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A new germacranolide from *Carpesium cernuum*

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Abstract: As a part of our interest in biologically active germacranolides from the genus *Carpesium* (Compositae), we have investigated the constituents of *Carpesium cernuum*. This paper reports the isolation and structural elucidation of a new germacranolide, cernolide A (Compound 1), from the herb. The structure of Compound 1 was determined as 2α,3β-dihydroxy-9-angeloxygermacra-4-en-6,12-olide on the basis of spectral evidence. The skeleton of Compound 1 was elucidation by IR, MS, ^{1}H and ^{13}C NMR, COSY, HMQC and HMBC experiments. The stereochemistry of Compound 1 was deduced by ROESY spectral data. Finally, the procedures of extraction and isolation were described in detail.

Key words: *Carpesium cernuum*, Compositae, Sesquiterpenoid, 2α,3β-dihydroxy-9-angeloxygermacra-4-en-6,12-olide
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INTRODUCTION

The genus *Carpesium* (Compositae) had been reported as a rich source of antifungal and antibacterial sesquiterpene lactones (Maruyama and Omura, 1977; Maruyama, 1990; Masao *et al*., 1983; Dong and Ding, 1988; Lin and Ou, 1996). Further research revealed that germacranolide type of compounds from the genus had cytotoxicity to human tumor cells (Kim *et al*., 1997). *Carpesium cernuum*, as a type species of the genus, is distributed all over China. But up to now, only 6 sesquiterpenoids were isolated from this plant (Yang *et al*., 2002). As a part of our interest in biologically active compounds from natural sources, we have investigated the constituents of *C. cernuum*. This paper reports the isolation and structural elucidation of a new germacranolide, cernolide A, from the herb.

Compound 1, colorless viscous, showed a molecular ion peak as *m/z* 363 $[M-H]^-$ in the negative FAB mass spectrum. In combination with ^{1}H and ^{13}C NMR (Table 1), its molecular formula was deduced to be $C_{20}H_{28}O_6$ (ESI-MS *m/z*: 364.1878 $[M]^+$, calcd. 364.1885). The IR spectrum presented hydroxy groups (3500 cm^{-1}), α,β-unsaturated-γ-lactone (1770 cm^{-1}), ester carbonyl (1700 cm^{-1}) and unsaturation (1650 cm^{-1}). The ^{1}H and ^{13}C NMR spectra showed the presence of four methyls (δ_C 12.5, 16.1, 20.8, 21.4), three methylenes (δ_C 29.6, 33.6, 119.2), eight methines (δ_C 30.1, 46.4, 75.6, 81.0, 82.1, 83.1, 126.8, 139.3), and five quaternary carbons (δ_C 129.2, 142.1, 143.6, 169.1, and 172.1). Comparison of the ^{1}H and ^{13}C NMR spectral data of 1 with that of nepalolide C, revealed that they were similar (Lin and Ou, 1996). The ^{1}H NMR spectral data at [δ_H 6.29 (1H, q, J=7.2 Hz), 1.97 (3H, d, J=7.0), and 1.88 (3H, s)] suggested existence of an angeloyl group. The germacranolide skeleton (Fig.1) deduced by the correlations between δ_H 1.44 (H-1) with δ_H 2.22 (H-10), δ_H 3.97 (H-2) with δ_H 4.99 (H-3), δ_H 4.87 (H-6) with δ_H 5.59 (H-5) and δ_H 2.63 (H-7), δ_H 1.57 (H-8) with δ_H 2.63 and δ_H 4.75 (H-9), δ_H 2.22 (H-10) with δ_H 1.17 (H-14) in ^{1}H-^{1}H COSY spectrum. And the inference was supported by the cross peaks between δ_H 1.17 (H-14) with δ_C 30.1

Table 1 ^{13}C and ^{1}H NMR spectral data for Compound 1 (500 Hz, in $CDCl_3$)

Position	C	H
1	33.6 (t)	1.44 (m) α; 1.73 (m) β
2	75.6 (d)	3.97 (m)
3	83.1 (d)	4.99 (d, J=7.4)
4	143.6 (s)	
5	126.8 (d)	5.59 (d, J=10.1)
6	82.1 (d)	4.87 (m)
7	46.4 (d)	2.63 (m)
8	29.6 (t)	2.06 (m) α; 1.57 (m) β
9	81.0 (d)	4.75 (m)
10	30.1 (d)	2.22 (m)
11	142.1 (s)	
12	172.1 (s)	
13	119.2 (t)	5.64 (brs), 6.75 (brs)
14	21.4 (q)	1.17 (d, J=6.8)
15	12.5 (q)	1.87 (s)
1′	169.1 (s)	
2′	129.2 (s)	
3′	139.3 (d)	6.29 (q, J=7.2)
4′	16.1 (q)	1.97 (d, J=7.0)
5′	20.8 (q)	1.88 (s)

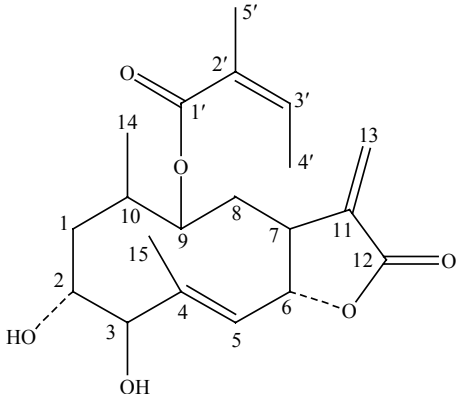

Fig.1 The structure of Compound 1

(C-10), 33.6 (C-1), 81.0 (C-9), and δ_H 1.87 (H-15) with δ_C 143.6 (C-4), 126.8 (C-5), 83.1 (C-3) in HMBC spectrum. The relative stereochemistry of 1 was assigned from the ROESY correlations shown in Fig.2 and supported by the coupling constants in the ^{1}H NMR spectrum (Table 1). The ROESY interaction between H-3 with H-10 showed that 3-OH had β configuration. And the coupling constant of H-3 (J=7.4) showed that H-2 was at the opposite side of H-3. Therefore, Compound 1 was elucidated as 2α, 3β-dihydroxy-9-angeloxygermacra-4-en-6,12-olide and named cernolide A.

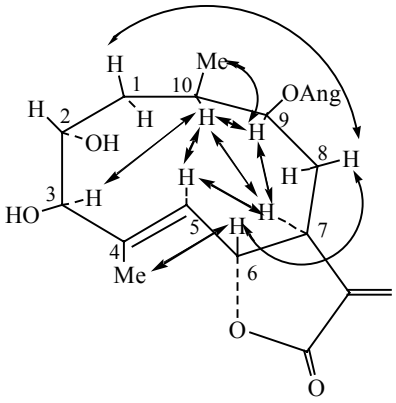

Fig.2 Correlation observed in the ROESY spectrum of Compound 1

EXPERIMENTAL DETAILS

General experimental procedures

Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectropolarimeter. ^{1}H, ^{13}C NMR and 2D NMR spectra were recorded on a DRX-500 MHz NMR spectrometer with TMS as internal standard. MS spectral data were obtained on a VG Autospec-3000 spectrometer. Si gel (200~300 mesh) for column chromatography and GF_{254} for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, China. Macroporous resin D1300 was obtained from the Bengbu Liaoyuan Resin Factory, Bengbu, China.

Plant material

The *C. cernuum* herb collected from Kunming, Yunnan Province, China, in August 2001, and was identified by Professor Li, H., Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, China.

Extraction and isolation

The dried *C. cernuum* herb (10.7 kg) was ex-

tracted three times with EtOH under reflux. After removal of the solvent in vacuo, the residue was partitioned in H_2O and extracted with petroleum ether, CH_3Cl, and *n*-BuOH three times respectively. The CH_3Cl fraction (173 g) was chromatographed over silica gel using $CHCl_3$-MeOH (from 10:0 to 7:3) as eluent to give 33 fractions. The fraction 6~10 (59.5 g) was chromatographed over silica gel using petroleum ether-acetone (from 8:1 to 2:1) as eluent to give 63 fractions. The fraction 10~11 (17 g) was chromatographed over macroporous resin D1300 and eluted with EtOH-H_2O (0:100~95:5) to give 4 fractions. Fraction 3 (0.7 g) was chromatographed over silica gel using $CHCl_3$-MeOH, $CHCl_3$-acetone and petroleum ether-acetone, respectively, as eluent to give Compound 1 (15 mg).

Compound 1 (2α,3β-dihydroxy-9-angeloxygermacra-4-en-6,12-olide)

Colorless viscous; $[\alpha]_D^{23.7}$-23.0 (*c* 0.15, $CDCl_3$), IR (KBr) ν_{max}: 3500, 1770, 1700, 1650, 1225, 1150, 1075, 1050, 975, 750 cm^{-1}; ^1H NMR and ^{13}C NMR spectral data, see Table 1; negative-ion FABMS *m/z*: 363 $(M-H)^-$ (20), 517 $(M-H+MNBA)^-$ (100); ESI-MS *m/z*: 364.1878 $[M]^+$ (Calcd for $C_{20}H_{28}O_6$ 364.1885, error: 1.9×10^{-6}).

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