The Journal of Antibiotics (2011) 64, 465–467

www.nature.com/ja

NOTE

Diphenyl etheric metabolites from *Streptomyces* sp. neau50

Dian-Liang Gong[1], Xiang-Jing Wang[1], Zhi-Dan Xiang[1], Ji-Dong Wang[2], Hui Zhang[2], Chong-Xi Liu[1], Ji Zhang[1] and Wen-Sheng Xiang[1]

The Journal of Antibiotics (2011) **64,** 465–467; doi:10.1038/ja.2011.25; published online 6 April 2011

Keywords: cytotoxic activity; diphenyl ether; *Streptomyces* sp. neau50; structure elucidation

Natural products are among the most important resources of the clinically used cancer chemotherapeutic agents. Many compounds with anticancer properties have been isolated from natural sources since actinomycin was discovered in 1940's. More than 60% of the currently known compounds with anti-neoplasic activity are natural products or their derivatives.[1–6] As part of the search for new and biologically active secondary metabolites produced by actinomyces from unexplored and underexplored ecological niches, an endophytic actinomycete *Streptomyces* sp. neau50 was isolated from healthy root of soybean. Initial screening of the crude extract exhibited cytotoxicity against certain cancer cell lines and inhibitory activity against phytopathogenic fungi. Subsequent isolation resulted in borrelidin as the major active component. To further investigate minor amount of active constituents in this strain, the fermentation was scaled up, and detailed fractionation of the crude extract led to the isolation of a novel diphenyl ether compound, methyl 2-hydroxy-4-(2-hydroy-3-methoxy-5-methylphenoxy)-6-methylbenzoate (**1**), together with a known one, methyl 2-hydroxy-4-(3-hydroxy-5-methylphenoxy)-6-methylbenzoate (**2**). In this paper, we describe the isolation, structure elucidation and bioactivities of **1** and **2**.

The producing strain, *Streptomyces* sp. neau50, was isolated by moist incubation and desiccation method from healthy soybean root in Harbin, Heilongjiang province, China.[7] The 16S rRNA was sequenced for taxonomic classification (GenBank Accession No: GQ 494994).

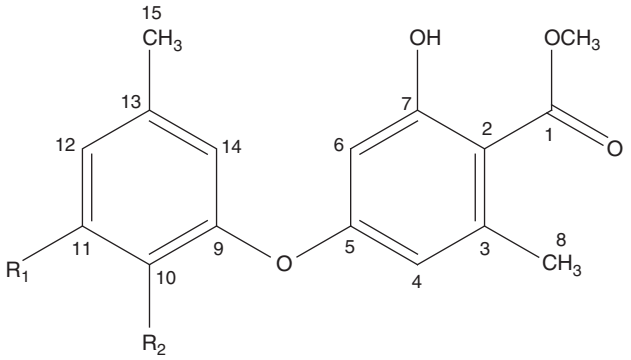
The strain was maintained in the medium containing 10 g glucose, 3 g maltose, 3 g yeast extract, 0.5 g K_2HPO_4 $3H_2O$, 0.5 g $MgSO_4$ $7H_2O$, 0.5 g NaCl, 1 g KNO_3 and 20 g agar in 1.0 l tap water, pH 7.0. Slant culture was incubated for 6–7 days at 28°C. The seed medium consisted of 4 g glucose, 10 g maltodextrin, 4 g yeast extract, 2 g $CaCO_3$ in 1.0 l water, pH 7.2–7.4. All the media were sterilized at 121°C for 20 min.

Fermentation was carried out in a 50-l first seed fermentor (containing 30 l seed culture) and a 500-l second fermentor (containing 300 l producing medium), successively. The producing medium comprised 1% glucose, 4% soluble amylum, 0.5% yeast extract, 2.5% soybean powder, 0.5% peptone, 0.2% $CaCO_3$, 0.8% $MgSO_4$ $7H_2O$, 0.6% $FeSO_4$ $7H_2O$, 0.2% $ZnSO_4$ $7H_2O$, 0.2% $MnSO_4$ H_2O, 0.05% $CoCl_2$ $6H_2O$, 0.2% Na_2MoO_4 $2H_2O$, pH 7.0. The fermentation was performed at 28°C for 7 days stirred at 100 r min^{-1} with an aeration rate of 30 m^3 of air per hour.

The final 300 l of fermentation broth was filtered to afford the mycelial cake. After it was washed with water, the mycelia was extracted twice with 100 l of EtOH for about 24 h. The EtOH extract was diluted to about 30% EtOH and subjected to a Diaion HP-20 resin column, which was eluted with 30, 40, 50, 60, 70 and 80% EtOH (each concentration eluted 2 bed volumes). The eluents at 70 and 80% EtOH were pooled and concentrated in vacuo at 50°C. Then part of the concentrated material (32 g) was chromatographed on a silica gel column, and successively eluted with a stepwise gradient of petroleum ether–acetone (100:0–50:50, v/v) to obtain five fractions (fractions I–V) based on the TLC profiles. Fraction III was chromatographed on another silica gel column and eluted with petroleum ether–acetone (90:10–70:30, v/v) to give three subfractions. Subfraction II was then subjected to a Sephadex LH-20 column (GE Healthcare, Glies, UK) and eluted with EtOH to obtain fractions A and B. Finally, fraction B was fractionated by semi-preparative HPLC with column Zorbax SB-C18 (5 μm, 250×9.4 mm) on Agilent 1100 system (Agilent, Palo Alto, CA, USA). It was eluted with CH_3OH–H_2O (85:15, v/v) at 1.5 ml min^{-1}. Two main compounds **1** (t_R 11.8 min, 8 mg) and **2** (t_R 12.7 min, 11 mg) (Figure 1) were isolated.

Compound **1** was obtained as colorless oil with UV (EtOH) λ_{max} nm (log ε): 211 (4.29), 264 (3.92), 299 (3.52). The absorption peaks in the IR spectrum of **1** suggested the presence of hydroxyl

[1]Life Science & Biotechnology Research Center, School of Life Science, Northeast Agricultural University, Harbin, PR China and [2]Department of New Drug Screening, Zhejiang Hisun Pharmaceutical, Co., Ltd, Zhejiang, PR China
Correspondence: Professor W-S Xiang, Life Science & Biotechnology Research Center, School of Life Science, Northeast Agricultural University, No. 59 Mucai Street, Xiangfang District, Harbin 150030, PR China.
E-mail: xiangwensheng@yahoo.com.cn
Received 14 October 2010; revised 3 March 2011; accepted 8 March 2011; published online 6 April 2011



1 $R_1 = OCH_3$, $R_2 = OH$

2 $R_1 = OH$, $R_2 = H$

Figure 1 Structures of compounds **1** and **2**.

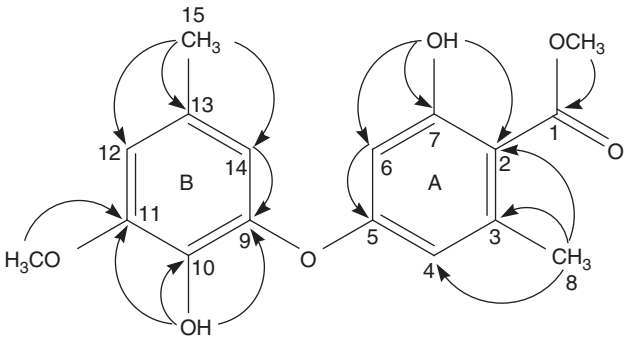


Figure 2 Key HMBC correlations of **1**.

Table 1 ^{1}H and ^{13}C NMR data of compounds 1 and 2

	1		*2*	
No.	δ_H *(mult., J in Hz)*	δ_C	δ_H *(mult., J in Hz)*	δ_C
1		172.1 s[a]		172.0 s
2		106.7 s		107.0 s
3		143.6 s		143.6 s
4	6.42 (1H, d, 2.4)	112.0 d	6.37 (1H, d, 2.5)	113.0 d
5		162.5 s		162.3 s
6	6.27 (1H, d, 2.4)	101.7 d	6.34 (1H, d, 2.5)	103.2 d
7		165.2 s		165.0 s
8	2.50 (3H, s)	24.3 q	2.50 (3H, s)	24.2 q
9		140.5 s		155.9 s
10		135.7 s	6.37 (1H, br s)	105.0 d
11		147.9 s		156.8 s
12	6.58 (1H, br s)	109.0 d	6.49 (1H, br s)	112.6 d
13		129.5 s		141.2 s
14	6.50 (1H, br s)	115.1 d	6.44 (1H, br s)	113.4 d
15	2.28 (3H, s)	21.2 q	2.28 (3H, s)	21.4 q
1-OCH_3	3.93 (3H, s)	51.9 q	3.94 (3H, s)	51.9 q
7-OH	11.65 (1H, s)		11.66 (1H, s)	
10-OH	5.32 (1H, s)			
11-OCH_3	3.91 (3H, s)	56.3 q		

[a]By DEPT sequence.

(3447 cm^{-1}) and conjugated carbonyl (1656 cm^{-1}) groups. Its molecular formula was established as $C_{17}H_{18}O_6$ on the basis of HRESIMS, which gave a quasi-molecular ion at *m/z* 341.1009 $[M+Na]^+$. ^{1}H NMR spectrum of **1** exhibited two methyl singlets at δ_H 2.28, δ_H 2.50, four aromatic protons at δ_H 6.27, δ_H 6.42, δ_H 6.50 and δ_H 6.58, two methoxy groups at δ_H 3.91 and δ_H 3.93, in addition to two hydroxyl signals at δ_H 5.32 and δ_H 11.65. Its ^{13}C NMR spectrum displayed 17 carbon resonances, including an ester carbonyl carbon, four sp^2 methines, eight sp^2 quaternary carbons, two methyls and two methoxy carbons. The detailed analysis of the ^{1}H NMR and ^{13}C NMR data (Table 1) of **1** indicated that it had two benzene rings. The full structure of **1** was established by the correlated signals in the HMBC spectrum. A methocarbonyl group was confirmed by the long-range coupling from the methoxy signal at δ_H 3.93 to δ_C 172.1 (Figure 2). The downfield proton signal of a hydroxyl at δ_H 11.65 (7-OH) showed the OH group was in ortho position to the methocarbonyl. In addition to an oxygen atom attachment to C-5 revealed by the ^{13}C chemical shift of C-5 (δ_C 162.5), the substituents of tetra-substituted ring A were assigned by the HMBC correlations from H_3-8 to C-2, C-3, C-4, from δ_H 11.65 to C-2, C-6, C-7 and from H-6 to C-5. The observed crossing peaks in HMBC spectrum of **1** between δ_H 3.91 and C-11, δ_H 5.32 (10-OH) and C-9, C-10, C-11, H_3-15 and C-12, C-13, C-14, H-12 and C-11, H-14 and C-9 demonstrated the presence of another tetra-substituted benzene ring and established the substituents of ring B as shown in Figure 2. The ether linkage of the two benzene rings through C-4 and C-9 was confirmed by the molecular formula of $C_{17}H_{18}O_6$. Furthermore, the ^{1}H and ^{13}C NMR data of **1** were very similar to those of gerfelin,[8] except the two methoxy groups in **1**. This finalized the study and assigned the structure of **1** as methyl 2-hydroxy-4-(2-hydroxy-3-methoxy-5-methylphenoxy)-6-methylbenzoate.

Compound **2** was also isolated as colorless oil with UV (EtOH) λ_{max} nm (log ε): 217 (4.50), 264 (4.12), 302 (3.72). The IR spectrum of **2** showed the presence of hydroxyl (3447 cm^{-1}) and conjugated carbonyl (1655 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** indicated five aromatic protons, two aromatic methyls, a downfield OH signal and a methoxy group. In the ^{13}C NMR spectrum of **2**, there were 12 sp^2 carbons in addition to three methyl carbons (one oxygenated one) and one carbonyl. These suggested the presence of two benzene rings in **2**. Comparison of the NMR data (Table 1) of **2** with those of cordyol B,[9] showed that compound **2** was identical to the aglycone of cordyol B and elucidated the structure of **2** as methyl 2-hydroxy-4-(3-hydroxy-5-methylphenoxy)-6-methylbenzoate.

We examined the inhibitory activity of compounds **1** and **2** against the growth of human lung adenocarcinoma cell line A549 using the CCK-8 colorimetric method as described in our preceding paper.[10] The result showed that compounds **1** and **2** inhibited the growth of A549 cells dose-dependently with an IC_{50} value of 5.3 and 15.2 μg ml^{-1}, respectively.

ACKNOWLEDGEMENTS

This work was supported by the National Key Project for Basic Research (No. 2010CB126102) and the National Natural Science Foundation of China (No. 30771427 and 31000884).

1 Schwartsmann, G. *et al*. Anticancer drug discovery and development throughout the world. *J. Clin. Oncol.* **20,** 47s–59s (2002).
2 Newman, D. J. & Cragg, G. M. Microbial antitumor drugs: natural products of microbial origin as anticancer agents. *Curr. Opin. Investig. Drugs.* **10,** 1280–1296 (2009).

3 Ma, X. & Wang, Z. Anticancer drug discovery in the future: an evolutionary perspective. *Drug. Discov. Today.* **14,** 1136–1142 (2009).
4 Coseri, S. Natural products and their analogues as efficient anticancer drugs. *Mini. Rev. Med. Chem.* **9,** 560–571 (2009).
5 Demain, A. L. & Sanchez, S. Microbial drug discovery: 80 years of progress. *J. Antibiot.* **62,** 5–16 (2009).
6 Flores-Bustamante, Z. R., Rivera-Orduña, F. N., Martínez-Cárdenas, A. & Flores-Cotera, L. B. Microbial paclitaxel: advances and perspectives. *J. Antibiot.* **63,** 460–467 (2010).
7 Emi, M., Youji, N., Yuzuru, I. & Masayuki, H. A new enrichment method for the selective isolation of *Streptomycetes* from the root surfaces of herbaceous plants. *Actinomycetologica.* **21,** 66–69 (2007).
8 Zenitani, S. *et al.* Gerfelin, a novel inhibitor of geranylgeranyl diphosphate synthase from *Beauveria feline* QN 22047 II structural elucidation. *J. Antibiot.* **56,** 658–660 (2003).
9 Taridaporn, B., Seangaroon, Y., Kamolphan, I. & Kanokarn, K. New diphenyl ethers from the insect pathogenic fungus *Cordycep* sp. BCC 186. *Chem. Pharm. Bull.* **55,** 304–307 (2007).
10 Wang, J. D. *et al.* HS071, A new furan-type cytotoxic metabolite from *Streptomyces sp.* HS-HY-071. *J. Antibiot.* **61,** 623–626 (2008).