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Two new compounds from the endophytic fungus *Colletotrichum* sp. L10 of *Cephalotaxus hainanensis*

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Two new compounds, 3-(4-methoxy-3-methyl-2-oxo-2H-pyran-6-yl)butyl pyroglutamate (**1**) and 2,2'-(1,1'-methylenebis(1H-indole-3,1-diyl))diethanol (**2**), were isolated from the endophytic fungus of *Cephalotaxus hainanensis*. Their structures were determined by means of chemical and spectroscopic analysis.

Keywords: *Cephalotaxus hainanensis* Li; endophytic fungus; dimer

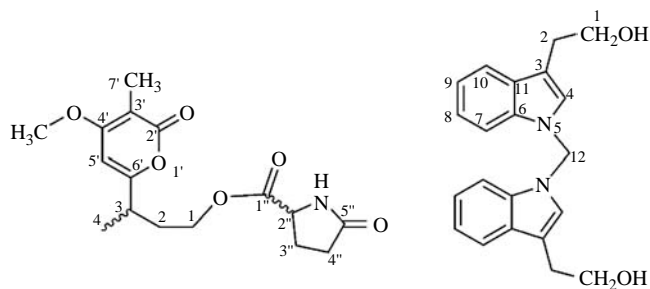
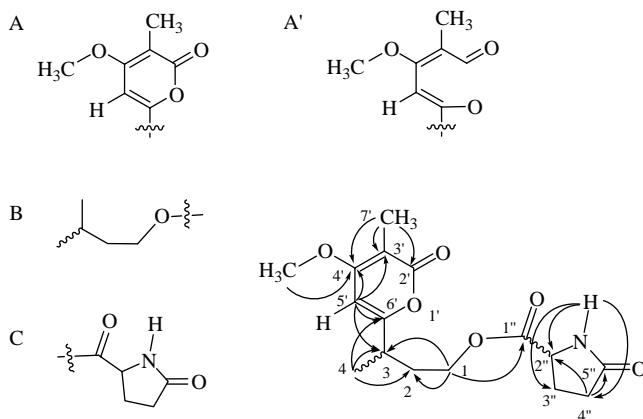
1. Introduction

Cephalotaxus hainanensis Li is a Chinese folk medicine with anticancer activity. The biological constituents are alkaloids [1]. Previous researches indicated that the compounds generated by endophytic fungus and compounds from the plants were structurally alike [2]. We investigated the endophytic fungus of *C. hainanensis* Li in order to isolate some alkaloids with anticancer function, which may solve the problem of natural resource of *C. hainanensis* Li prohibited from being developed as an endangered species of national level. In this paper, we report the isolation and structural elucidation of two new compounds: 3-(4-methoxy-3-methyl-2-oxo-2H-pyran-6-yl)butyl pyroglutamate (**1**) and 2,2'-(1,1'-methylenebis(1H-indole-3,1-diyl))diethanol (**2**) (see Figure 1).

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{20} + 8.6$ ($c = 0.29$, MeOH). The molecular formula was determined to be $C_{16}H_{21}O_6N$ by HR-FAB-MS at m/z 346.1260 $[M + Na]^+$ and the unsaturated degrees were determined to be 7. In HMBC spectrum of **1**, correlations between H-7' at δ 1.75 and C-3' at δ 99.1, C-2' at δ 164.5, C-4' at δ 166.3; $-OCH_3$ at δ 3.89 and C-4' at δ 166.3; H-5' at δ 6.47 and C-3' at δ 99.1, C-6' at δ 166.3, C-4' at δ 166.3 were observed (see Figure 2), indicating the existence of two double bonds at δ 166.3, 99.1 and δ 166.3, 94.2 as well as the fragment A' (see Figure 2). Besides the two double bonds in ^{13}C NMR spectrum, three carbonyl signals at δ 164.5, 173.0, and 177.0 were observed. So, two ring moieties must be in the structure of compound **1** according to the unsaturated degrees 7.

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Figure 1. Structures of compounds **1** and **2**.Figure 2. Fragments and key HMBC correlations of compound **1**.

In HMBC spectrum of **1**, correlations between H-4 at δ 1.20 and C-3 at δ 34.9, C-2 at δ 32.7; H-1 at δ 4.05 and C-2 at δ 32.7, C-3 at δ 34.9 were observed, which indicated the existence of fragment B (see Figure 2) and was further confirmed by correlations in $^1\text{H}-^1\text{H}$ COSY spectrum between H-1 at δ 4.05 and H-2 at δ 2.02, H-2 at δ 2.20 and H-3 at δ 2.85, H-3 at δ 2.85 and H-4 at δ 1.20.

Correlations between N-H at δ 7.99 and C-3'' at δ 24.5, C-2'' at δ 54.9, C-4'' at δ 28.9; H-4'' at δ 2.15 and C-2'' at δ 54.9, C-5'' at δ 177.0; H-3'' at δ 1.83 and C-2'' at δ 54.9, C-1'' at δ 173.0 were observed, which indicated the existence of fragment C (see Figure 2) and was also confirmed by correlations in $^1\text{H}-^1\text{H}$ COSY spectrum between H-2'' at δ 4.10 and H-3'' at δ 1.83, H-3'' at δ 1.83 and H-4'' at δ 2.15.

Besides the above evidence, six unsaturated degrees were assigned, according to the chemical shift of carbonyl C-2' at δ 164.5, the last unsaturated degree was assigned to fragment A' to form an α,β -unsaturated lactone (fragment A).

Fragments A, B, and C were connected by the correlations in HMBC spectrum between H-4 at δ 1.20 and C-6' at δ 166.3 as well as between H-1 at δ 4.05 and C-1'' at δ 173.0. And thus, the structure of compound **1** was finally determined as 3-(4-methoxy-3-methyl-2-oxo-2H-pyran-6-yl)butyl pyroglutamate.

Compound **2** was obtained as a white amorphous powder with $[\alpha]_D^{20} + 0.9$ ($c = 0.28$, MeOH). The molecular formula was determined to be $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2$ by HR-FAB-MS at m/z 357.1570 $[\text{M} + \text{Na}]^+$, suggesting that the unsaturated degrees were 12. Only 11 carbon signals were

observed in the ^{13}C spectrum of compound **2**, which suggested that compound **2** may be a dimer.

In addition to the carbon signals at δ 135.8, 128.3, 112.8, and 126.2, the proton signals at δ 7.76 (d, $J = 8.2$ Hz), 7.15 (t, $J = 7.4$ Hz), 7.01 (t, $J = 7.5$ Hz), and 7.49 (d, $J = 7.8$ Hz) indicated the existence of an O-di-substituted system in aromatic ring. Besides the signals above, carbon signals at δ 112.8, 126.2 suggested the existence of an indole ring, which was confirmed by the correlations in HMBC spectrum (see Figure 3) between H-8 at δ 7.15 and C-7 at δ 110.2, C-9 at δ 119.2, C-10 at δ 118.9; H-10 at δ 7.49 and C-3 at δ 112.8, C-11 at δ 128.3, C-6 at δ 135.8.

And the proton signals at δ 3.61 (2H, t, $J = 7.1$ Hz) and 2.78 (2H, t, $J = 7.1$ Hz) suggested the existence of a hydroxyethyl group, and the hydroxyethyl group was determined to be linked at C-3 of the indole ring by correlations in HMBC spectrum between H-1 at δ 3.61 and C-3 at δ 112.8 as well as between H-4 at δ 7.45 and C-2 at δ 28.6. Besides signals belonging to the indole ring and the hydroxyethyl group, another proton signal at δ 6.52 (1H, s) belonging to a methylene group was observed, and the methylene group was determined to be at N-5 for the

HMBC correlations between H-4 at δ 7.45 and C-12 at δ 55.0 as well as between H-12 at δ 6.52 and C-6 at δ 135.8.

All the proton and carbon signals were assigned, but the molecular weight of the structure we established was half of the HRMS we mentioned above and no group was found to be linked at the methylene group. So the structure of compound **2** was considered to be a dimer through the methylene and was finally determined as 2,2'-(1,1'-methylenebis(1H-indole-3,1-diyl))diethanol.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were measured on a Shimadzu UV-1601. IR spectra were taken on a Bruker IFS-55 infrared spectrophotometer. The NMR spectral data were recorded on Bruker AV-600 (600 MHz for ^1H and 150 MHz for ^{13}C) in $\text{DMSO}-d_6$ with TMS as the internal standard. The HR-FAB-MS data were obtained on the Micross Mass Autospec-UltimaE TOF mass spectrophotometer. Chromatography was performed on silica gel (200–300 mesh, Qingdao Haiyang Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, USA), reversed-phase HPLC (Shimadzu LC-8A vp, Tokyo, Japan).

3.2 Fungus material

C. hainanensis Li was collected in June 2005 in the Hainan Province of China. A voucher specimen (No. CE20050612) was identified by associate researcher Zengfu Dai and has been deposited in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences. The endophytic fungus was isolated from the phloem of the plant and was identified as *Colletotrichum* sp. The hyphostroma was brownish-black.

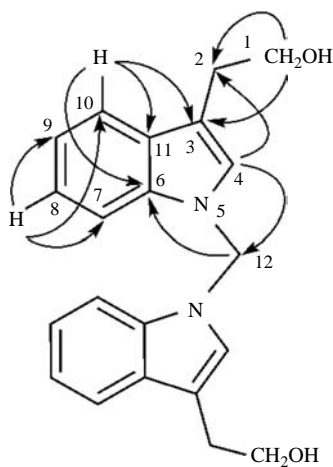


Figure 3. Key HMBC correlations of compound **2**.

The fresh mycelium grown on PDA medium was inoculated into 0.5 liter Erlenmeyer flasks containing 0.15 liter liquid medium (2% glucose, 2% maltose, 1% monosodium glutamate, 0.05% KH_2PO_4 , 0.03% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3% yeast extract, tap water, pH 6.5). After 2 days of the incubation on rotary shaker at 28°C at 180 rpm, a 7.5 ml culture liquid was transferred as a seed into each of 0.5 liter Erlenmeyer flask containing 0.15 liter of the same liquid medium. The flasks were subsequently incubated at the same conditions for 8 days.

3.3 Extraction and isolation

The fermentation broth of the endophytic fungus L10 (70 liters) was concentrated to 1200 ml, which was extracted with ethyl acetate and *n*-butanol, successively. The ethyl acetate soluble fraction (17.3 g) was subjected to silica gel column, eluted with CHCl_3 – CH_3OH (100:1–0:1), yielding 10 fractions. Fraction 3 (2 g) was purified by Sephadex LH-20 column chromatography (CH_3OH) and preparative

HPLC (CH_3OH – H_2O 50:100, flow rate 1 ml/min, wavelength 210 nm) to obtain compound **1** (11 mg, retention time 25 min). Fraction 2 (5 g) was purified by Sephadex LH-20 column chromatography (CH_3OH) and preparative HPLC (CH_3OH – H_2O 63:100, flow rate 1 ml/min, wavelength 210 nm) to obtain **2** (5 mg, retention time 36 min).

3.3.1 3-(4-Methoxy-3-methyl-2-oxo-2H-pyran-6-yl)butyl pyroglutamate (**1**)

White amorphous powder; $[\alpha]_D^{20} + 8.6$ ($c = 0.29$, MeOH); UV(MeOH) λ_{max} : 233 nm; IR (KBr) ν_{max} (cm^{-1}) 3127, 2947, 2875, 1708, 1687, 1546, 1260, 991. ^1H and ^{13}C NMR spectral data, see Table 1; HR-FAB-MS: m/z 577.2616 [$\text{M} + \text{Na}$] $^+$ (calcd for $\text{C}_{28}\text{H}_{42}\text{O}_{11}\text{Na}$, 577.2619).

3.3.2 2,2'-(1,1'-Methylenebis(1H-indole-3,1-diyl))diethanol (**2**)

White amorphous powder; $[\alpha]_D^{20} + 0.9$ ($c = 0.28$, MeOH); UV(MeOH) λ_{max} : 227 nm; IR (KBr) ν_{max} (cm^{-1}) 3390, 3325, 3058, 2919, 1632, 1518, 1457,

Table 1. The ^1H and ^{13}C NMR spectral data of compounds **1** and **2** (600 MHz for ^1H and 150 MHz for ^{13}C , *d*-DMSO).

1			2		
No.	δ_{H} (J in Hz)	δ_{C}	No.	δ_{H} (J in Hz)	δ_{C}
1	4.05 (m)	62.5	1	3.61 (t, $J = 7.1$)	61.4
2	1.95 (m), 2.02 (m)	32.7	2	2.78 (t, $J = 7.1$)	28.6
3	2.85 (m)	34.9	3	–	112.8
4	1.20 (d, $J = 9.0$)	18.2	4	7.45 (s)	126.2
1'	–	–	5	–	–
2'	–	164.5	6	–	135.8
3'	–	99.1	7	7.76 (d, $J = 8.2$)	110.2
4'	–	166.3	8	7.15 (t, $J = 7.4$)	121.7
5'	6.47 (s)	94.2	9	7.01 (t, $J = 7.5$)	119.2
6'	–	166.3	10	7.49 (t, $J = 7.8$)	118.9
7'	1.75 (s)	8.4	11	–	128.3
4'- OCH_3	3.89 (s)	56.8	12	6.52 (s)	55.0
1''	–	173.0			
2''	4.10 (m)	54.9			
3''	1.83 (m), 2.30 (m)	24.5			
4''	2.15 (m)	28.9			
5''	–	177.0			
N–H	7.99 (s)	–			

1235, 1272, 1069, 990, 687. ^1H and ^{13}C NMR spectral data, see Table 1; HR-FAB-MS: m/z 346.1260 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{16}\text{H}_{21}\text{O}_6\text{NNa}$, 346.1261).

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