Soraphinol A, a New Indole Alkaloid from *Sorangium cellulosum*

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Myxobacteria are unique gram-negative bacteria characterized by the gliding nature and forming fruiting bodies upon starvation. They were not yet intensively studied because of the difficulties in their isolation and cultivation. However, myxobacteria have recently been recognized as a new and rich source of secondary metabolites which produce novel lead compounds, such as well known anticancer compounds, epothilons.[1,2] In the course of our continuing search for novel secondary metabolites from myxobacteria, the myxobacterium *Sorangium cellulosum* JW1059 was found to produce a new indole alkaloid, named soraphinol A (**1**), along with a known compound kurasoin A (**2**).[3] Isolation and culture of the producing organism, *S. cellulosum* JW1059 were carried out by a general procedure that has been described elsewhere.[4] We describe here the isolation and structure elucidation of new metabolite by a combination of NMR techniques.

The bacterial cells and the adsorber resin were collected from culture broth and extracted with acetone. After partitioning the acetone extract between ethyl acetate and water, the concentrated organic phase was separated by silica gel column chromatography followed by Sephadex LH-20 chromatography to give **1** and **2**. These compounds were finally purified by diode-array detected HPLC. The total yield of **1** from a 10 liter fermentation was 16 mg (1.6 mg/L).

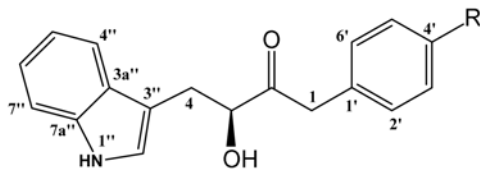
Soraphinol A (**1**) was isolated as optically-active colorless oil ($[\alpha]_D^{25}$ +28.1° (*c* 0.3, MeOH)) which analyzed for $C_{18}H_{17}O_3N$ by combined HRESIMS and ^{13}C NMR spectrometry. With this molecular formula eleven degrees of unsaturation are present in **1**. IR absorptions at 3410 (broad) and 1709 cm^{-1} revealed the presence of hydroxyl(s) and a ketonic carbonyl group, respectively. The presence of an indole and a phenyl moiety was evident from the observation of fourteen carbon signals in the region of δ158-110 in the ^{13}C NMR data as well as the corresponding proton signals at δ7.6-6.6 in the ^1H NMR data (Table 1). The UV spectrum also suggested the presence of an indole moiety in **1**. A combination of ^1H-^1H COSY, *g*HSQC, and *g*HMBC experiments revealed that indole was substituted at C-3" while phenyl was substituted at C-1' and C-4', respectively. The downfield shift of the C-4' carbon at δ157.4 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 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 δ213.3, coupled with the consideration of unit formula, established the partial structure of 3-hydroxy-2-butanone. Long range couplings between isolated methylene protons (δ 3.61, 3.56) and phenyl carbons at δ126.2 (C-1') and 131.9 (C-2', C-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 (δ3.18, 3.08) with indole carbons at 124.8 (C-2") and 111.1 (C-3") established a linkage between C-4 of 3-hydroxy-2-butanone moiety and C-3" of the indole (Table 1). Thus, the structure of compound **1** was established as 3-hydroxy-4-(3-indolyl)-1-(*p*-hydroxyphenyl)-2-butanone. A literature survey revealed that the optical rotation and NMR data of **1** were very similar to those of kurosoin B (**3**),[3] produced by a fungus *Paecilomyces* sp., except for a new hydroxyl group on C-4'. Although the stereochemical

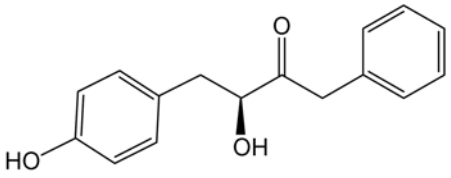
Table 1. ^1H, ^{13}C NMR data and principal HMBC correlations of **1**

Position	δ_C	δ_H	HMBC
1	46.3 t	3.56, 1H, d (16.5) 3.61, 1H, d (16.5)	C-2, C-1', C-2'
2	213.3 s		
3	77.7 d	4.51, 1H, dd (7.0, 5.5)	C-4, C-3"
4	31.3 t	3.08, 1H, dd (14.5, 7.0) 3.18, 1H, dd (14.5, 5.5)	C-2, C-2", C-3", C-3a"
1'	126.2 s		
2'(6')	131.9 d	6.79, 2H, d (8.0)	C-4'
3'(5')	116.3 d	6.66, 2H, d (8.0)	C-1', C-4'
4'	157.4 s		
2"	124.8 d	7.08, 1H, s	C-3", C-3a"
3"	111.1 s		
3a"	129.0 s		
4"	119.7 d	7.53, 1H, d (8.5)	C-6", C-7a"
5"	119.9 d	7.01, 1H, dd (7.0, 8.5)	C-3a", C-7"
6"	122.5 d	7.10, 1H, dd (7.0, 7.5)	C-4", C-7a"
7"	112.4 d	7.34, 1H, d (7.5)	C-3a", C-5"
7a"	138.1 s		

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.



1 R = OH
3 R = H



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assignment for the asymmetric carbon center in **1** was not established, the relative configuration at position 3 of **1** is likely to be the same as that of kurosoin B, because the ^{1}H coupling pattern of H-3 (1H, dd, J = 7.0, 5.5 Hz) was almost identical with that of kurosoin B (1H, dd, J = 6.8, 5.7 Hz).

Kurasoins A (**2**) and B (**3**) proved to inhibit protein farnesyl transferase (PFTase) in a dose-dependent manner. The IC_{50} values of these compounds against PFTase were 15.1 and 16.4 μg/mL, respectively.[5] On the basis of this information, **1** was evaluated for the PFTase inhibitor, conducted by the scintillation proximity assay. Unfortunately, soraphinol A (**1**) did not inhibit PFTase at concentration up to 100 μg/mL. Detailed investigation on other biological activities are now underway.

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) was used for column chromatography. HPLC was performed on Shimadzu liquid chromatography LC-10As with SPD-M10Avp diode array detector.

Organism and Culture Conditions. The *Sorangium cellulosum* strain JW1059 was isolated from a soil sample collected at Ansan, Korea. The strain is deposited in Applied Microbiology Lab., Division of Marine Environment & Bioscience, Korea Maritime University. This strain was cultivated in 2 L-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 ºC for 10 days on a rotary shaker at 160 rpm.

Extraction and Isolation. Cells and XAD-16 from a 10 L of culture broth (by centrifugation) were extracted with acetone. The acetone solution was dried *in vacuo* 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 λ 254 nm and TLC, and finally purified by HPLC on silica gel (YMC-Pack SIL, Hexane-EtOAc-iso-PrOH = 91:7.2:1.8) to yield **1** (16 mg) and **2** (14 mg).

Soraphinol A (1): Colorless oil; $[\alpha]_D^{25}$ +28.1 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε): 202 (4.46), 221 (4.53), 280 (3.82) nm; IR (KBr) ν_{max}: 3410, 1709, 1596, 1515, 1455, 1240, 1043, 746 cm^{-1}, ^{1}H and ^{13}C NMR: see Table 1; ESIMS: *m/z* 318 $[M+Na]^+$; HR-ESIMS: *m/z* 296.1303 $[M+H]^+$ (calcd for $C_{18}H_{18}O_3N$, 296.1281).

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