Chemical constituents from *Munronia sinica* and their bioactivities

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Abstract: Two new minor constituents, musinisins A (1) and B (2), together with five known compounds (3–7), were isolated from the aerial parts of *Munronia sinica*. Their structures were established by means of spectroscopic methods and the absolute stereochemistry of 1 was determined by single crystal X-ray experiment. Compound 4 showed antiangiogenic activity evaluated by a zebrafish model and apoptosis-inducing effect on A549 lung cancer cells.

Keywords: *Munronia sinica*, chemical constituent, musinisin, antiangiogenic activity

Introduction
The genus of *Munronia* (Meliaceae) is composed of about 15 species in the world, and 8 species of them were distributed in China.1 Up to now, some phytochemical studies have been reported on *M. delavayi*2–3 and *M. henryi*4–9, and tetranortriterpenoids and triterpenoids are their main components. Especially, tetranortriterpenoids exhibit chemical defensive function in nature, such as antibacterium, antifungal,2 and antifeeding3 activities. *M. sinica* Diels grows in Jinfoshan of Sichuan, which is low subshrubs and an endemic species of China. To the date, no chemical research has been published about this species. In this paper, we reported the isolation and structure elucidation of two new compounds, musinisins A (1) and B (2), together with five known compounds, including [glucosy] acetoxy-14,14-dienolide (4),11 cannabiside D (5),12 corchoionoside C (6),13 (+)-3-oxo-α-ionyl glucoside (7).14 Compounds 1–7 were tested for their antiangiogenic activities using a zebrafish and the antiproliferative activities using A549 lung cancer cells.

Results and Discussion
A 70% aqueous acetone extract prepared from the leaves and stems of *M. sinica* was partitioned between EtOAc and H2O. The EtOAc layer was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, and RP-18, to afford compounds 1–7. Their structures were shown in Figure 1, and 1H and 13C NMR spectroscopic data were listed in Tables 1 and 2.

Figure 1. The structures of compounds 1–7
Table 1. The NMR (DMSO-d$_6$, δ in ppm, J in Hz) data of 1

| position | δC  | δH  | position | δC  | δH  |
|----------|-----|-----|----------|-----|-----|
| 1        | 175.7 | C2 | 3'        | 18.1 | CH$_3$ | 0.79 (d, 6.6) |
| 2a       | 31.3 | CH$_3$ | 1       | 102.8 | CH | 4.13 (d, 7.8) |
| 2b       | 26.0 | CH$_3$ | 2        | 73.9 | CH | 2.90 (t, 8.5) |
| 3        | 44.7 | CH | 2'        | 76.9 | CH | 3.08 (m) |
| 4        | 75.9 | CH | 3'        | 70.3 | CH | 3.00 (m) |
| 5        | 21.4 | CH$_2$ | 4        | 76.9 | CH | 3.03 (m) |
| i-pr     | 26.7 | CH | 5'        | 61.3 | CH$_2$ | 3.63 (br, d, 11.3) |
| 1'       | 19.4 | CH | 2''       | 3.40 | (dd, 11.3, 4.4) |

Compound 1 was obtained as colorless crystals. Its molecular formula, C$_{26}$H$_{32}$O$_{15}$, was established on the basis of HRESIMS analysis ([M + H]$^+$, m/z 321.1543; calcld 321.1549) and its $^1$H and $^1$C NMR spectra. Analysis of the $^1$H and $^1$C NMR (Table 1), HSQC and HMBC spectra of 1 revealed the presence of 14 carbons, including one carboxylic acid group (δC 175.7), one i-pr group (δC 26.7), one methyl (δC 19.4), one methylene (δC 31.3), two methines (δC 44.7 and 75.9), and one hexose (δC 102.8, 76.9, 76.9, 73.9, 70.3, 61.3). In the $^1$H-$^1$H COSY spectrum (Figure 2), the correlation of H-2/H-3/H-4/Me-5 was observed, which established the direct connections of C-2, C-3, C-4 and C-5. The i-pr group locating at C-3 (δC 44.7) was determined by the HMBC correlations of H-3 (δH 1.87, m) with the carbon signals at δC 27.6 (C-1’) and 19.4 (C-2’). The linkage position of the hexose moiety was located at C-4 (δC 75.9) of its aglycone by the HMBC correlations of the anomeric proton at δH 4.13 with C-4. The anomeric center of the sugar moiety was determined to be β-configuration from the large $^3$J$_{HH}$ value (J = 7.8 Hz). Furthermore, we got a suitable crystal and the X-ray experiment was conducted using an anomalous dispersion with copper radiation (Figure 3), which not only determined the hexose to be D-glucose, but also established the absolute stereochemistry of 1. In combination with IUPAC sequence rule, the configuration of C-3 and C-4 were deduced as R and S, respectively. Thus, the structure of 1 was established as (3R,4S)-3-isopropyl-4-hydroxypentanoic acid 4-Oβ-D-glucopyranoside and given the name of musinisin A. The plausible biogenetic pathway of 1, starting from 3-isopropyl-4-methylcyclohexene was also discussed (Scheme 1).

Compound 2 was isolated as yellow powder with [α]$_D^{20}$ + 54.86 (c = 1.4, MeOH). The molecular formula, C$_{26}$H$_{32}$O$_{15}$, was deduced by HRESIMS ([M + Cl]$^+$) at m/z 395.1106; calcld 395.1108), with five degrees of unsaturation. The $^1$H NMR spectrum (Table 2) showed signals for two methoxyl groups. Comparison of the 1D and 2D NMR data of 2 with those of known compound glucoacetylsyringone (3) revealed that compound 2 was structurally similar to 3. The differences were resulted from the appearance of an oxygenated methine signal (δH 68.2, CH; δC 4.6, m), and the lack of a carbonyl signal in 2. This indicated that the carbonyl group at C-7 in 3 was replaced by an oxygenated methine group in 2, which was confirmed by the HMBC correlations of H-2 (δH 6.62), H-6 (δH 6.62) and Me-8 (δH 1.30) with C-7 (δC 68.2) and the $^1$H-$^1$H COSY correlation of H-7/Me-8 (Figure 2). Acid hydrolysis of 2 (5 mg) in 2 M HCl (3 mL) provided a D-glucose and an aglycone (C$_{10}$H$_{14}$O$_{6}$), an amorphous powder, [α]$_D^{20}$ +4.63). The glycosyl group was detected by TLC comparison with authentic sample using CHCl$_3$-MeOH (8:2) as a developing system (R$_f$ = 0.16). The anomeric center of the glucose moiety

![Figure 2. Key HMBC (→) and $^1$H-$^1$H COSY (→→) correlations of 1 and 2](image)

![Figure 3. X-ray structure of 1 showing absolute configuration](image)

Scheme 1. Hypothetical biogenetic pathway of 1
was determined to be β-configuration from the large \( J_{H4,1} \) value (7.3 Hz). As the aglycone showed a negative optical rotation value that was opposite to that of (1R)-1-phenyl-1-propanol,\(^2\) the absolute configuration at C-7 of 2 was suggested to be S. Thus, the structure of 2 was established and given the trivial name musinin B.

The antiangiogenic activities of compounds 1, 2, 4, 5 and 7 were evaluated using a zebrafish model, in terms of the inhibition ratio, **p < 0.01,

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(10 mg), 2 (15 mg), 3 (8 mg), 4 (20 mg), 5 (18 mg), 6 (18 mg), and 7 (13 mg).

**Musinisin A (1):** colorless crystal; mp 150–151 °C; [α]D20 = 23.95 (c 0.35, MeOH); IR (KBr) νmax 3490, 3347, 2985, 2901, 1698, 1430, 1403, 1282, 1201, 1105, 1015, 939, 637 cm⁻¹; H and ¹³C NMR data see Table 1; negative ion ESI-MS m/z 321 [M – H]; HRESIMS m/z 321.1543 (caled for C₄H₂O₇M [M – H], 321.1540).

**Musinisin B (2):** yellow powder; [α]D20 = 54.86 (c 0.14, MeOH); IR (KBr) νmax 3546, 3476, 3396, 3277, 2975, 2979, 2914, 1598, 1462, 1425, 1327, 1130, 1068 cm⁻¹; H and ¹³C NMR data see Table 2; negative ion ESI-MS m/z 395 [M + Cl]⁺; HRESIMS m/z 395.1100 (caled for C₉H₁₄O₂Cl [M + Cl]⁺, 395.1108).

**Crystallographic data for 1:** C₁₇H₂₀O₅, M = 322.36, orthorhombic, space group P2₁2₁2₁, a = 7.2561(4) Å, b = 9.5748(5) Å, c = 23.2846(14) Å, α = β = γ = 90°, V = 1617.71(16) Å³, Z = 4, d = 1.373 g cm⁻³, crystal size 0.30 mm × 0.52 mm × 0.54 mm, was used for measurements on a Bruker APEX DUO diffractometer with a graphite monochromator, Cu Kα radiation. The total number of independent reflections measured was 2753, of which 2740 were observed (|F|² ≥ 2σ(|F|²)). Final indices: R₁ = 0.0363, wR₂ = 0.0920 (w = 1/[σ²(|F|²)]). The crystal structure of 1 was solved and refined by the direct method SHELX-97 (Sheldrick, G. M. University of Gottingen: Gottingen, Germany, 1985). Crystallographic data for the structure of 1 has been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 853392). Copies of this can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

**Acid Hydrolysis of 2.** A solution of 2 (5 mg) in 2 M HCl (3 mL) was heated in a water bath at 70 °C for 6 h. After cooling, the reaction mixture was neutralized with NaHCO₃ and extracted with CHCl₃. Through TLC comparison with authentic sample using CHCl₃-MeOH (8:2) as a developing system, D-glucose was detected in the water layer (Rf = 0.16). The aqueous solution was further concentrated to dryness and subjected to a silica gel chromatography eluting with CHCl₃-MeOH (9:1) to give D-glucose (1 mg), [α]D²⁰ = +40 (c 0.2, MeOH). The CHCl₃ fraction was further subjected to a silica gel chromatography eluting with petroleum ether-EtOAc (4:1) to afford the aglycone (1.2 mg), [α]D²⁰ = –4.63 (c 0.17, MeOH).

**Antiangiogenesis Bioassay.** Stock solutions (10 mg/mL) of all samples were prepared by dissolving the test compounds in 100% DMSO. These solutions were diluted in sterile salt water (5 mM NaCl, 0.17 mM KCl, 0.4 mM CaCl₂, and 0.16 mM MgSO₄) to obtain solutions with the test compounds dissolved in 0.1% DMSO. These solutions were aliquot into 96-well plates, and embryos at 24 hpf (hours post fertilization) were also transferred randomly into the above wells. After 24 h of treatment, the intersegmental vessels of embryos were visualized with green fluorescent protein labeling and endogenous alkaline phosphatase staining. The antiangiogenic activities of compounds were calculated from the inhibition ratio of angiogenesis. PTK787 was used as the positive control.

**Antiproliferative Bioassay.** A549 lung cancer cells were cultured in RPMI 1640 medium at 37 °C with 5% CO₂ and 95% air, supplemented with 10% (v/v) bovine calf serum and 80 U/ml penicillin/streptomycin. The cells were seeded onto 96-well plates and treated with compounds at 3, 10, 30 and 100 μg/mL for 48 h, respectively. Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay. The light absorption was measured at 570 nm using Spectra MAX 190 microplate spectrophotometer (GMI Co., USA). Inhibition rate was calculated by the formula:

\[
\text{Inhibition (\%)} = 100\% - (\text{OD}_{\text{treatment}} - \text{OD}_{\text{blank}})/(\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}) \times 100\%
\]

The cells were incubated with compounds (100 μg/mL) for 48 h, and stained with 0.1 mg/mL of acridine orange (AO) at room temperature for 5 min. Then the cells were observed and photographed using the fluorescent stereo microscope (Olympus, Japan).

**Electronic Supplementary Material**

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s13659-012-0001-8 and is accessible for authorized users.

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**References**

[1] Yunnan Institute of Botany, Flora Yunnanica; Science Press: Beijing, 1977; Tomus. 1. pp 214–216.
[2] Lin, B. D.; Chen, H. D.; Liu, J.; Zhang, S.; Wu, Y.; Dong, L.; Yue, J. M. Phytotoxicology 2010, 71, 1596–1601.
[3] Cai, X. H.; Luo, X. D. Chin. J. Chem. 2007, 25, 986–988.
[4] Wu, B.; Li, W.; Lin, W. H.; Gao, H. Y.; Wu, L. J.; Jin, Z. S. Nat. Prod. Bioprospect. 2012, 2, 76–80.
[5] Cai, X. H.; Luo, X. D. Nat. Prod. Bioprospect. 2012, 2, 79.
[6] Zhang, H. P.; Bao, G. H.; Wang, H. B.; Qin, G. W. Nat. Prod. Res. 2004, 18, 415–419.
[7] Qi, S. H.; Wu, D. G.; Ma, Y. B.; Luo, X. D. Asian Nat. Prod. Res. 2003, 5, 215–221.
[8] Qi, S. H.; Chen, L.; Wu, D. G.; Ma, Y. B.; Luo, X. D. Tetrahedron 2003, 59, 4193–4199.
[9] Qi, S. H.; Wu, D. G.; Chen, L.; Ma, Y. B.; Luo, X. D. J. Agr. Food Chem. 2003, 51, 6949–6952.
[10] Itoh, T.; Ninomiya, M.; Nozawa, Y.; Oketsu, M. Bioorg. Med. Chem. 2010, 18, 7052–7057.
[11] Connelly, J. D.; Labbe, C.; Rycroft, S.; Taylor, D. A. H. J. Chem. Soc. Perkin Trans. 1 1979, 2959–2964.
[13] Yoshikawa, M.; Shimada, H.; Saka, M.; Yoshizumi, S.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1997**, *45*, 464–469.
[14] Cui, B.; Nakamura, M.; Kinjo, J.; Nohara, T. *Chem. Pharm. Bull.* **1993**, *41*, 178–182.
[15] IUPAC Nomenclature of Organic Chemistry, Pergamon: New York, 1979, Sections A–H. Recommendation for section A, Spiro hydrocarbons.

[16] Ishikawa, T.; Kondo, K.; Kitajima, J. *Chem. Pharm. Bull.* **2003**, *51*, 32–39.
[17] Li, X. L.; Li, Y.; Wang, S. F.; Zhao, Y. L.; Liu, K. C.; Wang, X. M.; Yang, Y. P. *J. Nat. Prod.* **2009**, *72*, 1001–1005.
[18] He, Q. X.; Zhu, X. S.; Shi, M.; Zhao, B. X.; Zhao, J.; Zhang, S. L.; Miao, J. Y. *Bioorg. Med. Chem.* **2007**, *15*, 3889–3895.