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Limonoids from *Munronia henryi* and their anti-tobacco mosaic virus activity

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ABSTRACT

Five new compounds (**1**–**5**), including three limonoids, one diterpenoid, and one phytosterol, along with four known limonoids (**6**–**9**), were isolated from the ethanolic extracts of whole plants of *Munronia henryi*. Their structures were elucidated by extensive spectroscopic analysis. In addition, the anti-tobacco mosaic virus (TMV) activities of all the isolated compounds were evaluated by the conventional half-leaf and leaf-disk methods along with Western blot analysis. Most of the tested compounds showed strong antiviral activities, with IC_{50} values in the range of 14.8–34.0 μg/mL, compared with ningnanmycin as a positive control (IC_{50} = 44.6 μg/mL), and their preliminary structure-activity relationships were also discussed.

1. Introduction

The plant disease caused by tobacco mosaic virus (TMV) has been found worldwide. TMV is known to infect members of nine plant families and at least 125 individual species, including tobacco, pepper, tomato, cucumbers, and a number of ornamental flowers [1]. However, there is no chemical treatment that can absolutely inhibit TMV once it does infect plants. On the other hand, plants have evolved multiple mechanisms to selectively suppress pathogens by the production of secondary metabolites with antimicrobials and/or antiviruses. Guided by such a principle, our previous research has identified a series of natural products from plants with an anti-TMV background [2–7].

Limonoids have attracted much attention in the fields of natural products and synthetic chemistry because of their significant insect antifeedant, growth-regulating, cytotoxic, and antiviral activities [8]. The genus *Munronia* Wight (Meliaceae), comprising 13–15 species, is naturally distributed in China, Sri Lanka, India, Indonesia and the Philippines; three species of this genus have been found in Yunnan province [9]. *Munronia henryi* Harms is a low, small semibush, which has been used for the treatment of several diseases such as tuberculosis, colds, indigestion, and sore throat as Chinese traditional medicine [10,11]. Previous phytochemical investigations on the genus *Munronia* have led to the identification of a series of limonoids, some of which showed significant biological properties such as antifeedant, insecticidal, cytotoxic, and antiviral activities [12–16], especially, anti-TMV (tobacco mosaic virus) activity of limonoids which has been firstly reported by our group [16].

In order to further investigate limonoids of this genus with higher anti-TMV activity, five new compounds, munronins O–S (**1**–**5**), and four known ones (**6**–**9**), were isolated from the EtOH extract of the whole bodies of *M. henryi*. All these isolates were evaluated for anti-TMV activity on *Nicotiana glutinosa* by the half-leaf method [17,18]. In this paper, the isolation and structure elucidation of the new compounds as well as the biological evaluation of these compounds are reported.

2. Experimental

2.1. General methods

Optical rotations were determined with a JASCO DIP-370 Digital Polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer, KBr disks, in cm^{-1}. ^{1}H and ^{13}C NMR and 2D-NMR spectra were obtained on an INOVA-400 MHz and INOVA-500 MHz NMR spectrometer with tetramethylsilane (TMS) as an internal standard; electrospray ionization-mass spectrometry (ESI-MS) and high-resolution (HR) ESI-MS spectra were measured with a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. Semi-preparative high performance liquid chromatography (HPLC) was performed on a

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Waters column (i.d. 10–100 mm). Column chromatography was performed on silica gel (90–150 μm; Qingdao Marine Chemical Inc.), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.), Lichroprep RP-C18 gel (40–63 μm; Merck, Darmstadt, Germany), and Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden). Thin-layer chromatography (TLC) plates were precoated with silica gel GF_{254} (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China). Spots on chromatograms were detected by spraying with 5% H_2SO_4–EtOH. Leaf disks were kept in a RXZ280B culture chamber (Ningbo, Zhejiang, China). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS — PAGE) and Western blotting were carried out using a Bio-Rad electrotransfer system (Bio — Rad, Hercules, CA).

2.2. Plant material

The whole plant of *M. henryi* was collected in Wenshan, Yunnan Province, People's Republic of China, and was identified by Prof. Zhi-Min Fu of Guiyang College of Traditional Chinese Medicine. A voucher specimen (DHL H20121120) was deposited at the Key Laboratory of Chemistry for Natural Product of Guizhou Province and Chinese Academy of Science.

2.3. Extraction and isolation

The air-dried powdered whole plant of *M. henryi* (20.0 kg) was extracted with 95% EtOH (20 L × 3) under reflux three times (4, 3, and 3 h, respectively). The combined EtOH extracts were concentrated under vacuum to give a crude residue (1.4 kg), which was suspended in water. The water layer was successively partitioned with petroleum ether (7 L × 3) and EtOAc (7 L × 4). The EtOAc portion (445 g) was subjected to a silica gel column and eluted with petroleum ether-acetone (from 1:0 to 0:1) to yield six fractions (A–F). Fr. D (21 g) was first applied to MCI gel column (MeOH–H_2O from 4:6 to 10:0) to obtain four subfractions (D1–D4). Fr. D3 (500 mg) was subjected to a silica gel column eluted with a gradient of petroleum ether-EtOAc (9:1 to 5:5) to obtain **4** (53 mg) and **7** (41 mg). Fr. E (18 g) was separated over an MCI gel column eluted with a gradient (MeOH–H_2O from 3:7 to 10:0) to give five fractions (E1 — E5). Fr. E2 (6.3 g) was chromatographed over a C18 silica gel column and eluted with a gradient MeOH–H_2O (60:40, 65:35, and 70:30) to afford four subfractions (E2a–E2d). Fr. E2a was purified by a Sephadex LH-20 CC (MeOH) and then chromatographed on a silica gel column ($CHCl_3$–Me_2CO, 10:1) to yield **2** (25 mg), **3** (31 mg) and **6** (12 mg). Subfraction E2b3 (2.4 g) was chromatographed over a silica gel column and eluted with a gradient of $CHCl_3$–Me_2CO (from 10:1 to 5:1) to afford **1** (15 mg) and **5** (7.8 mg).

2.3.1. Munronin O (*1*)

White amorphous powder; [α]21 D + 102.1 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) nm 220 (3.42); IR (KBr) ν_{max} 3465, 2991, 1749, 1233, 1126, 1046 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; positive ESIMS *m/z* 551 $[M + Na]^+$; HREIMS *m/z* 528.2352 $[M]^+$ (calcd for $C_{29}H_{36}O_9$, 528.2359).

Table 1
^1H and ^{13}C NMR data for **1–3** in $CDCl_3$ (δ in ppm, J in Hz).[a]

	1[a]		**2**[a]		**3**[b]	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	6.84 d (12.8)	148.6 CH	6.97 d (12.8)	148.6 CH	6.25 m	149.0 CH
2	6.22 d (12.8)	122.0 CH	6.31 d (12.8)	122.0 CH	6.23 m	121.9 CH
3		166.7 C		166.6 C		166.3 C
4		83.6 C		83.5 C		83.5 C
5	3.36 d (7.6)	49.9 CH	3.35 d (7.6)	50.0 CH	3.46 d (7.6)	49.2 CH
6α	2.20 m	34.9 CH_2	2.18 m	35.0 CH_2	2.18 m	34.9 CH_2
6β	2.27 m		2.29 m		2.35 m	
7		173.5 C		173.5 C		173.4 C
8		136.1 C		136.8 C		138.7 C
9	3.09 d (7.2)	53.1 CH	3.10, d (7.2)	53.3 CH	2.97 d (7.2)	52.7 CH
10		46.2 C		46.2 C		46.4 C
11	5.68 dd (10.8, 7.2)	70.3 CH	5.67 dd (10.8, 7.2)	71.1 CH	5.51 dd (10.8, 7.2)	71.9 CH
12	5.74 d (10.8)	73.6 CH	5.92 d (10.8)	74.3 CH	5.96 d (10.8)	76.3 CH
13		45.5 C		45.3 C		52.0 C
14		70.6 C		71.1 C		148.4 C
15	3.82 s	59.6 CH	3.88 s	59.6 CH	5.75 t (2.0)	123.6 CH
16α	1.78 m	33.2 CH_2	1.87 m	33.3 CH_2	2.42 m	37.7 CH_2
16β	2.38 m		2.25 m		2.69 m	
17	2.73 m	33.2 CH	3.07 m	37.8 CH	3.34 m	48.2 CH
18	1.05 s	13.3 CH_3	0.92 s	13.6 CH_3	0.87 s	15.3 q CH_3
19	0.97 s	22.7 CH_3	1.01 s	22.6 CH_3	1.03 s	23.1 CH_3
20		82.2 C		120.9 C		123.7 C
21	2.08 s	71.8 CH	7.04 s	140.5 CH	7.22 s	140.1 CH
22			6.10 s	111.2 CH	6.28 s	111.0 CH
23			7.23 s	142.2 CH	7.33 s	142.4 CH
28	1.30 s	30.2 CH_3	1.32 s	30.2 CH_3	1.32 s	30.0 CH_3
29	1.54 s	22.3 CH_3	1.56 s	22.4 CH_3	1.56 s	22.9 CH_3
30α	5.22 s	121.4 CH_2	5.23 s	120.9 CH_2	4.98 s	119.3 CH_2
30β	5.33 s		5.36 s		5.33 s	
7-OMe	3.73 s	52.4 CH_3	3.71 s	52.3 CH_3	3.71 s	52.3 CH_3
11-OAc		170.4 C		170.4 C		170.0 C
	2.16 s	20.3 CH_3	2.02 s	20.3 CH_3	1.81 s	20.6 CH_3
12-OAc		169.8 C				170.1 C
	1.98 s	20.5 CH_3			2.11 s	20.5 CH_3
1′				166.8 C		
2′				128.1 C		
3′			6.59 q (5.6)	137.7 CH		
4′			1.69 d (7.2)	14.3 CH_3		
5′			1.58 s	11.7 CH_3		

[a] Recorded at 400 and 100 MHz.
[b] Recorded at 500 and 125 MHz.