Spirodienal C, a New Spiroketal Produced by *Sorangium cellulosum* (Myxobacteria)

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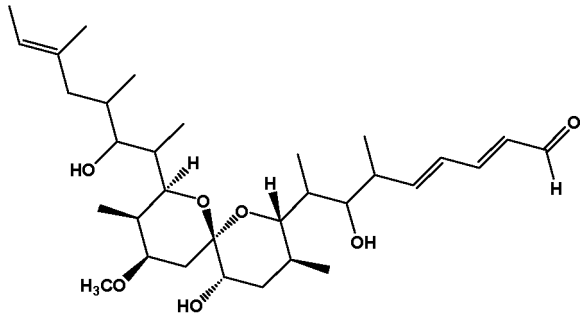
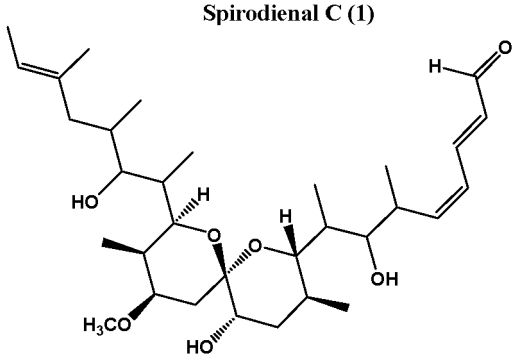
Myxobacteria are unique bacteria characterized by the gliding nature and forming fruiting bodies upon starvation. Because of the difficulties in their isolation and cultivation, these organisms are less well studied and generally less exploited than other groups of microbes. However, myxobacteria have recently been recognized as a new source of novel metabolites exhibiting a variety of bioactivities.[1-4] The author also has recently isolated new bioactive metabolites, spirodienal A and B from the myxobacterium *Sorangium cellulosum* KM1041.[5] The structures of these compounds were reported to be novel spiroketals. The isolation of spirodienal A and B from this strain in previous work encouraged us to do further search for this type of metabolites, which led to the isolation of a new derivative named as spirodienal C (**1**). 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 spirodienal A and a new derivative **1** (Fig. 1). These compounds were finally purified by C_{18} reversed-phase HPLC. The total yield of **1** from a 100 L fermentation was 2.8 mg (0.028 mg/L).



Spirodienal C (1)



Spirodienal A

Figure 1. Chemical structures of spirodienal A and C (**1**).

Spirodienal C (**1**) was obtained as a colorless oil with a specific rotation of +34.2° (*c* 0.35, MeOH, 25 °C). Its UV spectrum showed an absorption maximum at 275 nm, and the IR spectrum showed intense absorption bands at 3421(OH) and 1680 (C=O) cm^{-1}. These observations suggested the presence of a conjugated dienone system.[7]

Compound **1** was determined to have the molecular formula $C_{32}H_{54}O_7$ based on HRESIMS data ($C_{32}H_{54}O_7Na$, *m/z* 573.3763, calcd 573.3762), indicating that the molecular formulae of **1** and spirodienal A were identical. 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, which account for four of the six degrees of unsaturation. The remaining two degrees of unsaturation are attribute to two rings. It was also indicated that 51 protons were attached to 32 carbons, while the remaining three protons were those in hydroxyl groups.

The spectral data of **1** were very similar to those obtained for spirodienal A. However, the ^{13}C NMR data showed noticeable differences (2.0 ~ 5.5 ppm) in the chemical shifts of olefinic carbons at C-2 ~ C-6. Despite the spectral differences, combined 2D NMR experiments showed that **1** had the same proton-proton and proton-carbon correlations throughout the entire molecule as spirodienal A. The connectivity of two partial structures (C-1 ~ C-12 and C-14 ~ C-24) was established by ^1H-^1H COSY with an aid of HMBC as shown in Fig. 2. HMBC correlations of H-9 (δ 3.81), H-11α (δ 1.61), H-14β (δ 1.54), and H-17 (δ 3.82) with C-13 (δ 99.0) indicated connectivity between these partial structures and the presence of a 6,6-spiroketal moiety. The configuration of the $\Delta^{2,3}$ double bond was assigned as *E* on the basis of its vicinal coupling constant of 15.2 Hz, and the configuration of $\Delta^{22,23}$ double bond was shown to be *E* from NOESY correlations of H-23 (δ 5.30) with C-21 methylene protons (δ 2.49, 1.83). In the ^1H NMR data, the olefinic protons at δ 5.92 (1H, t, J = 10.4 Hz,

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

Position	δ_H (J Hz)	δ_C	HMBC
1	9.36 d (8.0)	193.0	C-2
2	5.92 dd (15.2, 8.0)	131.2	C-4
3	6.44 dd (15.2, 10.4)	151.9	C-1, C-5
4	5.91 dd (15.2, 10.4)	129.9	C-3, C-6
5	6.24 dd (15.2, 8.8)	146.9	C-3, C-7, 6-CH_3
6	2.44 m	40.5	C-5, 6-CH_3
7	3.79 m	75.8	C-5, C-9
8	1.69 m	39.6	C-7, 8-CH_3
9	3.81 m	74.1	C-13
10	1.92 m	25.0	
11	1.88 m; 1.61 m	36.5	C-9, C-10
12	3.38 br s	70.7	
13		99.0	
14	2.39 dd (13.6, 4.8); 1.54 t (12.8)	33.7	C-13, C-15, C-16
15	3.83 m	77.6	
16	2.16 m	32.8	
17	3.82 m	71.9	C-13
18	1.92 m	37.0	C-17, 18-CH_3
19	3.94 d (9.6)	76.3	C-18, C-20, C-21, 18-CH_3
20	1.62 m	35.3	
21	2.49 dd (13.6, 4.8); 1.83 dd (12.8, 8.0)	46.6	C-20, 22-CH_3
22		135.9	
23	5.30 q (6.4)	121.6	C-21, C-24, 22-CH_3
24	1.54 d (6.4)	13.9	C-23
6-CH_3	1.19 d (6.4)	19.3	C-6, C-7
8-CH_3	0.90 d (7.2)	9.8	C-7, C-8, C-9
10-CH_3	0.75 d (6.4)	18.2	C-9, C-10, C-11
16-CH_3	0.95 d (6.4)	4.5	C-15, C-16, C-17
18-CH_3	0.70 d (6.4)	7.8	C-17, C-18
20-CH_3	0.72 d (6.4)	16.5	C-19, C-20, C-21
22-CH_3	1.52 s	16.3	C-21
15-OCH_3	3.16 s	55.4	C-15

^1H and ^{13}C NMR were observed at 800 and 200 MHz, respectively.

H-4) and 6.18 (1H, t, J = 10.4 Hz, H-5) in spirodienal A were replaced by those at δ 5.91 (1H, dd, J = 15.2, 10.4 Hz, H-4) and 6.24 (1H, dd, J = 15.2, 8.8 Hz, H-5) in **1**. Accordingly, **1** possessed the 4*E* double bond instead of the 4*Z* geometry found in spirodienal A.

The cross peak of H-12 with H-14β defined the relative configuration at C-13. The coupling constant of $J_{14\beta,15}$ = 12.8 Hz suggested that the configuration of H-14β was axial. The relative stereochemistry of the C-9 to C-17 spiroketal moiety of **1** was deduced from NOESY data and coupling constants, and the results were consistent with those observed in spirodienal A (Fig. 3). Thus, the structure of **1** was determined as shown in Fig. 1.

Spirodienal C (**1**) was tested for antimicrobial activity by the paper disk method. Compound **1** showed moderate antifungal activity against *Botrytis cinerea* (inhibition zone at a concentration of 10 μg/8 mm disk: 13 mm), *Botryosphaeria dithidea* (11 mm), *Sclerotinia sclerotiorum* (13 mm), and *Trichophyton mentagrophyte* (10 mm), but no activity against the other fungi: *Pythium ultimum*, *Phytophthora capsici*, *Colletotrichum acutatum*, *Rhizoctonia solani*, *Fusarium oxysporum*, and

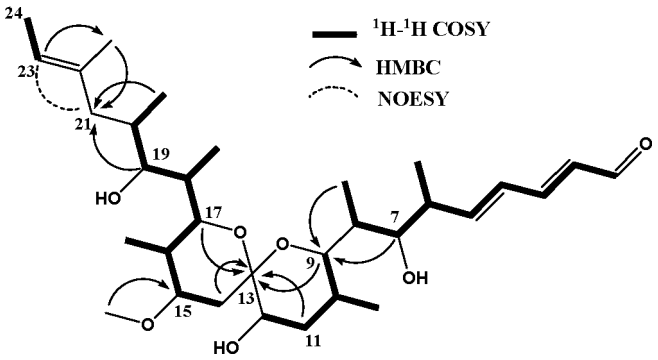


Figure 2. 2D-NMR correlations for 1.

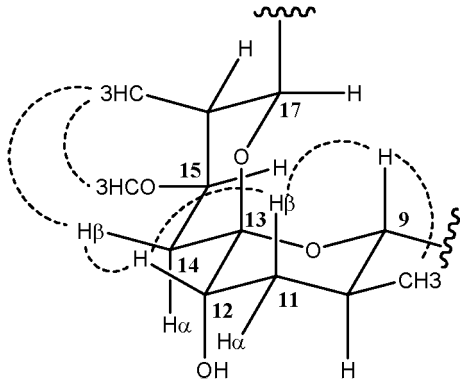


Figure 3. Key NOESY correlations for spiroketal moiety.

Candida albicans. It showed no antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

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 ^1H 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 2 L-Erlenmeyer flasks containing 400 mL of a medium[9] 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 160 rpm.

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 stepwise with 3 L 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 eluent. The fractions containing **1** were collected according to UV absorption at 280 nm and TLC, and finally purified by HPLC (Capcell Pak C_{18}, 10 × 250 mm, 70% aqeous MeOH, flow 4.0 mL/min) to yield 2.8 mg of **1** (t_R = 68 min) and 7.0 mg of spirodienal A (t_R = 80 min).

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