NOTES

GTRI-02, a New Lipid Peroxidation Inhibitor from *Micromonospora* sp. SA246

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Peroxidative disintegration of cells and organellar membranes by free radicals has been known to be involved in various pathological processes represented by the pathogenesis of diseases such as myocardial and cerebral ischemia, atherosclerosis, diabetes, rheumatoid arthritis, cancer-initiation, and aging processes.[1~3] Free radical scavengers are considered as protective agents against various diseases mentioned above. In our screening for free radical scavenging substances, a novel compound, GTRI-02 (**1**, Fig. 1), was isolated from a soil actinomycetes SA246, which was identified as *Micromonospora* sp. In this paper, we describe the isolation, structure determination, and free radical scavenging activity of compound **1**.

The producing organism was inoculated into 100 ml of seed culture medium, potato dextrose broth (Difco), in a 500 ml Erlenmeyer flask. After 24 hours of incubation at 27°C on a rotary shaker (250 rpm), 1 ml of this seed culture was transferred to 100 ml of the same medium in a 500 ml flask and incubated at 27°C for 96 hours on a rotary shaker (250 rpm).

The culture filtrate (5L) was extracted with EtOAc. The extract was concentrated under reduced pressure. The concentrated material was dissolved in a small amount of EtOAc and chromatographed on a silica gel column using a linear gradient elution of EtOAc-MeOH (100:10~100:50). The active fractions were collected and concentrated *in vacuo* to dryness to yield a brownish powder. This powder was rechromatographed on a silica gel column with $CHCl_3$-MeOH gradient elution (100:1~100:10). The active fractions were concentrated *in vacuo* and subjected to silica gel TLC developed with $CHCl_3$-MeOH (100:7). The active band with Rf value of 0.32 was scraped off and extracted with $CHCl_3$-MeOH (70:10) mixture. The extract was further purified by reverse phase TLC with 65% MeOH (Rf 0.58) as a developing solvent to give 3 mg of **1**.

Physico-chemical properties of **1** are summarized in Table 1. Compound **1**, obtained as a white powder was readily soluble in DMSO, MeOH and CH_3CN, and slightly soluble in EtOAc and $CHCl_3$. Its UV spectrum showed maxima at 274, 232 and 211 nm in CH_3CN. Its IR spectrum suggested the presence of hydroxyl (3470 cm^{-1}), α,β-unsaturated carbonyl (1693 cm^{-1}) and aromatic ring moiety (C=C, 1597 and 1573 cm^{-1}). Molecular formula was determined to be $C_{13}H_{14}O_4$ on the basis of FAB-MS spectrum in combination with ^1H and ^{13}C NMR spectral data (Table 2). ^1H NMR spectrum in DMSO-d_6 showed peaks attributed to aromatic methine at 6.60 ppm, oxygenated methine at 4.15 ppm, two methylenes at 3.03, 2.77, 2.71 and 2.45 ppm, and two methyls at 2.36 and 2.29 ppm. ^{13}C NMR and DEPT spectra revealed signals due to two carbonyls at 205.7 and 197.3 ppm, one oxygenated sp^2 quaternary carbon at 157.1 ppm, four sp^2 quaternary carbons at

Table 1. Physico-chemical properties of GTRI-02.

Appearance	White powder
Molecular formula	$C_{13}H_{14}O_4$
FAB-MS (m/z)	235 $(M+H)^+$
$[\alpha]_D^{20}$	$-10°$ (c 0.2, CH_3OH)
UV $\lambda_{max}^{CH_3CN}$ nm (ε)	274 (15,521), 232 (14,921), 211 (16,898)
IR ν_{max}^{KBr} cm^{-1}	3470, 3140, 1693, 1655, 1597, 1573, 1300, 1279, 1230, 1064
Rf value on TLC	0.32[a] 0.58[b]
Solubility	DMSO, MeOH, CH_3CN

[a] Silica gel: $CHCl_3$-MeOH (100:7).
[b] ODS: MeOH-H_2O (65:35).

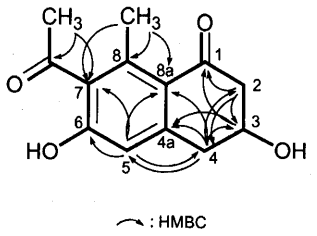
Table 2. ^{1}H and ^{13}C NMR spectral data for GTRI-02 in DMSO-d_6.

Positions	^{13}C chemical shifts, ppm	^{1}H chemical shifts, ppm
1	197.3	
2	49.2	2.71 (dd, 16.0, 3.6)[a] 2.45 (dd, 16.0, 6.8)
3	64.9	4.15 (m)
4	39.9	3.03 (dd, 16.0, 3.6) 2.77 (dd, 16.0, 6.8)
4a	145.5	
5	113.8	6.60 (s)
6	157.1	
7	130.9	
7-CO	205.7	
7-CH_3	32.4	2.36 (s)
8	137.7	
8-CH_3	18.5	2.29 (s)
8a	123.4	

[a] Proton resonance multiplicity and coupling constant (J=Hz) in parentheses.

145.5, 137.7, 130.9 and 123.4 ppm, one sp^2 methine at 113.8 ppm, one oxygenated methine at 64.9 ppm, two methylenes at 49.2 and 39.9 ppm and two methyls at 32.4 and 18.5 ppm. COSY spectrum gave a partial structure, $-CH_2-CH(-O)-CH_2-$. The structure of **1** was unambiguously assigned by the HMBC experiment (Fig. 1). It showed long-range correlations from 2-H (2.71, 2.45 ppm) and 3-H (4.15 ppm) to the carbonyl carbon at 197.3 ppm, and from 4-H at 3.03, and 2.77 ppm to three sp^2 carbons at 145.5, 123.4 and 113.8 ppm. Also long-range correlations from the methine proton at 6.60 ppm to carbons at 157.1, 130.9, 123.4 and 39.9 ppm and from the methyl proton at 2.29 ppm to three sp^2 quaternary carbons at 137.7, 130.9 and 123.4 ppm revealed that this compound had the skeleton of tetralone[4,5]. The methyl proton at 2.36 ppm showed long-range correlation with two quaternary carbons at

Fig. 1. Long-range couplings observed in the HMBC experiments on GTRI-02.



205.7 and 130.9 ppm indicating that an acetyl group must be attached to C-7. Thus the structure of **1** was determined to be 7-acetyl-3,6-dihydroxy-8-methyl tetralone.

Lipid peroxidation inhibitory activity was measured according to the methods of OHKAWA *et al.*[6] Compound **1** inhibited lipid peroxidation with a IC_{50} value of 1.89 μg/ml which is about half the activity of vitamin E (IC_{50}; 0.91 μg/ml).

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