The Journal of Antibiotics (2016) 69, 111–113

www.nature.com/ja

NOTE

Two rare quinone-type metabolites from the fungus *Septofusidium berolinense* and their biological activities

Güner Ekiz, Elif Esin Hameş, Ayşe Nalbantsoy and Erdal Bedir

The Journal of Antibiotics (2016) **69,** 111–113; doi:10.1038/ja.2015.84; published online 2 September 2015

Bioactive natural products can be produced by almost all types of living organisms, and especially soil bacteria and fungi are rich sources of these natural products.[1,2] Among them, fungi are remarkable organisms with unique biochemical pathways that produce a wide range of natural products such as penicillin, cyclosporin, statins, aflatoxins, trichothecenes and ergot alkaloids.[3,4] In the group of microscopic fungi, fungi imperfecti, the ascomycetes and several other filamentous and endophytic fungal species are the most frequent producers. Although fungi have proven to be source of diverse secondary metabolites, only a small portion of these have been cultivated and screened for new bioactive compounds. Due to the increasing demand for new bioactive natural products, the use of fungal diversity in the search for new bioactive molecules is crucial.[5]

In our ongoing research on bioactive secondary metabolites from filamentous fungi, a fungal strain identified as *Septofusidium berolinense* was isolated from a soil sample that was collected from Ayvacık, Çanakkale, Turkey, in 2006. Further investigation of this strain resulted in the isolation of a new metabolite (**1**), and a known compound (**2**) reported by Ymele-Leki *et al.*[6] in 2012. Herein, we report the isolation and structure elucidation of these secondary metabolites from *S. berolinense*. In addition, antimicrobial and cytotoxic activities of the compounds were evaluated. The compounds exhibited broad-spectrum antibacterial activity against clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 43300), *Escherichia coli* O157:H7 (RSKK 234), *Pseudomonas aeruginosa* (ATCC 27853) and vancomycin-resistant *Enterococcus faecium* (VREF, DSM 13590). Cytotoxicities of the isolated compounds were evaluated against five human cancer cell lines; CaCo-2, HeLa, MCF7, U87MG and PC3 as well as one non-tumorigenic cell line (Vero), using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2Htetrazolium bromide) assay. Compound **2** showed more potent cytotoxicity than compound **1** against the entire range of cell lines. This is the first report on bioactive secondary metabolites produced by the fungus *S. berolinense*.

The fungus was grown on malt extract agar plates at 25 °C for 7 days, after which the fresh culture was transferred to a 500 ml Erlenmeyer flask containing 100 ml malt extract broth. This culture was incubated on a rotary shaker at 150 r.p.m., 25 °C for 5 days and was used as seed culture. Solid substrate fermentation of *S. berolinense* was carried out in 10 × 1000 ml Erlenmeyer flasks, each containing zeolite 40 g, soybean flour 4 g, mannitol 4 g and distilled water 11 ml, all of which were inoculated with 10% inoculum. The cultures were then incubated at 25 °C, under static conditions for 14 days. After the incubation period, EtOAc (200 ml) was added to each flask and the resulting mixture was left overnight to stop cell growth, which was then filtered through filter paper and the mycelium was discarded. The culture filtrate was evaporated to dryness under vacuum to obtain the EtOAc extract.

The crude extract (2.215 g) was resuspended in MeOH and subjected to liquid–liquid partition with *n*-hexane. The *n*-hexane phase was evaporated to dryness (581.7 mg) and subjected to column chromatography on silica gel employing *n*-hexane/EtOAc, (90:10 to 60:40) and *n*-hexane/EtOAc/MeOH, (10:10:1 to 10:10:5) to provide 120 fractions. The fractions 81–100 (16.5 mg) were further chromatographed on Sephadex LH-20 eluting with MeOH to afford compound **1** (15.6 mg). The MeOH fraction from the liquid–liquid partitioning (240 mg) was chromatographed on silica gel using n-hexane/EtOAc (80:20 to 50:50) to yield compound **2** (50.2 mg).

Compound **1** was obtained as an amorphous yellow powder with the molecular formula $C_{10}H_{12}O_3$, based on the HR-time-of-flight-MS (*m/z* 179.0677 ($[M-H]^-$) and NMR data, implying five degrees of unsaturation. The ^{1}H NMR spectrum of **1** displayed signals attributable to a propyl group at δ_H 1.02 (t, J = 7.2 Hz, 3H), 1.65 (tt, J = 7.2, 8.0 Hz, 2H), 2.92 (t, J = 8.0 Hz, 2H) in the up-field region. On the other hand, two signals were observed at δ_H 6.72 (d, J = 8.8 Hz) and 7.00 (d, J = 8.4 Hz), indicating a 1,2,3,4-tetrasubstituted benzene ring with four degrees of unsaturation (Table 1). The ^{13}C NMR spectrum exhibited 10 resonances, six of which were readily attributable to the aromatic ring. In addition, three resonances in the up-field region showed characteristic signals for a propyl group (δ_C 14.1, 25.2 and 26.2). The last C-atom signal was observed in the low-field at δ_C 195.6 (Table 1). This data together with a singlet signal at δ_H 10.28 in the ^{1}H

Department of Bioengineering, Faculty of Engineering, Ege University, Izmir, Turkey
Correspondence: Professor E Bedir, Department of Bioengineering, Faculty of Engineering, Ege University, Bornova, 35100 Izmir, Turkey.
E-mail: erdal.bedir@ege.edu.tr
Received 28 April 2015; revised 19 June 2015; accepted 27 June 2015; published online 2 September 2015

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

	1		*2*	
Position	$\delta_{(H)}$	$\delta_{(C)}$	$\delta_{(H)}$	$\delta_{(C)}$
1	—	145.8	—	187.6
2	—	131.6	—	140.8
3	—	118.2	—	146.0
4	—	157.9	—	189.1
5	6.72 (d, *J*=8.8)	116.0	6.74 (d, *J*=10)	137.1
6	7.00 (d, *J*=8.4)	126.1	6.77 (d, *J*=9.6)	136.3
7	2.92 (t, *J*=8.0)	26.2	2.53 (t, *J*=7.6)	27.8
8	1.65 (tt, *J*=7.2, 8.0)	25.2	1.47 (tt, *J*=7.2, 7.6)	23.5
9	1.02 (t, *J*=7.2)	14.1	0.97 (t, *J*=7.2)	14.3
10	10.28 (s)	195.6	4.60 (s)	57.4

In $CDCl_3$ at 400 MHz; δ in p.p.m., *J* in Hz.

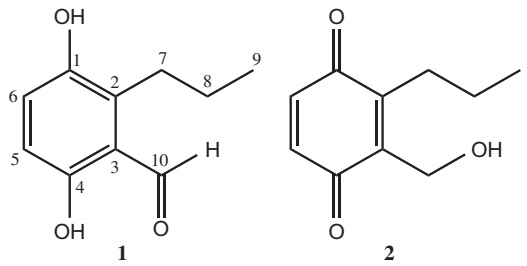

Figure 1 Structures of **1** and **2**.

NMR spectrum suggested the presence of an aldehyde group designating the remaining degree of unsaturation. Subtraction of the aforementioned groups (an aromatic ring, a propyl group and an aldehyde functionality) from the molecular formula revealed that the other substituents on the aromatic ring were hydroxyl groups.

Two spin systems including the propyl group and the aromatic protons were undoubtedly deduced from the Correlation spectroscopy (COSY) spectrum. Interpretation of the Heteronuclear multiple bond correlation (HMBC) spectrum allowed us to locate the substituents on the aromatic ring. Based on the key long-range correlations from C-1 and C-5 to the exchangeable proton on the C-6 hydroxy group, from C-6 to C-7 aldehyde proton, from C-3 to H-5 and H-1′ and from C-6 to H-4, the aldehyde and propyl substituents were positioned between phenolic C-atoms (C-3 and C-6).

Consequently, the structure of **1,** a new natural product, was elucidated as 3,6-dihydroxy-2-propylbenzaldehyde (Figure 1).

Compound **2** was obtained as a reddish-brown oil with the molecular formula of $C_{10}H_{12}O_3$. The detailed inspection of 1D- and 2D-NMR spectra of compound **2** and comparison of these data with those of **1**, indicated that **2** is the benzoquinone derivative of **1**. Although the propyl group is intact in the structure, compound **2** has a primary alcohol (δ_C 56.3; δ_H 4.60, s) instead of the aldehyde in **1.** Accordingly, the structure of **2** was determined as 2-(hydroxymethyl)-3-propylcyclohexa-2,5-diene-1,4-dione (Figure 1). This compound was previously reported in a high-throughput screening study of over 39 000 crude extracts derived from organisms that grow in the diverse ecosystems of Costa Rica.[6] In this study, Ymele-Leki *et al.* reported that compound **2** was isolated from an endophytic fungus that was closely related to *Septofusidium herbarum* and *Acremonium alternatum.*

Table 2 MIC values of compounds 1 and 2 against selected bacterial pathogens

	MIC ($\mu g\,ml^{-1}$)		
Test microorganisms	*1*	*2*	*Ampicillin*[a]
E. coli O157:H7 RSKK 234	40	40	5
MRSA (*S. aureus* ATCC 43300)	40	20	80
P. aeruginosa ATCC 27853	40	40	>160
E. faecium DSM 13590	20	20	160

[a]Positive control.

Table 3 Cytotoxic activities of compounds 1 and 2

	IC50 ($\mu g\,ml^{-1}$)		
Cell lines	*1*	*2*	*Doxorubicin*[a]
CaCo-2	25.2	10.2	10.9
HeLa	15.3	5.2	10.9
MCF7	>50	16.4	>27
U87MG	Nd	22.3	6.5
PC3	>50	16.2	25.5
VERO	nd	11.1	7

Abbreviation: nd, not determined.
[a]Positive control.

The antimicrobial activities of **1** and **2** against clinical strains of *E. coli* O157:H7, MRSA, *P. aeruginosa* and *E. faecium* were evaluated by the broth microdilution method.[7] As shown in Table 2, **1** and **2** exhibited antibacterial activity against all pathogenic bacteria, with MIC values in the range of 20–40 $\mu g\,ml^{-1}$. In particular, the compounds showed superior antibacterial activity over ampicillin toward MRSA, *P. aeruginosa* and *E. faecium* strains. Our results were conflicting with the previous study by Ymele-Leki *et al.*,[6] which reported no significant antimicrobial activity against clinical test strains.

Quinonic nature of compound **2** prompted us to carry out a cytotoxicity screening. The cytotoxic activities of the compounds and positive control doxorubicin were evaluated against five human cancer cell lines, namely CaCo-2, HeLa, MCF7, U87MG and PC3, as well as against the normal cell line (Vero), using MTT assay[8] (Table 3). As expected, **2** demonstrated notable cytotoxic activity against all cell lines, whereas **1** showed only weak activity toward the two cell lines CaCo-2 and HeLa. When the similar structures of **1** and **2** are taken into consideration, it could be inferred that the benzoquinone framework is essential for potent cytotoxic activity.[9,10]

In conclusion, this is the first isolation, structure elucidation and bioactivity screening study performed on the fungus *S. berolinense.* Although the chemistry encountered seems very simple, there has been no other report of this skeleton other than those reported by Ymele-Leki *et al.*[6] The rare character of the isolated compounds (**1** and **2**) warrants further studies to uncover the minor constituents of *S. berolinense* and other members of the genus *Septofusidium.*

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to TUBITAK (109S361) and EBILTEM (2012/BİL/028) for financial support.

1 Bérdy, J. Bioactive microbial metabolites. *J. Antibiot.* **58**, 1–26 (2005).
2 Brakhage, A. A. Regulation of fungal secondary metabolism. *Nat. Rev. Microbiol.* **11**, 21–32 (2013).
3 Keller, N. P., Turner, G. & Bennett, J. W. Fungal secondary metabolism-from biochemistry to genomics. *Nat. Rev. Microbiol.* **3**, 937–947 (2005).
4 Calvo, A. M., Wilson, R. A., Bok, J. W. & Keller, N. P. Relationship between secondary metabolism and fungal development. *Microbiol. Mol. Biol. Rev.* **66**, 447–459 (2002).
5 Demain, A. L. & Zhang, L. (eds) *Natural Products: Drug Discovery and Therapeutic Medicine*., (Humana Press Inc., Totowa, New Jersey, 3–29 2005)
6 Ymele-Leki, P. *et al.* A high-throughput screen identifies a new natural product with broad-spectrum antibacterial activity. *PLoS ONE* **7**, e31307 (2012).
7 Clinical and Laboratory Standards Institute. C.L.S.I. document M07-A8 (2009).
8 Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55–63 (1983).
9 Bolton, J. L., Trush, M. A., Penning, T. M., Dryhurst, G. & Monks, T. J. Role of quinones in toxicology. *Chem. Res. Toxicol.* **13**, 135–160 (2000).
10 Sevimli-Gur, C., Akgun, I. H., Deliloglu-Gurhan, I., Korkmaz, K.S. & Bedir, E. Cytotoxic naphthoquinones from *Alkanna cappadocica*. *J Nat Prod* **73**, 860–864 (2010).

Supplementary Information accompanies the paper on *The Journal of Antibiotics* website (http://www.nature.com/ja).