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A New Butyrolactone from the Fermentation Products of Endophytic Fungus *Aspergillus versicolor*

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A new butyrolactone, asperphenol C (**1**), was isolated from the fermentation products of an endophytic fungus *Aspergillus versicolor*. Its structure was elucidated by spectroscopic methods including extensive 1D- and 2D NMR techniques. Compound **1** was also tested for its antitobacco mosaic virus (anti-TMV) activity. The results showed that compound **1** exhibited anti-TMV activity with inhibition rate of 22.1 %.

Keywords: Asperphenol C, Butyrolactones, *Aspergillus versicolor*, Anti-TMV activity.

INTRODUCTION

The *Aspergillus (Moniliaceae)*, with over 180 species, has attracted considerable attention as a rich source of alkaloids, terpenoids, xanthones and polyketides, some of which showed antifungal, antibacterial, antifouling and cytotoxic activities[1-3]. Butyrolactones were mainly found as metabolites from fungi and high plants in nature[4]. They appeal to medicinal chemists because of their pronounced pharmacological effects including antibacterial[5,6], antitumor[7,8], anti-inflammatory[9,10], anti-virus[11,12], *etc*.

In previous studies, some butyrolactones were isolated from the fermentation products of fungus *Aspergillus*[13-16]. With the aim of searching for new bioactive metabolites from the fermentation products of microbe, an endophytic *Aspergillus versicolor* were isolated from the rhizome of *Paris polyphylla var. yunnanensis*, collected in Shizhong, Yunnan, P.R. China and the chemical constituents of it fermentation products were investigated. As a result, a new butyrolactones (**1**) were isolated. Its structure was elucidated on the basis of a comprehensive analysis of the ^{1}H and ^{13}C NMR. In addition, the antitobacco mosaic virus (anti-TMV) active of **1** was evaluated.

EXPERIMENTAL

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. 1D and 2D NMR spectra were recorded on a DRX-400 or 500 NMR spectrometer with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 5 % H_2SO_4 in EtOH.

Fungal material: The culture of *Aspergillus versicolor* was isolated from the rhizome of *Panax pseudoginseng Wall. var. notoginseng* collected from Shizhong, Yunnan, P.R. China, in 2011. The strain was identified by one of authors (Gang Du) based on the analysis of the ITS sequence. It was cultivated at room temperature for 7 days on potato dextrose agar at 28 °C. Agar plugs were inoculated into 250 mL Erlenmeyer asks each containing 100 mL potato dextrose broth and cultured at 28 °C on a rotary shaker at 180 rpm for 5 days. Large scale fermentation was carried out in 200 Fernbach asks (500 mL) each containing 100 g of rice and 120 mL of distilled H_2O. Each flask was inoculated with 5 mL of cultured broth and incubated at 25 °C for 45 days.

Extraction and isolation: The fermented substrate was extracted four times with 70 % MeOH (4 × 5 L) at room temperature and filtered. The crude extract (128 g) was applied to silica gel (200-300 mesh) column chromatography, eluting

with a $CHCl_3$-CH_3OH gradient system (9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-E. The further separation of fraction A (9:1, 22.6 g) by silica gel column chromatography, eluted with $CHCl_3$-$(CH_3)_2CO$ (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures A1-A5. Fraction A3 (7:3, 2.5 g) was separated by silica gel column chromatography and preparative HPLC (58 % MeOH, flow rate 12 mL/min) to give **1** (12.6 mg).

Asperphenol C (**1**), $C_{20}H_{18}NaO_7$, obtained as white amorphous powder; $[\alpha]_D^{24.8}$ + 62.3 (c 0.20, MeOH); UV (MeOH) λ_{max} (log *e*): 215 (4.06), 242 (3.46), 285 (3.68) nm; IR (KBr, ν_{max}, cm^{-1}): 3340, 3018, 2967, 2936, 1745, 1732, 1608, 1524, 1495, 1438, 1386, 1260, 1186, 1128, 1064, 1026, 908, 870, 760; ^1H and ^{13}C NMR (Table-1); ESIMS (positive ion mode) *m/z* 377 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 393.0962 $[M+Na]^+$ (calcd 393.0950 for $C_{20}H_{18}O_7$).

TABLE-1
^1H AND ^{13}C NMR DATA OF COMPOUND **1**
(δ in ppm, in C_5D_5N, 500 MHz)

No.	δ_C (m)	δ_H (m, *J*, Hz)
1	169.0 s	
2	138.2 s	
3	127.6 s	
4	85.0 s	
5	41.2 t	3.47, 3.56 d (14.5)
6	170.4 s	
1'	131.2 s	
2'	111.8 d	7.21 d (1.8)
3'	146.2 s	
4'	152.1 s	
5'	116.4 d	6.74 d (8.2)
6'	121.8 d	6.88 dd (8.2. 1.8)
1"	132.5 s	
2",6"	130.8 d	6.82 d (8.0)
3",5"	128.2 d	7.10 m
4"	127.5 d	7.05 d (7.6)
4'-OMe	56.2 q	3.80 s
6-OMe	53.0 q	3.72 s
3'-OH		11.42 s

RESULTS AND DISCUSSION

The fermented substrate was extracted with 70 % aqueous acetone. The extract was subjected repeatedly to column chromatography on silica gel, RP-18 and semi-preparative RP-HPLC separation to afford compound **1**. Its structure was shown in Fig. 1 and the ^1H and ^{13}C NMR data of **1** was listed in Table-1.

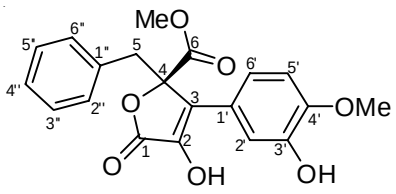

Fig. 1. Structure of compound **1**

Asperphenol C (**1**) was obtained as white solid. The molecular formula $C_{20}H_{18}O_7$ of **1** was determined by HRESIMS which showed pseudomolecular ion peaks $[M+Na]^+$ at 393.0962 (calcd. 393.0950). The IR spectrum showed the presence of ester/lactone carbonyl at 1745 and 1732 cm^{-1}, hydroxyl were evident at 3340 cm^{-1} and the presence of the absorptions at 1608, 1524 and 1495 cm^{-1} was suggestive of aromaticity in the molecule. The ^1H NMR signals revealed the presence of a monosubstituted benzene moiety (δ_H 6.82 d, *J* = 8.0, 2H; 7.10, m, 2H; 7.05, d, *J* = 7.6, 1H), a 1,3,4 disubstituted benzene moiety (δ_H 7.21, d, *J* = 1.8; 6.74, d, *J* = 8.2; 6.88, dd, *J* = 8.2, 1.8), a methylene protons (δ_H 3.47, 3.56, d each, *J* = 14.5), two methoxy proton (δ_H 3.72 s and 3.80 s) and a phenolic hydroxy group (δ_H 11.42). Its ^{13}C NMR showed the presence of monosubstituted benzene moiety [δ_C 132.5 s, 130.8 d (2C), 128.2 d (2C), 127.5 d], a 1,3,4-disubstituted benzene moiety [δ_C 131.2 s, 111.8 d, 146.2 s, 152.1 s, 116.4 d, 121.8 d] rings, one methoxycarbonyl group (δ_C 170.4 s, 53.0 q), one ester carbonyl (δ_C 169.0 s), a pair of olefenic carbon signals (δ_C 138.2 s and 127.6 s), one methylene carbon (δ_C 41.2 t) and one quaternary carbon quaternary carbon quaternary carbon (δ_C 85.0 s). The molecular formula $C_{20}H_{18}O_7$ requires 12 degrees of unsaturation. The presence of two aromatic rings accounts for eight, while two carbonyls and one olefenic carbons account for another three, which makes a total of eleven degrees of unsaturation. Therefore, **1** must possessing one aliphatic ring in addition to two aromatic rings. The tropical carbon signals (δ_C 169.0 s, 138.2 s, 127.6 s, 85.0 s, 41.2 t, 170.4 s) indicated that compound **1** should be a butyrolactone[15-17]. A methoxy group located at C-42 and a phenolic hydroxy group located at C-32 were also supported by the analysis of its HMBC collections. The structure of compound **1** is therefore determined as shown in Fig. 2.

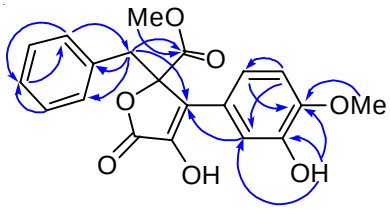

Fig. 2. Key HMBC (↷) correlations of compound **1**

Compound **1** was tested for their anti-TMV activities. The inhibitory activities of compound **1** against TMV replication were tested using the half-leaf method[18]. Ningnanmycin, a commercial product for plant disease in China, was used as a positive control. The results showed that compound **1** exhibited anti-TMV activity with inhibition rate of 22.1 %.

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