1:1) to obtain a target portion (2.2 g), which was further isolated and purified by MPLC (MeOH/H_2O) and Sephadex LH-20 ($CHCl_3$:MeOH=1:1) to afford compound 1 (32 mg, 30% MeOH for MPLC, 5.3 min in HPLC) and compound 2 (83 mg, 55% MeOH, 7.6 min).

Carabrolactone A (1), colorless crystal (MeOH), mp 170–172 °C; $[\alpha]^{17.8}_D$ −48.5° (*c* 0.44, $CHCl_3$); IR (KBr) cm^{-1}: 3440, 2975, 2939, 1770, 1638, 1465, 1391, 1216, 1045, 988, 947; ^1H and ^{13}C NMR data: see Table 1; EI–MS *m/z*: 283 $[M+H]^+$ (3), 264 $[M-H_2O]^+$ (0.3), 249 (2), 235 (1), 221 (5), 193 (10), 180 (66), 162 (33), 123 (49), 109 (59), 95 (92), 55 (100); ESI–MS (pos.): 305 $[M+Na]^+$; HR–ESI–MS (pos.): 305.1358 ($C_{15}H_{22}O_5Na$, calc. 305.1364).

Carabrolactone B (2), colorless oil; $[\alpha]^{18.6}_D$ −25.8 ° (*c* 0.40, $CHCl_3$); UV λ_{max} (MeOH): 214 nm; IR (KBr) cm^{-1}: 3439, 2962, 2921, 2876, 1766, 1640, 1461, 1379, 1263, 1111, 1085, 1050, 1013; ^1H and ^{13}C NMR data: see Table 2; EI–MS *m/z*: 248 $[M-H_2O]^+$ (62), 233 (25), 230 (47), 215 (33), 204 (100), 189 (55), 175 (45), 162 (49), 145 (48), 133 (58), 119 (42), 105 (57); ESI–MS (pos.): 289 $[M+Na]^+$; HR–ESI–MS (pos.): 289.1408 ($C_{15}H_{22}O_4Na$, calc. 289.1415).

3. Results and discussion

Compound 1, obtained as a structurally unstable colorless crystal, has a molecular formula of $C_{15}H_{22}O_5$ based on HR–ESI–MS (pos.), showing a quasi-molecular ion peak at *m/z* 305.1358 ($C_{15}H_{22}O_5Na$, calc. 305.1364). The IR spectrum showed absorption bands of hydroxyl (3440 cm^{-1}) and γ-lactone carbonyl (1770 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed the following clear signals: two oxygenated methine protons at δ 4.29 (ddd, *J*=11.1, 9.0, 1.7 Hz) and 3.47 (dd, *J*=11.5, 5.6 Hz), and three methyl resonances at δ 1.41 (s), 1.30 (s) and 1.25 (d, *J*=7.7 Hz). The ^{13}C NMR (DEPT) spectrum (Table 1) exhibited 15 carbon signals including a γ-lactone carbonyl resonance at δ

Table 2
NMR spectral data for compounds 2 and 3 in $CDCl_3$

No.	2			3
	δ_C	δ_H	HMBC[a]	δ_C
1	44.2 (d)	1.69 (m, H_α)	C-9, C-15	45.0 (d)
2	25.6 (t)	1.42 (m), 1.85 (m)	C-4, C-5	25.4 (t)
3	27.0 (t)	1.39 (m), 1.93 (m)	C-1, C-5	28.5 (t)
4	76.5 (d)	4.37 (dd, 10.3 ,8.3, H_α)	C-6, C-15	83.6 (d)
5	50.0 (s)	–	–	44.4 (s)
6	77.3 (d)	3.78 (d, 9.8, H_β)	C-1, C-11, C-15	37.9 (t)
7	51.9 (d)	2.84 (dddd, 9.8, 9.3, 3.4, 2.9, H_α)	C-9, C-13	47.6 (d)
8	77.0 (d)	4.30 (ddd, 11.7, 9.3, 2.9, H_β)	C-6	81.7 (d)
9	44.1 (t)	1.37 (m, H_α), 2.33 (ddd, 13.2, 4.4, 2.9, H_β)	C-1, C-7, C-14	44.2 (t)
10	30.9 (d)	1.65 (m, H_β)	–	30.1 (s)
11	139.3 (s)	–	–	140.8 (s)
12	170.8 (s)	–	–	170.2 (s)
13	123.0 (t)	6.16 (dd, 2.9, 1.0), 6.23 (dd, 3.4, 1.0)	C-7, C-12	119.5 (t)
14	20.5 (q)	0.94 (d, 6.4)	C-1, C-9	20.4 (q)
15	16.1 (q)	0.91 (s)	C-1, C-4, C-6	17.3 (q)

The assignments were unambiguously achieved by a combination of 1D- and 2D-NMR experiments. [a]Only three-bond correlations were listed.

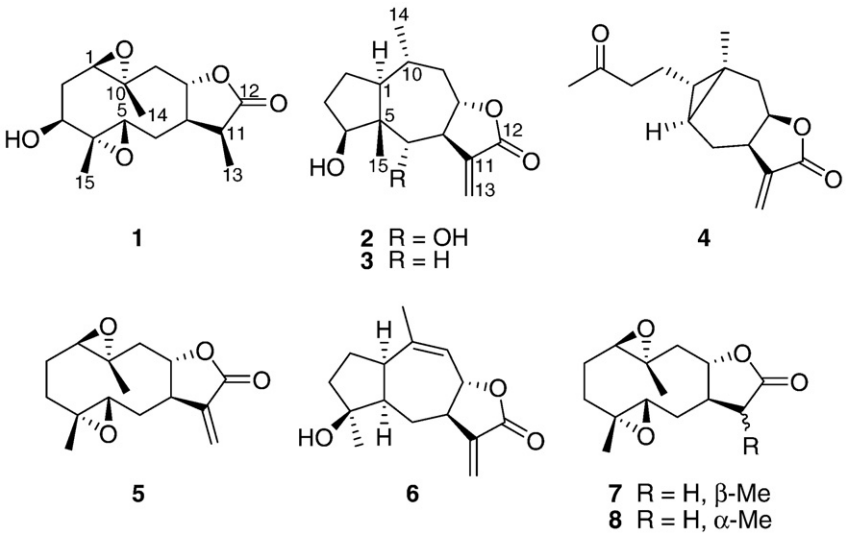

Fig. 1. Structures of compounds 1–8.

177.8 (s), two oxygen-bearing methine carbons at δ 81.8 (d) and 76.1 (d), and a set of signals at δ 57.6 (s), 61.7 (d), 61.8 (d), 64.0 (s) attributable to two oxirane functions. The above NMR character allowed us to conclude that compound 1 possessed a skeleton of sesquiterpene lactone containing only one alicyclic ring.

The ^{13}C NMR data of 1 were similar to those of ivaxillin (7) [7] (Table 1), previously isolated diepoxygermacranolide from *Iva axillaris*. Nevertheless there was an obvious difference: an up-field methylene resonance in 7 was absent and replaced by a newly arisen signal at δ 76.1 (d), indicating that the methylene group was necessarily substituted by a hydroxyl in 1. By analysis of the HMBC spectrum (Table 1), the position of the hydroxyl group was determined at C-3, in which the correlations from δ_H 3.47 (dd, J=11.5, 5.6 Hz, H-3) to δ_C 61.7 (d, C-1), 61.8 (d, C-5) and 10.7 (q, C-15) were observed. The correlation peaks in the ROESY spectrum (Fig. 2) between H-3 and H-1α, H-3 and H-5α, were clearly detectable, indicative of β orientation of the hydroxyl group. The configurational deduction was also supported by a typical γ-gauche effect: the ^{13}C NMR signal due to Me-15 was up-field shifted $\Delta\delta$ 5.6 ppm compared with that of 7. The stereochemistry of the methyl at the lactone ring was assigned to be β by comparison of the ^{13}C NMR data with those of C-11 epimers 7 [7] and 8 [5] (Table 1). Therefore, the structure of 1 was elucidated as shown in Fig. 1, named carabrolactone A.

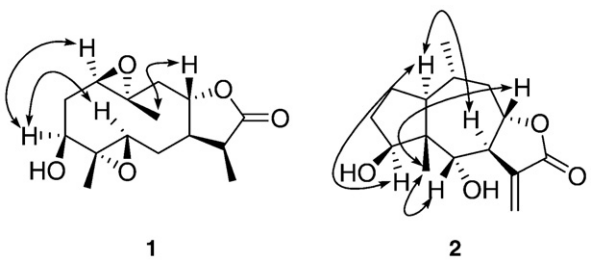

Fig. 2. Significant ROESY correlations of compounds 1 and 2.

Compound 2 was obtained as colorless oil, possessing a molecular formula $C_{15}H_{22}O_4$ based on HR–ESI–MS (pos.), showing a quasi-molecular ion peak at m/z 289.1408 ($C_{15}H_{22}O_4Na$, calc. 289.1415). The IR spectrum showed absorption bands of hydroxyl (3439 cm^{-1}), γ-lactone carbonyl (1766 cm^{-1}) and double bond (1640 cm^{-1}) groups. The ^{13}C NMR (DEPT) spectrum (Table 2) also exhibited 15 carbon signals, including an unsaturated γ-lactone carbonyl resonance at δ 170.8 (s), a set of signals at δ 139.3 (s), 123.0 (t) attributable to a terminal olefin, three oxygen-bearing methine carbons at δ 77.3 (d), 77.0 (d) and 76.5 (d), and two methyls at δ 20.5 (q), 16.1 (q), which suggested a skeleton of sesquiterpene lactone containing two alicyclic rings.

The ^{13}C NMR data of 2 were similar to those of 2-desoxy-4-*epi*-pulchellin (3) [8] (Table 2) isolated as a major sesquiterpene lactone from this plant. However, there was a set of newly arisen oxygenated methine signals at "δ_C 77.3 (d), δ_H 3.78 (d, J=9.8 Hz)" in the NMR spectra of 2, indicative of hydroxylation on a methylene in 3. The presence of the HMBC correlations (Table 2) from the proton δ_H 3.78 (d, J=9.8 Hz) to δ_C 44.2 (d, C-1), 139.3 (s, C-11) and 16.1 (q, C-15), allowed to determine the substituted position of the hydroxyl at C-6. The evident ROESY correlation (Fig. 2) between H-6 and Me-15, and a large coupling constant ^3J=9.8 Hz of H-6 were observed, indicative of α orientation of the hydroxyl group. Consequently, the structure of 2 was determined as 4β,6α-dihydroxy-11(13)-pseudoguaien-12,8-olide, named carabrolactone B.

Comparison of the spectroscopic and physical data with those published allowed us to establish the structures of known sesquiterpenes 3–6 as 2-desoxy-4-*epi*-pulchellin [8], carabrone [3], 11(13)-dehydroivaxillin [4] and 4-*epi*-isoinuviscolide [9], respectively. Among them, compounds 3 and 6 were reported for the first time from this plant.

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References

[1] Minato H, Nosaka S, Horibe I. J Chem Soc 1964:5503.
[2] Maruyama M, Shibata F. Phytochemistry 1975;14:2247.
[3] Maruyama M, Omura S. Phytochemistry 1977;16:782.
[4] Maruyama M, Karube A, Sato K. Phytochemistry 1983;22:2773.
[5] Dong YF, Ding YM. Acta Bot Sin 1988;30:71.
[6] Lee JS, Min BS, Lee SM, Na MK, Kwon BM, Lee CO, Kim YH, Bae KH. Planta Med 2002;68:745.
[7] Herz W, Prasad JS, Blount JF. J Org Chem 1982;47:3991.
[8] Zdero C, Bohlmann F. Phytochemistry 1989;28:1653.
[9] Bohlmann F, Mahanta PK, Jakupovic J, Rastogi RC, Natu AA. Phytochemistry 1978;17:1165.