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NOTE

JBIR-25, a novel antioxidative agent from *Hyphomycetes* sp. CR28109

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Active oxygen species cause many diseases such as atherosclerosis, inflammation, ischemia–reperfusion injury, rheumatoid arthritis and central nervous diseases.[1] Further, senility and cancer initiation as well as progression are also believed to involve active oxygen species.[2] Thus, it is expected that effective antioxidative agents may prevent the onset and development of these diseases. In the course of our screening program of novel antioxidants, we isolated a novel antioxidative agent, designated as JBIR-25 (**1**), from the culture of *Hyphomycetes* sp. CR28109. This paper describes the isolation, structural elucidation and briefly the biological activity of **1** (Figure 1).

Hyphomycetes sp. CR28109 was isolated from a soil sample collected in Ashigara, Kanagawa Prefecture, Japan, and cultured at 25 °C for 14 days in a 500-ml Erlenmeyer flask containing 80 g brown rice and 2 g oatmeal in static culture. The culture was extracted with 80% aq. Me_2CO (100 ml). After concentration *in vacuo*, the aqueous concentrate was extracted with EtOAc (three times). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue (0.51 g) was applied to normal-phase medium pressure liquid chromatography (Purif-Pack SI-60, Moritex, Tokyo, Japan) and eluted with a gradient system of *n*-hexane–EtOAc (0–30% EtOAc) and $CHCl_3$–MeOH (0–50% MeOH), successively. The 5% MeOH elute fraction (25.5 mg) was further purified by the preparative reversed-phase HPLC using a PEGASIL ODS column (Senshu Pak, 20 i.d.×150 mm, Senshu Scientific, Tokyo, Japan) with 50% MeOH–H_2O containing 0.1% formic acid (flow rate: 10 ml min^{-1}) to yield **1** (13.5 mg, Retention time (Rt), 10.5 min).

Compound **1** was isolated as a colorless oil that gave a $[M+H]^+$ ion at *m/z* 477.1504 in the high-resolution electrospray ionization-MS consistent with a molecular formula of $C_{22}H_{24}N_2O_{10}$ (calculated for $C_{22}H_{25}N_2O_{10}$, 477.1509), and displayed the UV and IR spectra as follows; UV (MeOH) λ_{max} (ε) 278 (2460) and 219 (13 140); IR (KBr) ν_{max} 3430 and 1720 cm^{-1}.

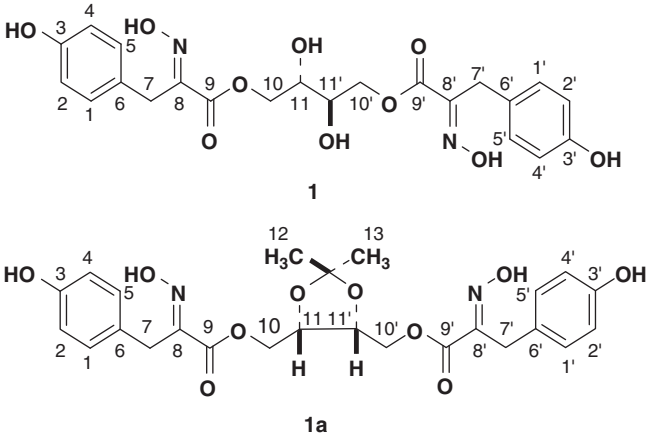

Figure 1 Structures of JBIR-25 (**1**) and 11,11′-acetonide JBIR-25 (**2**).

The ^1H and ^{13}C NMR spectral data for **1** are shown in Table 1. The completely symmetrical carbon signals were observed, indicating that **1** is a symmetric compound. The structural information on **1** was further obtained by the series of two-dimensional NMR analyses such as heteronuclear single quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC) and double quantum filtered correlation (DQF-COSY) spectra (Figure 2). A ^1H–^1H spin correlation was observed between doublet aromatic protons 1/5-H (δ_H 7.09) and 2/4-H (δ_H 6.65). In the HMBC spectrum, 2/4-H were strongly *m*-coupled to each other and coupled to an aromatic quaternary carbon C-6 (δ_C 127.3). Further, 1/5-H were also strongly *m*-coupled to each other, and coupled to an aromatic carbon C-3 (δ_C 155.8) and a methylene carbon C-7 (δ_C 29.2) in the HMBC spectrum. A singlet

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

	1		2	
No.	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)
1, 1′	130.0	7.09, d (8.3)	130.2	7.05, d (8.3)
2, 2′	115.0	6.65, d (8.3)	115.3	6.65, d (8.3)
3, 3′	155.8		157.0	
4, 4′	115.0	6.65, d (8.3)	115.3	6.65, d (8.3)
5, 5′	130.0	7.09, d (8.3)	130.2	7.05, d (8.3)
6, 6′	127.3		128.5	
7, 7′	29.2	3.84, s	30.3	3.81, s
8, 8′	151.4		152.3	
9, 9′	164.2		165.0	
10, 10′	66.9	4.40, dd (12.2, 4.3), 4.23, dd (12.2, 11.4)	61.5	3.68, dd (11.5, 5.0) 3.61, dd (11.5, 6.5)
11, 11′	69.5	3.76, m	78.8	4.22, m
12			25.5	1.33, s
13			28.2	1.42, s

^{13}C (125 MHz) and ^{1}H (500 MHz) NMR spectra were taken on a NMR system 500 NB CL (Varian, Palo Alto, CA, USA) in CD_3OD, and the solvent peak was used as an internal standard (δ_C 49.0, δ_H 3.30).

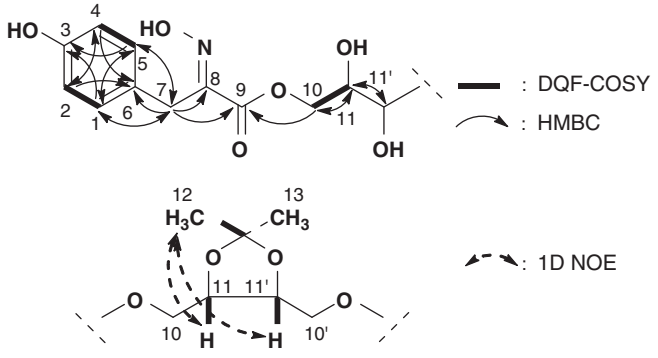

Figure 2 Key correlations in DQF-COSY (bold line) and HMBC (arrow) spectra of **1**, and 1D NOE correlations obtained from **2**.

methylene proton 7-H (δ_H 3.84) was long-range coupled to aromatic methine carbons C-1/5 (δ_C 130.0) and C-6. Thus, the methylene carbon C-7 was deduced to be substituted at the position of C-6. All the assignments of this disubstituted benzene ring moiety were established by ^{1}H–^{13}C long-range couplings, as shown in Figure 2. In addition, the ^{1}H–^{13}C long-range couplings from a methylene proton 7-H to an ester carbonyl carbon C-9 (δ_C 164.2) and an imino carbon C-8 (δ_C 151.4) and from methylene proton 10-H (δ_H 4.40, 4.23) to C-9 revealed a 3-(4-hydroxyphenyl)-2-iminopropanoate moiety. A ^{1}H–^{1}H spin coupling in DQF-COSY spectrum was observed between oximethine proton 11-H (δ_H 3.76, δ_C 69.5) and 10-H. Finally, 11-H was long-range coupled to an oximethine carbon C-11′ (δ_C 69.5), which is exactly the own carbon signal in the HMBC spectrum, indicating that **1** consisted of a symmetric structure at C-11, as shown in Figure 1. From the molecular formula of **1**, four hydroxyl groups were determined to be substituted at the position of C-3, C-3′, C-11 and C-11′, and remaining two hydroxyl groups were assigned to oxime functional groups at the imino moieties C-8 and C-8′. The geometries of the C-8 and C-8′ at oxime moieties were elucidated as *E* from the upfield ^{13}C chemical shift of C-7 and C-7′ (δ_C 29.2) due to the γ-effect of hydroxyl group in the oxime function. The difference in ^{13}C chemical shifts between *E* (δ_C 27.5) and *Z* (δ_C 35.7) was observed in (*E,Z*)-*N,N*′-bis (3-(3′-bromo-4′-hydroxyphenyl)-2-oximidopropionyl) cystamine[3] the positions of which corresponded to C-7 and C-7′ in **1**. This result supported the stereochemistry at C-8 and C-8′. The relative configurations of C-11 and C-11′ were established by preparation of its five-membered 11,11′-acetonide ring that was subjected to 1D NOE experiment, as shown in Figure 2. Compound **1** (1.0 mg) was dissolved in 0.2 ml of acetone, to which 0.1 ml of 2,2-dimethoxypropane and 0.8 mg of *p*-toluene sulfonate were added, and stirred at room temperature for 2 h to give **2**. The reaction mixture was then concentrated to dryness, and the residue was dissolved with 10 ml of $CHCl_3$. The $CHCl_3$ solution was washed twice with 5 ml of 5% $NaHCO_3$ solution and then twice with 5 ml of H_2O (pH 7). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The oily residue was purified by an L-column2 ODS column (20 i.d.×150 mm; Chemical Evaluation and Research Institute, Tokyo, Japan) with 60% MeOH–H_2O (flow rate: 10 ml min^{-1}) to yield 11,11′-acetonide JBIR-25 (**2**) (0.72 mg; Rt, 10.8 min). The assignments of ^{1}H and ^{13}C NMR data of **2** were determined by HSQC experiment, as shown in Table 1. The 1D NOE correlation of **2** was observed only between a singlet methyl proton 12-H (δ_H 1.33) and oxymethine protons 11-H and/or 11′-H (δ_H 4.22). On the basis of this data, the relative configuration of **1** was concluded to be 11*R** and 11′*S**, as shown in Figure 1. Moreover, the optical rotation value of **1** ($[\alpha]_D^{25}$ 0° (*c* 1.0, MeOH) indicated that **1** is the mixture of enantiomers at the ratio of 1:1. The monomeric structure of **1** was found to be structurally related to phenylpyruvic acid oxime isolated from a marine sponge, *Psammaplysilla purpurea*.[4] However, the symmetric structure such as that of **1** produced by a fungus is the first example.

We evaluated the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of **1**. A 96-well plate was used for the DPPH radical scavenging assay.[5] Compound **1** and α-tocopherol as a positive control were dissolved in MeOH as the stock solution (1 mM). In total, 90 µl of 200 µM DPPH dissolved in MeOH and 10 µl of sample were mixed in the microplate. After 1 h incubation at room temperature, the absorbance was measured at 540 nm. Compound **1** showed DPPH radical scavenging activity with an IC_{50} value of 79 µM, which was almost the same activity as that of α-tocopherol (IC_{50}=50 µM).

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