HETEROCYCLES, Vol. 104, No. 5, 2022, pp. 945 - 951. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 17th December, 2021, Accepted, 24th January, 2022, Published online, 28th January, 2022
DOI: 10.3987/COM-21-14615

A NEW MONOTERPENOID INDOLE ALKALOID FROM *UNCARIA RHYNCHOPHYLLA*

Guiyang Wang,[b†] Xiao Yu,[a†] Linlin Zhu,[a] Yan Liang,[a] Mengnan Zhou,[a] Bin Liu,[a]* and Wei Zhang[a]*

[a]School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 102488, People's Republic of China. [b]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China.
Email: zhangwei_bucm@163.com, liubinyn67@163.com
[†] Equal contribution to this article

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 3α-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.

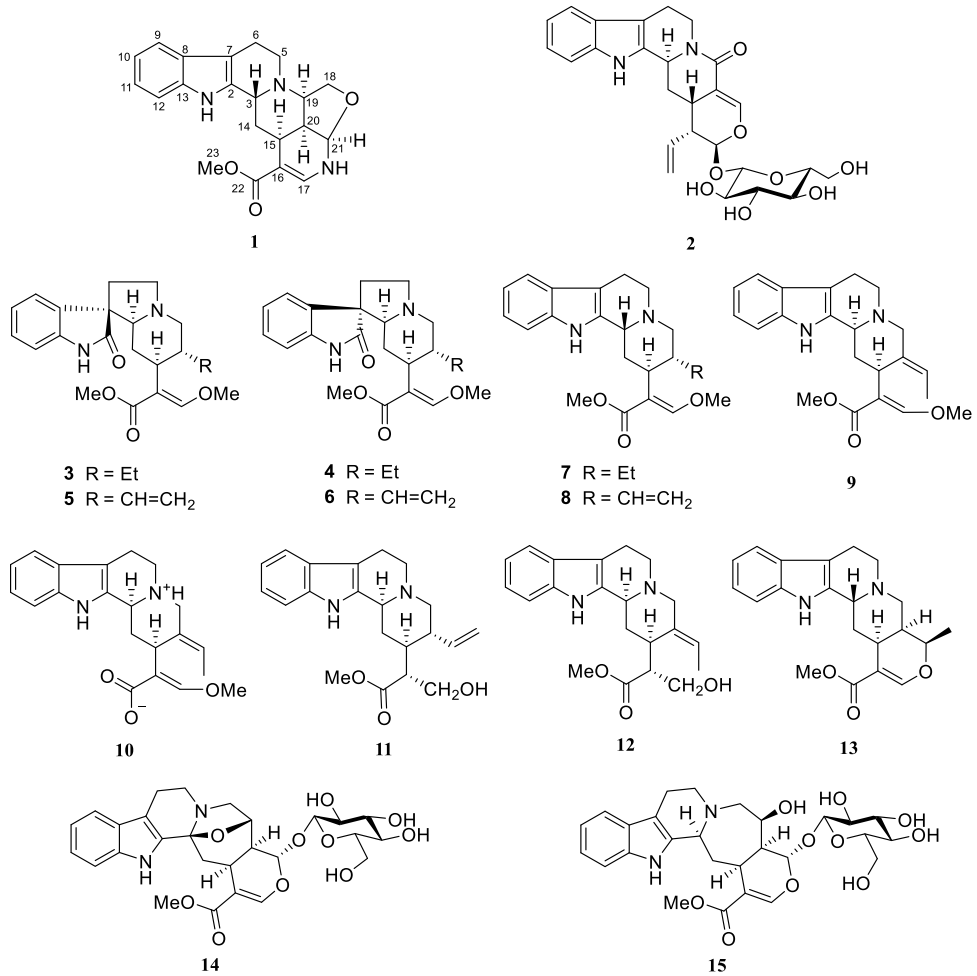

Figure 1. Chemical structures of compounds **1**-**15**

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.

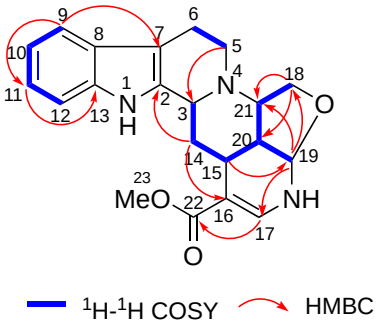

Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of **1**

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.

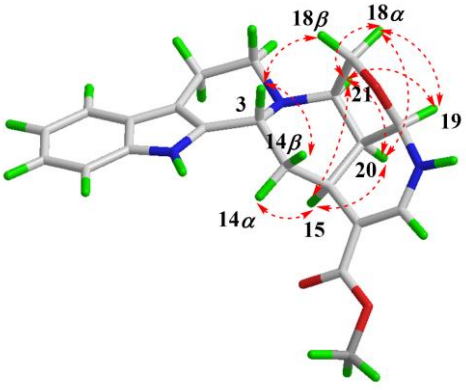

Figure 3. Key NOESY correlations of **1**

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*.

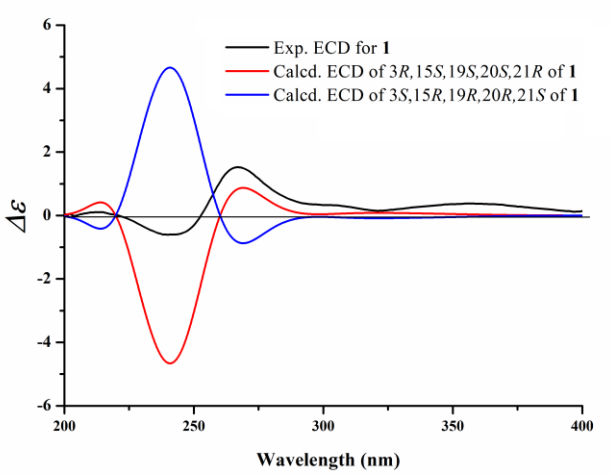

Figure 4. Experimental and calculated ECD spectra of **1**

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.

ACKNOWLEDGEMENTS

This investigation was supported by the Program for National Natural Science Foundation of China (No. 81803687) and the Young Scientist Cultivation Program of Beijing University of Chinese Medicine (BUCM-2019-QNKXJC008).

REFERENCES

1. Z. Chen, Z. H. Tian, Y. W. Zhang, X. Feng, Y. L. Li, and H. Q. Jiang, *Biochem. Syst. Ecol.*, 2020, **91**, 104057.
2. R. X. Li, J. T. Cheng, M. J. Jiao, J. Zhang, S. Chen, C. Guo, and A. Liu, *Chin. Tradit. Herbal Drugs*, 2017, **48**, 1499.
3. A. Ndagijimana, X. M. Wang, G. X. Pan, F. Zhang, H. Feng, and O. Olaleye, *Fitoterapia*, 2013, **86**, 35.
4. Y. Yang, W. G. Ji, Z. R. Zhu, Y. L. Wu, Z. Y. Zhang, and S. C. Qu, *Neuropharmacology*, 2018, **135**, 100.
5. Q. Zhang, J. J. Zhao, J. Xu, F. Feng, and W. J. Qu, *Ethnopharmacol.*, 2015, **173**, 48.
6. Q. Guo, X. L. Si, Y. T. Shi, H. S. Yang, X. Y. Liu, H. Liang, P. F. Tu, and Q. Y. Zhang, *J. Nat.*

Prod., 2019, **82**, 3288.

7. N. Qin, X. Lu, Y. J. Liu, Y. T. Qiao, W. Qu, F. Feng, and H. P. Sun, *Eur. J. Med. Chem.*, 2021, **210**, 112960.
8. Y. F. Xian, Z. X. Lin, M. Zhao, Q. Q. Mao, S. P. Ip, and C. T. Che, *Planta Med.*, 2011, **77**, 1977.
9. X. Yu, L. L. Zhu, J. Liu, Y. T. Hua, S. B. Guo, M. N. Zhou, Y. Y. Jiang, B. Liu, and W. Zhang, *Chin. Tradit. Herbal Drugs*, 2021, **52**, 6052.
10. G. C. Li, J. Y. Wu, and T. Liu, *Chin. J. Mod. Appl. Pharm.*, 2010, **27**, 1193.
11. B. Wang, D. Yuan, B. Ma, Y. Y. Xie, J. Yin, and Y. Kano, *Chin. J. Med. Chem.*, 2006, **6**, 369.
12. Y. Jiao, M. Q. Wang, D. Hua, C. Zhao, C. Tang, M. H. Jin, H. Q. Duan, and S. A. Tang, *J. Tianjin Med. Univ.*, 2013, **19**, 107.
13. J. N. Zheng and D. Y. Wang, *Guid. J. Tradit. Chin. Med. Pharm.*, 2009, **15**, 80.
14. W. B. Xin, G. X. Yu, and Z. T. Wang, *Chin. Tradit. Herbal Drugs*, 2009, **40**, 204.
15. H. Takayama, T. Watanabe, and H. Seki, *Tetrahedron. Lett.*, 1992, **33**, 6831.
16. Q. Guo, H. S. Yang, X. Y. Liu, X. L. Si, H. Liang, P. F. Tu, and Q. Y. Zhang, *Fitoterapia*, 2018, **127**, 47.
17. A. Ahmed, W. Li, F. F. Chen, J. S. Zhang, Y. Q. Tang, L. Chen, G. H. Tang, and S. Yin, *Fitoterapia*, 2018, **128**, 1.
18. X. Z. Wu, H. Q. Xie, X. H. Long, J. J. Zhang, T. Huang, X. J. Hao, and Y. H. Zhang, *Chin. Pharm. J.*, 2017, **52**, 631.
19. E. Wenkert, C. J. Chang, H. P. S. Chawla, D. W. Cochran, E. W. Hagaman, J. C. King, and K. Orito, *J. Am. Chem. Soc.*, 1976, **98**, 3645.
20. Z. M. Wang, L. Yang, Y. D. Fang, J. Chen, Y. Wang, R. S. Li, K. Wang, and F. Wang, *Chin. Tradit. Herbal Drugs*, 2019, **50**, 2802.
21. L. Wang, S. Lu, L. Wang, M. Xin, Y. Xu, G. Wang, D. Chen, L. Chen, S. Liu, and F. Zhao, *J Ethnopharmacol.*, 2021, **276**, 114186.
22. Y. Tian, S. Zhou, R. Takeda, K. Okazaki, M. Sekita, and K. Sakamoto, *Biomed. Pharmacother*, 2021, **141**, 111854.