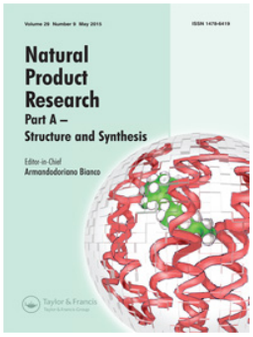


Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Geissoschizine methyl ether N-oxide, a new alkaloid with antiacetylcholinesterase activity from Uncaria rhynchophylla

Wei-Wei Jiang, Jia Su, Xing-De Wu, Juan He, Li-Yan Peng, Xiao Cheng & Qin-Shi Zhao

To cite this article: Wei-Wei Jiang, Jia Su, Xing-De Wu, Juan He, Li-Yan Peng, Xiao Cheng & Qin-Shi Zhao (2015) Geissoschizine methyl ether N-oxide, a new alkaloid with antiacetylcholinesterase activity from Uncaria rhynchophylla, Natural Product Research, 29:9, 842-847, DOI: 10.1080/14786419.2014.989847

To link to this article: http://dx.doi.org/10.1080/14786419.2014.989847

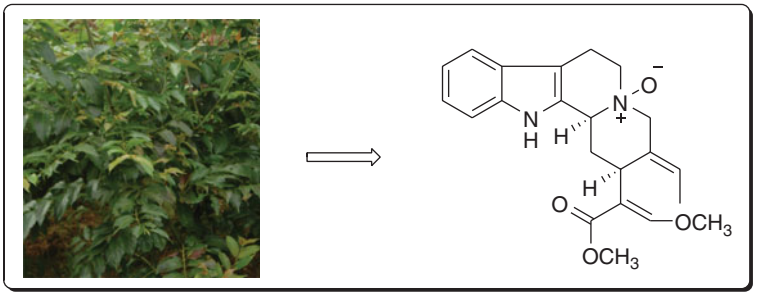
Natural Product Research, 2015
Vol. 29, No. 9, 842–847, http://dx.doi.org/10.1080/14786419.2014.989847

Geissoschizine methyl ether *N*-oxide, a new alkaloid with antiacetylcholinesterase activity from *Uncaria rhynchophylla*

Wei-Wei Jiang[ab], Jia Su[a], Xing-De Wu[a], Juan He[a], Li-Yan Peng[a], Xiao Cheng[a] and Qin-Shi Zhao[a]*

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China;* [b]*University of the Chinese Academy of Sciences, Beijing 100049, People's Republic of China*

(*Received 30 September 2014; final version received 16 November 2014*)



Geissoschizine methyl ether *N*-oxide, a new oxindole alkaloid, along with 14 known alkaloids, was isolated from the aerial part of *Uncaria rhynchophylla*. Their structures were identified by comprehensive spectral methods, including 2D NMR experiments, and confirmed by comparing with the literature data. *In vitro* acetylcholinesterase (AChE) inhibitory activity assay showed that the new compound exhibited anti-AChE activity with IC_{50} value of 23.4 μM.

Keywords: *Uncaria rhynchophylla*; Rubiaceae; alkaloids; geissoschizine methyl ether *N*-oxide; anti-AChE activity

1. Introduction

The hooks and stems of *Uncaria rhynchophylla* (Miq.) Jacks, a well-known traditional Chinese medicine, have been used to treat hypertension, dizziness, cerebral arteriosclerosis and convulsion (Heitzman et al. 2005). Previous phytochemical studies on the hooks and stems of this plant have resulted in the isolation of oxindole or indole alkaloids, triterpenic acids and phenolic compounds (Aimi et al. 1977; Yuan et al. 2008). Among them, the oxindole or indole alkaloids are commonly recognised as the major bioactive ingredients closely related to the pharmacological activities against the nervous system (Sakakibara, Terabayashi, Kubo, Higuchi, Komatsu, et al. 1999; Sakakibara, Terabayashi, Kubo, Higuchi, Sasaki, et al. 1999; Yang et al. 2012). As part of our effort to discover naturally bioactive metabolites from the traditional Chinese medicine, a phytochemical investigation of the aerial part of *U. rhynchophylla* has been carried out, which led to the isolation of a new oxindole alkaloid, geissoschizine methyl ether

*Corresponding author. Email: qinshizhao@mail.kib.ac.cn

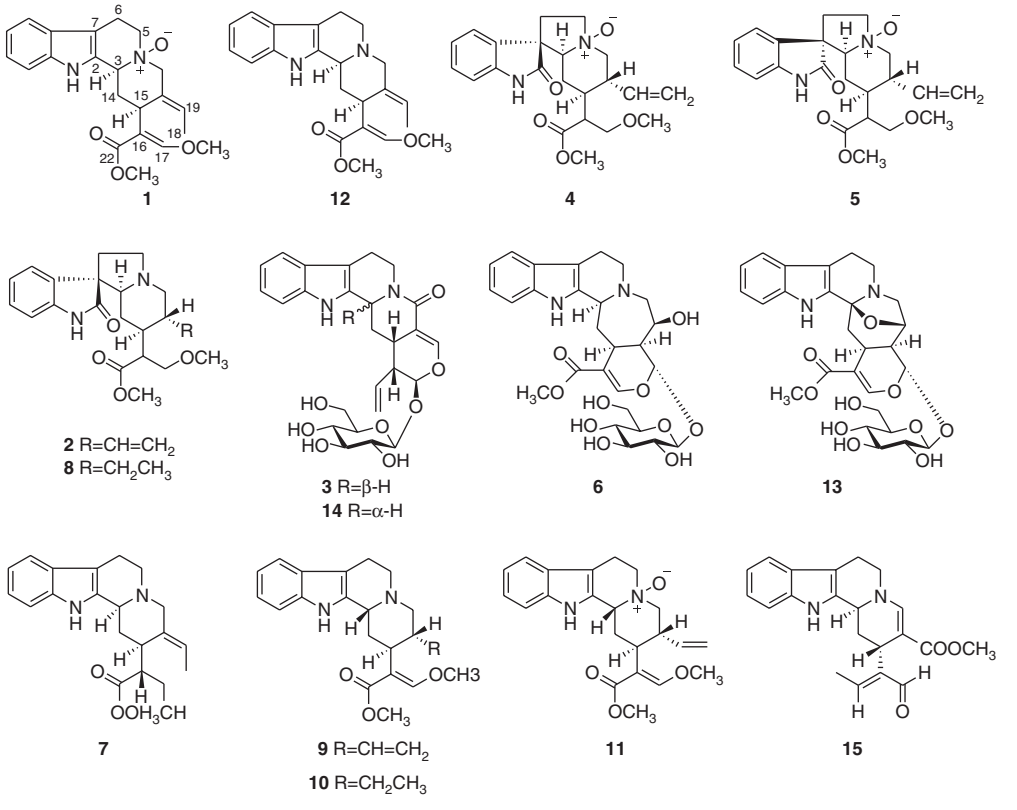


Figure 1. The structure of compounds **1**–**15**.

N-oxide (**1**), as well as 14 known compounds (Figure 1). Herein, we reported the isolation, structure elucidation and anti-acetylcholinesterase (AChE) activity evaluation of the new compound.

2. Results and discussion

2.1. *Chemistry*

Geissoschizine methyl ether *N*-oxide (**1**) was obtained as yellowish amorphous solid. The molecular formula of $C_{22}H_{26}N_2O_4$ was deduced from the HR-ESI-MS pseudo-molecular ion peak at *m/z* 383.1975 $[M + H]^+$(calcd for $C_{22}H_{27}N_2O_4$, 383.1971), indicating 11 degrees of unsaturation. The UV absorptions at 223 and 201 nm revealed the existence of an indole chromophore. And the IR absorptions implied the presence of amino (3433 cm^{-1}) and carbonyl (1699 cm^{-1}) functionalities. The ^1H NMR, ^{13}C NMR and DEPT spectra of compound **1** displayed unsubstituted A ring of indole chromophore [δ_C 130.9 (s, C-2), 106.8 (s, C-7), 127.4 (s, C-8), 119.2 (d, C-9), 120.4 (d, C-10), 123.1 (d, C-11), 111.2 (d, C-12), 138.5 (s, C-13); δ_H 7.47 (br d, $J = 7.8$ Hz, H-9), 7.02 (td, $J = 7.8$ and 1.0 Hz, H-10), 7.10 (td, $J = 7.8$ and 1.0 Hz, H-11), 7.29 (br d, $J = 7.8$ Hz, H-12)]. Besides the signals for the indole chromophore, the ^{13}C NMR spectrum displayed 14 additional carbon signals, which were classified by the chemical shifts and HSQC spectrum as three quaternary carbons (δ_C 169.3, 130.9, 111.8), four methine carbons (δ_C 161.7, 132.0, 74.2, 34.4), four methylene carbons (δ_C 77.3, 63.1, 33.5, 18.6), one methyl (δ_C 13.9) and two methoxy groups (δ_C 62.6, 51.9). The two methoxy groups were connected to C-17 and C-22, as deduced by the HMBCs of δ_H 3.66 (3H, s) and 3.89 (3H, s) with

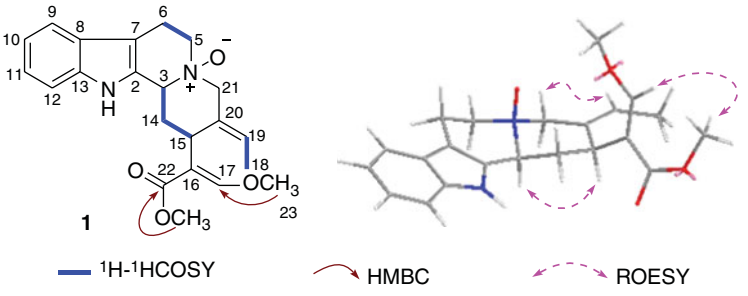


Figure 2. The structure and key 2D correlations of compound **1**.

δ_C 169.3 (C-22) and 161.7 (C-17), respectively (Figure 2). The $^1H-^1H$ COSY correlations established the linkages of C-5/C-6, C-3/C-14/C-15, and C18/C19 fragments, respectively, as shown in Figure 2. Detailed HMBCs analysis suggested that **1** possessed a similar carbon skeleton as that of geissoschizine methyl ether (**12**) (Takayama et al. 1992). The only difference was that **1** possessed one more oxygen atom compared with that of **12** which suggested that **1** should be an *N* oxide of geissoschizine methyl ether (**12**). Finally, **1** was elucidated as geissoschizine methyl ether *N*-oxide because of the downfield shifts of C-3 (δ_C 74.2, +15.6 ppm), C-5 (δ_C 63.1, +11.5 ppm) and C-21 (δ_C 77.3, +12.7 ppm).

The relative configuration of **1** was established by the ROESY spectrum. The correlation of H-3 with H-15 suggested that **1** possessed the same configuration as that of **12**. The *E* geometry of C(19)═C(20) was inferred from the ROESY correlation of H-19 with H-21 and the *Z* geometry of C(16)═C(17) was established by correlation of H-17 with OAc (Figure 2). Therefore, the structure of compound **1** was elucidated as geissoschizine methyl ether *N*-oxide.

The known compounds were identified as corynoxeine (**2**) (Wang et al. 2006), vincosamide (**3**) (Fan et al. 2010), (4*S*)-corynoxeine *N*-oxide (**4**) (Ma et al. 2009), isocorynoxeine *N*-oxide (**5**) (Ma et al. 2009), 3α-dihydrocadambine (**6**) (Xu et al. 2011), (16*R*)-*E*-isositrikine (**7**) (Lounasmaa et al. 1995), rhynchophyline (**8**) (Zhang et al. 1999), hirsuteine (**9**) (Xin et al. 2009), hirsutine (**10**) (Xin et al. 2009), hirsuteine *N*-oxide (**11**) (Sakai & Shinma 1978), geissoschizine methyl ether (**12**) (Sakakibara, Terabayashi, Kubo, Higuchi, Komatsu, et al. 1999), cadambine (**13**) (Xin et al. 2009), strictosamide (**14**) (Xin et al. 2009) and vallesiachotamine (**15**) (Shen et al. 1998) respectively, by comparison of their spectroscopic data with those reported in the literature (Figure 1).

2.2. *Biological activity*

Since geissoschizine methyl ether (**12**) was reported as a potential AChE inhibitor (Yang et al. 2012), compounds **1** and **12** have been estimated the AChE inhibitory activities using Amplex® Red reagent (Tacrine as positive control, $IC_{50} = 0.33$ μM). As a result, compound **1** showed a better anti-AChE activity with IC_{50} value of 23.4 μM, compared with an IC_{50} value of 82.8 μM as that of **12**. So compound **1** may be a potential AChE inhibitor.

3. Experimental procedure

3.1. *General experimental procedures*

Optical rotations were measured on a JASCO P-1020 digital polarimeter (JASCO, Tokyo, Japan). IR spectra were obtained on a Tensor 27 spectrometer (Bruker Optics, Ettlingen, Germany) with KBr pellets. UV spectra were recorded using a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). ESI-MS and HR-ESI-MS were recorded on an

Agilent 6530 Q-Tof spectrometer (Agilent, Santa Clara, CA, USA). 1D NMR and 2D NMR were performed on Bruker AVANCE III-600 spectrometers with TMS as internal standard (Bruker Optics, Ettlingen, Germany). Column chromatography (CC) was performed over silica gel (100–200 or 200–300 mesh, Qingdao Marine Chemical Co. Ltd, Qingdao, China), MCI gel (CHP 20P, 75–150 μM, Mitsubishi Chemical Corporation, Tokyo, Japan) and Sephadex LH-20 (GE Healthcare Bio-sciences AB, Sala, Sweden). Thin-layer chromatography was carried out on silica gel 60 F254 on glass plates (Qingdao Marine Chemical Inc.) using various solvent systems, and spots were visualised by spraying improved Dragendorff's reagent to the silica gel plates.

3.2. *Plant material*

The aerial part of *U. rhynchophylla* used in this study was collected from Puer, Yunnan province, P.R. China, in June 2012. The plant was identified by Prof. Xiao Cheng and a voucher specimen (20120608U01) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. *Extraction and isolation*

The air-dried aerial part of *U. rhynchophylla* (100 kg) was percolated with 50% aqueous EtOH (500 L × 24 h) for three times. The combined extract was concentrated under reduced pressure till without ethanol, which was then adjusted at pH 2 with 10% HCl and extracted with an equal volume of EtOAc. The acid-soluble fraction was collected and adjusted to pH 10 with NaOH solution. After exhaustive extraction with $CHCl_3$, the alkaloid fraction (100 g) was subject to reversed-phase MPLC (MCI) and eluted with MeOH–H_2O (3:7 → 5:5 → 8:2 → 95:5, each 8 L) to produced five fractions A–E. Fr. A (8 g) was chromatographed over silica gel CC, eluted with $CHCl_3$/MeOH (80:20 → 50:50), to obtain compound **2** (10 mg) and sub-fraction A-1. Sub-fraction A-1 (6 g) was then separated by silica gel columns (petroleum ether/acetone/diethylamine, 85:25:1 → 20:80:1) to give compound **3** (20 mg). Fr. B (15 g) was chromatographed over repeated silica gel columns ($CHCl_3$/MeOH, 90:10→50:50 and then petroleum ether/acetone/diethylamine, 85:15:1 → 50:50:1) to yield **4** (5 mg) and **5** (10 mg). Purification of Fr. C (35 g) over a silica gel column ($CHCl_3$/MeOH, 90:10 → 10:90) to give Fr. C-1, Fr. C-2 and Fr. C-3. Compound **6** (38 mg) was obtained from Fr. C-1 (8 g) after purification through a silica gel column (petroleum ether/EtOAc/diethylamine, 90:10:1). Fr. C-2 (10 g) was chromatographed over silica gel columns (petroleum ether/acetone, 90:10) and repeat Sephadex LH-20 columns (MeOH) to obtain **7** (29 mg) and **8** (3 mg). Sub-fraction C-3 (12 g) was applied to a silica gel column eluted with (petroleum ether/acetone, 80:20) and further separated by semipreparative HPLC (MeOH–H_2O, 40:60) to yield **10** (40 mg) and **11** (30 mg). Fr. D was eluted with $CHCl_3$/MeOH (95:5 to 50:50) on silica gel CC to yield **9** (40 mg), and sub-fractions D-1–D-3. Sub-fraction D-1 (10 g) was subjected to silica gel CC (petroleum ether/acetone/diethylamine, 80:20:1) to afford **12** (120 mg). Sub-fraction D-2 (2 g) was subjected to silica gel CC (petroleum ether/acetone, 70:30) to afford **1** (16 mg). Sub-fraction D-3 (4 g) was subjected to a silica gel column eluted with (petroleum ether/acetone, 70:30) and further separated by semipreparative HPLC (MeOH–H_2O, 45:55) to yield **13** (3800 mg) and **14** (42 mg). Fr. E (8 g) was purified by repeat silica gel CC ($CHCl_3$/MeOH, 95:5 → 50:50 and then petroleum ether/acetone, 80:20 → 50:50) to afford **15** (2 mg).

3.1.2. *Geissoschizine methyl ether* N-*oxide* (***1***)

Yellowish amorphous powder; $[\alpha]_D^{24.1}$ + 55.6 (c = 0.78, MeOH); UV (MeOH) λ_{max} (log ε): 201 (5.26) and 223 (4.85) nm. IR (KBr) ν_{max} 3433, 2943, 1699, 1634, 1453, 1437, 1246, 1115 and

744 cm^{-1}. ESI-MS *m/z* 383 $[M + H]^+$; HR-ESI-MS *m/z* 383.1975 $[M + H]^+$ (calcd for $C_{22}H_{27}N_2O_4$, 383.1971). ^1H NMR (CD_3OD, 600 MHz): δ_H 7.48 (1H, s, H-17), 7.47 (1H, br d, $J = 7.8$, H-9), 7.29 (1H, br d, $J = 7.8$, H-12), 7.10 (1H, td, $J = 7.8$ and 1.0, H-11), 7.02 (1H, td, $J = 7.8$ and 1.0, H-10), 5.81 (1H, q, $J = 6.8$, H-19), 4.97 (1H, d, $J = 12.9$, H-21a), 4.35 (1H, br d, $J = 11.8$, H-3), 4.16 (1H, dd, $J = 11.0$ and 6.6, H-15), 3.89 (3H, s, H-23), 3.82 (1H, d, $J = 12.9$, H-21b), 3.72 (1H, m, H-5a), 3.66 (3H, s, H—$COOCH_3$), 3.64 (1H, m, H-5b), 3.27 (1H, m, H-6a), 2.93 (1H, m, H-6b), 2.34 (1H, ddd, $J = 15.0$, 6.6, 2.3, H-14a), 2.25 (1H, dd, $J = 15.0$, 11.0, H-14b), 1.66 (3H, d, $J = 6.8$, H-18). ^{13}C NMR (CD_3OD, 125 MHz): δ_C 169.3 (s, C-22), 161.7 (d, C-17), 138.5 (s, C-13), 132.0 (d, C-19), 130.3 (s, C-2), 127.4 (s, C-8), 123.1 (d, C-11), 119.2 (d, C-9), 120.4 (d, C-10), 112.2 (d, C-12), 111.8 (s, C-16), 106.8 (s, C-7), 77.3 (t, C-21), 74.2 (d, C-3), 63.1 (t, C-5), 62.6 (q, C-23), 51.9 (q, C—$COOCH_3$), 34.4 (d, C-15), 33.5 (t, C-14), 18.6 (t, C-6), 13.9 (q, C-18).

3.5. *Anti-AChE assay*

Compounds were dissolved in DMSO. AChE inhibitory activities of the compounds were assayed by Amplex acetylcholinesterase assay kit (Life Technologies) using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex® Red reagent) as specified (Lau et al. 2013). AChE converts the acetylcholine substrate to choline, which is oxidised by choline oxidase to betaine and hydrogen peroxide, and then the hydrogen peroxide reacts with the Amplex® Red reagent to generate the fluorescent resorufin. The fluorescence of each well was measured by BioTek Synergy HT microplate reader with excitation and emission wavelengths of 535 and 590 nm, respectively.

Tacrine was used as positive control. The experiment was performed in triplicate. The percentage inhibition was calculated as follows: % inhibition $= (E - S)/E \times 100$ (E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound).

4. Conclusion

In summary, one new compound geissoschizine methyl ether *N*-oxide (**1**), together with 14 known compounds were isolated. *In vitro* AChE inhibitory activity assay showed that the new compound exhibited anti-AChE activity with IC_{50} value of 23.4 μM.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S10.

Funding

This work was financially supported by the National Natural Science Foundation of China [number U0932602] and the National Basic Research Program of China [973 Program No. 2011CB915503].

References

Aimi N, Yamanaka E, Shinma N, Fujiu M, Kurita J, Sakai S, Haginiwa J. 1977. Studies on plants containing indole alkaloids. VI. Minor bases of *Uncaria rhynchophylla* Miq. Chem Pharm Bull. 25:2067–2071.

Fan L, Fan CL, Wang Y, Zhang XQ, Zhang QW, Zhang JQ, Ye WC. 2010. Alkaloids from the leaves of *Nauclea officinalis*. Acta Pharm Sin. 45:747–751.

Heitzman ME, Neto CC, Winiarz E, Vaisberg AJ, Hammond GB. 2005. Ethnobotany, phytochemistry and pharmacology of *Uncaria* (Rubiaceae). Phytochemistry. 66:5–29.

Lau C, Kim Y, Chia D, Spielmann N, Eibl G, Elashoff D, Wei F, Lin YL, Moro A, Grogan T, et al. 2013. Role of pancreatic cancer-derived exosomes in salivary biomarker development. J Biol Chem. 288:26888–26897.

Lounasmaa M, Jokela R, hanhinen P, Miettinen J, Salo J. 1995. The Rhazimanine-Bhimberine enigma. J Nat Prod. 58:131–133.

Ma B, Wu C-F, Yang J-Y, Wang R, Kano Y, Yuan D. 2009. Three new alkaloids from the leaves of *Uncaria rhynchophylla*. Helv Chim Acta. 92:1575–1585.

Sakai S, Shinma N. 1978. The partial synthesis of heteroyohimbine alkaloids; akuammigine, 3-isorauniticine and corynanthe alkaloid; corynantheidine. Chem Pharm Bull. 26:2596–2598.

Sakakibara I, Terabayashi S, Kubo M, Higuchi M, Komatsu Y, Okada M, Taki K, Kamei J. 1999. Effect on locomotion of indole alkaloids from the hooks of *Uncaria* plants. Phytomedicine. 6:163–168.

Sakakibara I, Terabayashi S, Kubo M, Higuchi M, Sasaki H, Okada M. 1999. Evaluation of Gouteng (hooks and stems of Uncariae plants). III. Hypotensive principle from *Uncaria rhynchophylla* and alteration of its content on extraction process. Nat Med. 53:308–312.

Shen Z, Eisenreich W, Kutchan TM. 1998. Bacterial biotransformation of 3α(*S*)-strictosidine to the monoterpenoid indole alkaloid vallesiachotamine. Phytochemistry. 48:293–296.

Takayama H, Watanabe T, Seki H, Aimi N, Sakai S. 1992. Geissoschizine revisited – definite proof of its stereostructure. Tetrahedron Lett. 33:6831–6834.

Wang B, Yuan D, Ma B, Xie Y, Yin J, Kano Y. 2006. Chemical constituents of the leaves of *Uncaria rhynchophylla* (Miq.) Jacks. Chin J Med Chem. 16:369–372.

Xin W, Hao G, Wang Z. 2009. Alkaloids in *Uncaria rhynchophylla*. Chin Tradit Herb Drugs. 40:204–207.

Xu X, Li S, Song Q. 2011. Chemical constituents of the bark of *Anthocephalus chinensis*. Nat Prod Res Dev. 23:393–398.

Yang Zd, Duan DZ, Du J, Yang MJ, Li S, Yao XJ. 2012. Geissoschizine methyl ether, a corynanthean-type indole alkaloid from *Uncaria rhynchophylla* as a potential acetylcholinesterase inhibitor. Nat Prod Res. 26:22–28.

Yuan D, Ma B, Wu C, Yang J, Zhang L, Liu S, Wu L, Kano Y. 2008. Alkaloids from the leaves of *Uncaria rhynchophylla* and their inhibitory activity on NO production in lipopolysaccharide-activated microglia. J Nat Prod. 71:1271–1274.

Zhang J, Yang C, Wu D. 1999. Chemical constituents of *Uncaria rhynchophylla* (III). Chin Tradit Herb Drugs. 30:12–14.