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# Soraphinol C, a New Free-Radical Scavenger from *Sorangium cellulosum*

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**A new compound named soraphinol C (1) was isolated from myxobacteria *Sorangium cellulosum* KM1001 a soil isolate, together with a structurally related known compound, 4-hydroxysattabacin (2). These compounds were isolated by silica gel column chromatography and recycling preparative HPLC, consecutively. The structures of the compounds were determined on the basis of combined spectroscopic analyses. Compounds 1 and 2 exhibited antioxidant activity as a radical scavenger in the experiment using a hydrophilic free-radical initiator, 2,2'-azobis(2-amidinopropane)dihydrochloride with ORAC values of 0.956 and 0.617, respectively.**

**Keywords:** Soraphinol C, *Sorangium cellulosum*, myxobacteria, antioxidant

To find new compounds from microorganisms, it is valuable to isolate strains of morphologically and physiologically unusual microorganisms [12, 13]. Myxobacteria are common but unusual bacteria characterized by gliding behavior and fruiting body formation. They are not obtained by the routine method used in culturing bacteria and thus require special techniques for their isolation [4]. Myxobacteria especially attract many researchers, since they have many possibilities of producing undiscovered bioactive substances. So far, about 100 novel basic structures and 500 variants have been isolated from these organisms [10]. Many of these compounds are lipophilic polyketides and peptides. We also have recently reported new acyloin compounds from a cellulolytic myxobacterium, *Sorangium cellulosum* [1, 8]. In our search for additional bioactive metabolites from *S. cellulosum*, strain KM1001 was found to produce two closely related compounds. The major component, named soraphinol C (**1**), was found to be a new compound closely related to the minor component 4-hydroxysattabacin (**2**), previously identified as an antiviral compound [7]. We report here the isolation, structure elucidation, and bioactivity of these compounds.

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## Organism and Culture Conditions

*S. cellulosum*, designated strain KM1001, was isolated from a soil sample collected at Ansan, Gyeonggi-do, Korea. Isolation and identification of the strain were carried out by a general procedure [11]. The culture is deposited in the Korean Culture Center of Microorganisms with the accession number KCCM80040. This organism was cultivated in 2-l Erlenmeyer flasks containing 400 ml of a medium [5, 6] consisting of soyameal (Sigma) 0.4%, potato starch (Daejung) 0.8%, glucose (Junsei) 0.2%, $MgSO_4 \cdot 7H_2O$ (Junsei) 0.1%, $CaCl_2 \cdot 2H_2O$ (Junsei) 0.1%, HEPES (Sigma) 1.2%, and EDTA Fe(III)-Na salt (Junsei) 8 mg/l. The pH of the medium was adjusted to 7.4 before autoclaving. The production medium was the same as the seed medium, except that 1.5% (w/v) of Amberlite XAD-16 (Rhom and Haas) was added. The fermentation was performed on a rotary shaker (160 rpm) at 30°C for 10 days.

## Isolation and Purification

From 10 l of whole culture broth, bacterial cell mass and XAD-16 resin were collected by centrifugation and were extracted several times with 1 l of acetone for 24 h. The combined acetone extracts were concentrated *in vacuo*. The resulting aqueous residue was suspended with additional water and extracted three times with an equal volume of ethyl acetate. After concentration, the ethyl acetate extract (890 mg) was subjected to a silica gel column (2×30 cm, Silica gel 60, Merck), eluted with $CH_2Cl_2$-acetone (95:5, 300 ml), $CH_2Cl_2$-acetone-methanol (95:3:2, 300 ml), and $CH_2Cl_2$-methanol (95:5, 300 ml). The fraction that eluted with $CH_2Cl_2$-acetone-methanol (95:3:2) was further separated by silica gel column chromatography with *n*-hexane-*i*-propanol (9:1) to yield **1** (11 mg) and **2** (5 mg). These

**Table 1.** $^{1}H$, $^{13}C$ NMR data and principal HMBC correlations of compound **1**.

| Position | $\delta_C$ | $\delta_H$ | HMBC |
|---|---|---|---|
| 1 | 44.9 t | 3.74, 2H, s | C-2, C-2', C-6' |
| 2 | 213.2 s | | |
| 3 | 75.9 d | 4.18, 1H, dd (9.6, 4.2) | C-2, C-4, C-5 |
| 4 | 43.5 t | 1.45, 2H, m | C-2, C-3, C-5 |
| 5 | 25.7 d | 1.81, 1H, sept (6.6) | |
| 6 | 23.9 q | 0.93, 3H, d (6.6) | |
| 7 | 21.8 q | 0.92, 3H, d (6.6) | |
| 1' | 126.0 s | | |
| 2'(6') | 131.5 d | 7.00, 2H, d (6.7) | C-1, C-3', C-4', C-5' |
| 3'(5') | 116.1 d | 6.71, 2H, d (6.7) | C-1', C-4' |
| 4' | 157.1 s | | |

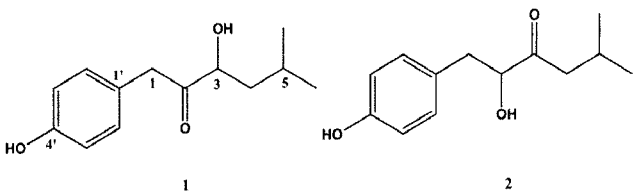
NMR data were obtained in $CD_3OD$ solution. Assignments were aided by a combination of $^{1}H$-$^{1}H$ COSY, *g*HSQC, and *g*HMBC experiments. The coupling constants (Hz) are in parentheses.

compounds were finally purified by recycling preparative HPLC (JAIGEL-GS310, 21.5×500 mm; detection, UV at 254 nm; mobile phase, methanol; flow rate, 5 ml/min).

### Structure Determination

Soraphinol C (**1**) was isolated as an optically active colorless oil $[[\alpha]_D^{25}$ -3.52 (*c* 0.17, MeOH)]. The molecular formula was deduced as $C_{13}H_{18}O_3$ from HRFABMS (measured: 245.1150; calcd: 245.1155 for $[M+Na]^+$ of $C_{13}H_{18}O_3$) and NMR spectral data (Table 1). **1** was thought to be an isomer of **2** because they possess the same molecular formula. The UV and IR spectra of **1** were very similar to those of **2**. Detailed examination of the NMR data revealed the presence of the same *p*-hydroxyphenyl, 3-hydroxy-2-butanone, and *iso*-propyl groups in **1** as found in **2**. However, proton signals of the 3-hydroxy-2-butanone moiety in the $^{1}H$ NMR data were significantly shifted between these compounds. The most noticeable differences in the $^{1}H$ NMR data were the significant downfield shifts of the C-1 methylene protons at δ 2.69 (1H, dd) and 2.92 (1H, dd) in **2** to those at δ 3.74 (2H, s) in **1**. The observed downfield shift of the C-1 methylene group referred to its link to a carbonyl group. These spectral changes were consistent with a migration of the carbonyl carbon from C-3 to C-2, as evidenced by the loss of direct spin-coupling between the C-1 methylene protons (δ 3.74) and the C-3 oxymethine proton (δ 4.18) in the *g*DQCOSY data of **1**, in which several correlations were observed between the C-4 methylene protons at δ 1.45 and their adjacent methine protons at δ 4.18 (H-3) and 1.81 (H-5). Thus, the structure of soraphinol C (**1**) was determined to be 3-hydroxy-1-(4-hydroxyphenyl)-5-methylhexan-2-one (Fig. 1).

Compound **2** was isolated as a colorless oil and analyzed as $C_{13}H_{18}O_3$ by HRFABMS and $^{13}C$ NMR spectrometry. IR absorptions at 3,390 (broad) and 1,710 $cm^{-1}$ revealed the presence of hydroxyl(s) and a ketonic carbonyl group,




1 2

**Fig. 1.** Chemical structures of compound **1** and 4-hydroxysattabacin (**2**).

respectively. The NMR data of **2** were in good accordance with those of a 4-hydroxysattabacin isolated previously from a *Bacillus* sp. [7]. The optical rotation of **2** $[[\alpha]_D$ -3.54 (c 0.5, MeOH)] and the reported for 4-hydroxysattabacin $[[\alpha]_D$ +14 (c 0.3, $CHCl_3$)], however, was different, suggesting that **2** was an enantiomer of the previously reported compound. The absolute stereochemistry of **1** and **2**, acyloin compounds of unknown stereochemistry, is currently under investigation using combined chemical and spectral methods, and the result will be reported in due course.

### Antioxidant Activity

The antioxidant activities of **1** and **2** were evaluated by the oxygen radical absorbance capacity (ORAC) method, which measures antioxidant scavenging activity against peroxy radicals induced by 2,2'-azobis(2-amidinopropane)dihydrochloride [2, 3]. The ORAC value is expressed as relative Trolox equivalent [9]. Compound **1** showed 0.956 ORAC, indicating that **1** possessed antioxidant potency comparable to Trolox (a water-soluble derivative of vitamin E), whereas compound **2** exhibited much weaker activity (0.617 ORAC). It is noteworthy that transposition of the α-hydroxyketone moiety in the acyloin compounds, as noticed between **1** and **2**, resulted in the significant difference of bioactivity.

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