

**Micranthin C, a Novel 13(12 → 11)abeo-Abietanoid from *Isodon lophanthoids* var. *micranthus***

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A novel 13(12 → 11)abeo-abietane skeletal diterpenoid, micranthin C (**1**), was isolated from *Isodon lophanthoids* var. *micranthus*. Its structure was determined by various spectroscopic techniques and finally confirmed by single-crystal X-ray diffraction.

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**Introduction.** – Recently, we have isolated several novel diterpenoids from *Isodon* plants [1–5]. *Isodon lophanthoids* (Buch.-Ham. Ex D Don) Hara has been used for treatment of acute hepatitis, cholecystitis, enteritis, dysentery, and trauma in folk medicine [6]. Some studies on this species and its variations have led to the isolation of a series of abietane diterpenoids [7–9]. In the course of our search for novel bioactive diterpenoids from *Isodon* species, we have investigated *I. lophanthoids* var. *micranthus* (C. Y. Wu) H. W. Li, a perennial herb collected in the southwest of Yunnan Province (China). The continuation of our previous work [10] resulted in the isolation of a minor constituent, a novel 13(12 → 11)abeo-abietanoid named micranthin C (**1**), along with three known abietane quinones, 16-acetoxy-7 $\alpha$ -hydroxyroyleanone (**2**) [11], 16-acetoxy-7 $\alpha$ -methoxyroyleanone (**3**) [12], and hyptol (**4**) [13]. Here, we present the isolation and structure elucidation of the unique compound **1**, accomplished by spectroscopic techniques, especially 1D and 2D NMR, as well as by X-ray diffraction analysis.

**Results and Discussion.** – Compound **1** was obtained as colorless needles. It showed a molecular-ion peak at  $m/z$  348 in the EI-MS, consistent with the molecular formula C<sub>20</sub>H<sub>28</sub>O<sub>5</sub>, as confirmed by HR-EI-MS (348.193665, calc.: 348.193674) and by NMR. The <sup>1</sup>H- and <sup>13</sup>C-NMR, HMQC, and HMBC spectra, as well as an X-ray crystal-structure, established that **1** corresponds to 7 $\alpha$ ,11 $\alpha$ -dihydroxy-14-oxo-13(12 → 11)abeo-abieta-8-en-12,16-olide.

The characteristic UV absorption at 239 nm and IR bands at 1738 and 1634 cm<sup>–1</sup> indicated the presence of an  $\alpha,\beta$ -unsaturated ketone. The <sup>13</sup>C-NMR signals indicated two C=O C-atoms, five quaternary C-atoms (including an oxygenated one and two tetrasubstituted olefinic ones), four methines (including an oxymethine), five



1 H, br. *d*,  $J = 11.3$  Hz)) and both the lactone C=O C-atom ( $\delta(\text{C})$  174.8 (*s*)) and C(13) ( $\delta(\text{C})$  62.3 (*d*)), as well as between H–C(15) ( $\delta(\text{H})$  2.52 (*m*)), C(11) ( $\delta(\text{C})$  78.9 (*s*)), and C(14) ( $\delta(\text{C})$  207.0 (*s*)) supported the above hypothesis.

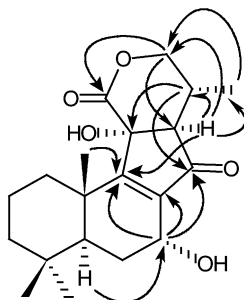
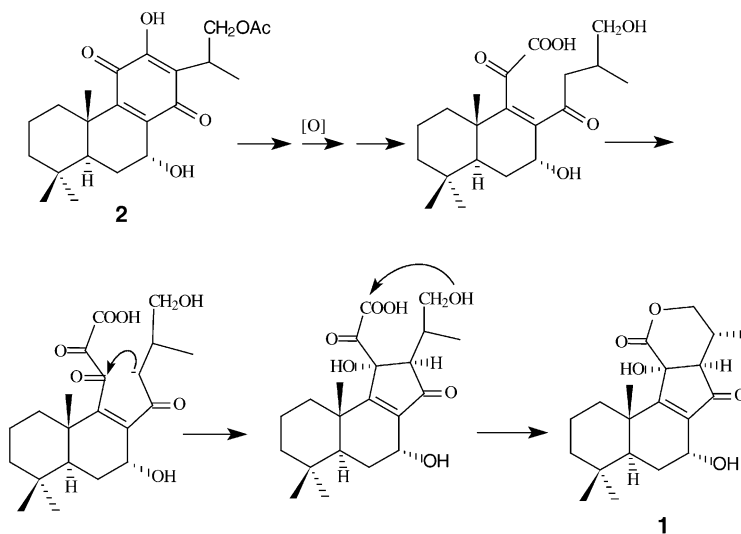


Fig. 1. Key correlations of **1** in the HMBC spectrum

The C(7)–OH group adopted an  $\alpha$ -orientation according to the absence of a correlation between H–C(7) and  $\text{H}_\alpha$ –C(5) in the ROESY spectrum. However, the relative configurations at C(11), C(13), and C(15) could not be settled at this stage. Fortunately, compound **1** could be finally crystallized. Single-crystal X-ray diffraction [14] then established not only the configuration of all stereogenic centers (OH(11), H–C(13), and Me(17) in  $\alpha$  position), but also confirmed the proposed skeleton (Fig. 2). Consequently, **1** corresponds to 7 $\alpha$ ,11 $\alpha$ -dihydroxy-14-oxo-13(12  $\rightarrow$  11)*abeo*-abietane-8-en-12,16-olide, which we have named *micranthin C*. Although the rearrangement in ring C of abietane diterpenoids has been reported before [14–18], compound **1** is an unprecedented natural diterpenoid with a 13(12  $\rightarrow$  11)*abeo*-abietane skeleton.

Scheme. Postulated Biogenetic Pathway Leading to *Micranthin C* (**1**)



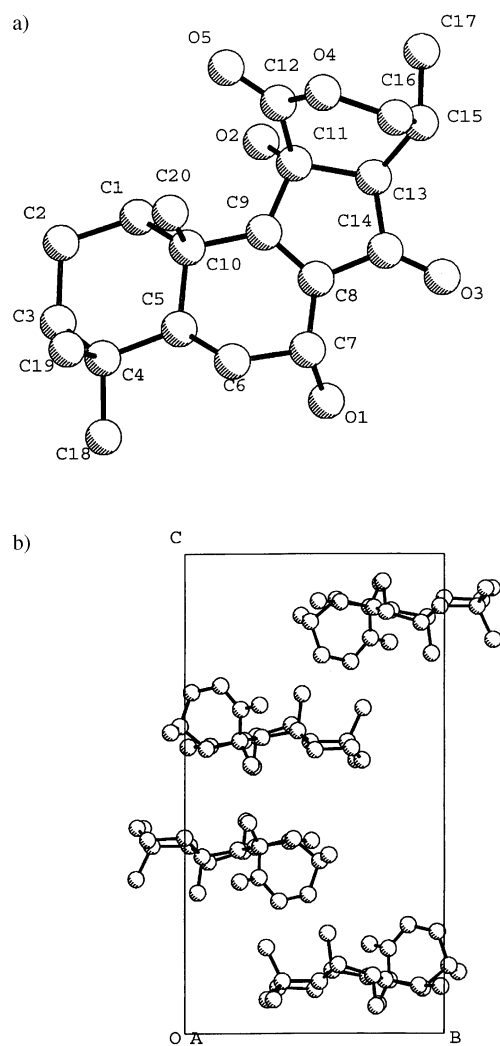


Fig. 2. a) X-Ray crystal structure of **1**. b) Lattice geometry.

From a biogenetic point of view, the precursor of **1** may be 16-acetoxy-7 $\alpha$ -hydroxyroyleanone (**2**), which has been isolated from the same plant. A plausible biogenetic pathway is shown in the *Scheme*, in which oxidation, nucleophilic ring closure, and esterification are probably responsible for the above transformation.

#### Experimental Part

*General.* M.p.: XRC-1 Micro-melting-point apparatus, uncorrected. Optical rotations: JASCO DIP-370 digital polarimeter. UV Spectra: Shimadzu UV-210A spectrometer;  $\lambda_{\max}$  in nm (log  $\epsilon$ ). IR Spectra: Bio-Rad FTS-135 spectrometer with KBr pellets; in  $\text{cm}^{-1}$ . Mass spectra: VG Autospec-3000 spectrometer; 70 eV for EI;  $m/z$

(rel. %). NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers;  $\delta$  in ppm,  $J$  in Hz,  $\text{SiMe}_4$  as internal standard, in  $(\text{D}_5)\text{pyridine}$ .

**Plant Material.** The aerial part of *I. lophanthoides* var. *micranthes* C. Y. Wu were collected in Tenchong, Yunnan (China) in October 2001 and identified by Prof. Zhong-Wen Lin. A voucher specimen (KIB 001-01) of this plant was deposited at the Kunming Institute of Botany, Kunming, China.

**Extraction and Isolation.** The air-dried plants (7.2 kg) were extracted with 70% aq. acetone at r.t. overnight ( $4\times$ ), and filtered. The filtrate was concentrated *in vacuo*, and the residue was partitioned between  $\text{H}_2\text{O}$  and  $\text{AcOEt}$ . The  $\text{AcOEt}$ -soluble fraction (60 g) was subjected to CC ( $\text{SiO}_2$ ;  $\text{CHCl}_3/\text{Me}_2\text{CO}$  (1:0  $\rightarrow$  9:1  $\rightarrow$  4:1  $\rightarrow$  7:3). Compound **1** (7 mg), **2** (29 mg), **3** (68 mg), and **4** (15 mg) were obtained by repeated CC ( $\text{SiO}_2$  and *Sephadex LH-20*;  $\text{CHCl}_3/\text{Me}_2\text{CO}$  1:0 and 9:1).

**Micranthin C** ( $=7\alpha,11\alpha$ -dihydroxy-14-oxo-13(12  $\rightarrow$  11)abeo-abieta-8-en-12,16-olide; **1**). Colorless needles. M.p. 174–176°.  $[\alpha]_D^{25} = -30.77$  ( $c = 0.13$ ,  $\text{MeOH}$ ). UV ( $\text{MeOH}$ ): 239 (3.50). IR (KBr): 3439, 2957, 2930, 2872, 1738, 1717, 1651, 1634, 1627, 1472, 1457, 1373, 1252, 1177, 1103, 1058, 1025, 997, 945, 742, 669, 528.  $^1\text{H-NMR}$  ( $\text{C}_5\text{D}_5\text{N}$ , 400 MHz): 4.95 ( $d$ ,  $J(7\beta-6\beta) = 4.0$ ,  $\text{H}_\beta-\text{C}(7)$ ); 4.19 (br.  $d$ ,  $J = 11.3$ ,  $\text{H}_\beta-\text{C}(16)$ ); 4.07 (br.  $d$ ,  $J = 11.3$ ,  $\text{H}_\alpha-\text{C}(16)$ ); 2.86 ( $m$ ,  $\text{H}_\beta-\text{C}(1)$ ); 2.84 ( $d$ ,  $J(13\alpha,15\beta) = 0.8$ ,  $\text{H}_\alpha-\text{C}(13)$ ); 2.52 ( $m$ ,  $\text{H}_\beta-\text{C}(15)$ ); 2.11 ( $d$ ,  $J(5\alpha,6\beta) = 13.3$ ,  $\text{H}_\alpha-\text{C}(5)$ ); 2.09 ( $m$ ,  $\text{H}_\alpha-\text{C}(1)$ ); 2.08 ( $d$ ,  $J(6\alpha,6\beta) = 13.3$ ,  $\text{H}_\alpha-\text{C}(6)$ ); 1.76 ( $dt$ ,  $J(6\beta,5\alpha) = J(6\beta,6\alpha) = 13.3$ ,  $J(6\beta,7\beta) = 4.0$ ,  $\text{H}_\beta-\text{C}(6)$ ); 1.55 ( $m$ ,  $\text{H}_\beta-\text{C}(2)$ ); 1.37 ( $m$ ,  $\text{H}_\alpha-\text{C}(2)$ ); 1.31 (br.  $d$ ,  $J = 10.3$ ,  $\text{H}_\alpha-\text{C}(3)$ ); 1.25 ( $s$ ,  $\text{Me}(20)$ ); 1.10 (overlapping,  $\text{H}_\beta-\text{C}(3)$ ); 1.05 ( $d$ ,  $J = 7.3$ ,  $\text{Me}(17)$ ); 0.94 ( $s$ ,  $\text{Me}(18)$ ); 0.82 ( $s$ ,  $\text{Me}(19)$ ).  $^{13}\text{C-NMR}$  ( $\text{C}_5\text{D}_5\text{N}$ , 100 MHz): 34.9 ( $t$ ,  $\text{C}(1)$ ); 18.6 ( $t$ ,  $\text{C}(2)$ ); 41.6 ( $t$ ,  $\text{C}(3)$ ); 33.4 ( $s$ ,  $\text{C}(4)$ ); 46.9 ( $d$ ,  $\text{C}(5)$ ); 28.7 ( $t$ ,  $\text{C}(6)$ ); 59.4 ( $d$ ,  $\text{C}(7)$ ); 142.5 ( $s$ ,  $\text{C}(8)$ ); 178.1 ( $s$ ,  $\text{C}(9)$ ); 41.5 ( $s$ ,  $\text{C}(10)$ ); 78.9 ( $s$ ,  $\text{C}(11)$ ); 174.8 ( $s$ ,  $\text{C}(12)$ ); 62.3 ( $d$ ,  $\text{C}(13)$ ); 207.0 ( $s$ ,  $\text{C}(14)$ ); 33.0 ( $d$ ,  $\text{C}(15)$ ); 70.7 ( $t$ ,  $\text{C}(16)$ ); 17.1 ( $q$ ,  $\text{C}(17)$ ); 33.7 ( $q$ ,  $\text{C}(18)$ ); 22.0 ( $q$ ,  $\text{C}(19)$ ); 18.7 ( $q$ ,  $\text{C}(20)$ ). EI-MS: 348 (52,  $M^+$ ), 330 (50,  $[M - \text{H}_2\text{O}]^+$ ), 312 (54,  $[M - 2\text{H}_2\text{O}]^+$ ), 302 (20), 286 (71), 271 (66), 247 (35), 233 (24), 217 (29), 202 (72), 187 (69), 173 (21), 163 (19), 140 (33), 123 (75), 109 (51), 95 (40), 83 (78), 69 (96). HR-EI-MS: 348.193665 ( $M^+$ ,  $\text{C}_{20}\text{H}_{28}\text{O}_5^+$ ; calc. 348.193674).

**X-Ray Crystal Structure of 1.** Colorless, transparent, column-like single crystal ( $0.15 \times 0.30 \times 0.60$  mm); formula (formula weight):  $\text{C}_{20}\text{H}_{28}\text{O}_5$  (348.44 g  $\text{mol}^{-1}$ ); crystal system: orthorhombic; space group:  $P2_12_12_1$ ; lattice parameters:  $a = 8.1830(2)$ ,  $b = 10.8520(5)$ ,  $c = 20.1230(10)$  Å,  $V = 1786.96(13)$  Å<sup>3</sup>;  $Z = 4$ ,  $d = 1.295$  g  $\text{cm}^{-3}$ ;  $\text{MoK}_\alpha$  radiation, *MAC DIP-2030K* diffractometer; maximum  $2\theta$  range:  $50.0^\circ$ ; total reflections observed (collected): 1784 (1780); data analysis:  $|F|^2 > 8\sigma|F|^2$ . Rings A and B are *trans*, in chair- and half-chair conformations, resp., whereas rings C and D are *cis*, in planar and boat conformations, resp.  $\text{HO}-\text{C}(7)$ ,  $\text{H}-\text{C}(13)$ , and  $\text{Me}(17)$  are all  $\alpha$ -oriented, i.e., they lie below the molecular plane. The crystallographic data (excluding structure factors) for **1** have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC 219060. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ UK (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

## REFERENCES

- [1] H. D. Sun, S. X. Qiu, L. Z. Lin, R. P. Zhang, Y. S. Zhou, Q. T. Zheng, M. E. Johnson, H. H. S. Fong, N. R. Farnsworth, G. A. Cordell, *J. Nat. Prod.* **1997**, 60, 203.
- [2] H. D. Sun, S. X. Qiu, E. B. Lobkovsky, L. Z. Lin, N. R. Farnsworth, J. Clardy, H. S. Fong, *Tetrahedron* **2001**, 57, 65.
- [3] X. M. Niu, Q. D. Zhao, Z. W. Lin, H. D. Sun, Y. Lu, Q. T. Zheng, *Tetrahedron Lett.* **2002**, 43, 661.
- [4] X. M. Niu, S. H. Li, Q. S. Zhao, Z. W. Lin, H. D. Sun, Y. Lu, Q. T. Zheng, *Tetrahedron Lett.* **2002**, 43, 5277.
- [5] H. D. Sun, Y. L. Xu, B. Jiang, 'Diterpenoids from *Isodon* Species', Beijing Academic Press, Beijing, 2001, p. 130.
- [6] C. Y. Wu, H. W. Li, 'Flora Republicae Popularis Sinicae', Beijing Academic Press, Beijing, 1979; Vol. 66, p. 479.
- [7] Y. L. Xu, Y. B. Ma, L. Zhou, H. D. Sun, *Phytochemistry* **1988**, 27, 3681.
- [8] Y. L. Xu, D. Wang, X. J. Li, J. Fu, *Phytochemistry* **1989**, 28, 189.
- [9] B. Jiang, Z. Q. Lu, H. J. Zhang, Q. S. Zhao, H. D. Sun, *Fitoterapia* **2002**, 71, 360.
- [10] A. H. Zhao, S. H. Li, Y. W. Li, Q. B. Han, Q. S. Zhao, Z. W. Lin, H. D. Sun, *Chin. Chem. Lett.* **2003**, 14, 591.
- [11] O. Batista, M. F. Simoes, A. Duarte, L. Valdeira, M. C. D. Torre, B. Rodriguez, *Phytochemistry* **1995**, 38, 167.
- [12] G. Y. Li, Y. L. Wang, W. Z. Song, Q. Y. Ji, *Acta Pharmaceutica Sinica* **1987**, 22, 269.
- [13] M. Takashi, T. Hiromitsu, W. Makoto, I. Sachihiko, *Bull. Chem. Soc. Jpn.* **1991**, 64, 2762.

- [14] A. Kelecom, T. C. D. Santos, L. B. Medeiros, *Phytochemistry* **1989**, 26, 2337.
- [15] A. Kedecom, T. C. D. Santos, *Tetrahedron Lett.* **1985**, 26, 3659.
- [16] R. C. Cambie, C. C. Liu, E. F. Rickard, P. S. Rutledge, *Aust. J. Chem.* **1998**, 51, 605.
- [17] M. J. S. Cuadrado, M. Bruno, M. C. De La Torre, F. Piozzi, G. Savona, B. Rodriguez, *Phytochemistry* **1992**, 31, 1697.
- [18] A. Ulubelen, G. Topcu, S. Olcal, *Phytochemistry* **1994**, 37, 1371.

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