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Complete assignments of ^{1}H and ^{13}C NMR spectra of Chivosazole F

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Abstract : The ^{1}H and ^{13}C NMR spectra of chivosazole F from *Sorangium cellulosum* were completely assigned by a combination of 1D and 2D NMR techniques. The configurations of double bonds were confirmed from the ROESY spectra. The stereochemistry at asymmetric carboncenters was partially assigned on the basis of the results of NOE analysis.
Key words: Chivosazole F, *Sorangium cellulosum*, Myxobacteria, 2D NMR techniques

INTRODUCTION

Various strains of *Sorangium cellulosum*(Myxobacteria) have proved to be rich source of potent antibiotics such as chivosazoles.[1] The chivosazoles are oxazole-containing macrolide glycosides active against yeast and, in higher concentrations, against filamentous fungi (0.12-10μg/ml)[2]. Furthermore, these metabolites are known to be highly cytotoxic (9 ng/ml) against mammalian cells[3]. Chivosazole A and its structural variants were originally isolated from *S. cellulosum*, strain So cel 2 [4].

In our search for cytotoxic metabolites from Korean soil microorganisms, we found chivosazole F from the strain of *Sorangium cellulosum* JW1045. However, the structure of this highly cytotoxic compound was elucidated with only partial structural units assigned in the other paper[5]. The NMR assignments reported for chivosazole F were made from the comparison of the ^{1}H and ^{13}C NMR data with those of chivosazole A, the representative metabolite of this series [5]. In this paper we report an unequivocal assignment for all of the hydrogen and carbon resonances as well as the determination of stereochemistry at several asymmetric carbon centers by an application of 1D and 2D NMR techniques. In addition, the cytotoxicity of this metabolite against various human cancer cell-lines is reported.

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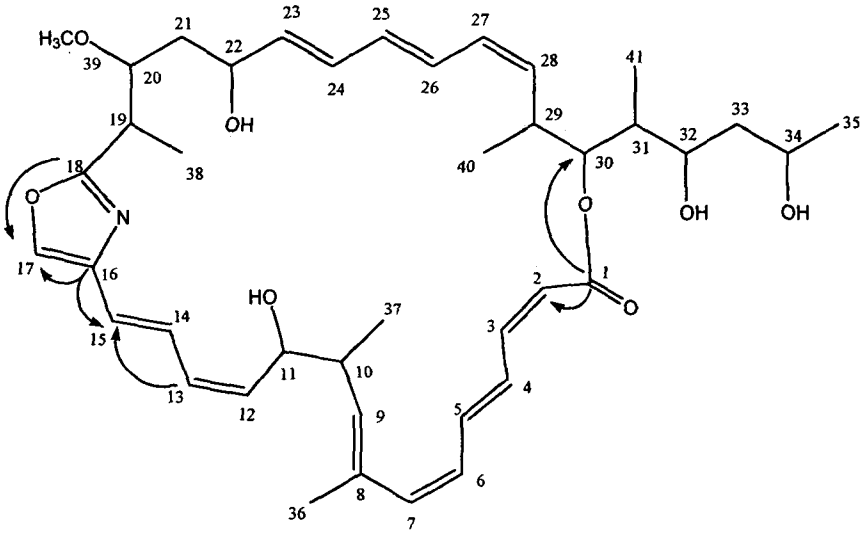
EXPERIMENTAL

Microorganism and Culture Conditions

The producing organism, *Sorangium cellulosum* JW1045 was isolated from a soil sample collected at Daejon, Korea. The culture is deposited in myxobacteria collection, KRICT. Seed cultures on yeast agar were inoculated into 300-ml Erlenmeyer flasks containing 50 ml of medium. The basic medium for growth and production had the following composition: soyameal 0.4%; potato starch 0.8%; glucose 0.2%; $MgSO_4 \cdot 7H_2O$ 0.1%; $CaCl_2 \cdot 2H_2O$ 0.1%; EDTA Fe(III)-Na salt 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) adsorber resin Amberlite XAD-16 (Aldrich) was added to promote the production of the active substance. The fermentation was performed on a rotary shaker (150 rpm) at 30℃ for 10 days.

Isolation

20-*l* fermentation broth cultivated in the presence of 300 g of Amberlite XAD-16 was filtered through a sieve to collect the Amberlite XAD-16 resin. The resin was transferred to an open gravity column and washed with methanol. Evaporation of the solvent from the eluent furnished 6.1 g of an oil, which was re-dissolved in 200 ml of methanol and extracted twice with 100 ml of *n*-heptane. The removal of methanol under reduced pressure yielded 5.2 g of brown oil which was fractionated by column chromatography. A solution of the oil in dichlomethane was applied onto a column of silica gel (500 ml), which was eluted with in



Chivosazole F

the order of elution 2.5 *l* of *n*-hexane/ethyl acetate (v:v=4:6), *n*-hexane/ethyl acetate 3:7, and *n*-hexane/ethyl acetate 8:2. The fraction eluted with 3:7 mixture of *n*-hexane/ethyl acetate (202 mg) was separated by three runs of Recycling preparative HPLC [column; JAIGEL 310, eluent; dichlomethane, flow rate; 4 ml/min, detection by UV at 280 nm] and gave a main fraction of 93 mg which was further separated by S*i*-HPLC [column; SenshuPak 8x250 mm, eluent; *n*-hexane/ethyl acetate 64:36, flow rate; 1.8 ml/min, detection by UV at 278 nm] to yield 9.6 mg of chivosazole F.

NMR spectra.

1D and 2D NMR spectra were measured on a Varian UNITY 500 spectrometer working at 500MHz for proton and 125MHz for carbon. The ^{1}H and ^{13}C NMR chemical shifts were referred to CD_3OD observed at 3.30ppm and 49.0ppm, respectively. For all experiments the temperature was stabilized at 297K. The pulse conditions for 2D NMR spectra were as follows: The gradient COSY spectra were collected with a spectral width 3834Hz in a 256(*t1*) ×1024(*t2*) matrix using the pulse gradient of 1ms duration with a stre-

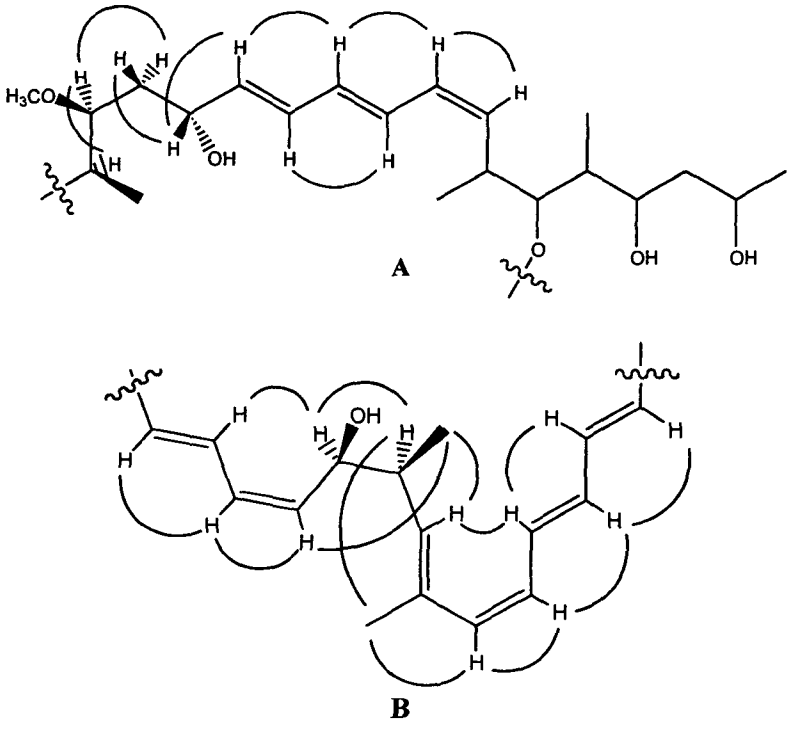


Fig. 1. Partial structures A and B with possible geometric configuration from the omonuclear 2D NMR experiments

Table 1. The ^{1}H and ^{13}C NMR assignments for chivosazole F.

#	δ_H	δ_C		HMBC
1		168.9	s	
2	5.42, 1H, d (11.7)	117.9	d	C-1, C-4
3	6.50, 1H, dd (11.7, 11.7)	145.3	d	C-1, C-5
4	7.06, 1H, dd (15.1, 11.7)	130.6	d	C-6
5	6.87, 1H, dd (15.1, 10.7)	139.9	d	C-3
6	5.90, 1H, dd (11.2, 10.7)	129.0	d	C-7
7	5.84, 1H, d (11.2)	139.8	d	C-9, C-36
8		134.3	s	
9	5.07, 1H, d (8.8)	136.3	d	C-7, C-36
10	2.83, 1H, m	40.4	d	C-8, C-9,C-11, C-12, C-37
11	4.72, 1H, dd (9.2, 5.9)	70.5	d	C-13, C-37
12	5.49, 1H, dd (10.3, 9.2)	132.4	d	C-14
13	6.21, 1H, dd (11.7, 10.3)	131.3	d	C-11, C-15
14	7.16, 1H, dd (15.1, 11.7)	127.3	d	C-16
15	6.36, 1H, dd (15.1)	122.0	d	C-13, C-16
16		140.1	s	
17	7.72, 1H, s	137.6	d	C-16, C-18
18		167.3	s	
19	3.49, 1H, m	36.3	d	C-38
20	3.94, 1H, ddd (10.5, 3.9, 1.7)	80.0	d	
21	1.06, 1H, ddd (14.2, 10.8, 1.7)	39.6	t	C-20
	1.66, 1H, ddd (14.2, 10.5, 2.7)			
22	4.33, 1H, br d (9.8)	68.1	d	
23	5.76, 1H, dd, (15.1, 3.5)	138.7	d	
24	6.38, 1H, ddd (15.1, 10.7, 1.7)	129.2	d	C-22
25	6.16, 1H, dd (14.6, 10.7)	134.4	d	C-27
26	6.54, 1H, dd (14.6, 11.3)	128.5	d	C-24
27	5.95, 1H, dd (11.3, 10.3)	130.2	d	C-25, C-29
28	5.17, 1H, dd (10.3, 10.3)	135.4	d	C-26
29	3.16, 1H, m	35.7	d	C-28, C-30, C-40
30	5.25, 1H, dd (10.2, 1.0)	78.1	d	C-1, C-29, C-32, C-41
31	1.78, 1H, m	41.8	d	C-28, C-32, C-41
32	3.44, 1H, m	70.4	d	
33	1.39, 1H, ddd (14.2, 10.3, 2.5)	44.6	t	C-34
	1.62, 1H, ddd (14.2, 9.7, 2.0)			
34	4.00, 1H, m	65.2	d	
35	1.16, 3H, d (6.3)	24.4	q	C-33, C-34
36	1.88, 3H, s	17.2	q	C-7, C-8, C-9
37	1.04, 3H, d (6.9)	14.3	q	C-9, C-10, C-11
38	1.35, 3H, d (6.9)	10.8	q	C-18, C-19, C-20
39	3.49, 3H, s	58.2	q	C-20
40	1.01, 3H, d (6.9)	17.8	q	C-28, C-29, C-30
41	0.97, 3H, d (7.4)	10.3	q	C-30, C-31, C-32

.ngth 10G/cm and processed with the sinebell function. The TOCSY and ROESY 2D NMR spectra were acquired with mixing times of 50ms and 350ms, respectively, in the phase-sensitive mode. The HSQC spectra were obtained in a 128(*t1*) ×1024(*t2*) matrix with one bond coupling constant parameter J_{CH}=140Hz and processed in a 256(*t1*) ×1024(*t2*) matrix by a linear prediction method. The HMBC experiment was optimized for long-range coupling constant of 8Hz and collected in a 256(*t1*) ×1024(*t2*) matrix. The HSQC and HMBC experiments were utilized by the pulse gradient of 1ms duration and 5 and 10 G/cm strength to reduce the artifact in the spectra.

Cytotoxicity Assay. Cytotoxicity was assessed using the sulforhodamine B (SRB)-assay method using human cancer cell lines of A549 (lung carcinoma), SK-OV-3(ovary carcinoma), SK-MEL-2 (melanoma), XF498 (CNS carcinoma), and HCT15 (colon carcinoma). The cells were incubated at 37℃ for 72h, at which time the SRB was added. The results are expressed as an ED_{50}, which is the drug concentration required to inhibit cell proliferation (absorbance at 520 nm) to 50% of that in untreated control cells.

RESULTS AND DISCUSSION

Chivosazole F was isolated as amorphous solid by combined chromatographic methods. The molecular formula of this compound was established as $C_{41}H_{57}NO_8$(*m/z* 691[M^+]) on the basis of HREIMS and ^{13}C NMR data. The structures of two partial structures A and B as well as the assignments of all of the ^{1}H signals were accomplished by the ^{1}H-COSY and TOCSY experiments (see Fig. 1). Also, their directly bound carbon signals were assigned from the gHSQC experiment. (Table 1). The connectivity at C-8 was secured by HMBC correlations from methyl protons at δ=1.88 to a quaternary carbon at δ=134.3 and two methine carbons at δ=136.3 and 139.8. The configurations of the double bond in structure A and B were deduced from the vicinal coupling constant of ca 11Hz for *Z* and 15Hz for *E* bonds. These were confirmed by a ROESY experiment as shown in the Figure 2. The ROESY experiment also suggested the relative stereochemistry at several asymmetric carbon centers as drawn in Fig. 1. Key NOE correlations obtained from the ROESY experiment were listed in Table 2.

Linkage of partial structure A with B was determined by several HMBC correlations of the remaining three quaternary carbons at δ=168.9, 140.1, 167.3 and one methine carbon at δ= 137.6 with neighboring protons. The methine proton at δ=7.72 showed HMBC correlations with two quaternary carbons at δ=140.1 and δ=167.3. In addition the coupling constant between this proton and its carbon at δ=137.6 was found to be J_{CH}=209 Hz, indicating the presence of an oxymethine in a heteroaromatic ring[6]. Together with a nitrogen atom they formed an 1,3-oxazole ring. Further correlations between the proton H-19 and a carbon at δ=167.3 and between proton H-15 and carbon at δ=140.1 revealed the attachment of partial structure A and B at the 2 and 4-position of oxazole ring, respectively.

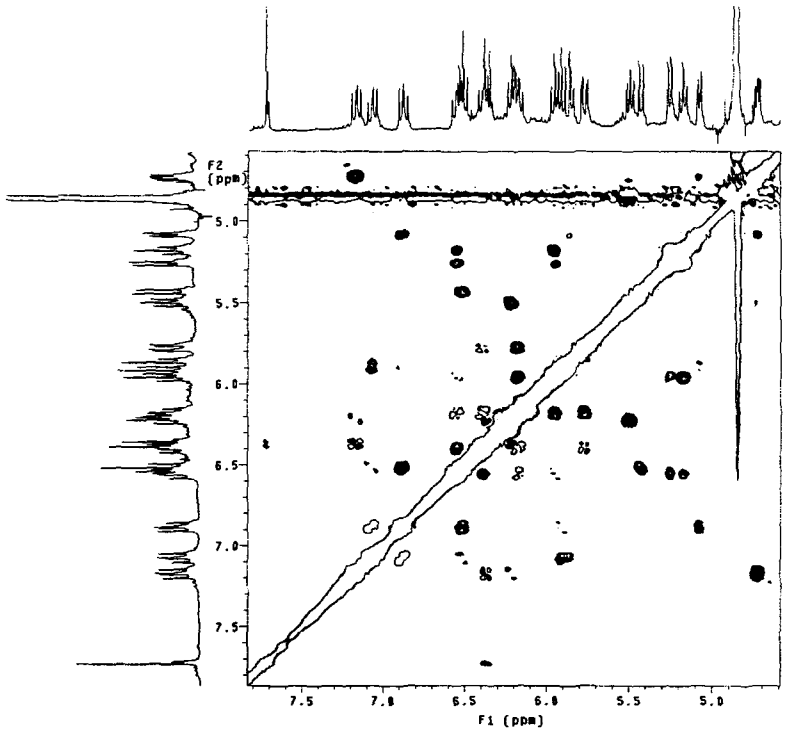


Fig. 2. Downfield region of ROESY spectrum of chivosazole F.

The carbonyl carbon at δ=168.9 was correlated with two protons at H-2 and H-30 from the HMBC experiment. The chemical shift of the carbon signal at δ=168.9, which was assigned to C-1, and carbon signal at C-30 implied the existence of an ester oxygen between C-1 and C-30. Thus, the structure of chivosazole F was determined as a macrolactone metabolite containing an oxazole ring. However, the relative stereochemistry for the gross structure was unable to be elucidated by NMR spectral data since reliable NOE signal was not observed between partial structure A and B.

The structure determined by this study was identical to that of chivosazole F reported previously[5]. The difference between two results is that our ROESY data evidently showed NOE correlation between H-9 proton at δ=5.07 and H-5 proton at δ=6.87. This finding results in a preference for the conformation in which H-9 is spatially close to H-5. However, this NOE correlation was not detected from the NOE difference experiment in the previous paper.

Table 2. The list of NOE data analyzed from the ROESY spectra of chivosazole F.

#	NOE correlation
H-2	H-3
H-3	H-2, H-5
H-4	H-6
H-5	H-3, H-9
H-6	H-4
H-7	H-36
H-9	H-5, H-6(w), H-7(w), H-12(w), H-11, H-37
H-10	H-11, H-36, H-37
H-11	H-10, H-14(w), H-36(w)
H-12	H-13, H-37
H-13	H-12
H-14	H-11
H-15	H-17
H-17	H-15
H-19	H-20, H-21(w, 1.65), H-22
H-20	H-19, H-21(1.07)
H-21, α	H-21(1.06), H-22, H-23
H-21, β	H-20, H-21(1.65)
H-22	H-21(1.67), H-23
H-23	H-22,H-25
H-24	H-26
H-25	H-23, H-27
H-26	H-29, H-30
H-27	H-25, H-28
H-28	H-26, H-27
H-29	H-26, H-40
H-30	H-26, H-27, H-31, H-40
H-36	H-7, H-10,H-11
H-37	H-9, H-10, H-11(w),H-12
H-38	H-19, H-21(1.65)
H-40	H-28
H-41	H-29, H-30, H-31, H-32

Table 3. Cytotoxicities of Chivosazole F and Antimycin A(reference)

Compound	ED_{50} [ng/ml]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
Chivosazole F	13.7	0.1	0.2	0.6	0.9
Antimycin A	480	5500	270	390	410

Chivosazole F was tested for *in vitro* cytotoxicity against several human cancer cells using antimycin A as a reference. The results were summarized in Table 3. Chivosazole F demonstrated potent cytotoxicity against human cancer cells, having ED_{50} values ranging from 0.1 to 13.7 ng/ml. Against human cancer cells such as SK-OV-3, the activity of chivosazole F was more than 50,000 times stronger than that of antimycin A in terms of ED_{50}.

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