New monoterpenoid alkaloids from the aerial parts of *Uncaria hirsuta*

Jun-Feng Jia\(^a\),\(^b\), Yuan Zhang\(^a\), Xiao-Jun Huang\(^a\),\(^b\), Sheng-Yuan Zhang\(^a\), Hai-Yan Tian\(^a\),
Lei Wang\(^a\),\(^b\)* and Wen-Cai Ye\(^a\),\(^b\)*

\(^a\)College of Pharmacy, Institute of Traditional Chinese Medicine & Natural Products, Jinan University,
Guangzhou 510632, P.R. China; \(^b\)JNU-HKUST Joint Laboratory for Neuroscience & Innovative Drug Research,
Jinan University, Guangzhou 510632, P.R. China

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To investigate the chemical constituents of medicinal plant *Uncaria hirsuta*, three new monoterpenoid alkaloids, named hirsutanines A–C (1–3), were isolated. Their structures with absolute configurations were elucidated by means of NMR, X-ray diffraction and CD analysis. Compound 3 was the first dimeric monoterpenoid alkaloid obtained from genus *Uncaria*.

**Keywords:** Rubiaceae; *Uncaria hirsuta*; alkaloids

1. Introduction

The plant *Uncaria hirsuta* Haviland (Rubiaceae) is mainly distributed in southern China, in areas such as Guangdong, Fujian provinces and the Guangxi Zhuang autonomous region. The stems of *U. hirsuta* have been used as a traditional Chinese medicine for the treatment of convulsions, numbness and hypertension (Chang et al. 1979; Yu et al. 1999). Although regarded as a commercial source of Ramulus Uncariae Cum Uncis (Chinese Pharmacopoeia Commission 2010), only a few alkaloids, flavones and triterpenoids had been reported from *U. hirsuta* (Wu & Chan 1994; Xin et al. 2008, 2009, 2011). The potential medicinal importance and our interest in the chemistry of alkaloids prompted us to investigate the chemical constituents of the title plant, which led to the isolation of three new monoterpenoid alkaloids (1–3) including a rare dimeric derivative (Figure 1). This article describes the isolation and structural elucidation of these new alkaloids. The absolute configurations of the new compounds were determined by X-ray diffraction, CD spectra and molecular modelling calculation.

2. Results and discussion

Hirsutanine A (1) was isolated as colourless prism. The molecular formula of 1 was deduced as C\(_{11}\)H\(_{15}\)NO\(_3\) by the quasi-molecular ion peak at \(m/z\) 210.1127 [M + H]\(^+\) (calcld for C\(_{11}\)H\(_{16}\)NO\(_3\) 210.1125) in the HR-ESI-MS. The UV spectrum displayed absorption maxima at 207 and 285 nm. The IR spectrum revealed the presence of amino (3390 cm\(^{-1}\)) and carboxyl (1672 cm\(^{-1}\)) groups. The \(^1\)H NMR spectrum of 1 revealed the signals for an amino proton [\(\delta_H\) 8.11 (1H, dd, J = 4.8, 4.8 Hz)], two oxygenated protons [\(\delta_H\) 4.16 (1H, m) and 4.24 (1H, m)], a methoxyl group [\(\delta_H\) 3.26 (3H, s)], an olefinic proton [\(\delta_H\) 7.39 (1H, dd, J = 6.0, 1.9 Hz)], a terminal vinyl group [\(\delta_H\) 5.26 (1H, td, J = 17.4, 8.7 Hz), 5.13 (1H, dd, J = 9.0, 3.6 Hz) and 5.22 (1H, dd, J = 17.4, 3.0 Hz)] and three aliphatic protons [\(\delta_H\) 2.74 (1H, m), 1.67 (1H, m), 1.46 (1H, m)].

*Corresponding authors. Email: chyewc@gmail.com; cpuwanglei@126.com

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The $^{13}$C NMR spectrum of 1 displayed 11 signals including 3 methylenes, 5 methines, 2 quaternary carbons, and a methoxy group, which suggested that 1 possessed a monoterpenoid alkaloid skeleton. With the aid of 1D and 2D NMR experiments, all the $^1$H and $^{13}$C NMR signals of 1 were assigned. Comparison of the NMR data of 1 with those of the aglycone of sweroside (Koichi et al. 1995; Zou et al. 2008) revealed that most signals were similar except for the oxygen atom between C-1 and C-3 was replaced by an NH unit in 1, which was confirmed by the correlation between H-2 ($\delta_1 8.11$) and H-3 ($\delta_1 7.39$) in the $^1$H–$^1$H COSY spectrum and the HMBC cross-peak between H-2 ($\delta_1 8.11$) and C-4 ($\delta_C 92.8$). In addition, the HMBC correlations between H-3 ($\delta_1 7.39$) and C-1 ($\delta_C 83.5$); C-5 ($\delta_C 134.3$), between H-1 ($\delta_1 4.16$) and C-8 ($\delta_C 53.6$); C-11 ($\delta_C 166.0$), and between H-7 ($\delta_1 4.24$) and C-11 ($\delta_C 166.0$) and between H-10 ($\delta_1 5.13, 5.22$) and C-9 ($\delta_C 41.5$) established the planar structure of 1 (see Supporting Information, Figure S30). The relative stereochemistry of 1 could be deduced from the NOESY spectrum. The NOE correlations between H-5 and H-9, between H-1 and H-8 (see Supporting Information, Figure S31) established the relative configuration of 1. Fortunately, the single crystals suitable for X-ray diffraction were obtained. The structure of 1 was further confirmed by X-ray diffraction analysis (CCDC 980271, Figure 2). To determine the absolute configuration of 1, a comparison between the experimental and calculated CD spectra by molecular modelling calculation was applied. The measured CD spectrum of 1 revealed negative Cotton effect at 285 nm ($\Delta \varepsilon = -15.3$) and positive one at 220 nm.
which were in accordance with the calculated CD spectrum for the isomer with 1S, 5S and 9R configurations (Figure 3). Thus, the structure of 1 was determined and named as hirsutanine A.

Hirsutanine B (2) was obtained as amorphous powder. The molecular formula of 2 was determined as C_{10}H_{13}NO_{3} by its HR-ESI-MS at m/z 196.0970 [M + H]^+ (calcd for C_{10}H_{14}NO_{3} 196.0969). The UV and IR spectra of 2 displayed similar absorptions to those of 1. Detailed analysis of $^1$H and $^{13}$C NMR spectra of 2 and 1 revealed their considerable structural similarity. The main difference was that 2 was substituted by a hydroxyl group instead of a methoxyl group at C-1 position in 1. The $^1$H–$^1$H COSY correlation between H-1 (δ_H 4.49) and OH (δ_H 5.96), and the HMBC correlation between OH (δ_H 5.96) and C-1 (δ_C 75.5)/C-9 (δ_C 44.0) further suggested that the hydroxyl group was attached to the C-1 position (see Supporting Information, Figure S30). Analysis of the NOESY spectrum of 2 revealed that the relative configuration of C-1, C-5 and C-9 in 2 was identical to that in 1. The CD spectrum of 2 displayed the same Cotton effects as 1, indicating the presence of 1S, 5S and 9R configurations (Figure 3). Assignment of the $^1$H and $^{13}$C NMR data of 2 was completed through the aid of $^1$H–$^1$H COSY, HSQC, HMBC and NOESY data. Consequently, the structure of 2 was determined and named as hirsutanine B.

Hirsutanine C (3) was also obtained as amorphous powder. The quasi-molecular ion at m/z 373.1755 [M + H]^+ in its HR-ESI-MS indicated that the molecular formula of 3 was C_{20}H_{24}N_{2}O_{5} (calcd for C_{20}H_{25}N_{2}O_{5} 373.1758). The UV and IR spectra of 3 revealed absorptions similar to those of 1 and 2. The NMR spectra of 3 displayed signals for 10 carbons including 3 methylenes, 5 methines and 2 quaternary carbons, which were similar to those of 2 except for the absence of the hydroxyl signal (δ_H 5.96). The molecular formula of 3 revealed twice as many carbons as the $^{13}$C NMR displayed, indicating that 3 was a dimeric derivative of 2 linked through oxygen atom. This conclusion was confirmed by the high-intensity fragment ion peak at m/z 194.0828 and 176.0699 in ESI-MS/MS (negative mode), which were formed by eliminating a monomer unit and a molecular of H$_2$O, respectively (see Supporting Information). The linkage position was further confirmed by the HMBC correlation between H-1 and C-1' (see Supporting Information, Figure S32). A comprehensive analysis of the $^1$H–$^1$H COSY, HSQC and HMBC spectra allowed the assignment of NMR data of 3. The absolute configuration of 3 was also deduced by CD experiment. The CD spectrum of 3 revealed similar Cotton effects to those of 1 and 2 (Figure 3). As a result, the structure of 3 was determined and named as hirsutanine C.
3. Experimental

3.1. General experimental procedures

HR-ESI-MS was performed on an Agilent 6210 ESI/TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). 1D (1H, 13C, and DEPT) and 2D (1H–1H COSY, HSQC, HMBC and NOESY) NMR spectra were recorded on a Bruker AV-300 spectrometer (Bruker, Switzerland) with TMS as internal standard, and chemical shifts were expressed in δ values (ppm). Optical rotations were measured by a Jasco P-1020 digital polarimeter at 25°C. IR spectra were conducted on a Jasco FT/IR-480 plus Fourier transform infrared spectrometer (Jasco, Tokyo, Japan). UV spectra were recorded on a Jasco-V-550 UV–vis spectrophotometer (Jasco, Tokyo, Japan). Silica gel (Qingdao, P.R. China) and Sephadex LH-20 (Pharmacia Biotec AB, Uppsala, Sweden) were used for column chromatographies (CCs). Preparative high-performance liquid chromatography (HPLC) was carried out on an Agilent chromatograph 1260 equipped with a G1310B pump and a G1365D UV detector using Cosmosil 5C18-MS-II column (20 mm × 250 mm). All solvents used in CC were of analytical grade (Tianjin Damao Chemical Plant, Tianjin, P.R. China).

3.2. Plant material

The aerial parts of *U. hirsuta* were collected in Conghua city, Guangdong province of P.R. China, and authenticated by Prof. Guang-Xiong Zhou (College of Pharmacy, Jinan University). A voucher specimen (No. 2012110501) has been deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, P.R. China.

3.3. Extraction and isolation

The air-dried and powdered materials (65.0 kg) were refluxed with 95% ethanol (100 L, each 3 h) for three times. The combined extract was concentrated under vacuum to afford a residue (6.5 kg). The crude extract was dissolved in 10% HCl (pH 2) and filtered. After removal of the neutral components by EtOAc extract, the acidic suspension was then basified with NH₄OH to pH 9 and re-extracted with CHCl₃ to obtain a total alkaloid part (60.0 g). Subsequently, the total alkaloid part was subjected to silica gel CC (CHCl₃–CH₃OH, 100:0–0:100) to give five major fractions (Frs 1–5). Fraction 3 was re-chromatographed on a silica gel column (CHCl₃–CH₃OH, 100:0–0:100) to afford 10 subfractions Frs 3A–3J. Fraction 3B was subsequently purified on Sephadex LH-20 (MeOH) and preparative HPLC [CH₃CN–H₂O (30:70, v/v), 6 mL/min] to yield 1 (20.0 mg), 2 (10.0 mg) and 3 (7.0 mg).

3.3.1. Hirsutanine A (1)

Colourless prism, [α]D₂⁵ 252.7° (c = 1.0, CH₂OH); UV (CH₂OH) λmax: 207, 285 nm; IR (KBr) νmax: 3390, 3235, 1671, 1584, 1067 cm⁻¹; HR-ESI-MS m/z: 210.1127 [M + H]+ (calcd for C₁₁H₁₆NO₃ 210.1125). ¹H NMR (300 MHz, DMSO-d₆): 4.16 (1H, m, H-1), 8.10 (1H, t, 4.8 Hz, H-2), 7.39 (1H, dd, J = 6.0, 1.9 Hz, H-3), 2.74 (1H, m, H-5), 1.67 (1H, m, H-6a), 4.24 (1H, m, H-7), 5.26 (1H, td, J = 17.4, 8.7 Hz, H-8), 2.47 (1H, m, H-9), 5.22 (1H, dd, J = 17.4, 3.0 Hz, H-10a), 5.13 (1H, dd, J = 9.0, 3.6 Hz, H-10b), 3.26 (3H, s, H-12); ¹³C NMR (75 MHz, DMSO-d₆): 83.5 (C-1), 141.6 (C-3), 92.8 (C-4), 28.7 (C-5), 25.3 (C-6), 66.6 (C-7), 134.3 (C-8), 41.5 (C-9), 118.8 (C-10), 166.0 (C-11), 53.6 (C-12).

3.3.2. Hirsutanine B (2)

Amorphous powder, [α]D₂⁵ 349.2° (c = 1.0, CH₂OH); UV (CH₂OH) λmax: 206, 285 nm; IR (KBr) νmax: 3412, 1671, 1588, 1402, 1102 cm⁻¹; HR-ESI-MS m/z: 196.0970 [M + H]+ (calcd
for C₁₀H₁₄NO₃ 196.0968). ¹H NMR (300 MHz, DMSO- d₆): 4.49 (1H, m, H-1), 7.87 (1H, m, H-2), 7.36 (1H, dd, J = 6.0, 1.2 Hz, H-3), 2.82 (1H, m, H-5), 1.66 (1H, m, H-6a), 1.48 (1H, qd, J = 12.6, 4.2 Hz, H-6b), 4.19 (2H, m, H-7), 5.23 (1H, td, J = 17.3, 9.8 Hz, H-8), 2.31 (1H, m, H-9), 5.17 (1H, dd, J = 17.8, 3.3 Hz, H-10a), 5.11 (1H, dd, J = 9.3, 3.3 Hz, H-10b), 5.96 (1H, d, J = 4.3, H-OH); ¹³C NMR (75 MHz, DMSO- d₆): 75.5 (C-1), 141.9 (C-3), 91.4 (C-4), 27.9 (C-5), 25.4 (C-6), 66.6 (C-7), 135.0 (C-8), 44.0 (C-9), 118.3 (C-10), 166.1 (C-11).

3.3.3. Hirsutanine C (3)

Amorphous powder, [α]D²⁵ − 279.5° (c = 1.0, CH₃OH); UV (CH₃OH) λmax: 207, 286 nm; IR (KBr) νmax: 3445, 1634 cm⁻¹; HR-ESI-MS m/z: 373.1755 [M + H]+ (calcd for C₂₀H₂₅N₂O₅ 373.1758).

¹H NMR (300 MHz, DMSO- d₆): 4.63 (2H, dd, J = 3.9, 2.0 Hz, H-1, 10a), 7.88 (2H, m, H-2, H-20), 7.45 (2H, dd, J = 5.7, 2.0 Hz, H-3, 3'), 7.28 (2H, m, H-5, 5'), 1.69 (2H, m, H-6a, 6a'), 1.47 (2H, qd, J = 12.2, 4.5 Hz, H-6b, 6b'), 4.22 (4H, m, H-7, 7'), 5.30 (2H, td, J = 16.8, 8.4 Hz, H-8, 8'), 2.40 (2H, m, H-9, 9'), 5.25 (2H, dd, J = 17.1, 3.0 Hz, H-10a, 10a'), 5.14 (2H, dd, J = 8.7, 3.3 Hz, H-10b, 10b'); ¹³C NMR (75 MHz, DMSO- d₆): 77.5 (C-1, 10a), 141.6 (C-3, 3'), 93 (C-4, 4'), 28.6 (C-5, 5'), 25.2 (C-6, 6'), 66.6 (C-7, 7'), 134.2 (C-8, 8'), 41.9 (C-9, 9'), 119.0 (C-10, 10'), 165.9 (C-11, 11').

4. Conclusions

Hirsutanines A–C (1–3) were three new monoterpenoid alkaloids from the aerial parts of U. hirsuta. The structures with absolute configurations of these new compounds were determined by NMR, X-ray diffraction, CD analysis and quantum chemical calculation.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S32 and Tables S1–S7.

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References

Chang CC, Tung LH, Chen RR, Chiueh CC. 1979. A study on the antihypertensive action of uncarine A, an alkaloid of Uncaria formosana used in Chinese herb medicine. J Formos Med Assoc. 78:61–69.

Chinese Pharmacopoeia Commission. 2010. Pharmacopoeia of the People’s Republic of China. Vol. I 2010 ed. Beijing: Chemical Industry Press; p. 240.

Koichi M, Junko A, Masao K. 1995. Caeruleosides A and B, bis-iridoid glucosides from Lonicera caerulea. Phytochemistry. 39:111–114.

Wu TS, Chan YY. 1994. Constituents of leaves of Uncaria hirsuta Haviland. J Chin Chem Soc. 41:209–212.

Xin WB, Chou GX, Wang ZT. 2008. Two new alkaloids from the leaves of Uncaria hirsuta Haviland. Chin Chem Lett. 19:931–933.

Xin WB, Chou GX, Wang ZT. 2009. Triterpenoids and saponins from the leaves of Uncaria hirsuta Haviland. Chin Chem Lett. 20:638–644.

Xin WB, Chou GX, Wang ZT. 2011. Bis(monoterpenoid) indole alkaloid glucosides from Uncaria hirsuta. Phytochem Lett. 4:380–382.

Yu ZB, Shu GM, Qin SY, Zhou Y. 1999. Survey on traditional medicinal resources of Uncaria distributed in China. China J Chin Mater Med. 24:198–204.

Zou LC, Zhu FT, Xiang H, Deng XM. 2008. New secoiridoid glycosides from the roots of Picrorhiza scrophulariiflora. Molecules. 13:2049–2057.