Two New Oplopanol Esters from *Cremanthodium ellisii*

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Abstract: **Two new sesquiterpenoids have been isolated from *Cremanthodium ellisii* Kitam. Their structures were established on the basis of spectral analysis (IR, MS, ^{1}H-^{1}H COSY, NOESY, ^{1}H-^{13}C COSY, and COLOC) and chemical evidence.**

Key words: *Cremanthodium ellisii*, **Compositae, sesquiterpenoids, 2D-NMR, oplopane derivatives.**

Introduction

So far, none of the 55 *Cremanthodium* species (Compositae, tribe Senecioneae), of which 47 are indigenous to Tibet and the Himalaya area (1), has been chemically investigated although other genera of the same tribe such as *Senceio* (2, 3), *Ligularia* (4–6), and *Kleinia* (7) have received much phytochemical attention. Some *Cremanthodium* plants like the title species have been used as traditional Tibetan medicines for anti-inflammation, detoxication, and relief of pain since ancient times (8). To the best of knowledge, the close morphological resemblance of *Cremanthodium* plants to species of the *Ligularia* and *Senecio* genera causes much more confusion and abuse to the local Tibetan and sometimes to an experienced botanist. These observations prompted us to investigate chemically *Cremanthodium ellisii* Kitam, a famous Tibetan herbal drug of a long history (8). The result is discussed in this paper.

Materials and Methods

Plant material

Cremanthodium ellisii Kitam was collected in Zhang county, Gansu Province, People's Republic of China, in August, 1990. A voucher specimen (no. LZB 2013) was identified by Prof. Z. X. Peng and deposited in Herbarium of Department of Biology, Lanzhou University.

Apparatus

EI- and HR-mass spectra were recorded on a VG ZAB-HS mass spectrometer, the ORD curve was obtained with a JASCO J-20C spectropolarimeter; IR spectra were determined on a 5DX-FTIR spectrometer; ^{1}H- and ^{13}C-NMR spectra were recorded at 400 MHz and 100.6 MHz, respectively, using a Bruker AM 400 FT-NMR spectrometer with TMS as internal reference. The usual pulse sequences of Bruker were used in ^{1}H-^{1}H COSY and ^{1}H-^{13}C-COSY experiments; for the heteronuclear correlations (^{1}H-^{13}C COLOC), coupling constants of 140 Hz (one-bond), 5 Hz and 10 Hz (long-range) were employed in measurements; NOESY spectra resulted from a $2 \times 512 \times 1024$ data matrix size using a 32 step phase cycle. Total measuring times were 9 h.

Extraction and isolation

Air-dried and powdered whole plants (including roots) of *C. ellisii* (7.0 kg) were extracted at room temperature with Me_2CO (6 days × 3). The resultant extract (300 g), which was subjected to CC (6 × 130 cm) over 1700 g silica gel (200–300 mesh) with a petroleum ether/Me_2CO (50 : 1 – 20 : 1 – 10 : 1 – 6 : 1 – 4 : 1 – 2 : 1 – 1 : 1 – 0 : 1) gradient, was separated into eight crude fractions (A–H, each fraction 3000 ml). Fraction D (petroleum ether/Me_2CO, 6 : 1, 23.5 g) was chromatographed on a silica gel (200–300 mesh, 400 g) column with C_6H_6-EtOAc (first: 6 : 1, 3000 ml, then 0 : 1, 1500 ml) and the fraction (C_6H_6-ETOAc, 0 : 1) was rechromatographed on silica gel (200–300 mesh, 250 g) with cyclohexane-EtOAc, 3 : 1, 2000 ml) to obtain a mixtures of compounds **1** and **2** (TLC: GF_{254}, with cyclohexane-EtOAc, 3 : 1, $R_f = 0.4$). The mixtures were finally purified with HPLC (reverse phase: ODS-3, MeOH-H_2O, 4 : 1) to yield 16 mg of crellisin (**1**) and 10 mg of crellisin (**2**).

Crellisin (**1**): Colourless gum; $[\alpha]_D^{20}$ − 31.7 ($CHCl_3$, *c* 0.37); EIMS: *m/z* (rel. int.) = 634 $[M]^+$ (0.5), 574 (1.0), 546 $[M - 88]^+$ (0.5), 486 (1), 462 $[M - 60 - 112]^+$, 375 (2), 318 (9), 290 (7), 272 (7), 230 (35), 212 (28), 183 (14), 173 (19), 155 (37), 145 (10), 129 (10), 113 [4-acetoxy-4-methylsenecioylic acid − HOAc]$^+$ (100), 57 (78), 43 (93); HRMS: *m/z* = 574.3148 [M − HOAc]$^+$ (calc. for $C_{32}H_{46}O_9$: 574.3142); IR: ν_{max} = 3086 (α,α-disubstituted oxirane ring), 2973, 2936, 2877, 1738 (C=O), 1656 (C=C), 1461, 1369, 1303, 1238, 1154, 1078, 914 cm^{-1}; ^{13}C-NMR ($CDCl_3$): δ = 71.9 (C-1), 71.8 (C-2), 46.0 (C-3), 45.8 (C-4), 45.8 (C-5), 33.6 (C-6), 73.0 (C-7), 140.5 (C-8), 44.7 (C-9), 113.1 (C-10), 57.0 (C-11), 56.5 (C-12), 16.2 (C-13), 69.3 (C-14), 16.2 (C-15); MeBu: 176.0 (C-1′), 41.6 (C-2′), 26.5 (C-3′), 11.9 (C-4′), 17.4 (C-5′); *i*-Bu: 176.5 (C-1″), 33.9 (C-2″), 19.2 (C-3″), 18.6 (C-4″); Ac: 169.9, 21.2; AcMeSen: 165.5 (C-1‴), 115.2 (C-2‴), 157.5 (C-3‴), 73.6 (C-4‴), 19.2 (C-5‴), 15.9 (C-3‴-Me), 169.5, 21.1 (C-4‴-OAc).

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Crellisin (**2**): Colourless gum; $[\alpha]_D^{20}$ −57.8 ($CHCl_3$, *c* 0.3); EIMS: *m/z* (rel. int.) = 620 $[M]^+$ (1), 560 $[M - 60]^+$ (2), 532 $[M - 88]^+$ (1), 472 $[M - 2 \times HOAc]^+$ (2), 448 $[M - 60 - 112]^+$ (5), 432 (1), 402 (2), 377 (3), 361 (9), 318 (11), 290 (10), 272 (9), 247 (3), 230 (48), 212 (40), 183 (18), 172 (28), 155 (47), 113 (100), 96 (75), 71 (64), 43 (80); HRMS: *m/z* = 560.3002 $[M - HOAc]^+$ (calc. for $C_{31}H_{44}O_8$: 560.2985); IR: ν_{max} = 3021 (an α,α-disubstituted oxirane ring), 2975, 2937, 2876, 1738, 1655, 1467, 1368, 1238, 1154, 1073, 942, 753 cm^{-1}; ^{13}C-NMR ($CDCl_3$): δ = 71.8 (C-1), 71.8 (C-2), 46.2 (C-3), 45.9 (C-4), 45.9 (C-5), 33.7 (C-6), 73.1 (C-7), 140.6 (C-8), 44.9 (C-9), 113.1 (C-10), 57.0 (C-11), 56.5 (C-12), 16.2 (C-13), 69.3 (C-14), 16.2 (C-15); 2 × *i*-Bu: 176.4, 176.5, 34.0, 34.3, 19.3, 19.1, 18.6; Ac: 169.9, 21.1; AcMeSen: 165.1 (C-1‴), 115.3 (C-2‴), 157.5 (C-3‴), 73.5 (C-4‴), 19.3 (C-5‴), 15.2 (C-3‴-Me), 169.5, 21.6 (C-4‴-OAc).

Alkali treatment of **1**: Compound **1** (6 mg) was dissolved in 3 ml of 2 M Na_2CO_3 methanolic solution and the mixture refluxed for 48 h; after evaporation of the MeOH, the residual material was extracted with 20 ml of *n*-butyl alcohol. The *n*-butyl alcohol phase was washed with 2 M HCl and saturated NaCl solution dried over $MgSO_4$, and the solvent removed under reduced pressure. The residue was submitted to silica gel CC eluted with petroleum ether-Me_2CO (2 : 1) to yield about 3 mg of **3**. ^{1}HNMR: see Table **1**.

Results and Discussion

Crellisin (**1**) was obtained as a colourless gum. The EI mass spectrum showed $[M]^+$ at *m/z* = 634, while the high resolution mass pectrum gave $[M - HOAc]^+$ at *m/z* = 574.3148 ($C_{32}H_{46}O_8$, calcd. 574.3142), in accordance with the formula $C_{34}H_{50}O_{11}$. The IR spectrum of **1**, exhibiting no hydroxy band, revealed the presence of ester group (1738 cm^{-1}) and double bond bands (1650 cm^{-1}). In the ^{1}H-NMR spectrum of **1**, there were an isobutyryl group, a 2-methylbutyryl, and a pair of acetoxy groups (Table **1**). The EIMS of **1** showed a base peak at *m/z* = 113 and fragment peaks at *m/z* = 574 and 462 produced by successive eliminations of units of acetic acid and 4-acetoxy-4-methylsenecioylic acid. Furthermore, the presence of 4-acetoxy-4-methylsenecioyl moiety was subsequently confirmed by the ^{1}H-NMR signals at δ = 5.75 (H-2‴), 5.20 (H-4‴), 2.09 (H-6‴) and 1.44 (H-5‴). This was further reinforced by the C-H long range correlations of C-3‴ with H-4‴ (δ = 5.20) and of C-1‴ with H-2‴ (δ = 5.75) in the COLOC spectrum.

The ten degrees of unsaturation of **1** ($C_{34}H_{50}O_{11}$), six of which were attributed to five ester groups and a double bond in the senecioyl-derived part, indicated that two of the four left were clearly due to an exomethylene and an epoxide (see below). Thus, the alcohol moiety of the ester was a bicyclic sesquiterpene. In the ^{1}H-NMR spectrum of **1**, an oplopane skeleton was clearly indicated by an oxygenated methyl doublet at δ = 1.32 (J = 6.6 Hz), broadened exomethylene singlets at δ = 5.16 and 4.75 as well as signals at δ = 1.24 (3H, s, H-13), 2.91 (1H, d, J = 4.0 Hz, H-12), and 2.89 (1H, d, J = 4.0 Hz, H-12′) arising from an α,β-epoxyisopropyl residue (7). The ^{1}H-^{1}H COSY spectrum of **1** also supported the deduction. The attachment of those ester groups to this oplopanol part was elucidated from the ^{1}H-^{13}C long range correlation NMR spectrum (COLOC) of **1**. The long range C-H couplings at δ = 176.0, δ = 5.65 (H-1), and δ = 2.43 (2-MeBu-H_1), δ = 176.0, δ = 1.76, and δ = 1.44 (2-MeBu-H_2), δ = 176.6 and δ = 5.54 (H-2), δ = 169.9, δ = 5.20 (H-14) and δ = 2.03 (acetyl-H), δ = 165.5 and δ = 5.50 (H-7) clearly indicated the positions of the 2-methylbutyryl, isobutyryl, acetyl, and 4-acetoxy-4-methylsenecioyl should be located at located at C-1, C-2, C-14, and C-7, respectively.

Table 1 ^{1}H-NMR spectral data for **1**–**3**[a].

H	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** (C_5D_5N)
1	5.65 dd	5.64 dd	5.12 dd
2	5.54 dd	5.56 dd	4.36 dd
3	2.62 ddd	2.63 ddd	2.84 ddd
4	1.93 br q	1.95 br q	2.54 br q
5	1.44 m	1.46 m	1.74 ddd
6	1.68 ddd	1.69 ddd	2.09 ddd
6α	2.16 dt	2.22 dt	2.42 ddd
7	5.50 t	5.52 t	4.59 dd
9	2.43[b]	2.42[b]	2.85 dd
10	5.16 s	5.17 s	5.30 s
10′	4.75 s	4.76 s	4.77 s
12	2.91 d	2.91 d	4.04 d
12′	2.89 d	2.90 d	3.65 d
13	1.24 s	1.25 s	1.44 s
14	5.20 m	5.23 m	4.44 dq
15	1.32 d	1.34 d	1.46 d
Me Bu		*i*-Bu	
2′	2.43 m	2.63 m	
3′	1.76 m, 1.44 m	1.21 d	
4′	0.96 t		
5′	1.18 d	1.22 d	
iBu			
2″	2.43 m	2.42 m	
3″	1.10 d	1.12 d	
4″	1.11 d	1.12 d	
Ac-H	2.03 s	2.04 s	
AcMeSen			
2‴	5.75 q	5.77 q	
4‴	5.20 m	5.23 m	
5‴	1.44 d	1.46 d	
3‴-Me	2.09 s	2.11 s	
4‴-Ac	2.09 s	2.10 s	

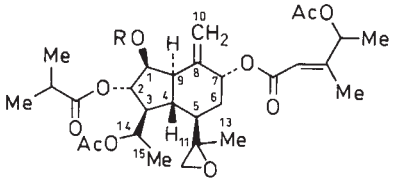
[a] *J* (Hz): Compound **1** and **2**: 1,9 = 2.5; 1,2 = 4.3; 2,3 = 10.5; 3,4 = 4,5 = 4,9 = 10.0; 6α,6β = 12.0; 5,6β = 12.8; 6α,7 = 6β,7 = 3.0; 12,12′ = 4.0; 3,14 = 4.3; 14,15 = 6.6; 2-MeBu: 2′,5′ = 7.0; 3′,4′ = 7.4; iBu: 2″,3″ = 2″,4″ = 7.0; AcMeSen: 2‴,6‴ = 1.3; 4‴,5‴ = 6.8. Compound **3**: 1,9 = 2.7; 1,2 = 5.0; 2,3 = 9.3; 3,4 = 4,5 = 4,9 = 11.6; 5,6β = 13.0; 5,6α = 2.2; 6α,6β = 10.3; 7,6β = 5.5; 7,6α = 3.3; 12,12′ = 11.9; 3,14 = 3.3; 4,15 = 6.0.

[b] Overlapped signals.

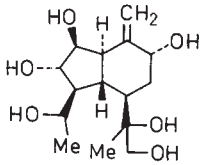
The relative stereochemistry of **1** was deduced by analyses of the ^{1}H-^{1}H coupling constants. The quartet signal with J = 10 Hz assignable to H-4 established that the H-4 proton is oriented in an *anti-trans*-axial direction with repect to the neighbouring three protons, H-3, H-5, H-9. Proton H-7 was in a β-orientation judging from the triplet signal with J = 3 Hz. This elucidation was consistent with the ^{1} H-^{1}H NOESY spectrum of **1** in which cross peaks of H-3 with H-5 and H-9 were discerned.

Mild alkaline hydrolysis of **1** with 2M Na_2CO_3 in MeOH at room temperature afforded a hexahydroxy derivative **3**. In the ^{1}H-NMR spectrum of **3**, the chemical shifts of H-1 (δ = 5.12), H-2 (δ = 4.36), H-7 (δ = 4.59) and H-4 (δ = 4.45) were upfield relative to **1** due to the deacylation. This further testified to the proposed structure.

Crellisin (**2**), a colourless gum, showed the IR absorptions at ν = 3021 (α,α-disubstituted oxirane ring), 1738 (C=O) and



1 R = 2-MeBu
2 R = isobutyryl



3

1655 cm^{-1} (C=C). The EIMS gave a molecular ion at m/z = 620, 14 mass units lower than that of compound **1**. The HRMS of **2** showed $[M - HOAc]^+$ at m/z = 560.3002 ($C_{31}H_{44}O_9$; calcd. 560.2985). Its molecular formula was determined as $C_{31}H_{44}O_{11}$. The ^{1}H-NMR spectrum of **2** was very similar to that of **1**. However, a methylene group (δ_C = 26.5, δ_H = 1.76 and 1.44) in the ^{13}C- and ^{1}H-NMR spectra of **1** was missing in those of **2**, while other spectral data in both sets were parallel. This indicated that the 2-methylbutyryl group in **1** was replaced by an isobutyryl group in **2**. Hydrolysis of **2** as in the case of **1** afforded compound **3**, too. Finally, the structure of **2** was extensively confirmed by ^{1}H-^{1}H COSY and NOESY spectra.

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References

[1] Northwest Institute of Botany, Academia, Sinica (1981) Flora Qinglingica, Science Press, Beijing 1 (5), 322.
[2] Bohlmann, F., Zdero, C., Gupta, P. K. (1981) Phytochemistry 20, 2024–2026.
[3] Nathan, P. D., Villagomez, J. R., Roman, L. U., Hernandez, J. D. (1989) Phytochemistry 28, 1207–1209.
[4] Jia, Z. J., Zhao, Y., Tan, R. X., Li, Y. (1992) Phyotchemistry 31, 199–201.
[5] Jia, Z. J., Zhao, Y., Tan, R. X. (1992) Planta Med. 58, 365–367.
[6] Jia, Z. J., Zhao, Y. (1994) J. Nat. Prod. 57, 146–150.
[7] Bohlmann, F., Ahmed, M., Jakupovic, J., Jeffrey, C. (1981) Phytochemistry 20, 251–256.
[8] North-Western Plateau Institute of Biology, Academia Sinica (1991) The Records of Tibetan Medicines, Qinghai People's Press, Xining, 389.