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A NEW MONOTERPENOID INDOLE ALKALOID FROM *UNCARIA RHYNCHOPHYLLA*

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Abstract – A new alkaloid, uncanidine A (**1**) and 14 known alkaloids (**2-15**) were isolated from the hook-bearing branches of *Uncaria rhynchophylla*. Their structures were elucidated by spectroscopic analyses, including 1D-, 2D-NMR, HR-ESI-MS, ECD, as well as comparison with the data reported in the literature. Noteworthily, uncanidine A (**1**) was a novel *Uncaria* alkaloid which possessed a 6/5/6/6/6/5 hexacyclic ring system. In addition, uncanidine A (**1**) was found to inhibit the generation of NO from RAW 264.7 cells stimulated by LPS.

Monoterpenoid indole alkaloids (MIAs) are well-known for their significant biological effects. The liana *Uncaria rhynchophlly* (Rubiaceae), widely distributed in South China, especially in the Guangdong, Fujian and Yunnan Provinces, is a rich source of MIAs known as *Uncaria* alkaloids.[1] The dried branches of *Uncaria rhynchophlly* (Miq.) Miq. ex Havil. is one of the most important traditional Chinese herbs, named “Gouteng” in Chinese, which has long been used in traditional Chinese medicine to relieve hypertension, epilepsy, headaches and dizziness.[2-4] From this plant, around 300 chemical constituents have been reported, covering alkaloids, flavonoids, triterpenoids, *etc*, of which MIAs are regarded as the predominantly bioactive constituents.[3,5-7] Modern pharmacological studies have shown that *Uncaria* alkaloids has biological activities such as lowering blood pressure, anti-convulsant, antiarrhythmia, sedative, and antithrombotic.[3,7-9] In a search for structurally diverse and bioactive alkaloids, we carried out a phytochemical investigation on the total alkaloid fraction of the hook-bearing branches of *Uncaria rhynchophylla*. A new alkaloid uncanidine A (**1**) and fourteen known ones were obtained by various chromatographic separation methods from the hook-bearing branches of *Uncaria rhynchophylla* using normal-phase silica gel, Sephadex LH-20 and semi-preparative HPLC. Uncanidine A (**1**) was a novel *Uncaria* alkaloid, which possess a 6/5/6/6/6/5 polycyclic system featuring a tetrahydrofuran unit and a 1, 2,3,4-tetrahydropyridine ring. The known compounds were identified as strictosamide (**2**),[2] rhynchophylline (**3**),[10] isorhynchophylline (**4**),[11] corynoxeine (**5**),[12] isocorynoxeine (**6**),[13] hirsutine (**7**),[14] hirsuteine (**8**),[15] geissoschizine methyl ether (**9**),[15] rhynchophyllionium D (**10**),[16] sitsirikine (**11**),[17] 16*R*, *E*-isositsirikine (**12**),[18] akuammigine (**13**),[19] cadambine (**14**)[20] and 3*α*-dihydrocadamine (**15**)[20] by comparison the NMR data with those reported in the literature. Among them, compound **12** is first discovered from *Uncaria rhynchophylla*. The structures of **1**-**15** are shown in Figure 1.

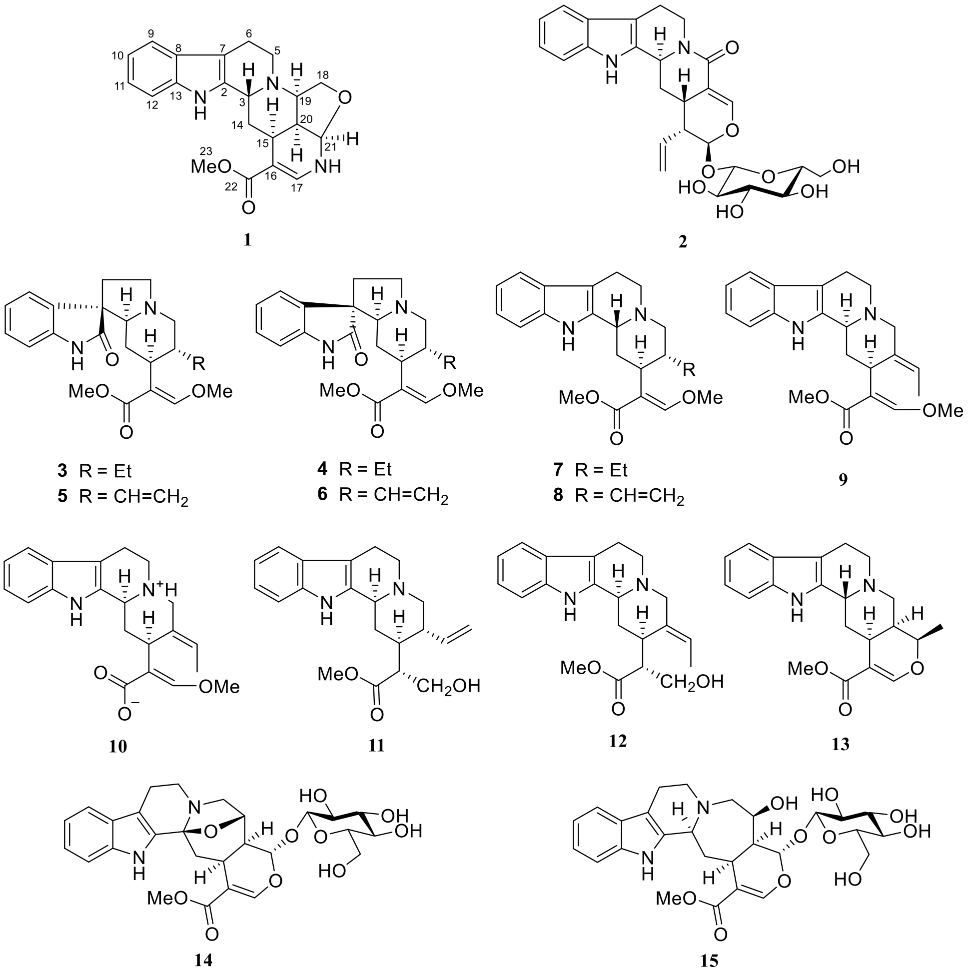

Figure 1. Chemical structures of compounds **1**-**15**

Uncanidine A (**1**) was isolated as yellow amorphous powder, $[\alpha]_D^{25}$ + 66.7 (*c* 0.06, MeOH). The molecular formula $C_{21}H_{23}N_3O_3$ was deduced on the basis of its HRESIMS (*m/z* 366.1813 $[M+H]^+$), which indicated the presence of three nitrogen atoms and twelve degrees of unsaturation. In the ^1H NMR spectrum, signals assignable to an *ortho*-disubstituted phenyl ring [δ_H 7.37 (1H, d, J = 7.8 Hz), 6.96 (1H, t, J = 7.8 Hz), 7.02 (1H, t, J = 7.8 Hz), 7.27 (1H, d, J = 7.8 Hz)], a methoxy group [δ_H 3.35 (3H, s)], an oxygenated olefinic proton [δ_H 7.52 (1H, s)], and seven methylene and methine proton signals possibly associated with heteroatoms [δ_H 4.74 (1H, d, J = 5.2 Hz), 4.36 (1H, dd, J = 9.0, 2.8 Hz), 3.57 (1H, dd, J = 9.0, 2.8 Hz), 4.16 (1H, m), 3.98 (1H, ddd, J = 9.7, 7.3, 2.5 Hz), 3.17 (1H, m), 2.89 (1H, m)] were observed. The ^{13}C NMR spectrum of **1** showed 21 carbon signals, one indole moiety including eight carbon signals (δ_C 138.1, 107.4, 128.4, 118.4, 119.7, 121.7, 111.9, 138.1), one ester carbonyl (δ_C 170.5), one double bond (δ_C 143.0, 101.9), and five carbon signals possibly attached to heteroatom (δ_C 63.8, 63.4, 52.4, 50.8, 51.3). Comparison of the MS and NMR data (Table 1) of **1** with those of the known compounds ajmalicine and akuammigine[19] revealed that their NMR signals were similar except for the absence of the terminal methyl signals, and the existence of signals for a methylene [δ_C 63.4, δ_H 3.57 (1H, dd, J = 9.0, 2.8 Hz), 4.36 (1H, dd, J = 9.0, 2.8 Hz), CH$_2$-18]. In addition, the chemical shift of C-17 (δ_C 143.0) was obvious different from those in ajmalicine (δ_C 154.5) and akuammigine (δ_C 154.8). At the same time, the molecular weight of **1** is odd, suggesting that it contains three nitrogen atoms, which is one more than those of ajmalicine and akuammigine.

Table 1. ^{1}H NMR and ^{13}C NMR spectroscopic data of **1**

No.	δ_C[a)]	δ_H[b)]
2	138.1	-
3	50.8	4.16 (1H, m)
5	52.4	α 3.17 (1H, m) β 2.89 (1H, m)
6	22.6	α 2.98 (1H, m) β 2.69 (1H, m)
7	107.4	-
8	128.4	-
9	118.4	7.37 (1H, d, J = 7.8 Hz)
10	119.7	6.96 (1H, t, J = 7.8 Hz)
11	121.7	7.02 (1H, t, J = 7.8 Hz)
12	111.9	7.27 (1H, d, J = 7.8 Hz)
13	138.1	-
14	31.1	α 2.16 (1H, m) β 1.95 (1H, m)
15	24.3	3.13 (1H, m)
16	101.9	-
17	143.0	7.52 (1H, s)
18	63.4	α 3.57 (1H, dd, J = 9.0, 2.8 Hz) β 4.36 (1H, dd, J = 9.0, 2.8 Hz)
19	84.8	4.74 (1H, d, J = 5.2 Hz)
20	36.7	2.39 (1H, m)
21	63.8	3.98 (1H, ddd, J = 9.7, 7.3, 2.5 Hz)
22	170.5	-
23	51.3	3.35 (3H, s)

[a)] Measured at 175 MHz. [b)] Measured at 700 MHz.

The ^{1}H-^{1}H COSY spectrum of **1** revealed the presence of three spin systems as shown in Figure 2. In the HMBC spectrum, the correlations between H-15 and C-19, between H-19 and C-17/C-18/C-21, between H-18 and C-20/C-21/C-19 suggested the presence of a CH_2 adjacent to C-21. According to the downfield chemical shifts of CH_2-18/CH-19, upfield chemical shift of CH-17, and the molecular weight information, it can be inferred that CH_2-18 and CH-19 may be linked to oxygen atom and form a five-membered heterocyclic ring with CH-20 and CH-21, as well as CH-17 may be linked to nitrogen atom rather than oxygen. Based on the above evidence, the planar structure of **1** was assigned as shown in Figure 2.

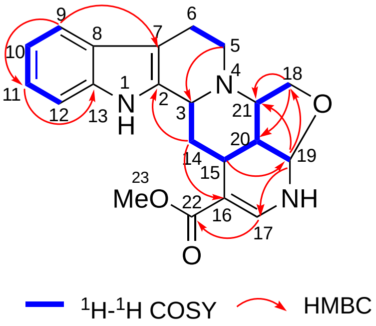

Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of **1**

In the NOESY spectrum, the correlations between H-3 (δ_{H} 4.16) and H-14β (δ_{H} 1.95)/H-18β (δ_{H} 4.36), between H-15 (δ_{H} 3.13) and H-14α (δ_{H} 2.16)/H-20 (δ_{H} 2.39)/H-21 (δ_{H} 3.98), between H-19 (δ_{H} 4.74) and H-18α (δ_{H} 3.57)/H-21 (δ_{H} 3.98), between H-20 (δ_{H} 2.39) and H-15 (δ_{H} 3.13)/H-21 (δ_{H} 3.98) indicated the relative configuration of **1** as shown in Figure 3. However, the orientation of H-20 could not be deduced directly from insufficient evidence in NOESY spectrum. In order to determine the orientation of H-20 and further confirmed the structure of **1**, models of the *s*-isomer and *r*-isomer (Figure S1) of **1** were investigated by quantum chemical calculation. The calculation ^{13}C NMR chemical shifts of **1** (*s*-isomer) at the B3LYP/6 31 G (2d, p) level with the PCM in CD_3OD agreed well with the experimental data (Figure S2), a correlation coefficient (R^2) of 0.9992 (Figure S3), indicating the structure with relative configuration of **1** shown in Figure 3 was rational.

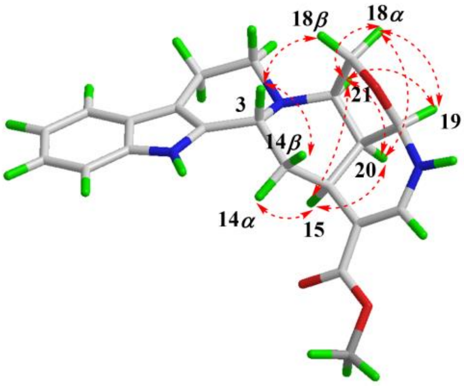

Figure 3. Key NOESY correlations of **1**

To determine the absolute configuration of **1**, ECD curves for the two possible enantiomers (3*R*, 15*S*, 19*S*, 20*S*, 21*R*-**1** and 3*S*, 15*R*, 19*R*, 20*R*, 21*S*-**1**) were calculated using the TD-DFT method. As shown in Figure 4, the experimental ECD spectrum of **1** showed a negative cotton effect at 243 nm and positive cotton effects at 265 nm, which were similar to the calculated one for the isomer with 3*R*, 15*S*, 19*S*, 20*S* and 21*R* configuration. Therefore, the absolute stereochemistry of **1** was assigned as 3*R*, 15*S*, 19*S*, 20*S* and 21*R*.

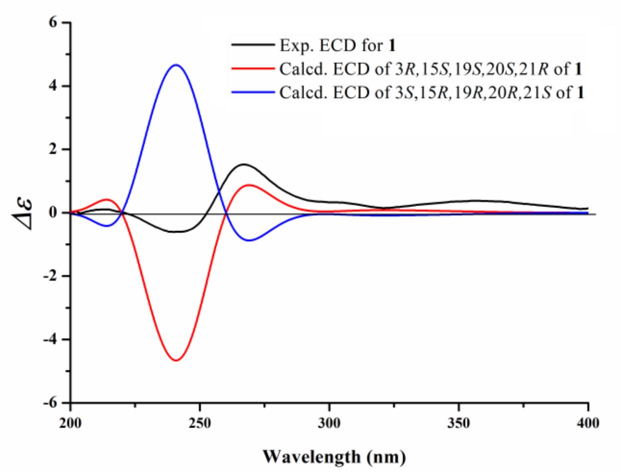

Figure 4. Experimental and calculated ECD spectra of **1**

Uncanidine A (**1**) was evaluated for its NO inhibitory effects in LPS-stimulated RAW 264.7 cells. The results showed that **1** could inhibit the generation of NO from RAW 264.7 cells stimulated by LPS.

EXPERIMENTAL

General experimental procedures. Optical rotations were determined on a JASCO P-2000 polarimeter. IR and UV spectra were measured on Nicolet 5700 and TU-1901 UV/Vis spectrophotometers, respectively. NMR spectra were recorded on a Bruker Avanced spectrometer at 700 MHz and 400 MHz, with TMS as an internal standard. HR-ESI-MS was acquired on a Waters Xevo-G2-S Q-TOF mass spectrometer. CD spectra were recorded with a Chirascan V100 spectropolarimeter. For column chromatography (CC), silica gel (200-300 mesh) was obtained from Qingdao Ocean Chemical Plant of Qingdao, China, and Sephadex LH-20 was obtained from Pharmacia Company of Switzerland. Semi-preparative HPLC analysis was carried out on an Agilent 1260 series system equipped with a diode array detector. TLC silica gel GF_{254} plates were purchased from Qingdao Ocean Chemical Plant and were visualized under a UV lamp at 254 nm or by spraying the Dragendorff’s reagent.

Plant material. The hook-bearing branches of *Uncaria rhynchophylla* (Miq.) Miq. ex Havil. (10.0 kg) were collected by Yanrong Zhang (Anguo Tongde Medicinal Materials, Hebei, China) from Hunan, China in September 2018, and were identified by Professor Yaojun Yang (Beijing University of Chinese

Medicine). A voucher specimen was deposited in the School of Traditional Chinese Materia Medica, Beijing University of Chinese Medicine.

Extraction and isolation. The powdered hook-bearing branches of *Uncaria rhynchophylla* (10.0 kg) were extracted with 90% EtOH (3 × 2 h). The extract was suspended in H_2O (6 L) and acidified with 10% HCl to pH 2, which was further partitioned with EtOAc to remove the neutral components. The aqueous layer was then basified with ammonia to pH 10 and re-extracted with CH_2Cl_2 to obtain a total alkaloid fraction (9.2 g). The alkaloid fraction was separated continuously by column chromatography over silica gel, Sephadex LH-20, ODS and HPLC to yield **1** (2.5 mg). Extraction and isolation of **2-15** see Supporting Information.

Uncanidine A (1): yellow amorphous powder; $[\alpha]_D^{25}$ + 66.7 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.27), 254 (2.89) nm; IR (KBr) ν_{max}: 3267, 2921, 2850, 1707, 1620, 1547, 1518, 1454, 1383, 1300, 1261, 1213, 1066, 918, 874, 703, 755 cm^{-1}; HRESIMS *m/z*: 366.1813 $[M+H]^+$ (Calcd for $C_{21}H_{24}N_3O_3^+$, 366.1812); CD (MeCN, $\Delta\varepsilon$) λ_{max} 243 (-1.19), 267 (+2.31), 300 (+0.50); ^1H NMR (CD_3OD, 700 MHz) and ^{13}C NMR data (CD_3OD, 175 MHz) are shown in Table 1.

***In vitro* anti-inflammatory Assay.** The inflammatory assay was employed to evaluate the activity of **1** related to the release of NO from macrophage cells as the literature reported.[21,22] Detailed experiments and results see Supporting Information.

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