

Micranolic Acids A and B: Two New Octanortriterpenoids from *Schisandra micrantha*

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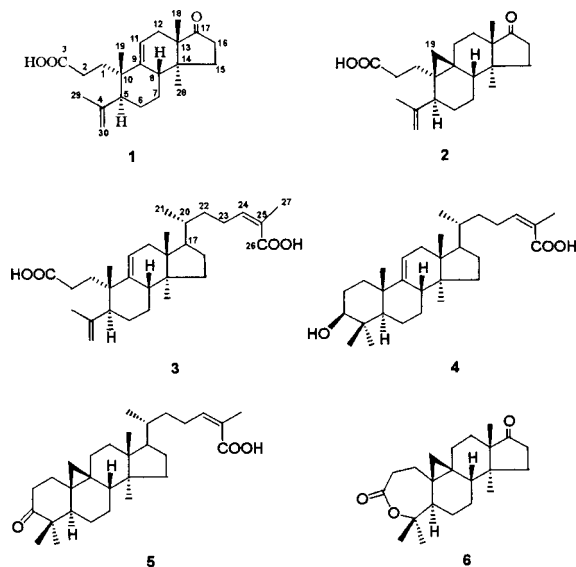
Two novel octanortriterpenoids, micranolic acids A (**1**) and B (**2**), along with three known compounds, kadsuric acid (**3**), 3 β -hydroxy-lanost-9(11),24(25)-dien-26-oic acid (**4**) and schizandronic acid (**5**), were isolated from the leaves and stems of *Schisandra micrantha*. The structures of **1** and **2** were determined by 1D and 2D NMR spectroscopic analysis. Micranolic acids A and B represent a new group of triterpenes in which the entire C-17 side chain has lost. This is the first report of octanortriterpenoids isolated from the family Schisandraceae.

Key words *Schisandra micrantha*; Schisandraceae; octanortriterpenoid; micranolic acid A; micranolic acid B

Much attention has been focused on the family Schisandraceae because lignans isolated from this family showed various biological activities.^{1,2} It has been reported that several species of this family contain triterpenoids.¹ Our previous phytochemical study on *Schisandra sphaerandra* resulted in the discovery of nigranolic acid (**7**), which showed activity on several anti-HIV reverse transcriptase and polymerase assays.³ In continuation of our investigation on Schisandraceae species, we examined the chemical constituents of *Schisandra micrantha* A. C. SMITH, a plant indigenous to Yunnan Province of China. In the preceding paper, we have described the isolation of a novel nortriterpene, micrandilactone A with a unique skeleton, from the EtOAc extract of the leaves and stems of this plant.⁴ Further studies on its analogues in the petroleum ether extract of the same source led to the isolation of two novel octanortriterpenes, micranolic acids A (**1**) and B (**2**), along with three known compounds: kadsuric acid (**3**),⁵ 3 β -hydroxy-lanost-9(11),24(25)-dien-26-oic acid (**4**)⁶ and schizandronic acid (**5**).⁷ The structural elucidation of **1** and **2** and proposed biogenetic pathways of these five compounds are reported in this paper.

Results and Discussion

Micranolic acid A (**1**) analyzed for C₂₂H₃₂O₃ by high reso-



lution electrospray ionization (HR-ESI)-MS (m/z 345.2427, $[M+H]^+$), a formula indicating seven degrees of unsaturation. The IR spectrum indicated the presence of ketone (1732 cm^{-1}), carboxylic acid ($3550\text{--}3250$, 1703 cm^{-1}), and double bond (1634 cm^{-1}) group. The ¹H-NMR spectrum (Table 1) showed the presence of an isopropenyl group (methylene protons at δ 4.67 and 4.82, H-30a and H-30b; an olefinic methyl group at δ 1.71, Me-29) and three tertiary methyl groups (δ 0.67, 0.82, 1.03). The ¹³C-NMR spectrum showed signals for 22 carbons, while distortionless enhancement by polarization transfer (DEPT) spectral data indicated the presence of four methyls, eight methylenes, three methines and seven quaternary carbons (including two olefinic carbons, a ketone carbon and a carboxylic acid group). A careful comparison of the ¹H- and ¹³C-NMR spectra of **1** with those of kadsuric acid (**3**)⁵ indicated that both of two

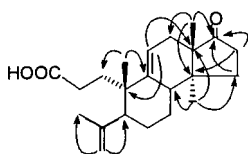
Table 1. ¹H- and ¹³C-NMR Data for Compounds **1** and **2** in CD₃OD^{a)}

No.	1		2	
	δ_{H} (mult; J , Hz)	δ_{C}	δ_{H} (mult; J , Hz)	δ_{C}
1	1.76 m, 2.36 m	29.9 t	1.44 m, 2.07 m	30.2 t
2	1.94 m, 2.22 overlap	33.6 t	2.29 m, 2.50 m	32.3 t
3	—	177.8 s	—	177.6 s
4	—	148.6 s	—	150.6 s
5	2.02 dd (2.7, 10.2)	50.4 d	2.55 m	46.8 d
6	1.54 m, 1.79 m	28.8 t	1.24 m, 1.58 m	28.2 t
7	1.21 m, 1.64 overlap	26.7 t	1.29 m, 1.51 m	24.5 t
8	2.22 m	42.7 d	1.88 m	46.9 d
9	—	144.7 s	—	22.6 s
10	—	43.1 s	—	29.2 s
11	5.36 br s	118.2 d	2.13 m, 2.19 m	27.3 t
12	1.79 overlap, 2.14 m	30.8 t	1.51 overlap, 1.75 m	26.1 t
13	—	52.4 s	—	53.8 s
14	—	43.9 s	—	45.5 s
15	1.63 m	31.4 t	1.79 m, 1.88 overlap	33.0 t
16	2.14 m, 2.38 overlap	34.9 t	2.25 m, 2.46, m	35.0 t
17	—	223.3 s	—	224.2 s
18	0.82 s	18.0 q	1.22 s	20.4 q
19	1.03 s	27.6 q	0.42 ABd (4.6), 0.92 ABd (4.6)	29.9 t
28	0.67 s	19.8 q	0.98 s	20.3 q
29	1.71 s	23.8 q	1.74 s	19.9 q
30	4.67 br s, 4.82 br s	114.7 t	4.78 br s, 4.89 br s	112.4 t

^{a)} Data were recorded on Bruker AM-400 MHz spectrometer (¹H, ¹³C); chemical shifts (δ) are in ppm; assignments were confirmed by ¹H-¹H COSY, HMQC and HMBC.

compounds existed the identical constituting rings A, B and C of which ring A has suffered an oxidative cleavage between C-3 and C-4. This assignment was confirmed by detailed analysis of the correlation spectroscopy (COSY), ^1H -detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) data for **1** (Fig. 1). Thus the structure of **1** was proposed as a lanostane derivative.

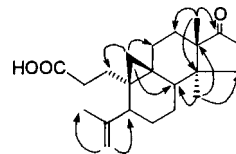
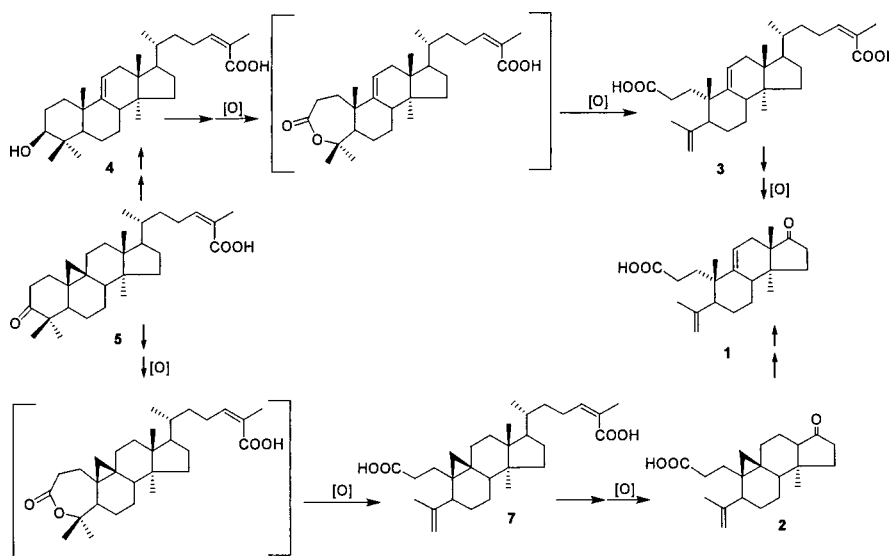
Two groups of HMBC correlations were observed to establish the structural features of compound **1**: one methyl singlet resonance at δ_{H} 0.82 corresponding to Me-18 showed cross peaks with a ketone carbon (C-17, δ_{C} 223.3), a methylene (C-12, δ_{C} 30.8), and two quaternary carbons (C-13, δ_{C} 52.4, and C-14, δ_{C} 43.9); another methyl singlet resonance at δ_{H} 0.67 (Me-28) correlated with a methine (C-8, δ_{C} 42.7), a methylene (C-15, δ_{C} 31.4), and also the two quaternary carbons (C-13, C-14). The above observations suggested that C-12, C-14 and C-17 all be attached to the carbon (C-13) bearing the methyl group, and another quaternary carbon (C-14) bearing the methyl group (Me-28) was situated between C-8 and C-15. HMBC correlations of H_2 -16 (δ_{H} 2.14/2.38) with C-14 and C-17, along with the ^1H - ^1H COSY spin system, H-15-H-16, permitted the closure of the D ring by linking C-16 with C-17. This connection was further supported by HMBC correlations of the H_2 -15 (δ_{H} 1.62/1.65) with C-14, C-16 (δ_{C} 34.9) and C-17. The compound (**1**) was, therefore, represented by octanorlanostane skeleton, with the absence of the side chain at C-17. Furthermore, the β -orientation of H-8 (δ_{H} 2.22) was deduced on the basis of rotating frame Overhauser enhancement spectroscopy (ROESY) correlations of Me-19/H-8, Me-18/H-8. In addition, H-5 (δ_{H} 2.02) showed no ROESY cross peaks with H-8 and Me-19 suggesting that H-5 was α -oriented.

Fig. 1. Key HMBC Correlations of Compound **1**

The electron impact (EI) spectrum of micranoic acid B (**2**) provided a molecular ion peak at m/z 344, the same as that of compound **1**. The ^1H - and ^{13}C -NMR spectral data of compound **2** (Table 1) were similar to those of **1**. The only significant differences included the presence of two up-field quaternary carbon signals at δ_{C} 22.6 and δ_{C} 29.2, and the absence of the C-9-C-11 double-bond (δ_{C} 118.2, 144.7) and tertiary methyl signal (Me-19, δ_{C} 29.6).

The differences between compounds **1** and **2** were established by analysis of ^1H -, ^{13}C -NMR and HMBC data. The ^1H -NMR spectrum of **2** exhibited the signals for two proton AB quartets at δ 0.42 and 0.92 (d, $J=4.6$ Hz) due to the characteristic C-19 cyclopropyl methylene protons. This was confirmed by the unusual far up-field quaternary carbon signals at δ 22.6 and δ 29.6 in the ^{13}C -NMR spectrum. Furthermore, H_2 -19 showed HMBC correlations (Fig. 2) with two methylenes: C-1 (δ_{C} 30.2), C-11 (δ_{C} 27.3), as well as with C-8 (δ_{C} 46.9) and C-9 (δ_{C} 22.6) of the ring B. The above spectroscopic evidence revealed that **2** differed from **1** in which the Me-19 was replaced by a cyclopropyl methylene group. Thus, the structure of compound **2** was elucidated as an octanorlanostane derivative.

The new compounds of **1** and **2** are highly degraded triterpenes in which fission of the C-17/C-20 bond is achieved *via* oxidation, while the closest analogue of micranoic acid B would appear to be buxaheptalactone (**6**), previously isolated from the roots of *Buxus papillosa*.⁸ Chart 1 outlined a potential biogenetic relationship existing between the five compounds. The precursor of the nucleus of micranoic acid A (**1**) can be envisaged as **4**. Oxidation of the hydroxyl group at C-3 of **4** yields a seven-membered lactone ring-A intermediate, which is converted to **3** by oxidative cleavage of ring A. Compound **1** could then be formed by oxidative fission be-

Fig. 2. Key HMBC Correlations of Compound **2**Chart 1. Proposed Biosynthetic Pathway for Compounds **1**–**5**

tween C-17 and C-20. In a similar manner, micranic acid B (**2**) could be biosynthesized from **5** as shown in Chart 1. As lanostanes and cycloartanes are biogenetic related,⁹ compounds **1** and **4** were then supposed to be derived from **2** and **5**, respectively.

This is the first report of the occurrence of these two unusual octanortriterpenoids isolated from the family Schisandraceae plant.

Experimental

General Melting points were measured on XRC-1 micro melting point apparatus and were uncorrected. Optical rotations were recorded on a Horiba SEPA-300 High Sensitive Polarimeter. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with HBr pellets. Mass spectra were obtained on a VG Auto Spec-3000 spectrometer, 70 eV for EI. 1D and 2D NMR experiments were performed on Bruker AM-400 and DRX-500 instruments with TMS as internal standard. Chemical shifts were (δ) expressed in ppm with reference to the solvent signals. Column chromatography was performed either on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China) or on silica gel H (10–40 μ , Qingdao Marine Chemical Inc., China). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in EtOH. HPLC separations were performed on a HP 1100 apparatus equipped with an RI detector and Zorbax SB-C-18 (Agilent, 9.4 mm \times 25 cm) column.

Plant Material The leaves and stems of *Schisandra micrantha* were collected in Weisan, Yunnan, People's Republic of China, in November 2001. A voucher specimen was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, the Chinese Academy of Sciences and was identified by Prof. Su-Gong Wu.

Extraction and Isolation The air-dried and powdered leaves and stems of *S. micrantha* (6.8 kg) were extracted three times with 70% aq. Me₂CO (3 \times 20 l) at room temperature for 24 h and filtered. The filtrate was evaporated *in vacuo* to give a residue which was suspended in water and partitioned successively with petroleum ether and EtOAc. The petroleum ether extract (75 g) was separated into four fractions through column chromatography over porous resin D101 (Chemical Factory of Tianjin University, Tianjin, China) by eluting with water and a gradient mixture of methanol–water from 70% to 100%. Fractions 3 (35 g) eluted by 80% MeOH/H₂O was subjected to column chromatography over Si gel and the column was eluted with petrol containing amounts of acetone (1:0, 19:1, 9:1, 8:2, 7:3) to give fractions 3a–f. Fraction 3b (6.5 g, eluted with 5% acetone) was chromatographed on Si gel using petrol–EtOAc (9:1) as elution to afford **5** (12 mg), as well as mixture of compounds **1** and **2**. The mixture was further chromatographed using RP-HPLC with 70% MeOH–H₂O (flow rate 3.0 ml/min) to yield 93 mg of **1** (t_R 30 min) and 73 mg of **2** (t_R 27 min). Fraction 3d was chromatographed over Si gel, eluting with CHCl₃–MeOH (1:0–100:1) to give **3** (25 mg) and **4** (41 mg).

Compound 1: Colourless needles from acetone; mp: 87–88 °C; $[\alpha]_D^{25} + 69.11^\circ$ ($c=0.25$, MeOH); UV (MeOH) λ_{max} (log ϵ): 203 nm (3.95); IR (KBr) cm^{-1} : 3550–3250, 1704 (br, COOH), 1732 (C=O), 1651, 1634 (C=C); EI-MS m/z (rel. int.%): 344 [M]⁺ (73), 329 (34), 311 (10), 301 (18), 283 (16), 271 (87), 245 (72), 227 (22), 203 (23), 185 (20), 173 (23), 163 (62), 145 (50), 133 (41), 119 (70), 105 (68), 91 (62); HR-ESI-MS m/z : 345.2427 ([M+H]⁺, Calcd for C₂₂H₃₃O₃, 345.2429); ¹H- and ¹³C-NMR: see Table 1.

Compound 2: Colourless prisms from acetone; mp: 80–81 °C; $[\alpha]_D^{24} + 121.74^\circ$ ($c=0.23$, MeOH); UV: (MeOH) λ_{max} (log ϵ): 202 nm (3.69); IR (KBr) cm^{-1} : 3560–3260, 1703 (br, COOH), 1720 (C=O), 1645 (C=C); EI-MS m/z (rel. int.%): 344 [M]⁺ (79), 329 (85), 311 (25), 301 (26), 271 (66), 245 (46), 163 (71), 133 (65), 119 (94), 105 (100), 91 (86), 81 (55), 67

(36); HR-ESI-MS m/z : 367.2236 ([M+Na]⁺, Calcd for C₂₂H₃₂O₃Na, 367.2249); ¹H- and ¹³C-NMR: see Table 1.

Compound 3: Colourless prisms from acetone; C₃₀H₄₆O₄, EI-MS m/z (rel. int.%): 470 [M]⁺ (6), 437 (5), 427 (9), 397 (26), 369 (15), 353 (17), 327 (17), 213 (35), 199 (37), 173 (64), 159 (82), 147 (100), 119 (82), 95 (100); ¹H-NMR (pyridine-*d*₅) δ : 5.52 (1H, d, $J=5.3$ Hz, H-11), 0.67 (3H, s, Me-18), 1.07 (3H, s, Me-19), 0.98 (3H, d, $J=6.3$ Hz, Me-21), 6.04 (1H, t, $J=7.0$ Hz, H-24), 2.13 (3H, s, Me-27), 0.75 (3H, s, Me-28), 1.80 (3H, s, Me-29), 4.95 (1H, s, H-30a), 4.89 (1H, s, H-30b); ¹³C-NMR (pyridine-*d*₅) δ : 29.9 (t, C-1), 34.0 (t, C-2), 176.8 (s, C-3), 148.2 (s, C-4), 49.3 (d, C-5), 28.2 (t, C-6), 26.8 (t, C-7), 42.9 (d, C-8), 142.7 (s, C-9), 42.7 (s, C-10), 118.8 (d, C-11), 37.9 (t, C-12), 47.4 (s, C-13), 44.2 (s, C-14), 33.3 (t, C-15), 26.7 (t, C-16), 51.2 (d, C-17), 14.9 (q, C-18), 26.9 (q, C-19), 36.4 (d, C-20), 18.4 (q, C-21), 27.2 (t, C-22), 27.1 (t, C-23), 142.9 (d, C-24), 128.7 (s, C-25), 170.7 (s, C-26), 21.6 (q, C-27), 18.4 (q, C-28), 23.6 (q, C-29), 114.1 (t, C-30).

Compound 4: White powders; C₃₀H₄₈O₃, EI-MS m/z (rel. int.%): 456 [M]⁺ (65), 441 (98), 423 (100), 395 (13), 369 (15), 316 (55), 301 (40), 273 (16), 215 (15), 203 (18), 187 (25), 175 (42), 160 (35), 147 (35), 135 (42), 119 (55), 95 (88); ¹H-NMR (pyridine-*d*₅) δ : 3.53 (1H, dd, $J=4.3$, 11.3 Hz, H-3), 5.28 (1H, br d, $J=6.3$ Hz, H-11), 0.70 (3H, s, Me-18), 1.09 (3H, s, Me-19), 1.00 (3H, d, $J=6.8$ Hz, Me-21), 6.03 (1H, t, $J=7.3$ Hz, H-24), 2.14 (3H, s, Me-27), 0.91 (3H, s, Me-28), 0.99 (3H, s, Me-29), 0.79 (3H, s, Me-30); ¹³C-NMR (pyridine-*d*₅) δ : 37.4 (C-1, t), 27.1 (C-2, t), 78.0 (C-3, d), 39.8 (C-4, s), 53.0 (C-5, d), 21.8 (C-6, t), 28.5 (C-7, t), 42.0 (C-8, d), 149.2 (C-9, s), 39.7 (C-10, s), 115.0 (C-11, d), 36.7 (C-12, t), 44.6 (C-13, s), 47.3 (C-14, s), 34.2 (C-15, t), 28.3 (C-16, t), 47.5 (C-17, d), 18.5 (C-18, q), 22.6 (C-19, q), 36.4 (C-20, d), 21.6 (C-21, q), 28.7 (C-22, t), 26.3 (C-23, t), 142.7 (C-24, d), 128.7 (C-25, s), 170.9 (C-26, q), 18.8 (C-27, q), 28.9 (C-28, q), 14.7 (C-29, q), 18.4 (C-30, q).

Compound 5: White powders; C₃₀H₄₆O₃, EI-MS m/z (rel. int.%): 454 [M]⁺ (65), 439 (39), 421 (14), 355 (30), 342 (15), 313 (50), 235 (30), 175 (42), 95 (100); ¹H-NMR (CDCl₃) δ : 0.89 (3H, s, Me-18), 0.55 (1H, d, $J=4.3$ Hz, H-19a), 0.76 (1H, d, $J=4.3$ Hz, H-19b), 0.87 (3H, d, $J=3.8$ Hz, Me-21), 6.07 (1H, t, $J=7.6$ Hz, H-24), 1.90 (3H, s, Me-27), 1.02 (3H, s, Me-28), 1.08 (3H, s, Me-29), 0.97 (3H, s, Me-30); ¹³C-NMR (CDCl₃) δ : 35.5 (t, C-1), 37.5 (t, C-2), 216.7 (s, C-3), 50.2 (s, C-4), 48.4 (d, C-5), 21.5 (t, C-6), 28.1 (t, C-7), 47.9 (d, C-8), 21.1 (s, C-9), 25.9 (s, C-10), 26.7 (t, C-11), 35.8 (t, C-12), 45.3 (s, C-13), 48.7 (s, C-14), 33.4 (t, C-15), 26.9 (t, C-16), 52.2 (d, C-17), 18.1 (q, C-18), 29.6 (t, C-19), 36.0 (d, C-20), 18.1 (q, C-21), 32.7 (t, C-22), 25.9 (t, C-23), 147.4 (d, C-24), 125.8 (s, C-25), 173.4 (s, C-26), 20.5 (q, C-27), 19.3 (q, C-28), 22.2 (q, C-29), 20.5 (q, C-30).

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