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Eudesmanolides, aromatic derivatives, and other constituents from *Carpesium cernuum*

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A new eudesmanolide 13-hydroxy-4αH-eudesman-5,7(11)-dien-12,8β-olide (**1**) and a new aromatic derivative 3-methyl-8-acetoxy-9,10-diisobutanoyloxy-*p*-cymene (**6**), along with ten known compounds were isolated from the roots of *Carpesium cernuum* L. Their structures were elucidated by spectral methods (IR, EIMS, FAB-MS, 1D and 2D NMR). Compound **2**, **3** and compound **10** exhibited moderate antibacterial activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus.*

1. Introduction

The genus *Carpesium* (Compositae) consists of about 21 species distributed throughout the world, 17 of them growing in China. *Carpesium* species have shown antifungal and antibacterial activity [1]. Sesquiterpene lactones are the most widespread secondary metabolites in the genus. *Carpesium cernuum* L. has long been used as Chinese folk medicine for anti-inflammatory, pain-relief, and detoxication properties [2, 3]. However, up to now, chemical studies of this plant have not been reported. In this paper, we describe the isolation and structural elucidation of the chemical constituents from the roots of *Carpesium cernuum*, and the antibacterial activities of ten compounds (**1–10**).

2. Investigations, results and discussion

From the methanol extracts of the roots of *Carpesium cernuum* L., a new eudesmanolide 13-hydroxy-4αH-eudesman-5,7(11)-dien-12,8β-olide (**1**) and a new aromatic derivative 3-methyl-8-acetoxy-9,10-diisobutanoyloxy-*p*-cymene (**6**) were isolated, together with four known eudesmanolides: 4αH-eudesman-5,11(13)-dien-12,8β-olide (**2**) [4], 11αH-eudesman-5-en-12,8β-olide (**3**) [4], eudesman-4,11(13)-dien-12,8β-olide (**4**) [5], 5αH-eudesman-4(15),11(13)-dien-12,8β-olide (**5**) [5], four known *p*-cymene derivatives: 3-methoxy-8-hydroxy-9,10-diisobutanoyloxy-*p*-cymene (**7**) [6], 3-methoxy-8-hydroxy-9-acetoxy-10-isobutanoyloxy-*p*-cymene (**8**) [6], 3,8-dihydroxy-9,10-diisobutanoyloxy-*p*-cymene (**9**) [7], 8,9-epoxy-3,10-diisobutanoyloxy-*p*-cymene (**10**) [7]; β-sitosterol (**11**) and β-sitosteryl-β-D-glucopyranoside (**12**). The structures of the known compounds were identified either by comparing their corresponding properties (m.p. MS, IR, ^{1}H NMR and ^{13}C NMR) with the reported values in the literature (**2–10**) or by comparing with authentic samples (**11–12**). Compounds (**1–5**) are eudesmaolides, which are characteristic for the genus. Compounds (**6–10**) are cymene derivatives and, to our knowledge, in the genus Carpesium, this kind of compounds had also been isolated from *Carpesium lipskyi* Winkl [6].

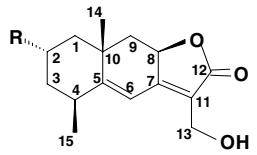
Compound **1** was obtained as colorless gum, with molecular formula $C_{15}H_{20}O_3$ deduced from EI-MS (248 $[M]^+$), ^{13}C NMR and DEPT data. Its IR spectra showed the presence of a carbonyl group (1743 cm^{-1}: C=CCO_2R), a hydroxyl group (3392 cm^{-1}) and double bond (1646 cm^{-1}). In the ^{1}H NMR and ^{13}C NMR spectra of **1**, there are one tertiary and one secondary methyl [$\delta_H = 1.32$ (s) and 1.29 (d, $J = 7.5$ Hz)], an α-en-γ-lactone moiety [$\delta_C = 174.4$ (C-12); $\delta_C = 158.8$ (C-7) and 118.4 (C-11)], one > C=CH− group [$\delta_H = 6.36$ (1 H, s); $\delta_C = 112.7$ (C-6) and 163.5 (C-5)], and a $-CH_2OH$ unit [$\delta_H = 4.44$ (2 H, s); $\delta_C = 55.4$ (C-13)]. Together with long-range (^{3}J) coupling cross peaks (Table 1): between C-8/H-6; C-10/H-4,

Table 1: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), DEPT and HMBC data of 1 ($CDCl_3$, TMS, δ, ppm)

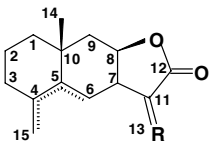
No.	^{1}H (α/β) **1**	^{1}H (α/β) **1a**	^{13}C **1**	DEPT **1**	HMBC **1**
1α	1.60 (m)/	1.59 (dd, 12.5, 10)/	39.7	CH_2	C-1/H-9, H-14
1β	1.68 (br.dd, 13.0, 4.0)	1.95 (dd, 12.5, 4.5)			
2	1.56* (m)/1.95 (m)	−/4.32 (m)	29.5	CH_2	C-2/H-4
3	1.56* (m)/1.74 (m)	1.53 (m)/2.05 (m)	34.0	CH_2	C-3/H-15
4α	2.78 (ddq, 7.5, 6.5, 2.0)	2.89 (ddq, 7.5, 6.5, 2.0)	40.6	CH	C-4/H-6
5	−	−	163.5	C	C-5/H-9, H-14, H-15
6	6.36 (s)	6.41 (s)	112.7	CH	−
7	−	−	158.8	C	C-7/H-9, H-13
8α	4.80 (dd, 13.0, 6.0)	4.80 (dd, 12.5, 5.5)	76.4	CH	C-8/H-6
9α	1.54 (t, 13.0, 13.0)/	1.51 (dd, 13.0, 12.5)/	43.2	CH_2	C-9/H-1, H-14
9β	2.18 (dd, 13.0, 6.0)	2.24 (dd, 13.0, 5.5)			
10	−	−	38.6	C	C-10/H-4, H-6
11	−	−	118.4	C	C-11/H-6
12	−	−	174.4	C	C-12/H-13
13	4.44 (br.s)	4.43 (br.s)	55.4	CH_2	−
14	1.32 (s)	1.31 (s)	18.0	CH_3	−
15	1.29 (d, 7.5)	1.29 (d, 7.5)	20.6	CH_3	−

Signal multiplicity and coupling constants (Hz) are in parentheses
* Overlapping signals

H-6; C-12/H-13 etc, in HMBC experiment, the structural elucidation of compound **1** was achieved. The stereochemistry of compound **1** was determined to be identical to that of the known compound **1a** [8] on the basis of the similar chemical shifts and coupling constants of H-4, 8, 9, 14, 15 (Table 1) observed in the ^{1}H NMR spectrum. Furthermore, the α-configuration of H-4 and H-8, and the β-orientations of 14-CH_3 and 15-CH_3 were also confirmed through the correlation between 9β-H/14-CH_3, H-6/H-4, and 14-CH_3/15-CH_3 in the ^{1}H-^{1}H NOESY experiment of **1**. Hence, the structure of 13-hydroxy-4αH-eudesman-5,7(11)-dien-12,8β-olide was suggested for compound **1**.



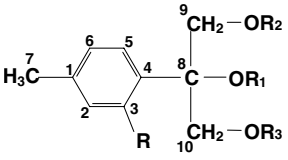
1 R = H
1a R = OH



	2	**3**	**4**	**5**
	Δ5	**Δ**5	**Δ**4	**Δ**4 (15)
R	CH_2	βCH_3	CH_2	CH_2
		αH		

Compound **6** was obtained as colorless crystals from acetone, m.p. 92–94 °C, and was assigned to the molecular formula $C_{21}H_{30}O_6$ by FAB-MS analysis (quasi-molecular ion peak: 385 $[M + Li]^+$, 401 $[M + Na]^+$, 379 $[M + 1]^+$), ^{13}C NMR and DEPT data. It consists of a 1,3,4-trisubstitued benzene moiety [$\delta_H = 7.29$ (1 H, d, J = 8 Hz), 7.08 (1 H, dd, J = 8, 2 Hz), and 6.90 (1 H, d, J = 2 Hz)], two methyls [$\delta_H = 2.38$ (3 H, s) and 2.35 (3 H, s)]; as well as a glyceryl-2-acetate-1,3-diisobutyrate (which was deduced on the signals of a glyceryl, an acetyl and double overlapped isobutanoyl in ^{1}H- and ^{13}C NMR spectra (Table 2), along with the cross peaks between carbonyl of isobutanoyl and H-9a, 9b, H-10a, 10b in HMBC experiments). Furthermore, the relative location of the three substitutions was established through the cross peaks (J^3) in HMBC experiments, such as C-1/H-5 and C-7/H-2, H-6 due to Me-7 at C-1; C-3/H-5 and Me-11 /H-2 due to Me-11 at C-3; C-8/H-5 and C-4/ H-2, H-6, H-9, H-10 due to the glyceryl-2-acetate-1,3-diisobutyrate at C-4. Therefore, the structure of compound **6** was established as 3-methyl-8-acetoxy-9,10-diisobutanoyloxy-*p*-cymene. Its ^{1}H- and ^{13}C NMR spectral chemical shifts were assigned by the HMQC and HMBC experiments.

Compounds 1–10 were screened for antibacterial activity. The results are given in Table 3.



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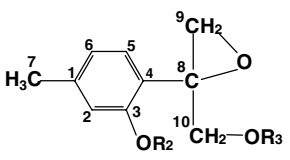

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Compound	**R**	**R_1**	**R_2**	**R_3**
6	Me-11	acetyl	isobutanoyl	isobutanoyl
7	OCH_3	H	acetyl	isobutanoyl
8	OCH_3	H	isobutanoyl	isobutanoyl
9	OH	H	isobutanoyl	isobutanoyl
10	–	–	isobutanoyl	isobutanoyl

Table 3: Antibacterial activity

Compound	*B. subtilis*	*S. aureus*	*E. coli*
1	–	+	+
2	++	–	+
3	+	+	++
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	+
10	+	++	+
Chloromycetin	+++	+++	+++

"–": Zone diameter of growth inhibition less than 10 mm, "+" equal to 11–13 mm, "++" equal to 14–15 mm, "+++" more than 15 mm
Concentration: 100 μg/ml, each cup 0.2 ml

3. Experimental

3.1. Apparatus

M.p.s.: Kofler Melting point apparatus, uncorr. Optical rotation: JASCO-20C automatic recording spectro-polarimeter, solvent $CHCl_3$. IR spectra were measured on a Nicolet 170SX FT-IR instrument. ^{1}H NMR (400.13 Hz), ^{13}C NMR (100.62 Hz) and 2D NMR spectra were record on a Bruker AM-400 FT-NMR spectrometer in $CDCl_3$ with TMS as int. standard. EI-MS was recorded on a HP 5988A GC/MS instrument and FAB-MS on a VG ZAB-HS mass spectrometer. Silica gel (200–300 mesh) was used for column chromatography, and silica gel GF_{254} for TLC were supplied by the Qingdao Marine Chemical Factory in China. Spots were detected on the TLC under UV light or by heating after spraying with 5% H_2SO_4.

3.2. Plant material

Carpesium cernuum was collected in Zhang county, Gansu province, P. R. China, in October, 1997 and identified by Prof. Guoliang Zhang, Department of Biology, Lanzhou University. A voucher specimen (NO.9701) was deposited in the Institute of Organic Chemistry, Lanzhou University.

Table 2: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT data of 6 ($CDCl_3$, TMS, δ, ppm)

No.	^{1}H	^{13}C	DEPT	No.	^{1}H	^{13}C	DEPT
1	–	140.0	C	isobutanoyl	–	176.0	C
2	6.90 (d, 2.0)	124.8	CH		2.55 (m)	33.9	CH
3	–	147.8	C		1.12 (d, 7.2)	18.8	CH_3
4	–	125.8	C		1.12 (d, 7.2)	18.8	CH_3
5	7.29 (d, 8.0)	127.6	CH	isobutanoyl	–	176.0	C
6	7.08 (dd, 8.0, 2.0)	126.9	CH		2.55 (m)	33.9	CH
7	2.35 (s)	21.2	CH_3		1.12 (d, 7.2)	18.8	CH_3
8	–	80.8	C		1.12 (d, 7.2)	18.8	CH_3
9	4.87 (d, 11.4)	62.7	CH_2	acetyl	–	169.3	C
	4.75 (d, 11.4)						
10	4.87 (d, 11.4)	62.7	CH_2		1.99 (s)	21.2	CH_3
	4.75 (d, 11.4)						
Me-11	2.38 (s)	20.8	CH_3				

Signal multiplicity and coupling constants (Hz) are in parentheses
Assignments from ^{1}H-^{1}H COSY and HMQC experiments

3.3. Extraction and isolation

The air-dried roots of *Carpesium cernuum* (480 g) were pulverized and extracted with MeOH at room temp. (7 days × 5 times). The combined extracts were evaporated giving a residue (37 g) which was chromatographed on a silica gel column (4.2 × 64 cm, 340 g) with petroleum ether (60–90 °C)-EtOAc gradient to give fractions 1–8: eluents of 20 : 1 (1600 ml), 15 : 1 (700 ml) were combined to be Fr. 1; eluents of 15 : 1 (2000 ml), 10 : 1 (1600 ml) to be Fr. 2; 8 : 1 (1800 ml), 6 : 1 (600 ml) to be Fr. 3; eluent of 6 : 1 (2600 ml) was Fr. 4; 4 : 1 (2000 ml) was Fr. 5; 2 : 1 (2200 ml) was Fr. 6; 1 : 1 (2200 ml) was Fr. 7; 0 : 1 (2200 ml) was Fr. 8, with acetone as eluent giving Fr. 9, and with MeOH giving Fr. 10. Compound **2** (68 mg) obtained from Fr. 2 by recrystallization in petroleum ether-EtOAc. The other part of Fr. 2 (1.6 g) was subjected to CC on silica gel (2.0 × 26 cm, 30 g) to afford four subfractions (Sfr. 1–Sfr. 4) with $CHCl_3$−EtOAc (100 : 1–10 : 1): Sfr. 1 (100 : 1) yielded **4** (12 mg, Rf = 0.45), **5** (7 mg, Rf = 0.32) by preparative TLC (cyclohexane-Et_2O, 3 : 1); Sfr. 2 (60 : 1) yielded **3** (34 mg) by recrystallization in petroleum ether-EtOAc and yielded **10** (12 mg, Rf = 0.26) by preparative TLC (cyclohexane-Et_2O, 6 : 1); Sfr. 3 (30 : 1) yielded **6** (15 mg, Rf = 0.3), **7** (8 mg, Rf = 0.38), **8** (5 mg, Rf = 0.48) by preparative TLC (petroleum ether-EtOAc, 2 : 1); and Sfr. 4 (10 : 1) yielded **9** (18 mg) by preparative TLC (cyclohexane-acetone, 5 : 1). Fr. 3 afforded **11** (28 mg) by recrystallization in petroleum ether-EtOAc. Fr. 6 was subjected to a silica gel CC eluted with $CHCl_3$−MeOH (45 : 1), and purified by preparative TLC ($CHCl_3$−EtOAc, 10 : 1) to get **1** (12 mg, Rf = 0.22). From Fr. 9 **12** (48 mg) was obtained by recrystallization in $CHCl_3$−MeOH.

3.4. 13-Hydroxy-5,7(11)-eudesmadien-12,8-olide (1)

Colorless gum, $[\alpha]_D^{20}$ +128.4° (C 0.43, $CHCl_3$). IR (film, cm^{-1}): 3392 (OH), 2924, 2851, 1743 (O=C−O), 1663, 1451, 1382, 1121. EI-MS (70 eV) m/z (rel.int.): 248 (32) $[M]^+$, 230 (62) $[M-H_2O]^+$, 215 (46), 187 (30), 173 (32), 159 (36), 105 (60), 91 (100), 55 (47). ^1H and ^{13}C NMR: see Table 1.

3.5. 3-Methyl-8-acetoxy-9,10-diisobutanoyloxy-p-cymene (6)

Colorless needle crystals (cyclohexane-Et_2O), mp. 92–94 °C. IR (KBr, cm^{-1}): 2972, 2937, 2876, 1742 (O=C−O), 1621, 1573 (phenyl), 1470, 1155 (C−O). FAB-MS m/z (rel.int.): 385 $[M + Li]^+$, 401 $[M + Na]^+$, 379 $[M + 1]^+$, 71 (isobutanoyl), 43 (isopropyl). ^1H and ^{13}C NMR: see Table 2.

3.6. Antibacterial activity

The compounds **1–10** were tested for their antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* using the cup-plate technique in the nutrient agar media by measuring the inhibition zone in mm. Choromycetin was used as a control. The test was performed at 100 μg/ml concentration in a cup of 8 mm diameter (each cup 0.2 ml). From antibacterial activity data it was found compounds **2**, **3** and **10** exhibited moderate activity against the microorganisms.

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