^{1}H NMR SPECTRAL EVIDENCE FOR THE STRUCTURE AND CONFORMATION OF PEPTIDE ANTIBIOTIC SIOMYCIN-A

Sir:

The chemical structures of the peptide antibiotic siomycin-A (**I**) isolated from *Streptomyces sioyaensis*[1] as well as thiostrepton (**II**) produced by *S. azureus*[2] had earlier been studied by chemical degradations[3,4]. In 1970, much of the structure of **II** was determined by an X-ray crystallographic analysis[5]. In 1976, we proposed the total structure of **II** on the basis of the X-ray analysis[5] and 15-MHz ^{13}C NMR spectroscopy[6], and also the total structure of **I** on the basis of the close relationships, between **I** and **II**, of the degradation products[3,4,7] and ^{13}C NMR spectra[6]. We further confirmed the structure relationship between **I** and **II** by the analysis of the 36.5-MHz ^{15}N NMR spectra[8].

However, the correlations between the structures and ^{1}H NMR spectra of **I** and **II** have not yet been elucidated in detail, which should provide some useful information about their stereostructures and conformations in solution. Recently the structures of similar antibiotics thiopeptins[9] were proposed from studies by 300-MHz ^{1}H and 75-MHz ^{13}C NMR spectroscopy[10]. We report here the detailed analyses of 270-MHz ^{1}H NMR spectra of **I**, **II**, and thiopeptin-B_a (**III**), which was isolated from commercially available thiopeptin mixtures[9], and propose the molecular conformation of these antibiotics in solution.

The ^{1}H signals were assigned by detailed spin-decoupling experiments and by comparisons of chemical shifts in various solvents. The OH and NH signals were revealed by the addition of a small amount of D_2O to $CDCl_3$ solutions. Additions of CD_3OD or CD_3COOD to $CDCl_3$ solutions did not completely exchange some of CONH protons by deuterons (see Fig. 1). All CONH protons were exchanged in pure CD_3COOD. However, **I** rapidly decomposed by the addition of CF_3COOD. The signal assignments with chemical shift data are listed in Table 1 and the coupling constants, J values, in Table 2.

Intramolecular interproton OVERHAUSER effects[11] were measured in a degassed $CDCl_3$ solution of **I** (at 23°C and 60°C), by observing the difference spectra between the selective on-resonance gated-^{1}H-irradiated (with NOE) and off-resonance gated-^{1}H-irradiated (without NOE) modes[12]. In preliminary experiments, about 25% positive NOE enhancement was observed at 100-MHz, between the two geminal $=CH_2$ signals of a dehydroalanine (Deala) residue of **I** in $CDCl_3$ at 30°C, although precise NOE measurements were not possible because of signal overlappings. However, the correlation time was roughly estimated as $6 \sim 8 \times 10^{-10}$ sec by 25-MHz ^{13}C NMR spectroscopy[13]. Accordingly, at 270-MHz, the NOE enhancements of proton resonances of **I** in $CDCl_3$ were much smaller (several % at most) and negative at 23°C, as expected for large globular molecules[14,15]. Therefore, we also measured the NOE

Fig. 1. 270-MHz ^{1}H NMR spectra of siomycin-A (recrystallized from $CHCl_3$–CH_3OH) in $CDCl_3$ (bottom) and siomycin-A (recrystallized from $CDCl_3$–CD_3OD) in $CDCl_3$–D_2O (8: 1) (upper trace).

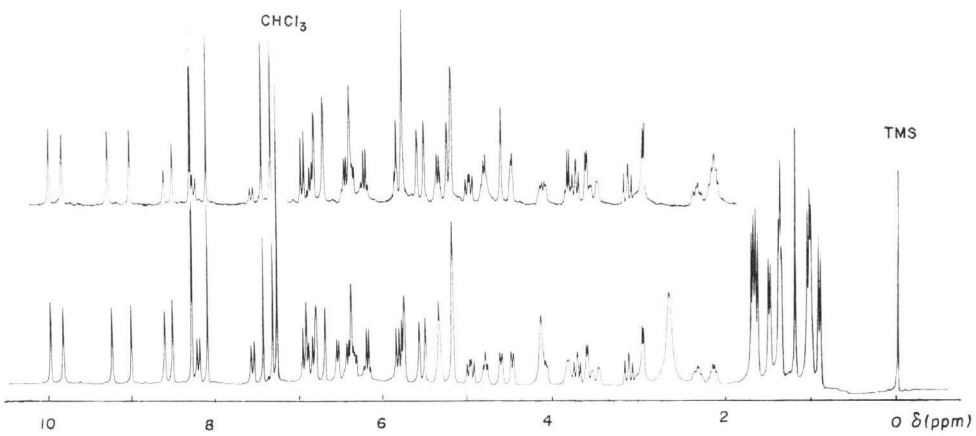


Table 1. ^{1}H Chemical shift data[a].

Assignments		I			II		III	
		$CDCl_3$	$CDCl_3$ / CD_3OD	CD_3CO_2D	$CDCl_3$	CD_3CO_2D	$CDCl_3$	CD_3CO_2D
Ala-1	βCH_3	1.48d	1.47d	1.42d	1.46d	1.44d	1.54d	1.63d
	αCH	4.80dq	4.81dq	4.87q	4.77dq	4.81q	4.96dq	4.98q
	CONH	6.54bd	7.35bd[b]	—	6.47bd	—	6.85bd	—
Deala-1	β=CH(c)	5.18bs	5.42bs	5.62bs	5.21bs	5.61bs	5.26bs	5.64bs
	β=CH(t)	5.76d	5.80d	5.85bs	5.78d	5.94bs	5.82d	5.94bs
	CONH	8.60bs	8.64bs[b]	—	7.80bs	—	7.87bs	—
Deala-2	β=CH(c)	5.18bs	5.17bs	5.30bs	—	—	—	—
	β=CH(t)	6.38bs	6.39bs	6.48bs	—	—	—	—
	CONH	9.23bs	9.37bs[b]	—	—	—	—	—
Ala-2	βCH_3	—	—	—	1.20d	1.19d	1.22d	1.20d
	αCH	—	—	—	3.83dq	4.35q	3.87dq	4.35d
	CONH	—	—	—	7.58bd	—	7.65bd	—
Val	γCH_3	0.91d	0.81d	0.83d	—	—	0.95d	0.78d
		1.04d	1.10d	1.09d	—	—	0.99d	1.11d
	βCH	2.12m	2.37m	2.35m	—	—	1.94m	2.39m
	αCH	2.94d	3.01d	3.22d	—	—	2.90d	3.31d
Ile	γCH_3	—	—	—	0.95t	0.89t	—	—
	γCH_3	—	—	—	0.90d	1.08d	—	—
	γCH_2	—	—	—	~1.3m	~1.6m	—	—
	βCH	—	—	—	~1.7m	~2.1m	—	—
	αCH	—	—	—	2.99d	3.34d	—	—
Q	3=CH	7.31s	7.31s	7.41s	7.31s	7.47s	7.36s	7.47s
	5=CH	6.94d	6.94d	6.98d	6.89d	6.97d	6.92d	6.96d
	6=CH	6.35dd	6.44dd	6.51dd	6.31dd	6.51dd	6.34dd	6.51dd
	7–CH	3.61d	3.60d	3.69d	3.63d	3.72d	3.62d	3.69d
	8–CH	4.61d	4.42s	4.69s	4.68d	4.64s	4.67d	4.60s
	11–CH	5.34bq	5.34q	5.47q	5.33bq	5.47q	5.37bq	5.47q
	11–CH_3	1.37d	1.40d	1.42d	1.37d	1.44d	1.37d	1.43d
	8–OH	6.91d	—	—	6.89d	—	6.85d	—
Thr-2	γCH_3	1.68d	1.69d	1.68d	1.74d	1.69d	1.77d	1.67d
	βCH	6.43q	6.37q	6.51q	6.39q	6.51q	6.56q	6.60q
	αCH	5.83d	5.80d	5.94s	5.83d	5.94bs	6.81d	6.77s
	CONH	8.19bd	8.73bd[b]	—	8.32bd	7.96bd[b]	9.80bd	9.63bd[b]
Thstn	γCH_3	1.35d	1.32d	1.31d	1.34d	1.42d	1.37d	1.41d
	γCH	3.83bq	3.82q	3.98q	3.82bq	4.00q	3.81bq	3.99q
	βCH_3	1.19s	1.18s	1.19s	1.19s	1.26s	1.21s	1.24s
	αCH	5.77d	5.78d	5.84s	5.76d	5.85s	5.77d	5.89s
	CONH	7.55bd	7.58bd	—	7.57bd	7.74bd[b]	7.56bd	8.10bd[b]
	Thz-4 =CH	8.27s	8.31s	8.55s	8.28s	8.56s	8.56s[e]	8.62s[e]
(+)Cys	βCH	3.12dd	3.18dd	3.25dd	3.11dd	3.25dd	3.11dd	3.24dd
	$\beta' CH$	3.72dd	3.66dd	3.71dd	3.70dd	3.72dd	3.71dd	3.71dd
	αCH	4.96dd	5.00dd	5.17dd	4.95dd	5.19dd	4.94dd	5.15dd
Debut	γCH_3	1.62d	1.64d	1.65d	1.63d	1.64d	1.62d	1.65d
	βCH	6.19q	6.23q	6.30q	6.19q	6.30q	6.20q	6.28q
	CONH	8.51bs	8.55bs	—	8.50bs	—	8.48bs	—
Thr-1	γCH_3	1.01d[e]	0.86d	1.04d	1.00d[e]	1.06d[e]	0.97d[e]	0.96d
	βCH	~1.25m	1.57dq	~1.60m	~1.05m	~1.30m	~1.10m	~1.30m
	αCH	4.48bd	4.43dd	4.58d	4.45bd	4.64d	4.44bd	4.58d
	CONH	6.83bd	7.09bd	—	6.84bd	7.17bd[b]	6.87bd	—
ThstA[d]	P–2–CH	—	—	—	—	—	4.15dd	4.35m
	P–3a–CH	2.97dddd	2.96dddd	[h]	2.96m	[h]	2.13m[i]	2.57m[j]
	P–3e–CH	3.51dddd	3.51dddd	[h]	3.48m	[h]	2.13m[i]	2.57m[j]
	P–4a–CH	2.30ddd	2.33ddd	2.53d	2.28ddd	2.54d	1.48m[i]	[j]
	P–4e–CH	4.11ddd	4.10ddd	4.15d	4.09ddd	4.16d	2.34m[i]	2.67m[j]
	P–6a–CH	5.18bs	5.32bs	5.49s	5.14bs	5.49s	4.46bs	5.28bs

(to be continued)

Table 1. (continued)

Assignments		I			II		III	
		$CDCl_3$	$\begin{cases}CDCl_3\\CD_3OD\end{cases}$	CD_3CO_2D	$CDCl_3$	CD_3CO_2D	$CDCl_3$	CD_3CO_2D
	CONH	9.81bs	9.81bs	—	9.83bs	—	9.76bs[g]	—
	Thz-1 =CH	8.26s	8.30s	8.52s	8.27s	8.53s	8.18s[e]	8.51s[e]
	Thz-2 =CH	8.08s	8.17s	8.43s	8.11s	8.42s	8.12s	8.43s
	Thz-3 =CH	7.43s	7.57s	7.69s	7.44s	7.68s	7.40s	7.70bs
Deala-S-1	β=CH(c)	5.58bs	5.63d[f]	5.74bs	5.57bs	5.72bs	5.57bs	5.70bs
	β=CH(t)	6.80d	6.71d	6.75bs	6.79bs	6.76bs	6.74bs	6.74bs[k]
	CONH	9.97bs	9.98bs[b]	—	9.96bs	—	9.89bs[g]	—
Deala-S-2	β=CH(c)	5.51bs	5.71d[f]	5.83bs	5.48bs	5.84bs	6.13bs	6.12bs
	β=CH(t)	6.69d	6.53d	6.50bs	6.69bs	6.52bs	6.74bs	6.70bs[k]
	CONH	8.99bs	9.14bs[b]	—	8.99bs	—	8.69bs	—

[a] The samples were dissolved in $CDCl_3$, $CDCl_3$–CD_3OD (4: 1), and CD_3COOD (*ca.* 10 mM) [not soluble in water or CD_3OD]. The 270-MHz ^1H NMR spectra of sample solutions at 23°C were measured by a Bruker WH-270 spectrometer equipped with a Nicolet 1180 computer system. The experimental conditions for the pulse FT measurements were: spectral width, 3,600 Hz; number of data points, 16K; flip angle, 90°; acquisition time, 2.26 sec; number of transients, 64~256. Chemical shifts (δ) were measured from the internal standard of tetramethylsilane. Data on **II** and **III** in $CDCl_3$–CD_3OD (4: 1) are not described here.

Abbreviations are as follows: Deala, dehydroalanine; Debut, dehydrobutyrine; P, piperidine ring; Q, quinaldic acid precursor[6]; Thstn, thiostreptine residue[6]; ThstA, thiostreptonic acid unit[6]; Thz, thiazole ring: for signal multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad signal.

[b] Signal intensities are reduced owing to partial deuterium exchanges.

[c] Second-order patterns.

[d] Abbreviations a and e are axial and equatorial, respectively.

[e~g] Assignments may be interchanged.

[h] Not observed because of the deuterium exchanges through the imine-enamine tautomerism[9].

[i~k] Not assigned.

Table 2. ^1H, ^1H Coupling constants (J in Hz) for **I** in $CDCl_3$*.

Ala-1	(αCH, CONH)=9.3
Val	(αCH, βCH)=4.0
Q	(5=CH, 6=CH)=9.7; (6=CH, 7-CH)=5.8; (7-CH, 8-CH)=1.8; (8-CH, 8-OH)=7.0
Thr-2	(αCH, βCH)<0.5; (αCH, CONH)=9.3
Thstn	(αCH, CONH)=10.2
(+)-Cys	(αCH, βCH)=11.5; (αCH, β'CH)=9.3; (βCH, β'CH)=−12.8
Thr-1	(αCH, βCH)=1.5; (αCH, CONH)=10.7
ThstA-P	(3a-CH, 3e-CH)=−19.0; (4a-CH, 4e-CH)=−12.5; (3a-CH, 4a-CH)=12.5; (3a-CH, 4e-CH)=6.0; (3e-CH, 4a-CH)=6.0; (3e-CH, 4e-CH)=1.5; |(3a-CH, 6a-CH)|≈1.5; |(3e-CH, 6a-CH)|≈0.5
Deala-S-1	[β=CH(c), β=CH(t)]=−2.2
Deala-S-2	[β=CH(c), β=CH(t)]=−2.5

* About ±0.5 Hz. All $^3J(CH_3, CH)$ values were about 6.6~7.2 Hz.

difference spectra of **I** in $CDCl_3$ at 60°C and, in fact, obtained all NOE's as positive enhancements at 270-MHz (25 and 15% NOE between the geminal $=CH_2$ of the Deala-S-1 residue), as shown in Table 3. These NOE observations were useful for establishing the signal assignments (Table 1) and further for elucidating the stereostructures as described below.

For investigating the correlations between the stereostructures and ^1H spectra, we constructed molecular models (CPK and Fieser models) on the basis of the stereographic drawing of **II** (Fig.

Table 3. Positive NOE enhancements observed for **I** in $CDCl_3$ at 60°C*.

^1H signal				NOE	^1H signal				NOE
Irradiated		Observed		(%)	Irradiated		Observed		(%)
Ala-1	αCH	Thr-2	αCH	5	Debut	CONH	Thr-1	αCH	slight
	αCH	ThstA	CONH	15	Thr-1	αCH	Debut	CONH	5
Deala-1	β=CH(c)	Q	3=CH	10		βCH	Thr-2	CONH	10
	β=CH(c)	ThstA	Thz-2 =CH	10		γCH_3	Debut	CONH	slight
	CONH	Deala-2	β=CH(c)	5		γCH_3	Thr-1	CONH	slight
Deala-2	β=CH(c)	Deala-1	CONH	10		CONH	Thr-1	βCH	5
Q	5=CH	Q	11-CH	5		CONH	ThstA	Thz-3 =CH	5
	7-CH	Val	αCH	5	ThstA	P-4a-CH	ThstA	P-6a-CH	15
	8-CH		αCH	10		P-6a-CH		Thz-3 =CH	10
	11-CH	Q	3=CH	15		CONH	Ala-1	αCH	5
	11-CH		5=CH	15		Thz-3 =CH	ThstA	P-6a-CH	slight
	11-CH	ThstA	Thz-2 =CH	15	Deala-S-2	β=CH(c)	Deala-S-1	CONH	10
	$11-CH_3$	Q	3=CH	5		CONH		β=CH(c)	5

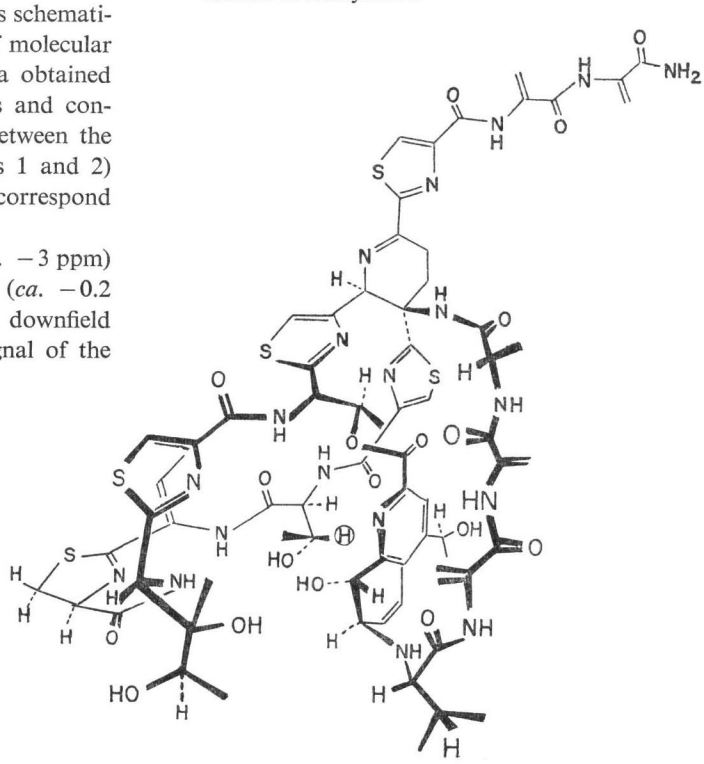
* Positive NOE's were observed between all mutually geminal and all mutually vicinal proton signals except those between mutually vicinal αCH and CONH signals, and are not described here.

The experimental conditions for NOE measurements were: ^1H-irradiation time, 1 sec; acquisition time, 0.56 sec; total number of transients, 256.

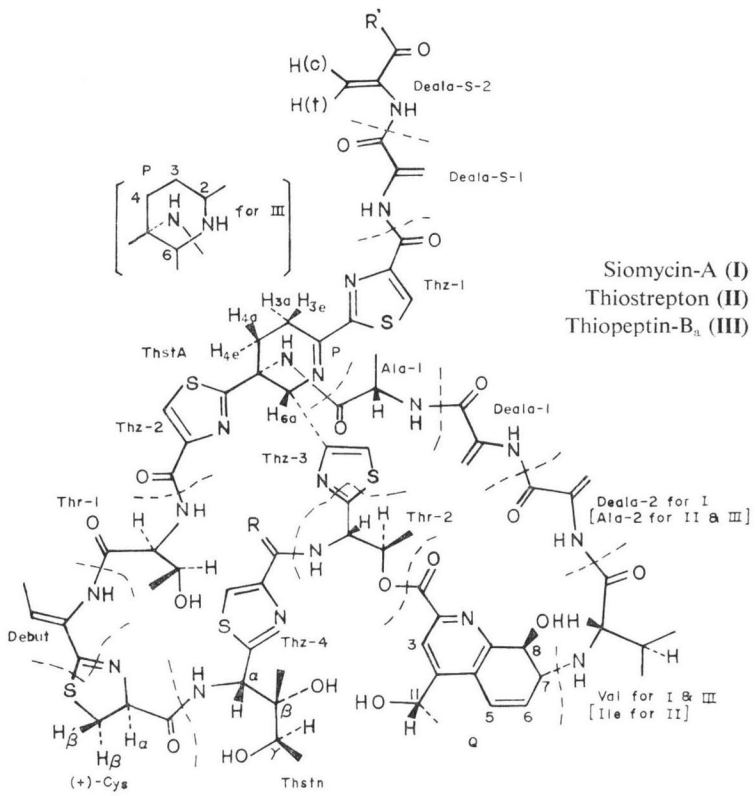
4 of Ref. 5), the dependence of $^3J_{H,H}$ (Table 2) upon dihedral angles[16], and NOE enhancements (Table 3). The molecular model of **I** is schematically shown in Fig. 2. Inspections of molecular models show that the ^1H NMR data obtained are all consistent with the structures and conformations. Detailed comparisons between the NMR data on **I**, **II**, and **III** (Tables 1 and 2) indicate that their spectral differences correspond to their structural ones.

The extraordinary upfield shift (*ca.* −3 ppm) of the βCH signal, the upfield shift (*ca.* −0.2 ppm) of the γCH_3 signal and the downfield shift (*ca.* +0.4 ppm) of the αCH signal of the Thr-1 residue from the ordinary positions[17] are satisfactorily explained by the ring-current anisotropy effects of the pyridine ring of the quinaldic-acid precursor[6] (Q) unit; the βCH proton is situated directly above the plane of the pyridine ring (see the circled H in Fig. 2). The pyridine ring also exerts the ring-current effects upon the β=CH signals of the Deala-1 and Deala-2 residues; these β=CH signals appear at appreciably higher fields than do those of the Deala-S-1 and Deala-

Fig. 2. A schematic view of the molecular conformation of siomycin-A.



Scheme 1.



	R	R'
Siomycin-A (**I**)	O	NH_2
Thiostrepton (**II**)	O	NH_2
Thiopeptin-B_a (**III**)	S	OH

S-2 residues in the side chain. Some downfield shifts of the Thr-2 signals are also due to the ring-current effects of the pyridine and the thiazole-3 (Thz-3) ring.

In conclusion, the 270-MHz ^{1}H NMR spectra of **I** and **II** were found to be consistent with the total structures proposed previously[6,8]. Furthermore, the conformations of the cyclic parts of these antibiotics in solution were found to be similar to the conformation of **II** in crystal[5].

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