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

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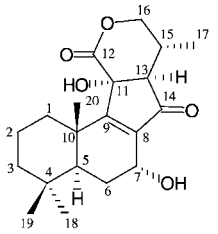
A novel 13(12 → 11)*abeo*-abietane skeletal diterpenoid, micranthin C (**1**), was isolated from *Isodon lophanthoids* var. *micranthus*. Its structure was determined by various spectroscopic techniques and finally confirmed by single-crystal X-ray diffraction.

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-7α-hydroxyroyleanone (**2**) [11], 16-acetoxy-7α-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 $C_{20}H_{28}O_5$, as confirmed by HR-EI-MS (348.193665, calc.: 348.193674) and by NMR. The ^{1}H- and ^{13}C-NMR, HMQC, and HMBC spectra, as well as an X-ray crystal-structure, established that **1** corresponds to 7α,11α-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^{-1} indicated the presence of an α,β-unsaturated ketone. The ^{13}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



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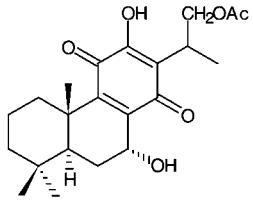

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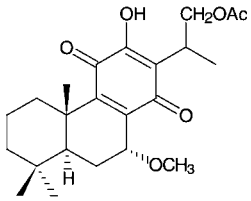

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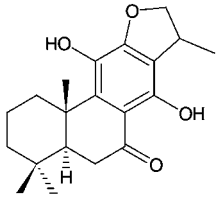

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methylenes (including an oxymethylene), and four Me groups, suggesting a diterpenoid skeleton. Consideration of the predominant abietane diterpenoids isolated from *Isodon lophanthoids* and its variations, together with the typical ^{1}H-NMR signals of three Me groups (next to tertiary C-atoms) at δ(H) 0.82, 0.94, 1.25 (3*s*) and one Me group (next to a secondary center) at δ(H) 1.05 (*d*, $J = 7.3$ Hz), one oxymethylene at δ(H) 4.07/4.19 (each 1 H, br. *d*, $J = 11.3$ Hz), indicated that **1** presumably had an abietane diterpenoid skeleton.

From HMQC and HMBC experiments, a long-range correlation between Me(20) at δ(H) 1.25 (*s*) and an olefinic C-atom at δ(C) 178.1 (*s*) was apparent, which led to the conclusion that the olefinic C-atom was C(9). From the electron distribution in the α,β-unsaturated ketone and due to another quaternary olefinic C-atom at relatively high field (δ(C) 142.5 (*s*)), the second olefinic C-atom was C(8), and the ketone C=O group was either at C(7) or C(14). Moreover, H–C(5) at δ(H) 2.11 (*d*, $J = 13.3$ Hz) exhibited a cross-peak with C(7) (δ(H) 59.4 (*d*)), and H–C(7) (δ(H) 4.95 (*d*, $J = 4.0$ Hz)) showed correlations with the two olefinic C-atoms C(8) and C(9) in the HMBC spectrum. This indicated that C(7) was hydroxylated and that the ketone C=O group was, therefore, attributable to C(14).

As typically observed in common abietane diterpenoids from *Isodon* plants, H–C(13) at δ(H) 2.84 (*d*, $J = 0.8$ Hz) of **1** showed HMBC couplings to C(15) (δ(C) 33.0 (*d*)), C(16) (70.7 (*t*)), and C(17) (17.1 (*q*)). However, surprisingly the long-range cross-peak between H–C(13) and C(9) was clearly observed in the HMBC spectrum of **1** (*Fig. 1*), which implied a dramatic difference between **1** and conventional abietane diterpenoids. This and further data analysis prompted us to assume that the C(12)–C(13) abietane bond was split and that a new bond was formed between C(11) and C(13) in **1**, leading to a 13(12 → 11)*abeo*-abietane skeleton. The new D-ring could then have formed through lactonization. In this way, the above difference could be nicely explained. The HMBC correlations between CH_2(16) (δ(H) 4.07/4.19 (each

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

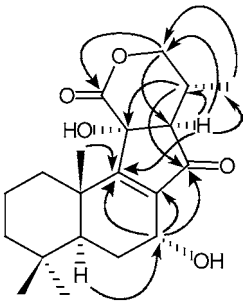
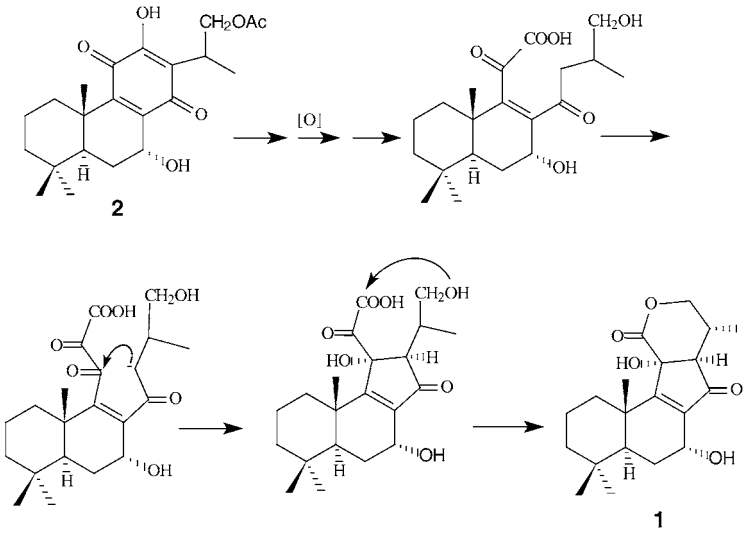


Fig. 1. *Key correlations of* **1** *in the HMBC spectrum*

The C(7)–OH group adopted an α-orientation according to the absence of a correlation between H–C(7) and H_α–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 α position), but also confirmed the proposed skeleton (*Fig. 2*). Consequently, **1** corresponds to 7α,11α-dihydroxy-14-oxo-13(12 → 11)*abeo*-abieta-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 → 11)*abeo*-abietane skeleton.

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



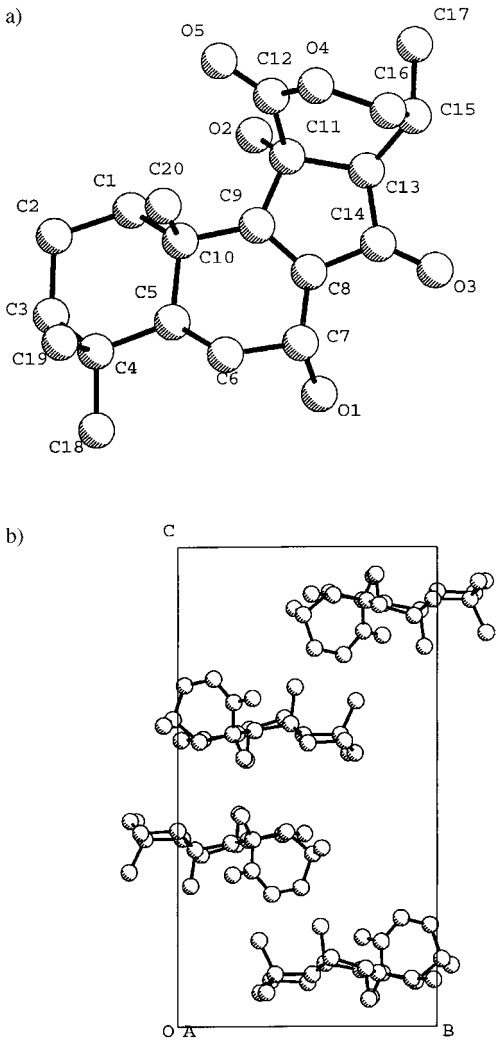


Fig. 2. a) *X-Ray crystal structure of* **1**. b) *Lattice geometry.*

From a biogenetic point of view, the precursor of **1** may be 16-acetoxy-7α-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; λ_{max} in nm (log ε). IR Spectra: *Bio-Rad FtS-135* spectrometer with KBr pellets; in cm^{-1}. Mass spectra: *VG Autospec-3000* spectrometer; 70 eV for EI; *m*/*z*

(rel. %). NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm, J in Hz, $SiMe_4$ as internal standard, in (D_5)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×), and filtered. The filtrate was concentrated *in vavcuo*, and the residue was partitioned between H_2O and AcOEt. The AcOEt-soluble fraction (60 g) was subjected to CC (SiO_2; $CHCl_3$/Me_2CO (1:0 → 9:1 → 4:1 → 7:3). Compound **1** (7 mg), **2** (29 mg), **3** (68 mg), and **4** (15 mg) were obtained by repeated CC (SiO_2 and *Sephadex LH-20*; $CHCl_3$/Me_2CO 1:0 and 9:1).

Micranthin C (=*7α,11α-dihydroxy-14-oxo-13(12 → 11)*abeo-*abieta-8-en-12,16-olide*; **1**). Colorless needles. M.p. 174–176°. $[\alpha]_D^{25} = -30.77$ ($c = 0.13$, MeOH). UV (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}H-NMR (C_5D_5N, 400 MHz): 4.95 (*d*, $J(7\beta\text{-}6\beta) = 4.0$, H_β–C(7)); 4.19 (br. *d*, $J = 11.3$, H_β–C(16)); 4.07 (br. *d*, $J = 11.3$, H_α–C(16)); 2.86 (*m*, H_β–C(1)); 2.84 (*d*, $J(13\alpha,15\beta) = 0.8$, H_α–C(13)); 2.52 (*m*, H_β–C(15)); 2.11 (*d*, $J(5\alpha,6\beta) = 13.3$, H_α–C(5)); 2.09 (*m*, H_α–C(1)); 2.08 (*d*, $J(6\alpha,6\beta) = 13.3$, H_α–C(6)); 1.76 (*dt*, $J(6\beta,5\alpha) = J(6\beta,6\alpha) = 13.3$, $J(6\beta,7\beta) = 4.0$, H_β–C(6)); 1.55 (*m*, H_β–C(2)); 1.37 (*m*, H_α–C(2)); 1.31 (br. *d*, $J = 10.3$, H_α–C(3)); 1.25 (*s*, Me(20)); 1.10 (overlapping, H_β–C(3)); 1.05 (*d*, $J = 7.3$, Me(17)); 0.94 (*s*, Me(18)); 0.82 (*s*, Me(19)). ^{13}C-NMR (C_5D_5N, 100 MHz): 34.9 (*t*, C(1)); 18.6 (*t*, C(2)); 41.6 (*t*, C(3)); 33.4 (*s*, C(4)); 46.9 (*d*, C(5)); 28.7 (*t*, C(6)); 59.4 (*d*, C(7)); 142.5 (*s*, C(8)); 178.1 (*s*, C(9)); 41.5 (*s*, C(10)); 78.9 (*s*, C(11)); 174.8 (*s*, C(12)); 62.3 (*d*, C(13)); 207.0 (*s*, C(14)); 33.0 (*d*, C(15)); 70.7 (*t*, C(16)); 17.1 (*q*, C(17)); 33.7 (*q*, C(18)); 22.0 (*q*, C(19)); 18.7 (*q*, C(20)). EI-MS: 348 (52, M^+), 330 (50, $[M - H_2O]^+$), 312 (54, $[M - 2\,H_2O]^+$), 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^+, $C_{20}H_{28}O_5^+$; calc. 348.193674).

X-Ray Crystal Structure of **1**. Colorless, transparent, column-like single crystal (0.15 × 0.30 × 0.60 mm); formula (formula weight): $C_{20}H_{28}O_5$ (348.44 g 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^3; $Z = 4$, $d = 1.295$ g cm^{-3}; MoK_α radiation, *MAC DIP-2030K* diffractometer; maximum 2θ range: 50.0°; 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. HO–C(7), H–C(13), and Me(17) are all α-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 CB21EZ UK (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

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