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Antiviral anthraquinones and azaphilones produced by an endophytic fungus *Nigrospora* sp. from *Aconitum carmichaeli*

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ABSTRACT

A new hydroanthraquinone derivative, 6-*O*-demethyl-4-dehydroxyaltersolanol A (**1**), and two new azaphilones, 8,11-didehydrochermesinone B (**6**) and (7*S*)-7-hydroxy-3,7-dimethyl-isochromene-6,8-dione (**8**), along with five known analogues (**2**–**5** and **7**), were isolated from the culture broth of *Nigrospora* sp. YE3033, an endophytic fungus obtained from *Aconitum carmichaeli*. Their structures were elucidated on the basis of spectroscopic analyses. Biological activity test indicated that compounds **1**–**3**, and **7** exhibited the inhibitory effects on influenza viral strain of A/Puerto Rico/8/34 (H1N1) with the IC_{50} values of 2.59, 8.35, 7.82, and 0.80 μg/mL, respectively, while the low cytotoxicity of **7** with the CC_{50} value of 184.75 μg/mL, displaying a promising potential of **7** in the development of anti-influenza A virus drugs.

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1. Introduction

Endophytic fungi colonizing inside the normal tissues of healthy host plants have attracted considerable attention due to their ecological and biotechnological potential [1]. They are promising sources for the discovery of structurally diverse and various bioactive secondary metabolites [2,3]. Fungi belonging to the genus *Nigrospora* have proved to producing bioactive compounds with diverse chemical structures, such as plant growth-inhibiting nigrosporolide [4] and phomalactone [5], phytotoxic and antibacterial nigrosporins [6], antibacterial anthraquinones [7], antifungal griseofulvin [8], antiviral heptaketides [9], and cytotoxic 4-dehydroxyaltersolanol A [10] and cochlioquinone [11].

During the course of our search for biologically active substances from endophytic fungi, we found a culture broth of the fungal strain *Nigrospora* sp. YE3033 isolated from the root of *Aconitum carmichaeli*, exhibiting inhibitory activity against H1N1 influenza virus. Our current chemical study on the culture broth of this fungal strain led to the isolation of one new hydroanthraquinone derivative, 6-*O*-demethyl-4-dehydroxyaltersolanol A (**1**), and two new azaphilones, 8,11-didehydro-chermesinone B (**6**) and (7*S*)-7-hydroxy-3,7-dimethyl-isochromene-6,8-dione (**8**), together with five known analogues, 4-dehydroxyaltersolanol A (**2**) [10], altersolanol B (**3**) [12,13], macrosporin (**4**) [13–15], 1, 3, 7-trihydroxy-6-methylanthraquinone (**5**) [16], and chermesinone B (**7**) [17] (Fig. 1). Herein we report the isolation, structure elucidation, and biological activities of these compounds.

2. Experimental

2.1. General experimental procedures

Optical rotations were determined with a Jasco P-2000 polarimeter. UV spectra were recorded on Shimadzu double-beam 210A spectrophotometer. IR spectra were obtained on a Bio-Rad FTS spectrometer, with KBr pellets. CD spectra were acquired with Applied Photophysics Chirascan Circular Dichroism spectrometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using TMS as an internal standard. HRESIMS were measured using an Agilent 3250AA LC-MSD TOF mass spectrometer. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Factory, China), Sephadex LH-20 (Amersham Pharmacia Biotech), and RP-18 silica gel (40–63 μm, Merck). Precoated silica gel plates (Qingdao Marine Chemical Factory,

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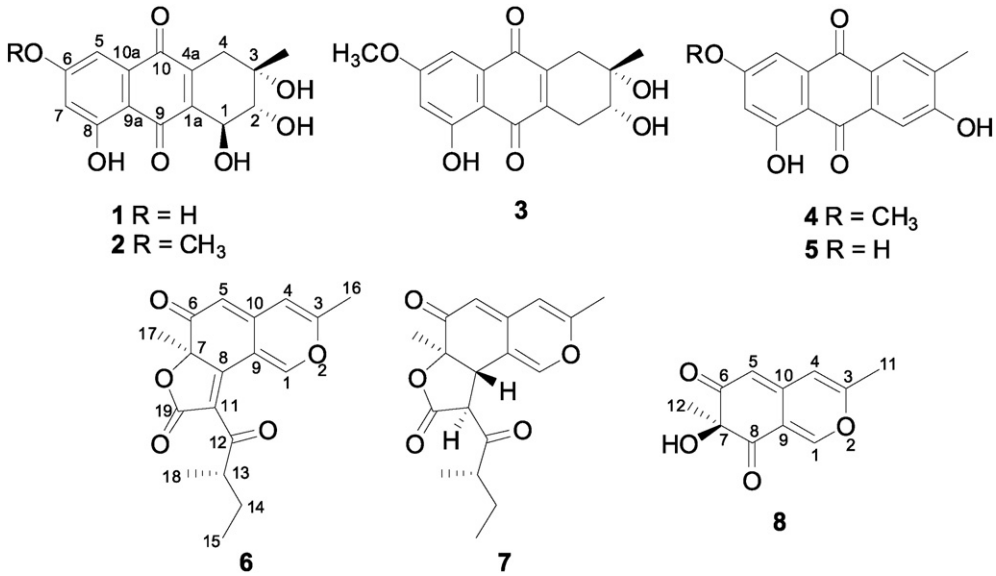

Fig. 1. Chemical structures of compounds **1**–**8**.

China) were used for TLC. Detection was done by spraying the plates with 5% H_2SO_4, followed by heating.

2.2. Fungal material

The fungal strain *Nigrospora* sp. YE3033 was isolated from the root of *A. carmichaeli* collected in August 2011 in Lijiang County, Yunnan Province, P. R. China. The strain was identified as *Nigrospora* sp. based on its morphological characteristics, combined with the biological protocol by DNA amplification and the sequence analysis of rDNA ITS (internal transcribed spacer) region. The sequence of the ITS region of *Nigrospora* sp. YE 3033 has been submitted to GenBank (Accession No. KU987454). By using BLAST (nucleotide sequence comparison program) to search the GenBank database, *Nigrospora* sp. YE 3033 shared a similarity of 99% with *Nigrospora oryzae* Z19–18 (Accession No. HQ262527). The strain was deposited in Yunnan Institute of Microbiology, Yunnan University, China.

2.3. Fermentation, extraction, and isolation

The fresh mycelium was inoculated into 250 mL Erlenmeyer flasks containing 50 mL of PDB medium (200 g potato and 20 g dextrose in 1 L H_2O), and then incubated at 28 ± 1 °C on a rotary shaker at 200 r.p.m. for 3 days as seed cultures. Each of the seed cultures (50 mL) was transferred into 2000 mL Erlenmeyer flasks (×50) containing 500 mL of PDB medium. The following cultivation was carried out at 28 ± 1 °C for 7 days on a rotary shaker at 200 r.p.m. The cultures were filtered to remove mycelia. The filtrate was concentrated under reduced pressure to a volume of 10 L and then exhaustively extracted with EtOAc (4 × 10 L), and the combined organic phase was concentrated to obtain a brown gum of broth extract (26.3 g). The extract was chromatographed on silica gel column, eluting with a gradient of $CHCl_3$/MeOH from 1:0 to 0:1 (v/v) to afford six fractions. Fraction 2 (1.4 g) was resubjected to silica gel CC (petroleum ether/Me_2CO, 95:5) to provide three subfractions: Fr. 2.1–2.3. Fr. 2.1 was separated by silica gel CC (petroleum ether/EtOAc, 85:15) and further purified by RP (MeOH/H_2O, 6:4, 7:3) to afford **6** (2.1 mg) and **7** (8.5 mg). Fraction 3 (2.7 g) was subjected to silica gel CC (petroleum ether/EtOAc, 8:2, 7:3, 6:4) to afford Fr. 3.1–3.3. Fr. 3.2 was purified by silica gel CC ($CHCl_3$/acetone, 9:1) to afford **8** (2.3 mg). Fr. 3.3 was purified by RP CC (MeOH/H_2O, 4:6, 1:1, 6:4, 7:3) to afford **4** (8.2 mg) and **5** (7.4 mg). Fraction 4 (1.2 g) was subjected to silica gel CC ($CHCl_3$/MeOH, 10:1) and then purified by Sephadex LH-20 (MeOH) to give **3** (6.5 mg). Fraction 5 (1.8 g) was subjected to RP CC (MeOH/H_2O gradient system, 3:7 → 6:4 *v*/v) and further purified by silica gel ($CHCl_3$/MeOH, 9:1, 8:2) to yield **1** (3.6 mg) and **2** (5.8 mg).

2.3.1. 6-O-demethyl-4-dehydroxyaltersolanol A (*1*)

Yellow powder; $[\alpha]^{20}_D$ + 38.4 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 218 (2.17), 279 (1.96) nm; IR (KBr) ν_{max} 3422, 2915, 2847, 1661, 1609, 1472, 1384, 1226, 1135, 996 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 305.0667 $[M\text{-}H]^-$ (calcd for $C_{15}H_{13}O_7$, 305.0661).

2.3.2. 8,11-Didehydrochermesinone B (*6*)

Yellow powder; $[\alpha]^{20}_D$ + 257.2 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 263 (3.08), 355 (3.64) nm; IR (KBr) ν_{max} 2981, 2923, 2869, 1762, 1703, 1647, 1548, 1372, 1296, 1245, 1169, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 337.1054 $[M + Na]^+$ (calcd for $C_{18}H_{18}O_5Na$, 337.1052).

2.3.3. (7S)-7-hydroxy-3,7-dimethyl-isochromene-6,8-dione (*8*)

White powder; $[\alpha]^{20}_D$ + 54.7 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 251 (3.17), 287 (3.82) nm; IR (KBr) ν_{max} 3435, 2967, 2872, 1668, 1584, 1541, 1147, 956 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 229.0482 $[M + Na]^+$ (calcd for $C_{11}H_{10}O_4Na$, 229.0477).

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1**.

Position	δ_H (*J* in Hz)	δ_C
1	4.84 (d, 5.8)	70.5
2	3.62 (d, 5.8)	78.2
3		71.5
4	2.79 (d, 19.1)	36.5
	2.61 (d, 19.1)	
5	7.00 (d, 2.1)	109.5
6		163.0
7	6.50 (d, 2.1)	108.5
8		166.9
9		189.3
10		185.5
1a		143.2
4a		143.7
9a		109.8
10a		135.2
3-Me	1.36 (s)	25.7

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