Soraphinol B, A New Acyloin Compound Produced by *Sorangium cellulosum*

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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. Myxobacteria have been recognized as producers of novel secondary metabolites, many of the compounds showing interesting and sometimes rare mechanisms of action. So far, about 100 different basic structures and nearly 500 variants have been isolated from these organisms.[1-3] We also have recently reported a new indole alkaloid named soraphinol A(3) from a cellulolytic myxobacterium *Sorangium cellulosum* JW1059.[4] In our search for additional novel metabolites from the strain, we have found a new compound, named soraphinol B(**1**), along with a known compound kurasoin A (**2**).[5] These are acyloin compounds. We describe here the isolation and structural elucidation of soraphinol B.

Isolation and culture of the producing organism, *S. cellulosum* JW1059 were carried out by a general procedure that has been described elsewhere.[6,7] The fermentation of the producing strain was performed with XAD-16 resin. At harvest the resin and bacterial cell mass were collected by centrifugation and were extracted several times with acetone. The combined acetone extracts were partitioned between ethyl acetate and water, then the former layer re-partitioned between methanol and *n*-heptane. The methanol layer (1.4 g) was further separated by silica gel column chromatography followed by Sephadex LH-20 chromatography to give **1** and **2**. These compounds were finally purified by normal-phase HPLC. The total yield of **1** from a 10 L fermentation was 5.3 mg (0.53 mg/L).

The molecular formula of **1** was determined to be $C_{16}H_{16}O_3$ by combined high-resolution FABMS and ^{13}C NMR spectrometry. With this molecular formula nine degrees of unsaturation are present in **1**. The IR spectrum showed absorption bands at 3390 (-OH), 1715 (C=O), 1600, 1516, and 1450 cm^{-1}. The spectral data for **1** were very similar to those obtained for **2**.[5] In particular, signals of protons and carbons of the phenyl moieties in the NMR data were almost identical to each other, indicating the presence of the same monosubstituted and *p*-disubstituted phenyl moieties as **2** in **1**. The presence of two phenyl moieties in **1** was evident from the observation of twelve carbon signals in the region of δ 116-158 in the ^{13}C NMR data as well as the corresponding proton signals at δ 6.7-7.3 in the ^1H NMR data (Table 1). A combination of ^1H-^1H COSY, *g*HSQC, and *g*HMBC experiments revealed that one phenyl was substituted at C-1" while the other was substituted at C-1' and C-4', respectively. The downfield shift of the C-4' carbon at δ 157.5 in the 1',4'-disubstituted phenyl placed a hydroxyl group at this position.

The remaining portion of the molecule, having the unit formula of $C_4H_6O_2$, was also determined by combined 2-D NMR analysis. The ^1H-^1H COSY and *g*HMBC data (Table 1) indicated that the remaining protons were assigned to be -CH_2-CH-O- and isolated CH_2. Long range correlations of these protons with the carbonyl carbon at δ 212.6, coupled with the consideration of unit formula, established the partial structure of 3-hydroxy-2-butanone. Long range couplings between isolated methylene carbon (δ 45.6) and phenyl protons at δ 6.95 (H-2', H-6') in the *g*HMBC data located a 4'-hydroxyphenyl moiety at C-1 of 3-hydroxy-2-butanone. Similary, long-range correlation of H-4 methylene protons (δ 2.80, 3.05) with phenyl carbons at 139.0 (C-1") and 130.7 (C-3", C-6") established a linkage between C-4 of 3-hydroxy-2-butanone moiety and C-1" of monosubstituted phenyl moiety. Thus, the structure of compound **1** was established as 3-hydroxy-1-(*p*-hydroxyphenyl)-4-phenyl-2-butanone (Fig. 1). Both **1** and **2** show very similar ^1H NMR

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

Position	δ_C	δ_H	HMBC
1	45.6 t	3.66, 1H, brs 3.69, 1H, brs	
2	212.6 s		
3	78.4 d	4.39, 1H, dd (4.5, 8.0)	C-4
4	41.1 t	2.80, 1H, dd (8.0, 14.5) 3.05, 1H, dd (4.5, 14.5)	C-2, C-3, C-1", C-2", C-6"
1'	126.1 s		
2'(6')	131.8 d	6.95, 2H, d (8.5)	C-1, C-4'
3'(5')	116.4 d	6.71, 2H, d (8.5)	C-1', C-4'
4'	157.5 s		
1"	139.0 s		
2"(6")	130.7 d	7.21, 2H, d (7.5)	C-3", C-4", C-5"
3"(5")	129.4 d	7.26, 2H, t (7.5)	
4"	127.6 d	7.20, 1H, d (7.5)	

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

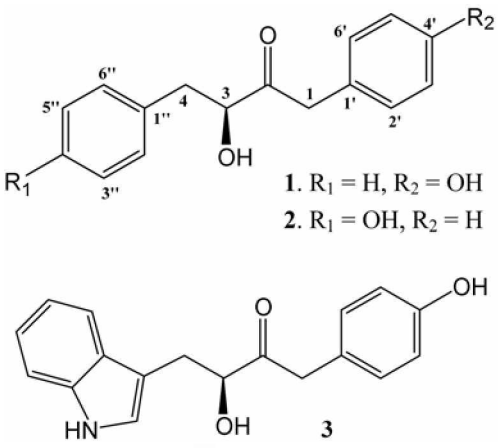

Figure 1. Structures of acyloin compounds **1-3**.

data (H-3) and CD spectra, indicating that the absolute stereochemistry of **1** is identical with that of **2**. Although diverse compounds belonging to various chemical classes have been identified as myxobacterial products,[1,8] the identification of these acyloin compounds further extends the demonstrated biosynthetic diversity of the myxobacteria.

Kurasoin A (**2**) proved to inhibit protein farnesyl transferase (PFTase) in a dose-dependent manner. The IC_{50} value of this compound against PFTase was 15.1 μg/mL.[5] On the basis of this information, soraphinol B(**1**) was evaluated for the PFTase inhibitor, conducted by the scintillation proximity assay. Unfortunately, **1** did not inhibit PFTase at concentration up to 100 μg /mL.

Experimental Section

General Methods. Optical rotation was measured on a JASCO P-1020 polarimeter. UV spectrum was recorded on a Agilent 8453 spectrophotometer and IR spectrum on a Bruker IFS-66/S FTIR spectrometer. ESIMS was obtained on a Agilent 1100LC/MSD trap SL mass spectrometer and HRESIMS was obtained on a high resolution tandem mass spectrometer (JMS-HX110/110A). NMR spectra were measured on a Varian UNITY 500 spectrometer working at 500 MHz for proton and 125 MHz for carbon. The ^{1}H and ^{13}C NMR chemical shifts were referred to CD_3OD observed at 3.30 ppm and 49.0 ppm, respectively. TLC analysis were performed on Kieselgel $60F_{254}$ (Merck) plates. Silica gel (230-400 mesh) and Sephadex LH-20 (Amersham Pharmacia Biotech) were used for column chromatography. HPLC was performed on Shimadzu liquid chromatography LC-10As with SPD-M10Avp diode array detector.

Organism and Culture Conditions. The isolation of strain JW1059 from soil has been described previously.[4] The organism was identified as a strain of *Sorangium cellulosum* on the basis of cellulose degradation, myxospore, swarm and fruiting body morphology according to Reichenbach and Dworkin.[6] The strain is currently on deposit in the Microbial Collection, Korea Maritime University. This strain was cultivated in 2L-Erlenmeyer flasks containing 400 mL of a medium consisting of Potato starch 0.8%, Soyameal 0.2%, Glucose 0.2%, Yeast extract 0.2%, $CaCl_2{\cdot}2H_2O$ 0.1%, $MgSO_4{\cdot}7H_2O$ 0.1%, Fe-EDTA 0.0008%, HEPES 1.2%, XAD-16 1.5%, pH 7.4. The flasks were incubated at 30 °C for 10 days on a rotary shaker at 160 rpm.

Extraction and Isolation. A 10 L fermentation of the producing strain was performed with XAD-16 adsorber resin. At harvest the resin was recovered by sieving and rinsed with water to remove cells and fermentation broth. The resin was extracted with 1 L and a further three times with 500 mL of acetone. The combined acetone extracts were concentrated and then partitioned with EtOAc and water, EtOAc soluble portion further partitioned between MeOH and *n*-Heptane. The MeOH layer was concentrated *in vacuo* to afford 470 mg of a dark brown gum, which was separated by silica gel column chromatography. A solution of the gum in CH_2Cl_2 was applied onto a column of silica gel (25 g), which was eluted stepwised with 150 mL of CH_2Cl_2-acetone 95:5 (fraction 1.1), CH_2Cl_2-acetone-MeOH 95:3:2 (fraction 1.2), and CH_2Cl_2-MeOH 95:5 (fraction 1.3). Fraction 1.2 (99 mg) was further separated by Sephadex LH-20 chromatography using CH_2Cl_2-MeOH (1:4) as solvent. The fractions containing **1** were collected according to UV absorption at λ254 nm and TLC, and finally purified by HPLC on silica gel (YMC-Pack SIL, *n*-hexane-EtOAc-iso-PrOH=91:7.2:1.8) to yield **1** (5.3 mg) and **2** (15.2 mg).

Soraphinol B (1): Colorless oil; $[\alpha]_D^{25}$ +1.3° (c 0.07, MeOH); UV λ_{max} (MeOH) nm (ε): 208 (8500), 221 (5500), 280 (1200); ^{1}H and ^{13}C NMR: see Table 1; ESIMS: *m/z* 279 $[M+Na]^+$, 257 $[M+H]^+$; HR-FABMS: *m/z* 279.0996 $[M+Na]^+$ (calcd for $C_{16}H_{16}O_3Na$, 279.0998); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 295 (+8.0), 220 (−7.9).

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