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A New Tropolone from the Insect Pathogenic Fungus *Cordyceps* sp. BCC 1681

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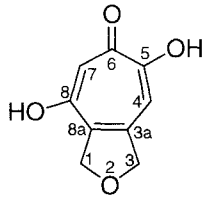
In our continued search for novel bioactive compounds from insect pathogenic fungi, collected from various parts of Thailand, we came across a new antimalarial tropolone, named cordytropolone (**1**), in the culture broth of *Cordyceps* sp. BCC 1681. Herein we report the isolation, structural elucidation and biological activities of this compound.

Cordyceps sp. was collected at Khao Soi Dao Wildlife Sanctuary, Chantaburi province, Thailand, on Coleoptera-elaterid, and identified by Dr. NIGEL L. HYWEL-JONES of the Mycology Research Unit, BIOTEC. The fungus is deposited at the Thailand BIOTEC Culture Collection (registration code: BCC 1681).

An isolated culture of the strain BCC 1681 was grown on potato dextrose agar (PDA) at 22°C for 7 days, before inoculation as several pieces of discs (5 mm diameter) into 20×1 liter Erlenmeyer flasks each containing 250 ml of potato dextrose broth (PDB). The cultures were incubated at 22°C for 19 days. The fermentation broth was filtered, the filtrate (5 liters) was thoroughly extracted with EtOAc (5 liters), and washed with H_2O. The combined EtOAc solution was concentrated under reduced pressure to obtain a light brown solid (550 mg) which was then triturated twice in EtOAc (25 ml, room temperature, 15 hours) to obtain pure cordytropolone (**1**; 382 mg) as a colorless powder.



1

The physico-chemical properties of **1** are shown in Table 1. The molecular formula of **1** was determined by HRMS (ESI-TOF, negative) as $C_9H_8O_4$. ^{13}C NMR spectrum of **1** showed nine carbon signals. DEPTs analyses revealed that this molecule consists of five aromatic quaternary carbons, two aromatic methine carbons and two methylene carbons. Analyses of HMQC and HMBC spectra (Table 2) revealed the structure **1** whose IR and UV spectra were in good agreement with the tropolone skeleton as compared to the data of related compounds.[1,2]

In order to confirm the presence of the two acidic hydroxyl groups, compound **1** was treated with excess MeI/K_2CO_3 in DMSO for polymethylation, and the crude mixture was subjected to separation and purification by preparative HPLC using a reversed-phase column (MeOH/H_2O) to obtain two products. The major component was identified as 5-*O*-8-*O*-dimethyl analog of **1** (compound **2a**) and minor was assigned as compound **2b** (**2a** : **2b**=77 : 23). Structures of these products were assigned by HRMS and NMR (^1H, ^{13}C, NOESY, HMQC and HMBC) analyses. The HMBC and NOESY correlations nicely revealed positions of the two methoxy groups in both molecules (Fig. 1). Although another

Table 1. Physico-chemical properties of cordytropolone (**1**).

Appearance	Colorless powder
MP (°C)	>300 °C
Molecular formula	$C_9H_8O_4$
HRMS (ESI-TOF)	
Found (*m/z*)	179.0341[M-H]$^-$
Calcd.	179.0345
UV λ_{max} nm (ε) in MeOH	254 (47,900), 342 (11,700)
IR ν_{max} (KBr) cm^{-1}	3192, 1607, 1523, 1441, 1386, 1279, 1228, 1178, 919, 770, 713

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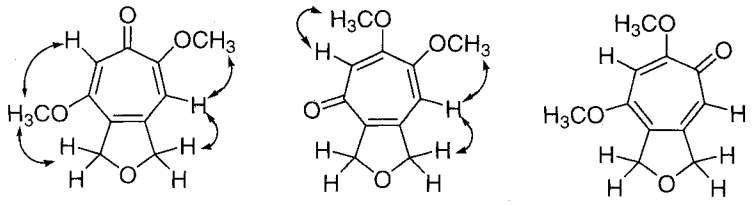
Fig. 1. NOESY correlations of **2a** and **2b**.



2a **2b** **2c**

Table 2. NMR data (DMSO-d_6) for compound **1**.

position	^{13}C NMR δ_C	^{1}H NMR δ_H	HMBC (^{1}H to ^{13}C)
1	75.1 (t)	4.97 (d, 2.8)	C3a, C8a
3	77.9 (t)	5.02 (d, 2.8)	C3a, C8a
3a	145.6 (s)	-	
4	106.6 (d)	6.81 (s)	C3, C5, C6, C8a
5	164.4 (s)	-	
6	173.1 (s)	-	
7	113.2 (d)	6.79 (s)	C5, C6, C8, C8a
8	162.1 (s)	-	
8a	127.5 (s)	-	

plausible product, **2c**, was not detected, the experimental evidence demonstrated the dihydroxytropolone structure of the parent compound, cordytropolone (**1**). As known for related dihydroxytropolones, cordytropolone (**1**) should also exist as an equilibrium structure corresponding to desmethyl **2a**, **2b**, **2c**. The structure of cordytropolone is related to those of some other natural tropolones such as sepedonin[1] (from *Sepedonium chrysospermum*), BMY-28438[2] (3,7-dihydroxytropolone; from *Streptomyces tropolofacience*), puberulic and puberulonic acids[3,4] (from *Penicillium puberulum* and several other *Penicillium* spp.).

Cordytropolone exhibited *in vitro* antimalarial activity (*Plasmodium falciparum* K1 strain)[5] with an IC_{50} value of 2.2 μg/ml. However, this compound also showed cytotoxicity[6] against two cancer cell-lines (KB and BC-1) and vero cells with IC_{50} values of 17, 2.2 and 11 μg/ml, respectively.

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