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RAPID COMMUNICATION

One novel alkaloid from the stems of *Tinospora crispa*

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ABSTRACT

The 6-methoxy-cannabisin I (**1**), a new alkaloid, together with five known compounds oleraisoindole A (**2**), cannabisin F (**3**), apigenin (**4**), syringin (**5**) and ethyl-syringin (**6**) were isolated from *Tinospora crispa* stems. Their structures were identified by the analysis of spectroscopic data. Compound **2** was isolated from *T. crispa* for the first time. Anti-inflammatory activity of compound **1** was detected against NO production in LPS-activated RAW 264.7 macrophages. However, no activity was observed.

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KEYWORDS
Tinospora crispa; alkaloid; anti-inflammatory activity

1. Introduction

Tinospora crispa Hook. f. & Thomson, a vine plant, is one species of *Tinospora* genus (Menispermaceae family) and mainly distributed in Xishuangbanna and Hainan Island in China (Praman et al. 2013), which was also called "hei luo he" by the minority of Dai people in Xishuangbanna district and used for treating edoema and rheumatism. Many studies showed that the crude extracts of the *T.* crispa displayed a wide range of biological activities, such as anti-inflammatory (Haque et al. 2020), anti-cholinesterase (Yusoff et al. 2014), immunomodulatory, antioxidant (Amom et al. 2009), antibacterial (Ramadani et al. 2018) and anti-diabetic (Gao et al. 2016) activities, etc. The previous chemical investigations of *T. crispa* revealed that it possessed diterpenoids (Lam et al.

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2012), triterpenes (Kongkathip et al. 2002), lactones (Praman et al. 2012), steroids, flavonoids, lignans and alkaloids (Alex et al. 2016), of which alkaloid is one of its important components and included three major types (aporphines, furquinolone and protoberberine) (Waqas et al. 2016). In our continuous studies on chemical constituents of Dai medicinal plants, a new alkaloid and five known compounds were isolated from *T. crispa* stems (Figure 1). Additionally, the anti-inflammatory activity of compound **1** also was investigated by NO inhibition assay.

2. Results and discussion

2.1. Structure elucidation

The 95% ethanol extract of *T. crispa* stems was dissolved in water and firstly partitioned with petroleum ether to exclude fatty acid, then treated with hydrochloric acid and ammonia water to give non-alkaloid and alkaloid fractions. The further separation and purification of non-alkaloid and alkaloid fractions by extensive column chromatography yielded one new compound (**1**) and five known compounds, oleraisoindole A (**2**) (Ma et al. 2021), cannabisin F (**3**) (Sakakibara et al. 1995), apigenin (**4**) (Inada et al. 1989), syringin (**5**) (Lee and Seo 1990) and ethyl-syringin (**6**) (Bu et al. 2012) (Figure 1).

Compound **1** was obtained as the pale yellow gum. Its molecular formula was identified as $C_{27}H_{21}NO_7$ on the basis of HR-ESI/MS spectrum (m/z 472.1396 $[M+H]^+$, calcd, for $C_{27}H_{22}NO_7^+$, 472.1391), indicating 18 degrees of unsaturation (DOUs). The IR absorption spectrum revealed the existence of hydroxyl (3388 cm^{-1}) and carbonyl (1752 and 1690 cm^{-1}) groups. Analysis of ^1H NMR (Table S1) revealed that the signals for ten aromatic protons (from δ_H 6.64 to δ_H 8.12), one methoxy group (δ_H 4.04, s), two methylenes [δ_H 2.82, t (J = 7.4 Hz) and δ_H 3.76, t (J = 7.4 Hz)]. Further inspection of ^{13}C NMR spectrum indicated that there were a total of 27 carbons, which was classified by the DEPT spectrum to fourteen quaternary carbons (including two carbonyl carbons at δ_C 169.9 and 169.2), two methylenes (including one oxygenated at δ_C 40.5), ten methines and one methoxy group (δ_C 56.5). Those aforementioned

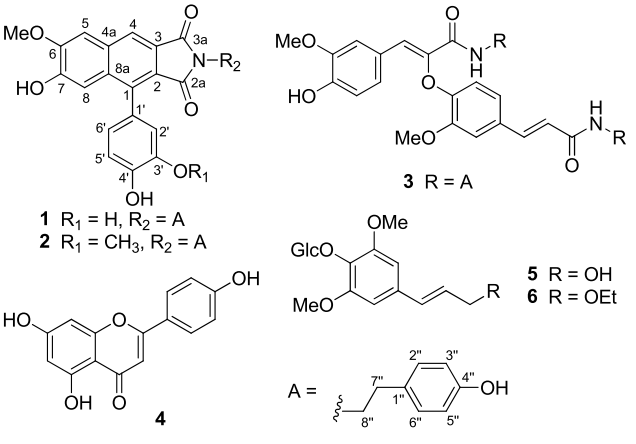

Figure 1. Isolated compounds of the stems of *T. crispa*.

functionalities account for 14 out of 18 DOUs, implying that compound **1** was tetracyclic.

The planar structure of **1** was established by the elucidation of 2D NMR spectra (Figure S1), which shared the same carbon skeleton with that of cannabisin I (Iwao et al. 1991; Borujot et al. 2016) by the comparison of their similar ^{1}H and ^{13}C NMR spectra. The major difference between them was the presence of an additional methoxy group (δ_H 4.04, s; δ_C 56.5) in compound **1** at C-6 position, which was verified by the HMBC correlation (from $OC\underline{H}_3$-6 to C-6) and ROESY cross peaks (from H-4 to H-5 and from $OC\underline{H}_3$-6 to H-5) (Figure S1). Thus, the structure of **1** was established as shown and named 6-methoxy-cannabisin I.

2.2. NO inhibitory activity assessment

The NO inhibitory activity of compound **1** was investigated using Griess system (Schaus 1956). However, no activity was observed. (Table S2)

3. Experimental

3.1. General experimental procedures

NMR spectra were obtained with Bruker-DRX-500 spectrometer. Mass spectra data were measured on a MS Waters AutoSpec Premier P776 mass spectrometer (EI-MS) and a Micro Q-TOF MS (HRESIMS). UV spectra were measured on a Shimadzu-UV-2401A spectrophotometer with methanol as solvent. Infra-red (IR) spectra were recorded on a Bio-Rad-FTS-135 spectrometer in KBr pellets. Mass spectra were obtained on a MS Waters AutoSpec Premier P776 mass spectrometer (EI-MS) and a Micro Q-TOF MS (HRESIMS), respectively. The size of silica gel used in column chromatography were 80–100 mesh and 200–300 mesh (Qingdao Marine Chemical Inc., P.R. China)

3.2. Plant material

The dried stems of *T. crispa* were obtained from Xishuangbanna Dai Hospital, Xishuangbanna Dai Autonomous Prefecture, Yunnan Province, China in August 2018, and identified by Dr. Qishi Song. A voucher specimen (No. TC-0701) was deposited in the herbarium of Xishuangbanna Tropical Botanical Garden.

3.3. Extraction and isolation

The dried stems of *T. crispa* (10 kg) were extracted by heating reflux with 95% ethanol and three times for 5 h each time. The concentrated extract was suspended in water and partitioned with petroleum ether, ethyl acetate (EtOAc) and n-butanol in sequence and when the EtOAc was used to partition, we used the 'alkali precipitation and acid extraction method' to obtain two fractions– non-alkaloid fraction (570 g) and alkaloid fraction (11 g). The non-alkaloid fraction was eluted by silica gel column chromatography with Chloroform-methanol ($CHCl_3$-MeOH) gradient (60:1, 30:1, 15:1, 7.5:1 3:1,

1:1) to obtain six fractions (Fr.1-6). Fr.3 was separated by macroporous resin column chromatography with ethanol-water (EtOH-water) gradient (30%, 50%, 70%, 80%) to obtain four sub-fractions (Fr.3-1 ~ 4). Fr.3-3 was subjected to microporous resin (MCI) column chromatography eluted with EtOH-water gradient (50%, 70%, 80%) to obtain three parts. The 80% EtOH-water part was eluted by silica gel column chromatography with $CHCl_3$-MeOH gradient (100:1 to 1:1), medium pressure preparative liquid chromatography (MPLC) with MeOH-water (50%, 70%, 80%). The 70% EtOH-water part was eluted by silica gel column chromatography with petroleum ether-acetone gradient (10:1 to 1:1) and Sephadex LH-20 to yield compounds **4** (3.1 mg), **6** (2.3 mg) and **5** (36.2 mg).

The alkaloid fraction was eluted by silica gel column chromatography with $CHCl_3$-MeOH gradient (50:1 to 1:1) to give seven fractions (Fr.8-14). Fr.11 was separated by Sephadex LH-20 with $CHCl_3$-MeOH (1:1, v/v) and HPLC with MeOH-water (56:44, v/v) to yield compound **2** (1.0 mg) and **3** (6.9 mg). Fr.10 was eluted by silica gel column chromatography with petroleum ether-acetone gradient (10:1 to 1:1) to give five sub-fractions (Fr.10-1 ~ 5). Fr. 10-3 was purified by Sephadex LH-20 (MeOH) and HPLC with MeOH-water (67:33, v/v) to obtain compound **1** (4.7 mg).

The 6-methoxy-cannabisin I **(1)**: pale yellow gum; IR (KBr) ν_{max} 3388, 2930, 1752, 1690, 1608, 1514, 1428, 1405, 1355, 1269, 1207 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table S1. HR-ESI-MS: *m/z* 472.1396 $[M+H]^+$ (calcd. for $C_{27}H_{22}NO_7^+$, *m/z* 472.1391).

3.4. NO inhibitory assay

RAW264.7 cells were incubated in 96-well plates and induced stimulation with 1 μg/mL LPS, add with 40 μM L-NMMA (NG-Monomethyl-L-arginine) or 40 μg/mL compound **1** at the same time. After cultured 24 h, the supernatant of the cell culture was collected to determine the inhibitory of NO, and the absorbance was measured at 570 nm. Add MTS to the remaining medium for cell viability testing to eliminate the toxic effect of compound **1** on cells (Goodwin et al. 1995). The data of NO inhibition ratio and cell viability were expressed as mean ± SD.

4. Conclusion

In conclusion, one new alkaloid, 6-methoxy-cannabisin I (**1**), together with five known compounds (**2**–**6**) were isolated from *T. crispa* stems, of which compound **2** was isolated from *T. crispa* for the first time. Their structures were established on the basis of spectroscopic data. Anti-inflammatory activity of compound **1** was detected against NO production in LPS-activated RAW 264.7 macrophages. However, no activity was observed.

Disclosure statement

No potential conflict of interest was reported by the authors.

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