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A New Phenyl Derivated Butyrolactone from Fermentation Products of Endophytic Fungus *Aspergillus terreus*

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A new phenyl derivated butyrolactone, terrephenol C (**1**) was isolated from the fermentation products of a fungus *Aspergillus terreus*. Its structure was elucidated by spectroscopic methods, including extensive 1D NMR and 2D NMR techniques. The antitobacco mosaic virus (anti-TMV) activity of **1** was evaluated and showed anti-TMV activity with inhibition rates of 16.7 %.

Keywords: Butyrolactone, Fungus *Aspergillus terreus*, Anti-tobacco mosaic virus activity.

INTRODUCTION

In recent years, numerous metabolites possessing potent bioactivities have been isolated from strains of bacteria and fungi collected from diverse environments[1,2]. Fungi belonging to *Aspergillus* genera are one of the major contributors to the secondary metabolites of fungal origin[3]. *Aspergillus terreus*, is a fungus (mold) found worldwide in soil, decomposing vegetation and dust. It is commonly used in industry to produce important organic acids, such as itaconic acid and *cis*-aconitic acid as well as enzymes, like xylanase[4]. In addition, some metabolites produced by *A. versicolor* have been received more and more attentions from medicinal chemists because they exhibited various biological activities[3-7].

Butyrolactones were mainly found as metabolites from fungi and high plants in nature[8]. They have potential pharmacological effects including antibacterial[9,10], cytotoxicity[11,12], anti-inflammatory[13,14], antivirus[10,15], *etc*. With the aim of multi-purpose utilization endophytic fungus and identify bioactive natural products, the phytochemical investigation on fermentation products of the endophytic fungus *Aspergillus terreus* was carried out. As a result, a new phenyl derivated butyrolactones (**1**) was isolated. Its structure was elucidated on the basis of analysis of the ^{1}H NMR, ^{13}C NMR and 2D NMR spectra.

EXPERIMENTAL

Optical rotations were measured in a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H NMR, ^{13}C NMR and 2D NMR spectra were recorded on Bruker 500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10-40 μm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

The culture of *Aspergillus terreus* was isolated from the rhizome of *Panax pseudoginseng Wall. var. notoginseng*, collected from Dali, Yunnan, P.R. China, in 2012. 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 100 Fernbach asks (500 mL) each containing 100 g of rice and 120 mL of distilled water. Each flask was inoculated with 5 mL of cultured broth and incubated at 25 °C for 45 days.

Extraction and isolation. The fermentation products were extracted four times with 70 % acetone (4 × 10 L) at room temperature and filtered. The crude extract (115 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a $CHCl_3$-$(CH_3)_2CO$ gradient system (20:1, 9:1,

8:2, 7:3, 6:4, 5:5), to give six fractions A-F. Further separation of fraction B (9:1, 11.2 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures B1–B5. Fraction B2 (8:2, 6.27 g) was subjected to preparative HPLC (68 % MeOH, flow rate 12 mL/min) to give **1** (13.3 mg).

Terrephenol C (1): m.f.: $C_{26}H_{28}O_8$, obtained as a yellow gum; $[\alpha]_D^{24.8}$ + 61.2 (c 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 220 (4.11), 252 (3.54), 308 (3.87) nm; IR (KBr, ν_{max}, cm^{-1}): 3460, 3022, 2973, 2897, 1736, 1726, 1610, 1533, 1479, 1440, 1391, 1275, 1143, 1085, 970, 867, 761; ^{1}H NMR and ^{13}C NMR (500 and 125 MHz, in $(CD_3)_2CO$) (Table-1); ESIMS (positive ion mode) *m/z* 491 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 491.1689 $[M+Na]^+$ (calcd. 491.1682 for $C_{26}H_{28}O_8Na$).

TABLE-1
^{1}H NMR AND ^{13}C NMR DATA OF COMPOUND **1** IN $(CD_3)_2CO$ AT 500 MHz

No.	δ_C (m)	δ_H (m, *J*, Hz)
1	169.5 s	
2	140.2 s	
3	127.6 s	
4	85.2 s	
5	40.3 t	3.35, 3.46 d (14.6)
6	170.1 s	
1'	121.2 s	
2',6'	130.9 d	7.60 d (8.8)
3',5'	115.4 d	6.72 d (8.8)
4'	160.5 s	
1"	133.2 s	
2"	114.4 d	6.65 d (2.2)
3"	151.6 s	
4"	143.8 s	
5"	131.0 s	
6"	124.4 d	6.92 d (2.2)
7"	27.5 t	3.23 d (7.0)
8"	120.6 d	5.15 t (7.0)
9"	131.8 s	
10"	17.9 q	1.58 s
11"	25.2 q	1.72 s
6-OMe	52.3 q	3.66 s
4'-OMe	56.1 q	3.81 s
3"-OMe	55.9 q	3.79 s
4"-OH		11.12 s

RESULTS AND DISCUSSION

A 70 % aq. acetone extract prepared from fermentation products of the endophytic fungus *Aspergillus terreus* was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compound **1**. The structure of **1** was shown in Fig. 1 and the ^{1}H NMR and ^{13}C NMR data of compound **1** was listed in Table-1.

Compound **1** was obtained as pale yellow gum. The molecular formula was determined to be $C_{26}H_{28}O_8$ by high resolution-electrospray ionization-mass spectra (HR-ESIMS), *m/z* 491.1689 $[M+Na]^+$ (calcd. 491.1682 for $C_{26}H_{28}O_8Na$). The IR spectrum showed broad and intense absorption bands for hydroxy (3460), ester/lactone carbonyl (1736, 1726) and aromatic rings (1610, 1533, 1479). The ^{1}H NMR signals revealed the presence of a 1,4-disubstituted benzene moiety δ_H [7.60 d (8.8) 2H and 6.72 d (8.8) 2H], a 1,3,4,5-tetrasubstituted benzene moiety δ_H [6.65 d (2.2) and 6.92 d (2.2)], a prenyl group δ_H [3.23 d (7.0) 2H, 5.15 t (7.0) 1H, 1.58 s 3H and 1.72 s 3H], a methylene protons δ_H [3.35, 3.46 d (14.6)], three methoxy protons δ_H (3.66 s, 3.81 and 3.79 s) and one phenolic hydroxy proton (11.12 s). Its ^{13}C NMR showed the presence of 1,4-disubstituted benzene moiety δ_C [121.2 s, 130.9 d (2C), 115.4 d (2C), 160.5 s], a 1,3,4,5-tetra substituted benzene moiety δ_C (133.2 s, 114.4 d, 151.6 s, 143.8 s, 131.0 s, 124.4 d), a prenyl group (27.5 t, 120.6 d, 131.8 s, 17.9 q, 25.2 q), one methoxy carbonyl group (δ_C 170.1 s, 52.3 q), one ester carbonyl δ_C (169.5 s), a pair of olefenic carbon signals δ_C (140.2 s and 127.6 s), one methylene carbon δ_C (40.3 t) and one oxidated quaternary carbon δ_C (85.2 s). The molecular formula $C_{26}H_{28}O_8$ requires 13 degrees of unsaturation. The presence of two aromatic rings accounts for eight while two carbonyls and two olefenic carbons account for another four, which makes a total of twelve degrees of unsaturation. Therefore, compound **1** must posses one aliphatic ring in addition to two aromatic rings. The typical carbon signals δ_C (169.5 s, 140.2 s, 127.6 s, 85.2 s, 40.3 t, 170.1 s) indicated that compound **1** should be a phenyl derivated butyrolactone[10,13]. The HMBC correlations (Fig. 2) of methoxy protons (δ_H 3.81 and 3.79) with C-42 (δ_C 160.5) and C-322 (δ_C 151.6) suggested the position of methoxy group at C-42 and C-322, respectively. The prenyl group located at C-522 was supported by the HMBC correlations of H-722 (δ_H 3.22) with C-422 (δ_C 143.8), C-522 (δ_C 131.0) and C-622 (δ_C 124.4) and of H-622 (δ_H 6.92) with C-722 (δ_C 27.5). Finally, a phenolic hydroxy group was assigned to C-422 on the basis of HMBC correlations between the hydroxy proton (δ_H 11.12) and C-322 (δ_C 151.6), C-422 (δ_C 143.8) and C-522 (δ_C 131.0). The structure of compound **1** is therefore determined and gives the trivail name of terrephenol C.

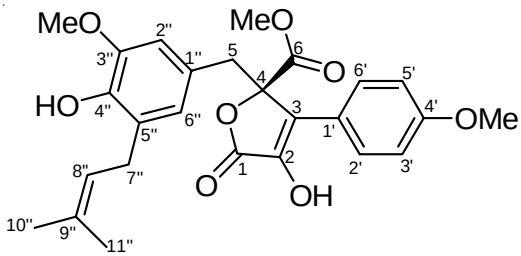

Fig. 1. Structure of compund **1**

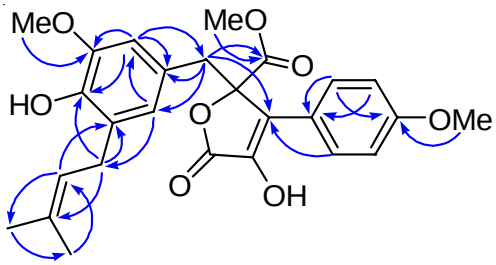

Fig. 2. Key HMBC (↷) correlations of compound **1**

Since some butyrolactones are known to exhibit potential anti-virus activities[10,15], compound **1** was tested for their anti-TMV activities. The anti-TMV activities were tested using the half-leaf method[16,17]. Ningnanmycin (a commercial product

for plant disease in China), was used as a positive control. The results revealed that compound **1** showed anti-TMV activities with inhibition rate of 16.7 %.

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REFERENCES

1. H. Laatsch, A Data Base for Rapid Structural Determination of Microbial Natural Products and Annual Updates, Chemical Concepts, Weinheim, Germany (2010).
2. Z.K. Wang, Y.S. Yang, A.T. Stefka, G. Sun and L.H. Peng, *Aliment. Pharmacol. Ther*., **39**, 751 (2014).
3. S. Martins, S.I. Mussatto, G. Martinez-Avila, J. Montanez-Saenz, C.N. Aguilar and J.A. Teixeira, *Biotechnol. Adv.*, **29**, 365 (2011).
4. R.M.P. Gutierrez, A.M.N. Gonzalez and A.M. Ramirez, *Curr. Med. Chem.*, **19**, 2992 (2012).
5. A.S. Awaad, A.J.A. Nabilah and M.E. Zain, *Phytother. Res.*, **26**, 1872 (2012).
6. Y. Wang, J.K. Zheng, P.P. Liu, W. Wang and W.M. Zhu, *Mar. Drugs*, **9**, 1368 (2011).
7. R.R. Parvatkar, C. D'Souza, A. Tripathi and C.G. Naik, *Phytochemistry*, **70**, 128 (2009).
8. J.A. Joule and K. Mills, Heterocyclic Chemistry, Blackwell Science Publishing: Oxford, UK, edn 4 (2000).
9. W. Wang, H. Kim, S.J. Nam, B.J. Rho and H. Kang, *J. Nat. Prod.*, **75**, 2049 (2012).
10. R. Haritakun, P. Rachtawee, R. Chanthaket, N. Boonyuen and M. Isaka, *Chem. Pharm. Bull. (Tokyo)*, **58**, 1545 (2010).
11. D.H. Li, T.J. Zhu, H.B. Liu, Y.C. Fang, Q.Q. Gu and W.M. Zhu, *Arch. Pharm. Res.*, **29**, 624 (2006).
12. Z.Q. Bai, X.P. Lin, Y.Z. Wang, J.F. Wang, X.F. Zhou, B. Yang, J. Liu, X.W. Yang, Y. Wang and Y.H. Liu, *Fitoterapia*, **95**, 194 (2014).
13. K.V. Rao, A.K. Sadhukhan, M. Veerender, V. Ravikumar, E.V.S. Mohan, S.D. Dhanvantri, M. Sitaramkumar, J. Moses Babu, K. Vyas and G. Om Reddy, *Chem. Pharm. Bull. (Tokyo)*, **48**, 559 (2000).
14. J.J. Qin, J.X. Zhu, Q. Zeng, X.R. Cheng, Y. Zhu, S.D. Zhang, L. Shan, H.Z. Jin and W.D. Zhang, *J. Nat. Prod.*, **74**, 1881 (2011).
15. H.-J. Zhang, N.V. Hung, N.M. Cuong, D.D. Soejarto, J.M. Pezzuto, H.H. Fong and G.T. Tan, *Planta Med.*, **71**, 452 (2005).
16. Q.F. Hu, B. Zhou, J.M. Huang, X.M. Gao, L.D. Shu, G.Y. Yang and C.T. Che, *J. Nat. Prod.*, **76**, 292 (2013).
17. Q.-F. Hu, B. Zhou, Y.-Q. Ye, Z.-Y. Jiang, X.-Z. Huang, Y.-K. Li, G. Du, G.-Y. Yang and X.-M. Gao, *J. Nat. Prod.*, **76**, 1854 (2013).