Spirodienal, a New Spiroketal from *Sorangium cellulosum*

Jong-Woong Ahn

Division of Marine Environment & Bioscience, Korea Maritime University, Busan 606-791, Korea
E-mail: jwahn@hhu.ac.kr
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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, epothilones.[1,2] We also have recently reported new bioactive metabolites from cellulolytic myxobacteria.[3,4] In the course of our continuing search for novel secondary metabolites from myxobacteria, the myxobacterium *Sorangium cellulosum* KM0141 was found to produce a new spiroketal, named spirodienal (**1**), along with a known compound spirangien A (**2**).[5] In this paper, the isolation, structure determination and biological activity of **1** are described.

Isolation and culture of the producing strain, *S. cellulosum* KM0141 were carried out according to the procedure reported elsewhere.[6] The fermentation of the producing strain was performed with the adsorbent resin XAD-16. At the end of fermentation, wet cell mass and XAD-16 resin were harvested by centrifugation and were extracted several times with acetone. After partitioning the acetone extract between ethyl acetate and water, the concentrated organic phase was separated by silica and ODS column chromatography to give **1** and **2** (Fig. 1). These compounds were finally purified by C_{18} reversed-phase HPLC. The total yield of **1** from a 100 L fermentation was 7 mg (0.07 mg/L).

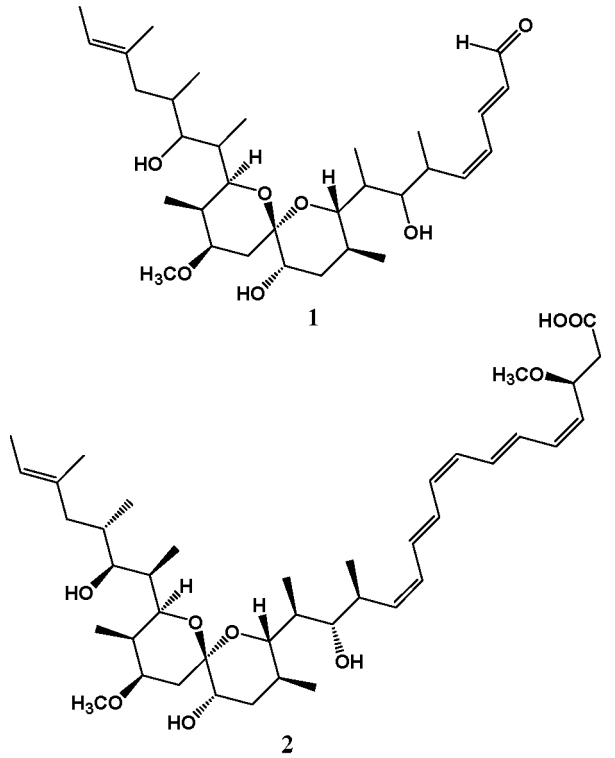

Figure 1. Chemical structures of spirodienal (1) and spirangien A (2).

Spirodienal (**1**) was isolated as optically-active colorless oil ($[\alpha]_D^{20}$ +39.4°(*c* 0.7, MeOH)) which analyzed for $C_{32}H_{54}O_7$ by combined HRESIMS and ^{13}C NMR spectrometry. Six degrees of unsaturation were inferred from the molecular formula. The spectral data for **1** were very similar to those obtained for **2**, suggesting that they are structurally related to each other. The IR spectrum of **1** showed absorption bands at 3437 (-OH), 2926, 1680 (C=O), and 1116 cm^{-1}. From ^1H and ^{13}C NMR data (Table 1), this compound was shown to possess an aldehyde, 1 sp^2 quaternary carbon, 5 sp^2 methines, 1 sp^3 quaternary carbon, 12 sp^3 methines including 6 oxymethines, 3 sp^3 methylenes, and 9 methyl carbons. The COSY spectrum of **1** indicated four ^1H-^1H spin coupling systems (Fig. 2). Spin systems A and B were linked by HMBC correlations of H-23 (δ 5.31), H-25 (δ 1.53), and H-26 (δ 0.72) to C-21 (δ 46.8). HMBC correlations from H-11 (δ 1.62), H-14 (δ 1.52), and H-17 (δ 3.83) to C-13 (δ 98.9) indicated connectivity between spin systems B and C and the presence of 1,7-dioxaspiro[5.5]undecan-5-ol. Spin systems C and D were connected through HMBC correlations from H-7 (δ 3.79) and H-31 (δ 0.86) to C-9 (δ 74.2). The configurations of the double bonds of the diene were assigned on the basis of the vicinal coupling constants, indicating (*Z*) configuration of the $\Delta^{4,5}$ double bond, while the configuration of the $\Delta^{2,3}$ double bond was (*E*)

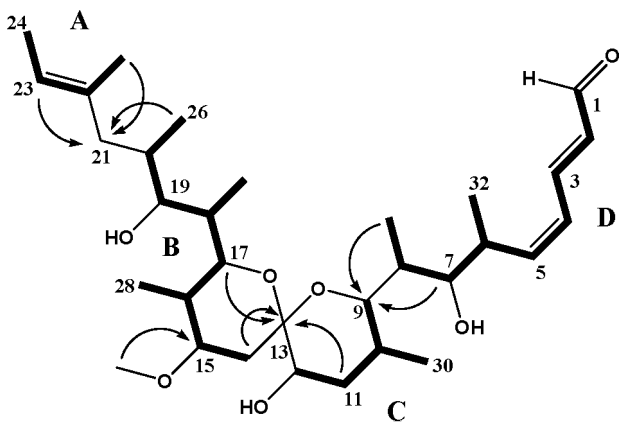

Figure 2. 2D-NMR correlations for **1**. Bold lines show ^1H-COSY correlations, and arrows show HMBC correlations.

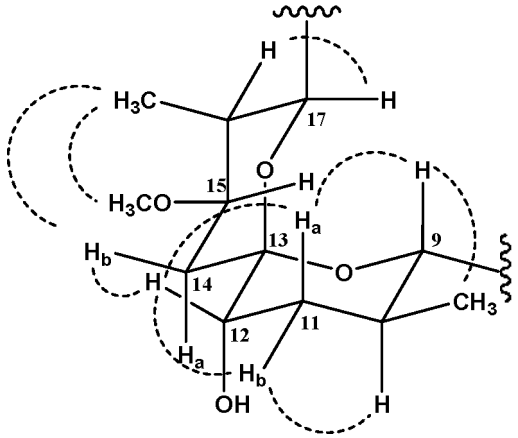

Figure 3. Key ROESY correlations for spiroketal moiety.

on the basis of its vicinal coupling constant of 15.2 Hz. The configuration of the $\Delta^{22,23}$ double bond was shown to be (*E*) from ROESY correlation between H-21 (δ 1.84/2.46) and H-23. The relative stereochemistry of the spiroketal moiety of **1** was deduced from ROESY and coupling constant data, and the results were consistent with those obtained for **2** (Fig. 3). Thus, the structure of **1** was determined as shown in Fig. 1.

Spirodienal (**1**) was tested for antimicrobial activity by the paper disk method.[7] **1** showed moderate antifungal activity against *Botrytis cinerea* (inhibition zone at a concentration 10 μg/8mm disk: 12 mm), *Botryosphaeria dithidea* (12 mm), *Sclerotinia sclerotiorum* (10 mm), and *Trichophyton mentagrophyte* (10 mm), but no activity against the other microorganisms: *Staphylococcus aureus*, *Escherichia coli*, *Pythium ultimum*, *Phytophthora capsici*, *Colletotrichum acutatum*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Candida albicans*.

Experimental Section

General Methods. Optical rotation was measured on a Perkin Elmer 341 polarimeter using a 1 cm cell. NMR spectra were measured on a Bruker Avans 800 spectrometer working at 800 MHz for proton and 200 MHz for carbon. Chemical shifts are shown in δ values (ppm) relative to C_6D_6 at 7.15 ppm for ^{1}H NMR and at 128.0 ppm for ^{13}C NMR. Mass spectral data were provided by the Korea Basic Science Institute, Ochang, Korea. UV and IR spectra were measured with a JASCO V-670 spectrophotometer and a JASCO FT/IR-4100 spectrometer, respectively. HPLC was performed on Shimadzu LC-10AS with SPD-M10AVP diode array detector. All solvents used were spectral grade or were distilled from glass prior to use.

Organism and Culture Conditions. The producing strain KM0141 was isolated from a soil sample collected in Ansan, Korea. The organism was identified as a strain of *Sorangium cellulosum* by morphological and cultural characteristics.[8] The strain is currently on deposit in the Korean Collection for Type Cultures with the accession number KCTC 11426. 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 7.2. The flasks were incubated at 30 °C for 10 days on a rotary shaker at 160rpm.

Table 1. NMR spectral data of spirodienal (1) in C_6D_6

No.	δ_H (*J* Hz)	δ_C	HMBC
1	9.48 d (8.0)	192.8 d	C-2, C-3
2	6.00 dd (15.2, 8.0)	133.2 d	C-1, C-4
3	7.13 dd (15.2, 11.2)	145.6 d	C-1, C-5
4	5.92 t (10.4)	129.4 d	C-2, C-6
5	6.18 t (10.4)	144.0 d	C-3, C-6, C-7, C-32
6	2.88 m	36.0 d	C-5, C-32
7	3.79 m	75.6 d	C-5, C-8, C-9
8	1.68 m	39.5 d	C-10, C-31
9	3.80 m	74.2 d	
10	1.90 m	24.9 d	C-9
11	1.88 m; 1.62 m	36.4 t	C-9, C-10, C-12, C-13
12	3.38 br s	70.6 d	C-10
13		98.9 s	
14	2.36 dd (12.8, 4.8); 1.52 m	33.5 t	C-13, C-15, C-16
15	3.84 m	77.6 d	
16	2.16 m	32.9 d	C-14, C-15
17	3.83 m	71.9 d	C-13
18	1.93 m	36.9 d	C-17, C-27
19	3.93 d (9.6)	76.4 d	C-20, C-21, C-26, C-27
20	1.65 m	35.3 d	C-19, C-26
21	2.46 dd (12.8, 4.8); 1.84 m	46.8 t	C-19, C-20, C-22, C-23, C-25
22		136.1 s	
23	5.31 q (6.4)	121.6 d	C-21, C-24
24	1.54 d (6.4)	13.8 q	C-22, C-23
25	1.53 s	16.3 q	C-21
26	0.72 d (7.2)	16.5 q	C-19, C-20, C-21
27	0.70 d (8.8)	7.7 q	C-17, C-19
28	0.94 d (7.2)	4.5 q	C-15, C-16, C-17
29	3.17 s	55.4 q	C-15
30	0.74 d (8.0)	18.1 q	C-9, C-10, C-11
31	0.86 d (7.2)	9.7 q	C-7, C-8, C-9
32	1.15 d (7.2)	19.8 q	C-5, C-6, C-7
7-OH[a]	5.03, d (5.5)		C-8
12-OH[a]	4.61, d (5.0)		C-13
19-OH[a]	4.42, d (7.0)		C-20

^{1}H and ^{13}C NMR were observed at 800 and 200 MHz, respectively. [a]Data were obtained in DMSO-d_6 solutions.

Extraction and Isolation. At the end of fermentation (100 L), wet cell mass and adsorbent resin XAD-16 were harvested by centrifugation and 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 16 g 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 (500 g), which was eluted stepwised with 3L of CH_2Cl_2 (fraction 1.1), CH_2Cl_2-MeOH 95:5 (fraction 1.2), and CH_2Cl_2-MeOH 90:10 (fraction

1.3). Fraction 1.2 (397 mg) was further separated by RP-18 column chromatography using MeOH-H_2O (9:1) as solvent. The fractions containing **1** were collected according to UV absorption at λ 280 nm and TLC, and finally purified by HPLC (CAPCELL PAK C18, 10 × 250 mm, 70% aqeous MeOH) to yield **1** (7 mg) and **2** (5.2 mg).

Spirodienal (1): UV (MeOH) λ_{max} (ε): 277 (23200) nm; ^{1}H and ^{13}C NMR: see Table 1; ESIMS: *m/z* 573 $[M+Na]^-$; HR-ESIMS: *m/z* 573.3785 $[M+Na]^+$ (calcd for $C_{32}H_{54}O_7Na$, 573.3767)

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