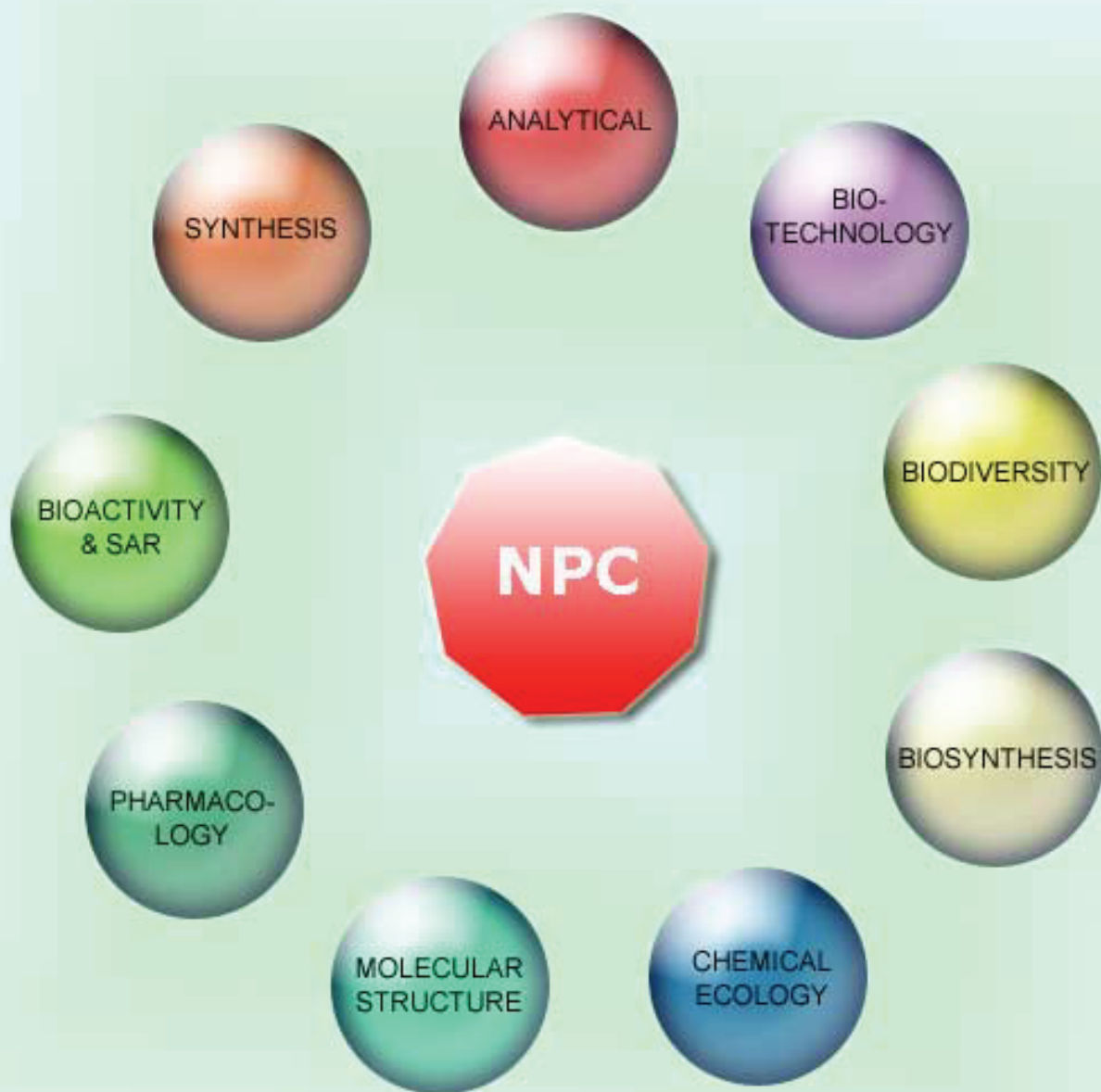


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(±)-Perforison A, A Pair of New Chromone Enantiomers from *Harrisonia perforata*

Wen-Juan Yuan^{a,b}, Wen-Fen Gao^d, Jia-Hui Zhang^c, Pei Cao^c, Yu Zhang^c, Duo-Zhi Chen^c, Shun-Lin Li^c, Ying-Tong Di^{c,*} and Xiao-Jiang Hao^{a,b,*}

^aXinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, 830011, Xinjiang, P. R. China

^bUniversity of Chinese Academy of Sciences, Beijing, 100049, P. R. China

^cState Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, Yunnan, P. R. China

^dYunnan Institute for Food and Drug control, Kunming, 650201, Yunnan, P. R. China

haoxj@mail.kib.ac.cn, diyt@mail.kib.ac.cn

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Dedicated to Prof. Lisheng Ding on the occasion of his 60th birthday.

(+)-Perforison A and (–)-perforison A, a new pair of chromone enantiomers, along with four known compounds, were isolated from the leaves and stems of *Harrisonia perforata*. Their structures and absolute configurations were determined on the basis of extensive analysis of spectroscopic data and electronic circular dichroism (ECD) calculations. The cytotoxic activities *in vitro* of these compounds were evaluated, but none showed significant activity.

Keywords: *Harrisonia perforata*, Simaroubaceae, Chromone, Electronic circular dichroism, Perforison A.

The genus *Harrisonia* (Simaroubaceae) comprises four species and is mainly distributed in south-east Asia, Africa and Oceania [1]. *H. perforata* (Blanko) Merr. is the only species growing in China [1], and has been used as a folk medicine against malaria and boils [2]. Several chromones [3-6], limonoids [4, 7-11], quassinoids [12, 13], and polyketides [14] have been isolated from *H. perforata*. As part of our effort to search for biologically active constituents of this species [7, 12, 15], a new pair of chromone enantiomers, perforisone A, along with four known compounds, peuceenin-7-methyl ether (2) [16], umtatin (3) [17], greveichromenol (4) [17], and saikochromone A (5) [18], were isolated from the leaves and stems of the title plant. Perforisone A was resolved to (+)-perforisone A (1a) and (–)-perforisone A (1b) by HPLC using a chiral column, and their absolute configurations were determined by computational methods via calculation of their electronic circular dichroism (ECD) spectra. Herein, we describe the structural elucidation and biological evaluation of the isolated compounds.

Perforisone A (1) was obtained as a colorless solid and had the molecular formula C₁₆H₁₈O₆ by the positive HRESIMS (*m/z* 329.0996 [M + Na]⁺, calcd: 329.0996), requiring 8 degrees of unsaturation. Its IR spectrum showed absorption bands for hydroxyl (3435 cm⁻¹), carbonyl (1653 cm⁻¹), and aromatic (1582, 1559, 1490 and 1457 cm⁻¹) groups. In the ¹H NMR spectrum, two aromatic H-atoms (δ_H 6.32, s, 6.58, s), one spin system (δ_H 2.95, dd, *J* = 13.1, 7.6 Hz, δ_H 2.86, dd, *J* = 13.1, 6.8 Hz, δ_H 4.34, t, *J* = 7.1 Hz), one singlet methyl (δ_H 1.80, s), together with one methoxyl (δ_H 3.89, s) were observed (Table 1). The ¹³C NMR (DEPT) spectrum exhibited 16 carbon resonances, assigned to one aryl ketocarbonyl (δ_C 184.2), seven sp² quaternary carbons (δ_C 171.6, 165.5, 160.0, 158.2, 148.7, 111.1, 106.1), one sp² methylene (δ_C 111.3), two sp² methines (δ_C 106.9, 91.0), two sp³ methylenes (δ_C 61.4, 29.4), one methoxy (δ_C 56.6), and one methyl (δ_C 17.3). These groups accounted for six out of eight indices of unsaturation.

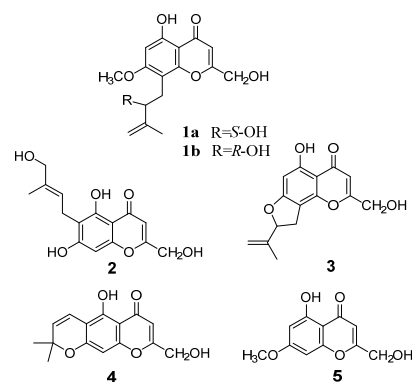


Figure 1: Structures of compounds 1-5.

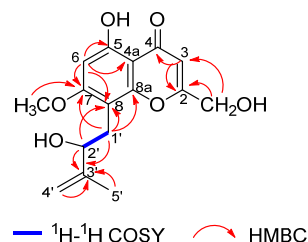


Figure 2: Selected ¹H-¹H COSY (bold) and key HMBC correlations (Arrow, H→C) of 1.

From the ¹H-¹H COSY correlation of H₂-1' and H-2', combined with the HMBC correlations of Me-5'/C-3', H-2'/C-3', H₂-1'/C-3', and H-2'/C-3', the side chain was assigned as a 2-hydroxy-3-methyl-3-butenyl group (Figure 2). The attachment of this unit at C-8 was confirmed by HMBC correlations from H₂-1' to C-7, C-8, and C-8a. The NMR data of 1 were similar to those of perforamone C [6], except for the presence of a hydroxymethyl group instead of a methyl group in the latter. The position of the hydroxymethyl group

Table 1: ^1H and ^{13}C NMR data (600 and 125 MHz, resp.) of compound **1** in MeOD.

No	^{13}C	^1H
2	171.6	
3	106.9	6.32 (1H, s)
4	184.2	
4a	106.1	
5	158.2	
6	91.0	6.58 (1H, s)
7	165.5	
8	111.1	
8a	160.0	
1'	29.4	2.95 (1H, dd, $J = 13.1, 7.6$) 2.86 (1H, dd, $J = 13.1, 6.8$) 4.34 (1H, t, $J = 7.1$)
2'	76.0	
3'	148.7	
4'	111.3	4.61 (1H, br s) 4.60 (1H, br s)
5'	17.3	1.80 (3H, s)
2- CH_2OH	61.4	4.48 (2H, s)
7- OCH_3	56.6	3.89 (3H, s)

at C-2 was supported by HMBC correlations of 2- CH_2OH (δ_{H} 4.48) to C-2 (δ_{C} 171.6) and C-3 (δ_{C} 106.9). Thus, the structure of **1** was established as 5-hydroxy-7-methoxy-2-hydroxymethyl-8-(2-hydroxy-3-methyl-3-butenyl)chromone. It is worth noting that the CD spectrum of **1** is almost a straight line, implying a racemic nature of **1**, which was in accordance with its optical activity ($[\alpha]_{\text{D}}^{25} = 0$). Chiral resolution of **1** was achieved on a CHIRALPAK IC column; the enantiomers **1a** and **1b** were well separated.

Compounds **1a** and **1b** had opposite optical rotations (**1a**: $[\alpha]_{\text{D}}^{25} = +21.5$; **1b**: $[\alpha]_{\text{D}}^{25} = -24.4$) and Cotton effects in their CD spectra. The final assignment of absolute configurations of **1a** and **1b** was achieved by comparison of the calculated electronic circular dichroisms (ECDs) with experimental data [19]. In detail, their ECDs calculated at the b3lyp/6-311++g(d,p)//b3lyp/6-311++g(d,p) level showed good agreement with the experimental spectra (Figure 3).

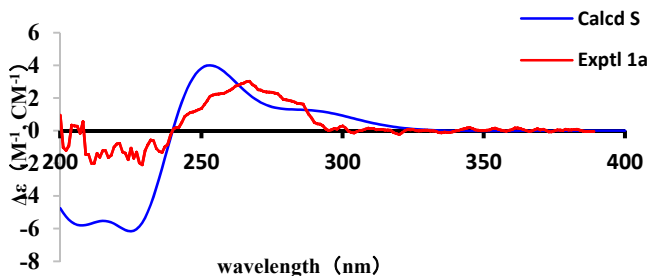


Figure 3: Calculated and experimental ECD spectra of **1a** (blue, at the B3LYP-SCRF (PCM)/6-311+G(2d,p)//B3LYP/6-311+G(2d,p) level in MeOH; red, experimentally observed in MeOH).

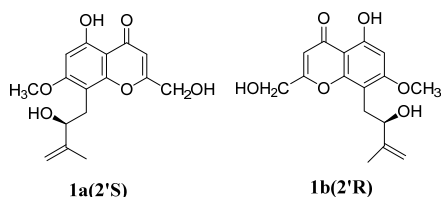


Figure 4: Absolute configuration for **1a** (2'S) and **1b** (2'R).

From the above evidence, the absolute stereochemistry for **1a** (2'S) and **1b** (2'R) were unambiguously determined as shown (Figure 4).

All compounds were evaluated for their *in vitro* growth inhibitory effects against five human cancer cell lines, namely, HL-60 (human

promyelocytic leukemia cell line), SMMC-7721 (human hepatocellular carcinoma cell line), A-549 (human lung cancer cell line), MCF-7 (human breast cancer cell line), and SW480 (colorectal cancer cell line) using MTS method [20]. However, none of them exhibited significant bioactivity ($\text{IC}_{50} > 40 \mu\text{M}$).

Experimental

General experimental procedures: Optical rotations were measured with a Jasco P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for IR spectra as KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker AV-500 spectrometer with TMS as internal standard. HRESIMS were obtained on a triple quadrupole mass spectrometer. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with either a Waters X-Bridge Prep Shield RP18 ($10 \times 150 \text{ mm}$) column or a CHIRALPAK IC ($10 \times 250 \text{ mm}$) column. Column chromatography (CC) was performed using silica gel (100-200 mesh and 300-400 mesh, Qingdao Marine Chemical, Inc., Qingdao, P. R. China) and Sephadex LH-20 (40-70 μm , Amersham Pharmacia Biotech AB, Uppsala, Sweden). MCI gel CHP 20P (75-150 μm , Mitsubishi Chemical Industries, Tokyo, Japan)

Plant material: The dried leaves and stems of *H. perforata*, collected in Hainan Province, P. R. China, in October 2014, were identified by Prof. Yanhui Fu (Hainan Normal University). A voucher specimen (HXJ20141208) was deposited at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Science (CAS).

Isolation and purification: The air-dried powdered leaves and stems (19.0 kg) were extracted 3 times (4, 3, and 3 h) with 95% ethanol (20 L \times 3) at room temperature and concentrated *in vacuo* at 60°C to give a residue (2.5 kg). This was dispersed in water (4.0 L) and extracted with light petroleum and ethyl acetate in turn. The light petroleum and ethyl acetate portions (1 kg) were combined and then passed over a silica gel column, eluting with a gradient of light petroleum-acetone (from 1:0 to 0:1), to yield 5 major fractions (1-5). Subsequently, Fr.3 (200 g) was separated over an MCI column (MeOH- H_2O from 3:7 to 10:0) to obtain 4 sub-fractions (3A-3D). Fr.3B (20 g) was then chromatographed on a silica gel column eluted with chloroform-acetone (100:1 to 5:1), to afford 5 sub-fractions (3B1-3B5). Fr.3B2 (2.2 g) was purified by Sephadex LH-20 (methanol) to obtain **4** (55 mg) and a major fraction (Fr.3B21). Fr.3B21 (200 mg) was separated by semi-preparative HPLC ($\text{CH}_3\text{CN}-\text{H}_2\text{O}$, 4:6) to give **2** (3 mg) and **3** (5 mg). Fr.3B2 (2.2 g) was purified by Sephadex LH-20 (methanol) to obtain Fr.3B22 and Fr.3B24. Fr.3B22 (500 mg) was then chromatographed on a silica gel column eluted with chloroform-acetone (25:1) to obtain **1** (6 mg). Compound **1** was finally subjected to chiral HPLC to afford **1a** ((+)-perforisone A, 1.1 mg) and **1b** ((-)-perforisone A, 2.3 mg) (*n*-hexane/isopropanol, 85:15). Fr.3B24 (200 mg) was separated by semi-preparative HPLC ($\text{CH}_3\text{CN}-\text{H}_2\text{O}$, 5:5) to give **5** (10 mg). The purities of compounds **1-5** were 95%, as determined by TLC and HPLC.

Calculation methodology: Calculations were performed using the Gaussian 03 program package. Geometries were fully optimized with the density functional theory methods of B3LYP at the 6-31+G* level. Harmonic vibrational frequency of the conformation was then calculated at the same level to confirm their stability. Lastly, the “self-consistent reaction field” method (SCRF) was employed to perform the ECD calculation of compounds **1a** and **1b** in methanol at the B3LYP-SCRF/6-311++G (d, p) level.

(±)-Perforison A

Colourless solid.

[[α]_D²⁵: + 21.5 (c 0.18, MeOH); **1a**]; [[α]_D²⁵: -24.4 (c 0.16, MeOH); **1b**]UV (MeOH) λ_{\max} (log ϵ): 312 (3.78), 292 (3.91), 258 (4.16), 232 (4.24) nmIR (KBr) ν_{\max} : 3435, 1669, 1653, 1624, 1582, 1559, 1540, 1490, 1457, 1411 cm⁻¹.¹H and ¹³C NMR: Table 1.ESI-MS (*m/z*): 329 [M + Na]⁺, 635 [2M + Na]⁺;HR-ESI-MS: *m/z*: 329.0996, [M + Na]⁺ (calcd for C₁₆H₁₈O₆Na, 329.0996).**Supplementary data:** ¹H NMR, ¹³C NMR, 2D NMR spectra data of new compounds can be found in the online version.**Acknowledgments** - This research was supported financially by grants from the National Science Foundation of China (21432010, 21372228, and 81573323), Technological leading talent project of Yunnan (2015HA020), Central Asian Drug Discovery and Development Center of Chinese Academy of Sciences (CAM201402, CAM201302), the Science Foundation of Yunnan (2014A050), and the Xibuzhiguang Project (grant to Ying-Tong Di).**References**

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