Natural Product Research

Formerly Natural Product Letters

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

A new indole-type alkaloid from the roots of *Clematis florida var. plena*

Kai-Hui Sun, Xin-Hua Ma, Xian-Ming Zeng, Zhong-Yue Lin, Yu-Mei Cai, Hai-Tao Zhang, Xiao-Yan Lin, Shi-Biao Feng, Tian-Hua Zhong & Yong-Hong Zhang

To cite this article: Kai-Hui Sun, Xin-Hua Ma, Xian-Ming Zeng, Zhong-Yue Lin, Yu-Mei Cai, Hai-Tao Zhang, Xiao-Yan Lin, Shi-Biao Feng, Tian-Hua Zhong & Yong-Hong Zhang (2018): A new indole-type alkaloid from the roots of *Clematis florida var. plena*, Natural Product Research, DOI: 10.1080/14786419.2018.1510396

To link to this article: https://doi.org/10.1080/14786419.2018.1510396

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2018.1510396

A new indole-type alkaloid from the roots of *Clematis florida var. plena*

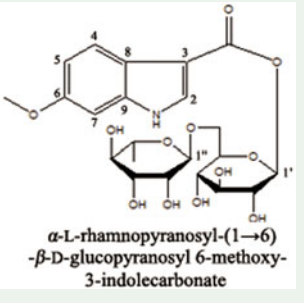
Kai-Hui Sun[a], Xin-Hua Ma[a], Xian-Ming Zeng[a], Zhong-Yue Lin[a], Yu-Mei Cai[a], Hai-Tao Zhang[a], Xiao-Yan Lin[a], Shi-Biao Feng[a], Tian-Hua Zhong[b] and Yong-Hong Zhang[a]

[a]Key Laboratory of Natural Drug Pharmacology in Fujian Province, School of Pharmacy, Fujian Medical University, Fuzhou, P. R. China; [b]Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Xiamen, P. R. China

ABSTRACT

One new indole-type alkaloid, α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl 6-methoxy-3-indolecarbonate (**1**), together with three known alkaloids (**2**–**4**), one aromatic acid (**5**) and five known saponins (**6**–**10**), was isolated from the roots of *Clematis florida var. plena*. Their structures were established by NMR spectroscopic analysis and acid hydrolysis. In *in vivo* anti-inflammatory activity, *n*-butanol extract was found to be potent against ear edema in mice, with inhibition rate of 48.7% at a dose of 800 mg/kg. Furthermore, compounds **8** and **9** obtained from the *n*-butanol extract exhibited significant anti-inflammatory activities with inhibition rates of 50.9% and 54.7% at a dose of 200 mg/kg.

C. florida var. plena



α-L-rhamnopyranosyl-(1→6)
-β-D-glucopyranosyl 6-methoxy-
3-indolecarbonate

ARTICLE HISTORY
Received 1 May 2018
Accepted 12 July 2018

KEYWORDS
Clematis florida var. plena; alkaloid; saponins; anti-inflammatory

1. Introduction

Clematis florida Thunb. *var. plena* D. Don is a perennial herbal plant belonging to the family Ranunculaceae and the genus *Clematis* and is a variety of *Clematis florida* Thunb. (Editorial Committee of Flora of China 1980). This variety has long been used as a folk remedy named Shiershichen in She Ethnopharmacy, and is widely distributed in southeast China. The roots and rhizomes of *C. florida var. plena* were popularly used

CONTACT Yong-Hong Zhang zhangyh@mail.fjmu.edu.cn; Tian-Hua Zhong zhongtianhua@tio.org.cn
Supplemental data for this article can be accessed here at https://doi.org/10.1080/14786419.2018.1510396.

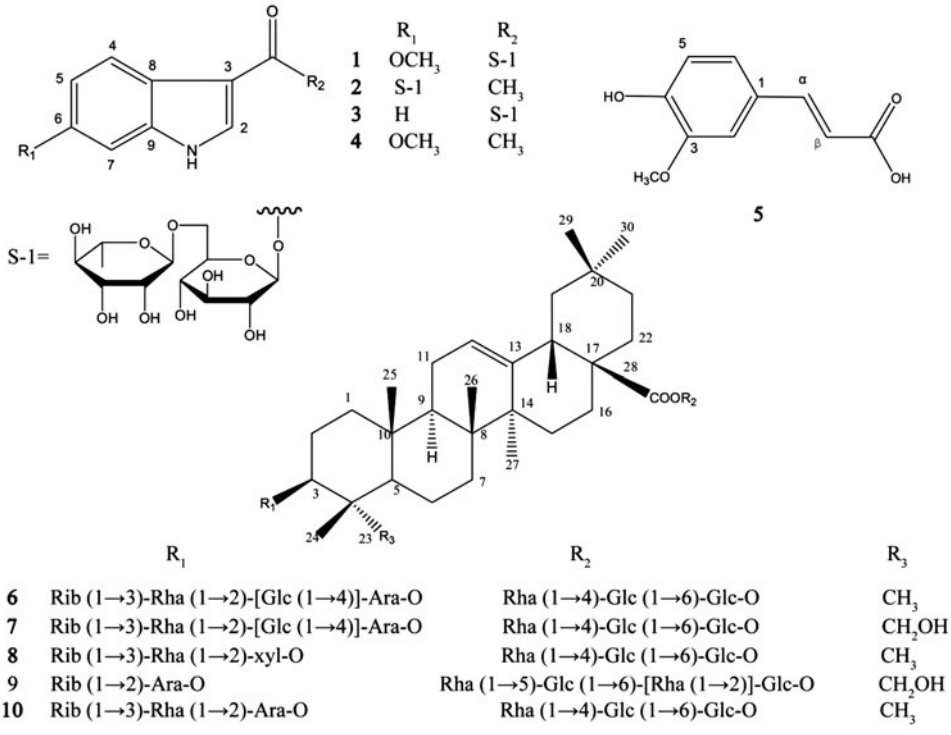


Figure 1. Chemical structures of compounds **1**–**10**.

as an anti-inflammatory, anti-rheumatism and analgesic agent in She Ethnopharmacy in Fujian Province, China (Huang et al. 2013). Chemical investigations of this genus afforded triterpene saponins (Zhao et al. 2016), alkaloids (Li et al. 2013), flavonoids (Chang et al. 2017), lignans (Xiong et al. 2014), coumarins (Xiong et al. 2014), macrocyclic compounds (Qiu et al. 2017), and phenolic glycosides (Zhang et al. 2015). In order to search for anti-inflammatory active ingredients, we have performed systematically chemical and pharmacological investigations of *C. florida var. plena*. Here we report the isolation and structural elucidation of one new indole alkaliod (**1**), together with three known analogues (**2**–**4**), one aromatic acid (**5**) and five known triterpene saponins (**6**–**10**) given in Figure 1, as well as the anti-inflammatory activities of the extracted fraction and isolated compounds.

2. Results and discussion

Compound **1** was obtained as a light brown gummy material. The positive-ion HRESIMS (Figure S1, supplementary material) showed the molecular ion $[M+Na]^+$ at *m/z* 522.1576, corresponding to the formula $C_{22}H_{29}NO_{12}$. Its IR spectrum showed the absorption of ester carbonyl group at 1695 cm^{-1} and aromatic ring absorptions at 1529 cm^{-1}. The ^1H NMR spectrum (Figure S2, supplementary material) indicated the presence of an ABX system of protons at δ_H 7.86 (1 H, d, $J = 8.8$ Hz), 6.76 (1 H, dd, $J = 2.2$, 8.8 Hz), 6.87 (1 H, d, $J = 2.2$ Hz), a singlet aromatic proton at δ_H 7.87 (1 H, s) and a methoxy group at δ_H 3.73 (3 H, s) (Table S1, supplementary material). Its ^{13}C NMR

spectrum exhibited eight aromatic carbons and an ester carbonyl carbon (δ_C 165.5) (Figure S3 and Table S1, supplementary material). All investigations suggested that compound **1** had a similar indole molecular skeleton to compound **4**, however, an ester methoxy group in **4** was found to be replaced by two hexoses in **1**. The two anomeric protons arising from the sugar moieties appeared at δ_H 5.60 (1 H, d, $J = 7.8$ Hz) and 4.63 (1 H, d, $J = 1.6$ Hz), which correlated respectively with signals at δ_C 95.2 and 102.2 ppm in the ^{1}H-^{13}C HSQC spectrum (Figure S4, supplementary material). The spin systems associated with monosaccharides were identified by a ^{1}H-^{13}C HSQC experiment with the aid of ^{1}H-^{1}H COSY spectra (Figure S6, supplementary material). In the ^{1}H-^{13}C HMBC spectrum (Figure S5, supplementary material), the anomeric proton at δ_H 5.60 showed long-range correlation with the carbon at δ_C 165.5 (C-10), suggesting the attachment of the glucosyl moiety at C-10. The anomeric proton at δ_H 4.63 indicated interaction with the carbon at δ_C 67.8 (C-6 of the glucose) confirming that the rhamnosyl unit was linked to C-6 of the glucosyl unit. The ^{1}H-^{13}C HMBC and ^{1}H-^{1}H COSY correlations are shown in Figure S7, supplementary material. The sugars were confirmed by acid hydrolysis and comparison with authentic sugars on TLC. Consequently, the structure of **1** was elucidated as α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl 6-methoxy-3-indolecarbonate.

By comparing the physical and spectral data with the literature data, 9 known compounds (**2**–**10**) were identified as: α-L-rhamnopyranosyl-(1→6)-β-D- glucopyranosyl - 3-indolecarbonate (**2**) (Shi et al. 2006), methyl 6-O-α-L- rhamnopyranosyl -(1→6)-α-D-glucopyranosyl 3-indolecarbonate (**3**) (Shi et al. 2006), methyl 6-methoxy-3-indolecarbonate (**4**) (Shi et al. 2006), Trans-isoferulic acid (**5**) (Prachayasittikul et al. 2009), 3-*O*-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl - (1→2) -[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**6**) (Zhao et al. 2014), Tomentoside A (**7**) (Wang et al. 2013), Huzhangoside C (**8**) (Kizu et al. 1995), Clematangoticoside B (**9**) (Zhao et al. 2016), Huzhangoside B (**10**) (Kizu et al. 1995), respectively. All compounds were isolated from *C. florida var. plena* for the first time. The ^{1}H and ^{13}C spectral data of the isolated compounds (**1**–**10**) are given in Table S1 and Table S2.

In order to identify the biological activity, an *in vivo* anti-inflammatory model was used, the results showed that the *n*-butanol extract of the plant exhibited potent anti-inflammatory activity against ear edema *in vivo*, with inhibition rate of 48.7% at a dose of 800 mg/kg (as shown in Table S3). The *n*-butanol subfraction (Fr. B4) exhibited potent anti-inflammatory activity with inhibition rate of 47.6% at a dose of 600 mg/kg. Furthermore, the results showed that compounds **8**–**9** (triterpene saponins) obtained from the *n*-butanol subfractions (Fr. B4) displayed significant anti-inflammatory activity with inhibition rate of 50.9% and 54.7% at a dose of 200 mg/kg. However, compound **1** (alkaloid) exhibited weak anti-inflammatory activity with inhibition rate of 27.0% at a dose of 200 mg/kg.

3. Experimental

3.1. General experimental procedures

Column chromatography (CC): silica gel 60 (SiO_2, 100–200 mesh and 200–300 mesh; *Qingdao Marine Chemical Factory*), *Sephadex LH-20* gel (40–70 μm; *GE Healthcare,*

Sweden). TLC: silica gel G (*Qingdao Marine Chemical Factory*). Prep. HPLC (with *SPD-20A* detector): *ODS* columns (25 cm × 10 mm i.d.) at r.t. on a Pegasil ODS-*II* 5 μm column (*Waters Scientific Co., Ltd.*, USA).Optical rotation: TU-1901 spectrometer. UV Spectra: *UV-210A* spectrometer; λ_{max} in nm. IR spectra: *Nicolet 170 SX* FT-IR spectrometer; KBr pellets; ν in cm^{-1}. NMR spectra: *Bruker NMR* spectrometer; at 400 (^{1}H) and 100 MHz (^{13}C); δ in ppm, *J* in Hz. HR-ESI-MS: *Bruker APEX II* mass spectrometer, ESI-MS: LCMS-2020 mass spectrometer, in *m/z*.

3.2. Plant material

The roots of *Clematis florida* Thunb. *var. plena* D. Don were purchased from Ningde, Fujian Province of China in May 2016 and identified by Prof. Yonghong Zhang, Fujian Medical University. *Clematis florida* Thunb. *var. plena* D. Don is a variety of *Clematis florida* Thunb., and there are obvious difference in the shape of stamens between them. The stamens in *C. florida var. plena* are petal-like, while wide linear in *C. florida* (Editorial Committee of Flora of China 1980). A voucher specimen (*No*. zyh 20160502) has been deposited in the Laboratory of the Natural Products, Fujian Medical University.

3.3. Preparation of the methanol extract and the fractions

Dried roots of *C. florida var. plena* (25 kg) were ground and extracted with methanol at room temperature. The solvent was evaporated under vacuum to afford 1100 g crude extract (yield, 4.4%). The methanol extract of *C. florida var. plena* (MCF) was then suspended in water and partitioned successively with petroleum ether, ethyl acetate and *n*-butanol. Each fraction was evaporated *in vacuo* to yield the residues of petroleum ether 85.5 g (7.8%), ethyl acetate 93.1 (8.5%), *n*-butanol 391.7 g (35.6%), respectively.

3.4. Evaluation of anti-inflammatory activity

3.4.1. Animals

Male ICR mice (approximately 20 g each) were obtained from the Laboratory Animal Center, Fujian Medical University and housed in temperature-controlled rooms (22–23 °C). The food and water were supplied ad libitum and each experimental group contained 10 animals. Animals required adaptive feeding for a week, and fasting for 12 h before the experiment. All experimental protocols were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication *No*. 85-23, revised 1985) and were approved by Committee of Ethics of Fujian Medical University, China.

3.4.2. Ear edema induced by xylene

MCF and the fractions were suspended in a 0.5% (w/v) sodium carboxyl methylcellulose (CMC-Na). The doses employed were expressed as mg of the dried extract per kg body weight. All the treatments involved intragastric administration (i.g.) to the animals in weight-based dosages. Aspirin was used as the standard anti-inflammatory

drug. After the administration of extracts or standard drug for 1 h, each mouse was externally treated with a test substance at 40 μL xylene solvent on surface of the right ear, and the left ear served as the control. After 1 h of xylene application, the mice were sacrificed by cervical dislocation. Circular sections (diameter: 6 mm) of both ears of each mouse were removed and weighted using an electronic analytical balance with 0.1 mg precision to calculate the inhibition of ear edema. The difference in weight between the two plugs was taken as a measure of edematous response. The percentage of protection was calculated using the following ratio:

$$(\text{control mean} - \text{treated mean}) \times 100\%/\text{control mean}$$

3.4.3. Statistical analysis

The data represent the means ± SD (n = 10). Differences between experimental groups and control group were tested using student's ***t*** test; $\boldsymbol{P} < 0.01$ were considered significant.

3.5. Purification

A portion of ethyl acetate extract (93.1 g) was isolated by silica gel (100–200 mesh) column chromatography (8 × 50 cm, 750 g) and eluted with petroleum ether/EtOAc in gradient mode (from 100:0 to 0:100, v/v) to afford eighteen fractions (Fr. A1-Fr. A18); Fr. A11 (8.3 g) was subjected to *C-18ODS* (solvent: MeOH/H_2O, 5:95-100:0, v/v) to give six subfractions (Fr. A11-1 - Fr. A11-6); the subfraction Fr. A11-4 (60% MeOH, 1.9 g) was seperated by *Sephadex LH-20* (CH_3Cl/MeOH 1:1) and purified by semi-PHPLC (CH_3CN/H_2O 33:67, 2.0 mL·min^{-1}) to yield compound **4** (12.1 mg) and compound **5** (37.4 mg). The *n*-butanol extract (391.7 g) was fractionated by MCI gel column and eluted with H_2O (Fr. B1), 20% MeOH (Fr. B2), 50% MeOH (Fr. B3), 70% MeOH (Fr. B4), 90% MeOH (Fr. B5) and MeOH (Fr. B6). The Fr. B2 (27.3 g) was further subjected to *Sephadex LH-20* eluted with CH_3Cl/MeOH (1:1) and purified by semi-PHPLC (CH_3CN/H_2O 15:85, 3.0 mL·min^{-1}) to yield compounds **1** (55.2 mg), **2** (35.7 mg), **3** (33.4 mg). The Fr. B4 (60.2 g) was subjected to *C-18ODS* (solvent: MeOH/H_2O, 5:95-100:0, v/v) to give six subfractions (Fr. B4-1 - Fr. B4-6); the subfraction Fr. B4-4 (60% MeOH, 19.2 g) was purified by semi-PHPLC (CH_3CN/H_2O 29:71, 3.0 mL·min^{-1}) to afford compounds **6** (79.5 mg), **7** (77.3 mg), **8** (1131.6 mg) and **9** (1193.2 mg). The Fr. B5 (33.0 g) was seperated using a semi-PHPLC (MeOH/H_2O 55:45-100:0, 3.0 mL·min^{-1}) to yield compound **10** (161.7 mg).

3.6. Acid hydrolysis of compound 1

Compound **1** and the authentic sugars were dissolved in methanol and spotted on a silica gel plate, then the plate was fumigated by the steam of hydrochloric acid for 30 min, after this period, the plate was expanded by *n*-butanol/EtOAc/2-propanol/HOAc/H_2O (7:20:12:7:6). D-Glc and L-Rha were detected from compound **1** by comparing with the authentic sugars. The R_f of the D-Glc and L-Rha were 0.53 and 0.70, respectively.

α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl 6-methoxy-3-indolecarbonate (**1**): light brown gum; $[\alpha]^{20}_{D}$-121.5 (c 2.0, MeOH), UV (MeOH): λ_{max} 286, 232, 211 nm; IR (KBr): ν_{max} 3357, 2926, 1695, 1529, 1433, 1170, 1068, 980 cm^{-1}; HRESI-MS *m/z*: 522.1576 $[M + Na]^+$ (calcd for $C_{22}H_{29}NO_{12}Na$, 522.1582); ^1H and ^{13}C NMR data, see Table S1.

4. Conclusion

The result of xylene ear inflammation in mice showed that *n*-butanol extract and its subfractions of powdered root of *Clematis florida var. plena* exerted anti-inflammatory activity *in vivo* against ear edema. Bioactivity-guided isolation of the *n*-butanol yielded one new alkaloid **1**, together with three known analogues **2**–**4**, one aromatic acid **5** and five known triterpene saponins **6**–**10**. The *n*-butanol fraction exhibited significant anti-inflammatory activity between 600 and 800 mg/kg, in a dose-dependent manner. At 200 mg/kg dose, the anti-inflammatory activity of triterpene saponins (**8**, **9**) was superior to that of alkaloid (**1**) (50.9%, 54.7% *vs* 27.0%) and the content of triterpene saponins was high in the plant, indicating that the triterpene saponins might be the anti-inflammatory constituents. The triterpene saponins isolated from the genus *Clematis* have been reported for their anti-inflammatory activity (Fu et al. 2010). *C. florida var. plena* was recognized to be a good source for anti-inflammatory activity may be attributed to these constituents. The results demonstrate the anti-inflammatory properties of *C. florida var. plena* which might justify the use of this plant for the treatment of inflammatory disease in Chinese, folk and herbal medicine.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the Natural Science Foundation of Fujian Province [grant number 2016J01369], [grant number 2018J01847]; the Open Project of National Marine Bureau Key Laboratory of Marine Biogenetic Resources [grant number HY201807], [grant number HY201604]; the Science and technology innovation platform project of Fujian Province [grant number 2018Y2001].

References

Chang YX, Zhang P, Zhang X, Chen JP, Rausch WD, Gula A, Bao BQ. 2017. Cytotoxic activities of flavonoids from a traditional Mongolian medicinal herb *Clematis aethusifolia* Turcz. Nat Prod Res. 31(10):1223–1227.

Editorial Committee of Flora of China. 1980. Chinese flora. Beijing: Science Press. Vol. 28; p. 209–211.

Fu Q, Zan K, Zhao MB, Zhou SX, Shi SP, Jiang Y, Tu PF. 2010. Triterpene saponins from *Clematis chinensis* and their potential anti-inflammatory activity. J Nat Prod. 73(7):1234–1239.

Huang ZH, Zhang YL, Shen XJ. 2013. Pharmacognostic Identification of *Clematis florida var. plena*. Subtrop Plant Sci. 42(2):104–108.

Kizu H, Shimana H, Tomimori T. 1995. Studies on the constituents of *Clematis* species. VI. The constituents of *Clematis stans* Sieb. et Zucc. Chem Pharm Bull. 43(12):2187–2194.

Li WT, Yang BY, Zhu W, Gong MH, Xu XD, Lu XH, Sun LL, Tian JK, Zhang L. 2013. A new indole alkaloidal glucoside from the aerial parts of *Clematis terniflora* DC. Nat Prod Res. 27(24):2333–2337.

Prachayasittikul S, Suphapon S, Worachartcheewan A, Lawung R, Ruchirawat S, Prachayasittikul V. 2009. Bioactive metabolites from *Spilanthes acmella* Murr. Molecules. 14(2):850–867.

Qiu L, Yuan HM, Liang JM, Cheng XL, Wang P, Du YF, Qiang F. 2017. Clemochinenosides C and D, two new macrocyclic glucosides from *Clematis chinensis*. J Asian Nat Prod Res. 23:1–7.

Shi SP, Tu PF, Dong CX, Jiang D. 2006. Alkaloids from *Clematis manshurica* Rupr. J Asian Nat Prod Res. 8(1–2):73–78.

Wang Y, Kang W, Hong LJ, Hai WL, Wang XY, Tang HF, Tian XR. 2013. Triterpenoid saponins from the root of *Anemone tomentosa*. J Nat Med. 67(1):70–77.

Xiong J, Bui VB, Liu XH, Hong ZL, Yang GX, Hu JF. 2014. Lignans from the stems of *Clematis armandii* ("Chuan-Mu-Tong") and their anti-neuroinflammatory activities. J Ethnopharmacol. 153(3):737–743.

Zhang LJ, Huang HT, Huang SY, Lin ZH, Shen CC, Tsai WJ, Kuo YH. 2015. Antioxidant and anti-inflammatory phenolic glycosides from *Clematis tashiroi*. J Nat Prod. 78(7):1586–1592.

Zhao M, Da-Wa Z-M, Guo D-L, Fang D-M, Chen X-Z, Xu H-X, Gu Y-C, Xia B, Chen L, Ding L-S, et al. 2016. Cytotoxic triterpenoid saponins from *Clematis tangutica*. Phytochemistry. 130:228–237.

Zhao M, Ma N, Qiu F, Hai WL, Tang HF, Zhang Y, Wen AD. 2014. Triterpenoid saponins from the roots of *Clematis argentilucida* and their cytotoxic activity. Planta Med. 80(11):942–948.