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XANTHOTHONE, A NEW NEMATICIDAL N-COMPOUND FROM *Coprinus xanthothrix*

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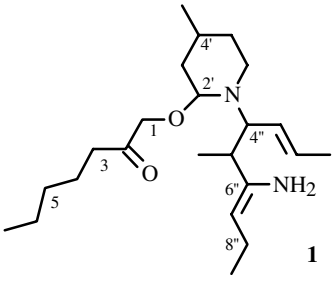
Coprinus xanthothrix *was found to have nematicidal activity. Xanthothone was isolated from culture extract guided by activity assay, which was identified as a novel natural product. Two other compounds were also isolated. These compounds showed nematicidal activity, with* LD_{50} *value of 125-250 ppm both against* Panagrellus redivivus *and* Meloidogyne incognita.

Key words: *Coprinus xanthothrix*, nematicidal activity, *N*-compounds, xanthothone.

Nematophagous fungi greatly contribute to the biological control of plant and animal parasitic nematodes. The production of nematotoxins by these fungi aids in the rapid immobilization and killing of nematodes. At present, more than 90 toxins have been isolated from various fungi [1, 2]. After it was reported that *Coprinus comatus* has nematocidal property [3], three metabolites were cultured, extracted, and isolated from *C. xanthothrix*, and they were tested for their nematicidal activity. They were identified as xanthothone (**1**), 7,8,11-drimanetriol (**2**), and 2-(1H-pyrrol-1-yl) ethanol (**3**).



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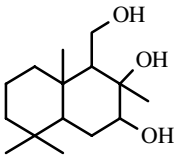

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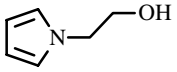

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Compound **1** was obtained as colorless crystals, the formula was determined to be $C_{23}H_{42}O_2N_2$ (*m/z*: 401.3119[M+Na]$^+$, calcd: 401.3143) by HRESI$^+$-MS. The IR spectra revealed the presence of NH_2 (3439, 2931 cm^{-1}) and double bonds (1726, 1645 cm^{-1}). The formula suggests that there are two unsaturations. Along with the numbers of methyl and methylene, compound **1** should have a chain. The compound could be colorized by $BiKI_2$, suggesting that N atoms exist in it.

The ^{13}C NMR and DEPT spectra of compound **1** showed 23 signals (Table 1), including two quaternary carbon atoms (one ketonic carbon). Methylene (δ 68.14 ppm) shifted downfiled corresponds to a joined hetero atom. The three methines positioned downflied belong to a double bond carbon. One N atom exists on a piperidine ring and the other N atom is amine. Furthermore, HMBC revealed a methylene joined to a hetero atom, and the ketonegroup is on the same chain, beyond another one containing a double bond. The ketone and methylene with the hetero atom were separated by two methines. Two of the five methyls were substituted.

Based on the above analysis, compound **1** was elucidated as 1-(1-((2*E*,6*Z*)-6-amino-5-methylnona-2,6-dien-4-yl)-4-methylpiperidin-2-yloxy)heptan-2-one, a novel structure named xanthothone.

Compound **2** was identified as the known structure 7,8,11-drimanetriol [4]. Compound **3** was elucidated as 2-(1H-pyrrol-1-yl) ethanol [5].

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TABLE 1. 1H (500 MHz) and ^{13}C NMR Data (125 MHz) of Compound **1** (in $CDCl_3$)

C atom	^{13}C	^{1}H	HMBC
1	68.14 (t)	4.24	H-3
2	200.18 (s)		H-1, H-3
3 - 6	23.10-33.66 (t)	1.29 - 2.59	
7	14.05 (q)	0.93	
2′	80.42 (d)	4.20	H-4′, H-3′
3′	35.36 (t)	1.75	
4′	24.77 (d)	1.88	
5′	32.80 (t)	1.45	
6′	57.37 (t)	1.25	H-5′, H-2′
4′-CH_3	26.07 (q)	1.04	
1″	25.76 (q)	1.33	
2″	128.79 (d)	7.69	H-4″
3″	130.89 (d)	7.54	
4″	55.26 (d)	3.96	H-5″, H-6″
5″	38.68 (d)	2.59	
6″	162.97 (s)		H-5, H-7, H-8
7″	120.39 (d)	6.33	
8″	22.97 (t)	2.19	
9″	19.57 (q)	1.06	
5″-CH_3	10.94 (q)	1.18	

TABLE 2. Nematicidal Activity of Isolated Compounds

Compound	Nematicidal activity, µg/mL			
	Meloidogyne incognita		*Panagrellus redivivus*	
	LD_{50}	LD_{90}	LD_{50}	LD_{90}
1	250	1000	250	1000
2	1000	>1000	1000	>1000
3	125	250	125	250

The nematicidal activity of xanthothone is shown in Table 2. The compound was active with an LD_{50} value of 250 ppm against both *Panagrellus redivivus* and *Meloidogyne incognita*. When the concentration was increased to 1000 ppm, almost all nematodes were killed. Compound **3** also showed strong nematicidal activity at 125 ppm. Compound **2**, however**,** showed weak activity against the tested nematodes.

There is no report on any previous phytochemical investigation on *C. xanthothrix* available to date. In *Coprinus* genus, only *C. comatus* was studied for its nematicidal compounds. But there are big differences between the compounds isolated from *C. comatus* and *C. xanthothrix*. The toxins found in *C. comatus* are *O*-containing heterocyclic compounds, whereas the toxins isolated from *C. xanthothrix* are *N*-containing compounds [5]. The *O*-containing heterocyclic toxins isolated from cultures of *C. comatus* have stronger nematicidal activity than the *N*-containing compounds from *C. xanthothrix.* As *Coprinus* genus has more than 744 records, there is a high potential of diversity of nematicidal components in them. More active compounds should be investigated on such a group of nematophagous fungi in further work.

EXPERIMENTAL

General Procedures. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were obtained on a Shimadzu double-beam 210A spectrometer. MS were recorded on a VG Auto Spec-3000 spectrometer. 1D- and

2D-NMR spectra were run on Bruker AM-400 and DRX-500 instrument with TMS as internal standard, respectively. TLC was performed on plates precoated with Silica gel (Qingdao Marine Chemical Ltd. China).

Producing Organism. *C. xanthothrix* 4916, was purchased from Direkt Marketing Szovetseg, Germany, and cultivated on potato dextrose medium. The slant strain is deposited in the culture collection of Key Laboratory for Conservation and Utilization of Bio-resource, Yunnan University.

Culture and Isolation. The strain was grown in 20 L potato dextrose agar and subsequently extracted twice with 15 L methanol. The organic phase was reduced to an oily extract (59 g). The extract was dissolved in water and extracted twice with ethyl acetate. All extracts and residues were tested for nematicidal activity. As a result, the ethyl acetate extract shows active properties, and the residue is inactive. The active extract (4g) was subjected to liquid chromatography on a silica gel (Meijing 200-300 μm, column 55 × 450mm) and stepwise elution with petroleum-acetone (5:1 and 2:1). The eluants were combined according to the TLC results and tested for nematicidal activity. The active fraction was purified by liquid chromatography. Three compounds were isolated.

Nematicidal Assay. Worms of the root-knot nematode *M. incognita* were cultivated on tomato plants in the greenhouse at 25°, and second stage juveniles were extracted and stored according to Kerry's method [6]. The nematode *P. redivivus* was cultured on 20% oatmeal medium at 25° for 7 days. The nematodes were separated from the culture medium by the Baerman funnel technique [7]. The assay for nematicidal activity was carried out as described by Stadler et al. [8].

Xanthothone (1): $C_{23}H_{42}O_2N_2$. IR (KBr, ν, cm^{-1}): 3439, 2931, 1726, 1654, 1461, 1382, 1274, 1072, 744, 534; HRESI-MS (*m/z*: 401.3119[M + Na]$^+$, calcd: 401.3143); EI-MS (70eV) *m/z*: 378[M]$^+$, 301 (27), 245 (15), 202 (20), 145 (10), 91 (13), 77 (7).The NMR data are listed in Table 1.

7,8,11-Drimanetriol (2): $C_{15}H_{28}O_3$, $[\alpha]_D^{28}$ –21.38° (0.00252g/mL); IR (KBr, ν, cm^{-1}): 3406, 2928, 2899, 2884, 2527, 2495, 1638, 1461, 1440, 1387, 1052, 997; EI-MS (70eV, *m/z)*: 241 [M]$^+$, 238 (45), 177 (100), 109 (60), 95 (45), 69 (33). ^{1}H NMR (500 MHz, CD_3OD, δ): 1.44 (H-1), 3.46 (H-3), 01.97(H-4), 1.26 (H-6), 1.68 (H-7), 1.58 (H-8), 1.39 (H-4a), 1.11 ($2CH_3$), 0.84 ($5CH_3$), 0.86 ($5CH_3$), 1.31 (8a-CH_3), 3.87 (CH_2OH); ^{13}C NMR (125 MHz, CD_3OD, δ): 55.49 (d, C-1), 76.69 (s, C-2), 76.13 (d, C3), 26.94 (t, C-4), 39.35 (s, C-5), 43.24 (t, C-6), 19.52 (t, C-7), 40.89 (t, C-8), 47.49 (d, C-4a), 33.73 (s, C-8a), 16.88 (q, 2-CH_3), 22.19 (q, $5CH_3$), 27.65 (q, $5CH_3$), 33.82 (q, 8a-CH_3), 59.96 (d, CH_2OH).

2-(1H-Pyrrol-1-yl) ethanol (3): C_6H_9ON, IR (KBr, ν, cm^{-1}): 3422, 2927, 2879, 1616, 1513, 1451, 1364, 1232, 1105, 1052, 1014, 818, 555; HRESI-MS (*m/z*: 110.0185[M-H]$^-$, calcd: 110.0187); FAB$^-$ MS *m/z*: 110[M-H]$^-$; ^{1}H NMR (500 MHz, $CDCl_3$,δ): 3.69 (H-1), 2.72 (H-2), 7.02 (H-3), 6.70 (H-4), 7.02 (H-5), 6.70 (H-6); ^{13}C NMR (125MHz, $CDCl_3$,δ): 64.54 (t, C-1), 39.39 (t, C-2), 130.81 (d, C-3), 116.16 (d, C-4), 130.81 (d, C-5), 116.16 (d, C-6).

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