Communications to the editor

THE STRUCTURE OF SIOMYCIN-D_1, PEPTIDE ANTIBIOTIC ISOLATED FROM *STREPTOMYCES SIOYAENSIS*

Sir:

Sulfur-containing peptide antibiotic siomycin (SIM) isolated from *Streptomyces sioyaensis*[1] belongs to the thiostrepton group of antibiotics and was shown to consist of one major component (SIM-A) and two minor components (SIM-B and -C)[2]. The structures of the thiostrepton group antibiotics are too complex to be elucidated by chemical degradation methods. While much of the structure of thiostrepton (TST) has been proposed by X-ray crystallographic analysis[3], no crystal of SIM-A was suitable for X-ray analysis. The chemical structure of SIM-A (**I**) was elucidated by ^{13}C NMR spectroscopic comparison with TST on the basis of X-ray analysis of TST; the ^{13}C NMR study also determined the total structure of TST[4]. The chemical structures of **I** and TST were finally confirmed by a 270-MHz ^1H FT NMR spectral study including nuclear OVERHAUSER effect difference FT NMR spectroscopy[5]. The structures of SIM-B and -C were also revealed[6]. Further search for other minor components led us to the isolation of several components including SIM-D_1. We report here the isolation of SIM-D_1 (**II**) and its structure elucidation by ^1H and ^{13}C NMR spectroscopy.

Siomycin complex was separated by column chromatography on silica gel using a mixture of $CHCl_3$ and CH_3OH as an eluting solvent. After SIM-C, -B, and -A were eluted, fractions containing SIM-D_1 were obtained. SIM-D_1 was purified by repeated column chromatography or preparative thin-layer chromatography and recrystallized from a mixture of $CHCl_3$ and CH_3OH: mp *ca.* 260°C (decomp.); $[\alpha]_D$ −69.9° (dioxane); Rf 0.13 (silica gel, $CHCl_3$ - CH_3OH, 95:5); UV (EtOH) a plain curve ascending to 205 nm with shoulders at 250 and 285 nm; IR ν_{max}^{KBr} 1730, 1680 cm^{-1} (Fig. 1); amino acid analysis, Ammonia, 6.76; Thr, 0.84; Ala, 1.00; Val, 1.01. These physicochemical properties are similar to those of SIM-A. SIM-D_1 exhibited *in vitro* antibacterial activity comparable to SIM-A against Gram-positive bacteria.

In the 270-MHz ^1H NMR spectrum of **II** in $CDCl_3$ (Fig. 2), the doublet due to the Q-12 CH_3 and the quartet due to the Q-11 CH, which were respectively seen at δ_H 1.37 and 5.34 in that of **I**, were not observed, and an ABX pattern assignable to a CH_2OH grouping was observed around δ_H 4.5 and 5.0. On addition of D_2O, the signal at δ_H 5.0 disappeared, and the ABX-type signal was changed into an AX-type signal at δ_H 4.43 and 4.99. Furthermore, all signals due to protons proximate to the $CH(OH)CH_3$ grouping of the Q residue of **I** were shifted to lower or higher fields in **II** (see Table 1).

The ^{13}C signal assignments at 15-MHz reported in our preceding paper[4] were very tentative. Further investigations of 25 and 50-MHz ^{13}C NMR spectra of **I** and SIM derivatives made it possible to assign almost all signals. Since almost all ^1H signals of **I** were assigned at

Fig. 1. Infrared spectra of siomycin-A (**I**) and siomycin-D_1 (**II**).

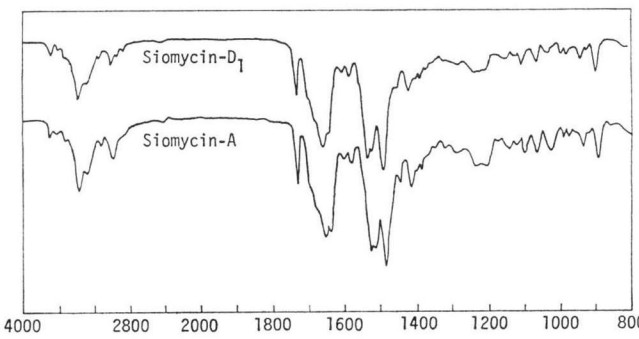


Fig. 2. 270-MHz ^1H FT NMR spectra of siomycin-A (**I**) (lower trace) and siomycin-D_1 (**II**) (upper trace) in $CDCl_3$.

FT measurement conditions: spectral width, 3600 Hz; pulse width, 12 μs (90°); acquisition time, 2.26 s; number of data points, 16 K; number of transients, 256; 5-mm spinning tube; concentration, 40 mg/ml; 23°C.

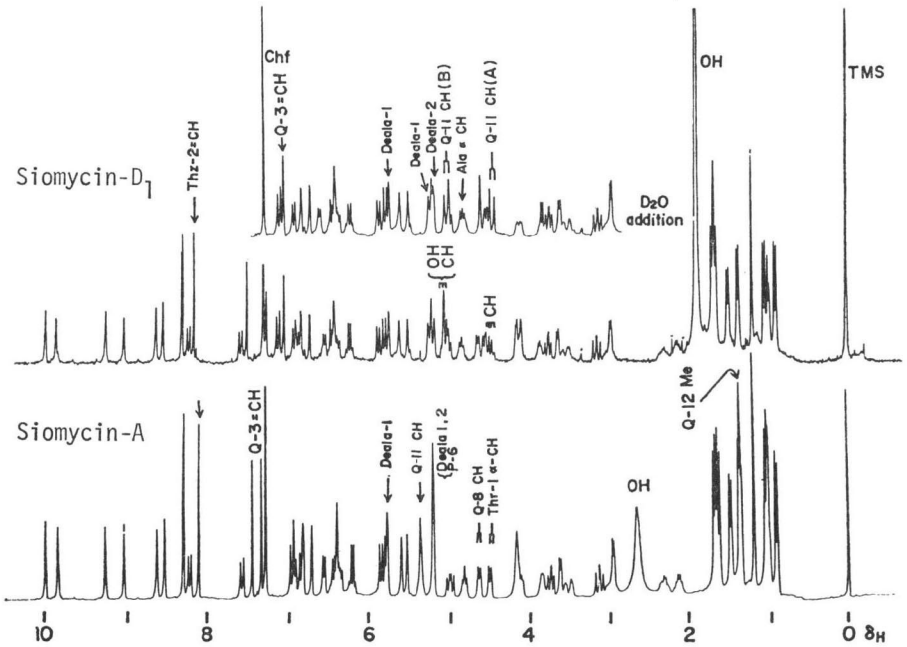


Fig. 3. 25-MHz ^1H complete-decoupled ^{13}C NMR spectra of siomycin-A (**I**) (lower trace) and siomycin-D_1 (**II**) (upper trace) in $CDCl_3$-CD_3OH (4:1).

FT measurement conditions: spectral width, 5500 Hz; pulse width, 18 μs (43°); acquisition time, 0.7 s; number of data points, 8 K; number of transients, 200 K; 5-mm spinning tube; concentration 120 mg/ml; 70°C.

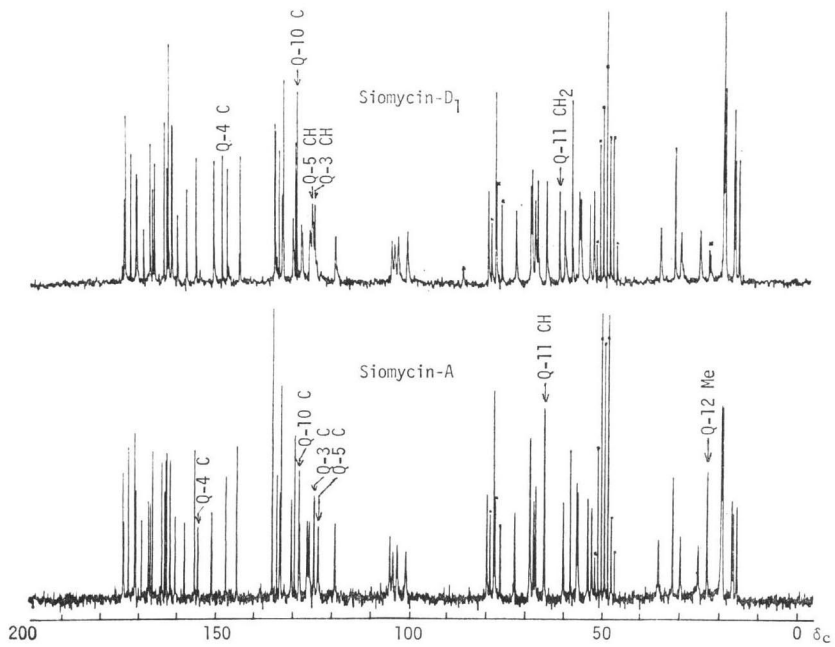


Table 1. Chemical shift data for siomycin-D_1 (II)[a].

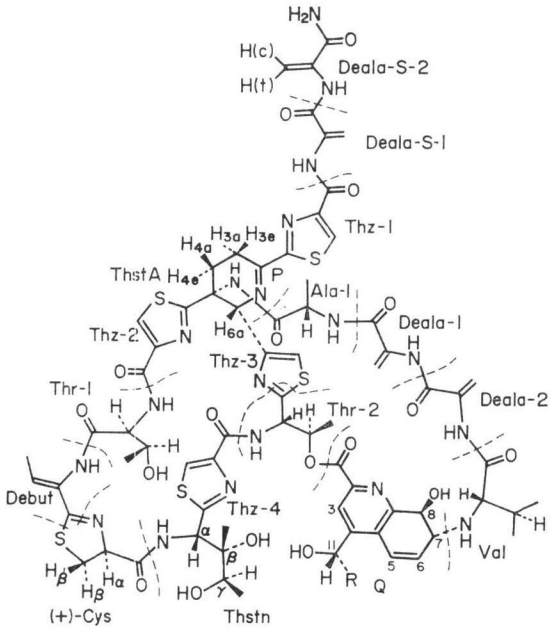
Assignment		δ_H	Assignment		δ_H	Assignment		δ_H
Ala-1	βCH_3	1.49d	Q	11CH(B)	4.99dd (−0.35)	Thr-1	γCH_3	0.97d
	αCH	4.79dq		$12CH_3$	—[b]		βCH	1.22m
	NHCO	6.50bd		8OH	6.85d		αCH	4.51dd
Deala-1	β=CH(c)	5.21bs	Thr-2	γ-CH_3	1.67d		NHCO	6.86dd
	β=CH(t)	5.71d (−0.05)		βCH	6.42d	Thst A[c]	P-3aCH	2.96dddd
	NHCO	8.61bs		αCH	5.84d		P-3eCH	3.50dddd
Deala-2	β=CH(c)	5.13bs (−0.05)		NHCO	8.20bd		P-4aCH	2.27ddd
	β=CH(t)	6.39bs	Thstn	δCH_3	1.36d		P-4eCH	4.09ddd
	NHCO	9.22bs		γCH	3.81bs		P-6aCH	5.17bs
Val	γCH_3	0.89d		γCH_3	1.19s		NHCO	9.84bs
		1.03d		αCH	5.76d		Thz-1=CH	8.28s
	βCH	2.12m		NHCO	7.55bd		Thz-2=CH	8.14s (+0.06)
	αCH	2.95d		Thz-4=CH	8.29s		Thz-3=CH	7.48s (+0.05)
Q	3=CH	7.02s (−0.29)	Cys	βCH	3.12dd	Deala-S-1	β=CH(c)	5.58bs
	5=CH	7.08d (+0.14)		β'CH	3.71dd		β=CH(t)	6.81d
	6=CH	6.35dd		αCH	4.97dd		NHCO	9.97bs
	7CH	3.60d	Debut	γCH_3	1.64d	Deala-S-2	β=CH(c)	5.47bs
	8CH	4.59d		β=CH	6.20q		β=CH(t)	6.70d
	11CH(A)	4.43dd (−0.91)		NHCO	8.52s		NHCO	9.00bs

Assignment		δ_C	Assignment		δ_C	Assignment		δ_C
Ala	βCH_3	19.6[d]	Thr-2	γCH_3	19.6[d]	Thst A	P-3 CH_2	25.5
	αNCH	52.7		αNCH	56.2		P-4 CH_2	30.3
	CO	163.7		βOCH	72.7		P-5 NC	58.5
Deala-1	$\beta=CH_2$	103.0	Thstn	δCH_3	16.6		P-6 CH	65.0
	α=C	132.9		γCH_3	19.2		P-2 C=N	162.9
	CO	162.5		αNCH	53.8		Thz-1 SCH=	125.4[h]
Deala-2	$\beta=CH_2$	100.6		γOCH	69.0		NC=	157.8[i]
	α=C	134.9[e]		βOC	77.9		CO	162.5
	CO	161.6		Thz-4 SCH=	126.0[h]		SC=N	169.0
Val	γCH_3	16.9		NC=	150.8		Thz-2 SCH=	128.1
		19.2[d]		CO	162.5		NC=	147.7[i]
	βCH	31.8		SC=N	167.4		CO	161.7[i]
	αNCH	68.7	Cys	βSCH	35.4		SC=N	170.8
	CO	173.7		αNCH	79.7		Thz-3 SCH=	119.4
Q	$12CH_3$	—[b]		CO	172.4		NC=	150.8[i]
	7NCH	60.4	Debut	γCH_3	15.7		SC=N	174.0
	11OCH	61.7 (−3.4)		α=C	129.4	Deala-S-1	$\beta=CH_2$	104.0
	8OCH	67.9		β=CH	133.1		α=C	135.1[e]
	3=CH	125.1 (+1.8)		SC=N	170.9[g]		CO	160.2
	5=CH	124.8	Thr-1	γCH_3	19.4[d]	Deala-S-2	$\beta=CH_2$	104.7
	10=C	129.7 (+1.4)		αNCH	56.6		α=C	134.0
	6=CH	130.4		βOCH	67.3		CO	166.7
	2=C	144.2[f]		CO	166.2			
	4=C	148.7 (−5.7)						
	9=C	155.4[f]						
	COO	170.7[g]						

[a] Differences in chemical shifts from those of **I** were designated in parentheses ($\delta_{II}-\delta_I$) when they were over ± 0.05 ppm (δ_H) or ± 0.5 ppm (δ_C). [b] See text. [c] Abbreviations a and e are axial and equatorial, respectively. [d~i] Assignments may be interchanged.

Fig. 4. Chemical structure of siomycin-A (**I**) and siomycin-D_1 (**II**): **I**, R=CH_3; **II**, R=H.

Deala, dehydroalanine; Debut, dehydrobutyrine; P, piperidine ring; Q, quinaldic acid precursor; Thstn, thiostreptine residue; Thst A, thiostreptonic acid unit; Thz, thiazole ring.



270 MHz in $CDCl_3$ - CD_3OD (4 : 1)[5], ^1H single-frequency off-resonance decoupling ^{13}C spectra in $CDCl_3$ - CD_3OD (4 : 1) at 70°C gave ^{13}C signal assignments for all protonated carbon signals except those for some overlapping Me signals. Most of the nonprotonated carbon signals were assigned by chemical-shift comparisons between the derivatives. The deuterium isotope substitution effects[7] upon these ^{13}C signals from $CDCl_3$ - CD_3OH (4 : 1) to $CDCl_3$ - CD_3OD (4 : 1) also provided useful information about the signal assignments[6]. The ^{13}C spectrum of **II** in $CDCl_3$-CD_3OH (4 : 1) (Fig. 3) exhibited a signal at δ_C 61.7 due to the Q-11 CH_2 instead of signals at δ_C 65.1 due to the Q-11 CH and 23.2 due to the Q-12 CH_3 in **I**. ^{13}C Signals due to Q-3 CH and Q-10 $-\overset{|}{\underset{|}{C}}-$ were shifted to lower fields and the signal of Q-4 $-\overset{|}{\underset{|}{C}}-$ was shifted to a higher field compared with those of **I**. These shift data are consistent with the effects of methyl substitution at Q-11 CH_2 of **II**. The ^{13}C NMR data for **I** and **II** are listed in Table 1. On the basis of these observations, the structure **II** was assessed for SIM-D_1. The structures of other minor components are under investigation.

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