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Reticulone, a Novel Free Radical Scavenger Produced by *Aspergillus* sp.

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Bioassay-guided fractionation of the culture broth of *Aspergillus* sp. FN070449 (KCTC 26428) using a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay led to the isolation of two compounds: reticulone (1) and reticulol (2). Their chemical structures were elucidated on the basis of UV, IR, NMR, and MS spectroscopic analyses. Compound 1 exhibited more potent free radical scavenging activity on $ABTS^{\cdot+}$ (2,2'-azino-bis [3-ethylbenzthiazoline-6-sulphonic acid]) and DPPH radicals than did butylated hydroxyanisole (BHA) and caffeic acid.

Keywords: *Aspergillus* sp., reticulone, DPPH, ABTS

Free radicals, inducing oxidative damage of cellular lipids, nucleic acids, and proteins, are thought to be one of the major risks for cancer, atherosclerosis, diabetes mellitus, coronary heart disease, inflammation, cerebral ischemia, skin damages, and various other degenerative diseases [3, 6]. Thus, free radical scavenging antioxidants have the potential as protective agents against various diseases caused by oxidative damage [4, 5]. In the past few years, the use of synthetic antioxidants has become more restricted owing to their health risks and toxicity. Thus, the importance of exploiting natural antioxidants from various sources and replacing synthetic antioxidants with natural ingredients have attracted increasing attention. Most of the natural antioxidants such as traditional nutrients, polyphenols, and flavonoids are obtained from plants [1, 11]. Recently, microbial secondary metabolites have been reported to harbor a host of bioactive substances [2]. The study of known and new natural derivatives in fungi might also support the development of new drugs, as well as health-promoting substances. We have searched for new biologically active substances from various natural resources [12]. In the course of our screening program for free radical scavengers, we isolated a new compound (**1**) along with a known compound (**2**, reticulol) [8, 9] from the fermentation broth of *Aspergillus* sp. FN070449 (KCTC 26428). In this paper, we report upon the isolation and structure elucidation of **1** primarily by extensive NMR experiments. The free radical scavenging activities of **1** against DPPH and $ABTS^{\cdot+}$ are also described.

The producing strain FN070449 (KCTC 26428) was cultivated in a producing medium consisting of 2% glucose, 0.5% polypeptone, 0.2% yeast extract, 0.1% KH_2PO_4, and 0.05 % $MgSO_4 \cdot 7H_2O$ (pH 5.8–6.2) for 7 days at 28°C on a reciprocal shaker. After cultured broth (5.8 l) was extracted with ethyl acetate, the organic layer was concentrated and applied to a column of silica gel eluted with methanol–chloroform [1:100–1:1 (v/v)]. The active eluate was then chromatographed on a Sephadex LH-20 column eluted with MeOH. Finally, **1** and **2** were obtained by HPLC using a YMC-pack ODS-A column (4.6 mm i.d.×150 mm) eluted with 20% acetonitrile.

The physicochemical properties of **1** are summarized in Table 1. Compound **1** was obtained as a brown powder and its molecular formula, $C_{11}H_{12}O_5$, was determined from its high-resolution ESI–MS spectrum [$(M+H)^+$, *m/z* 224.0681 (–0.4 mmu error)]. The UV spectrum of **1** showed two absorption maxima at 238 and 291 nm. The IR spectrum

Table 1. Physicochemical properties of **1**.

Compound	Reticulone
Appearance	Brown powder
Molecular formula	$C_{11}H_{12}O_5$
Molecular weight	224
HR–ESI–MS (*m/z*)	
found	224.0681 $[M+H]^+$
calcd.	224.0684
UV λ_{max}^{MeOH} nm (ε)	238 (4,301), 291 (2,471)
IR ν_{max}^{KBr} cm^{-1}	3,427; 2,947; 1,696; 1,653; 1,321; 1,270; 1,086

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Table 2. ^{1}H and ^{13}C NMR data of **1** in methanol-d_4.

Position	δ_C	δ_H (J in Hz)	HMBC
1	130.6		
2	120.9		
3	155.5		
4	138.3		
5	154.1		
6	116.7	6.40 (1H, s)	C-2, C-4, C-5
7	49.6	3.88 (2H, s)	C-1, C-2, C-6, C-8
8	209.3		
9	29.9	2.21 (3H, s)	C-7, C-8
OCH_3	62.9	3.90 (s)	C-3
CHO	191.9	10.1 (s)	C-1, C-2

^{1}H and ^{13}C NMR spectra were acquired at 400 and 100 MHz, respectively; TMS was used as the internal standard; assignments were based on ^{1}H-^{1}H COSY, DEPT, HMQC, and HMBC spectra.

revealed characteristic absorption bands for the hydroxyl group at 3,427 cm^{-1} and the carbonyl group at 1,696 cm^{-1}. The ^{1}H NMR spectrum of **1** displayed one aldehyde proton at δ_H 10.1 (1H, s, CHO), one aromatic proton at δ_H 6.40 (1H, s, H-6), one methoxyl group at δ_H 3.90 (3H, s, OMe-3), one methylene group at δ_H 3.88 (2H, s, H-7), and one methyl signal at δ_H 2.21 (3H, s, H-9). The ^{13}C NMR spectrum of **1** exhibited 11 carbon resonances consisting of two methyls, one sp^3 methylene, one sp^2 methine, five quaternary aromatic carbons, and two carbonyl groups. All protonated carbons and their protons were assigned by HMQC (heteronuclear multiple quantum correlation) experiments. Therefore, the above-mentioned spectroscopic data suggested compound **1** was a benzalaldehyde derivative substituted with a 2-oxopropyl group, and the gross structure was further confirmed by HMBC (heteronuclear multiple-bond correlation) experiments (Table 2). The HMBC correlations of H-7 of δ_H 3.88 with C-1 at δ_C 130.6, C-2 at δ_C 120.9, C-6 at δ_C 116.7, and C-8 at δ_C 209.3, suggested that 2-oxopropyl group was at C-6. The methoxyl group was assigned at C-3 by HMBC correlations of the methoxy protons at δ_H 3.90 with C-3 at δ_C 155.5. The aldehyde proton was long-range coupled to C-1 and C-2 in the HMBC spectrum. The ROESY (rotational frame nuclear Overhauser effect spectroscopy) correlations of H-7 with C-6 and aldehyde proton with

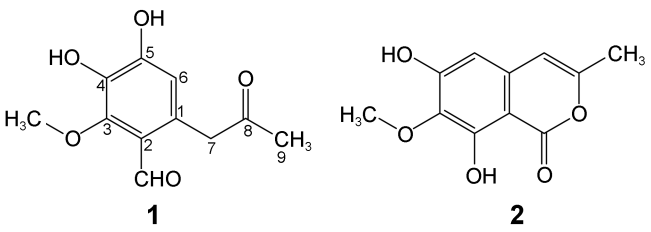

Fig. 1. Chemical structures of **1** and **2**.

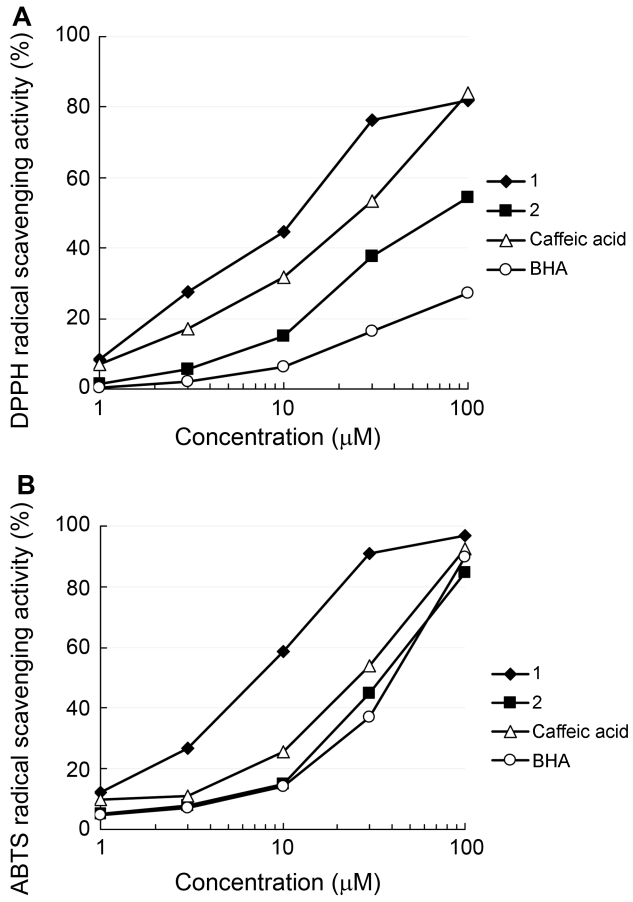

Fig. 2. DPPH and ABTS radical scavenging activities of **1** and **2**.

methoxy protons indicating an aldehyde group should be assigned at C-2. In addition, HMBC correlations of the methyl protons at δ_H 2.21 (3H, s, H-9) with C-7 at δ_C 49.6 and C-8 were observed. On the basis of the above spectral data, the structure of **1** was established to be 3,4-dihydroxy-2-methoxy-6-(2-oxopropyl) benzaldehyde, and named as reticulone (Fig. 1).

The antioxidant activity of **1** was evaluated by DPPH and ABTS assays as previously described [7, 10]. As a result, **1** exhibited potent radical scavenging activity with IC_{50} values of 6.91 and 3.05 mM in the ABTS and DPPH assay, respectively (Fig. 2).

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