STRUCTURES OF SIOMYCIN-B AND -C AND THIOSTREPTON-B DETERMINED BY NMR SPECTROSCOPY AND CARBON-13 SIGNAL ASSIGNMENTS OF SIOMYCINS, THIOSTREPTONS, AND THIOPEPTIN-B_a

Sir:

For several years, we have been studying the total chemical structures of thiostrepton (TST), a sulfur-containing peptide antibiotic isolated from *Streptomyces azureus*[1] and siomycin-A (SIM-A) and -D_1 (SIM-D_1), the major antibiotic and a minor antibiotic produced by *S. sioyaensis*, respectively[2], using ^1H, ^{13}C, and ^{15}N NMR spectroscopies[3~6]; much of the TST structure was revealed by X-ray crystallographic analysis[7].

The isolation and physicochemical properties of siomycin-B (SIM-B) and -C (SIM-C), minor components of the SIM complex, have also been reported but without elucidation of their structures[8].

Fig. 1. ^{13}C FT NMR spectra of (a) SIM-A (**I**), (b) SIM-B (**II**), and (c) SIM-C (**III**), and (d) ^{13}C PRFT NMR spectra of SIM-A (**I**) in $CDCl_3$-CD_3OD (4: 1) at 25 MHz.

FT measurement conditions: see footnote *a* of Table 1. ^{13}C PRFT measurement conditions: spectral width, 5000 Hz; pulse width, 11 μs (90°); pulse interval, 0.1 s; repetition time, 3 s; number of data points, 4K; number of transients, 17K.

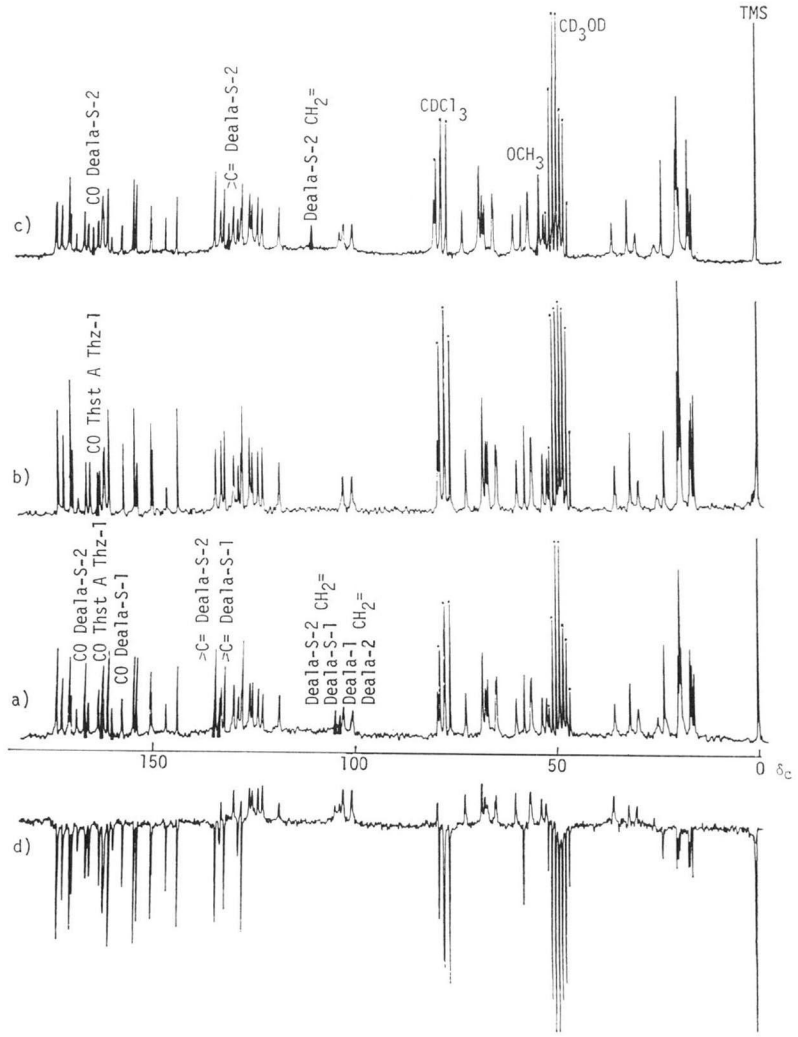
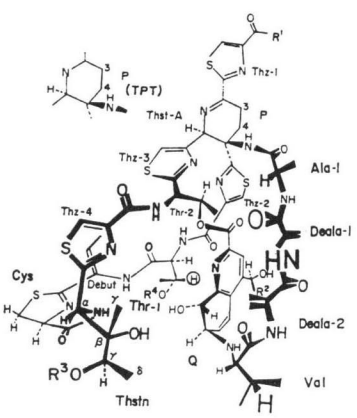
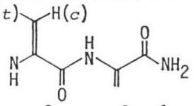


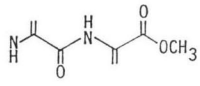
Fig. 2. Chemical structures of SIM-A (I) and related compounds.

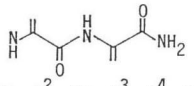


Deala-S-1 Deala-S-2 H(t) H(c)

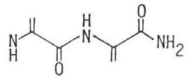
Siomycin-A (I) R^1 =  $R^2 = CH_3$ $R^3 = R^4 = H$

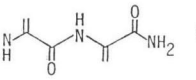
Siomycin-B (II) $R^1 = NH_2$ $R^2 = CH_3$ $R^3 = R^4 = H$

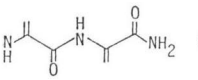
Siomycin-C (III) R^1 =  $R^2 = CH_3$ $R^3 = R^4 = H$

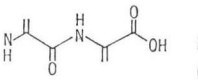
Thiostrepton (IV) R^1 =  $R^2 = CH_3$ $R^3 = R^4 = H$
Val-Deala → Ile-Ala (Ala-2)

Thiostrepton-B (V) $R^1 = NH_2$ $R^2 = CH_3$ $R^3 = R^4 = H$
Val-Deala → Ile-Ala (Ala-2)

Siomycin-D_1 R^1 =  $R^2 = R^3 = R^4 = H$

Siomycin-A monoacetate R^1 =  $R^2 = CH_3$ $R^3 = COCH_3$ $R^4 = H$

Siomycin-A diacetate R^1 =  $R^2 = CH_3$ $R^3 = R^4 = COCH_3$

Thiopeptin-B_a R^1 =  $R^2 = CH_3$ $R^3 = R^4 = H$
CO → CS (Thz-4)
Val-Deala → Val-Ala (Ala-2)

In this communication, we wish to report the chemical structures of SIM-B and -C elucidated by 270-MHz ^1H and 25-MHz ^{13}C NMR spectroscopy, together with the structure of thiostrepton-B (TST-B), which was newly isolated from the culture broth of *S. azureus* and might be an artifact from TST. We also report here the ^{13}C signal assignments of TST and SIM as well as thiopeptin-B_a (TPT)[3] since we can now compare the ^{13}C signals of several derivatives.

In a previous paper[3], we reported almost all the ^1H NMR signal assignments for SIM-A, TST, and TPT at 270 MHz in $CDCl_3$, $CDCl_3$-CD_3OD (4: 1), and CD_3COOD. In the 270-MHz ^1H spectra of SIM-B in these solvents, all signals arising from the Deala-S-1 and -S-2 residues disappeared completely, and some signals due to protons spatially proximate to the side chain were shifted: $\delta_H(CDCl_3)$ Ala βCH_3 1.47, CONH 6.56, ThstA P-3a 2.89, and P-3e 3.35. The other signals remained essentially unchanged from those of SIM-A. The 25-MHz ^{13}C spectrum in $CDCl_3$-CD_3OD lacked six ^{13}C signals including those of two $=CH_2$ at δ_C 104.0 and 105.1 in the Deala-S-1 and -S-2 residues in comparison with that of SIM-A; further, one signal at δ_C 162.7 was shifted downfield by +0.9 ppm, whereas the other signals remained unchanged from those of SIM-A (Fig. 1). From these observations, the structure of SIM-B was determined to be **II**.

The ^1H spectra of SIM-C in the solvents mentioned above showed a distinct OMe singlet at $\delta_H(CDCl_3)$ 3.92 and considerable shifts of the Deala-S-1 and -S-2 residue signals compared with those of SIM-A: $\delta_H(CDCl_3)$ Deala-S-1 $\beta=CH_2$(t) 6.74, $\beta=CH_2$(c) 5.53, Deala-S-2 $\beta=CH_2$(t) 6.06, $\beta=CH_2$(c) 5.23, and CONH 8.58. The other signals remained essentially unchanged from those of SIM-A. In the ^{13}C spectrum, the OMe signal appeared at δ_C 53.4 and signals of the Deala-S-2 residue were shifted considerably, whereas the other signals remained unchanged (Fig. 1). From these findings, the structure of SIM-C was assessed as **III**.

In a similar manner, the structure of TST-B was determined easily by comparisons of ^1H and

Table 1. ^{13}C Chemical shift data.[a)]

Carbons		SIM-A	SIM-B	SIM-C	SIM-D_1	SIM-A-Ac	SIM-A-Ac_2	TST	TST-B	TPT	Assignment Bases
Ala-1	αNCH	52.6	52.5	52.5	52.7	52.6	52.4	52.5	52.5	52.6	d, C, D
	βCH_3	19.6[b)]	19.6[b)]	19.6[b)]	19.6[b)]	19.6[b)]	19.5[b)]	19.5[b)]	19.4[b)]	19.5[b)]	q
	CONH	164.0	163.8	163.9	163.7	163.7	163.7	163.7	164.0	163.4	s, D
Deala-1	α=C	132.9	132.6	132.9	132.9	132.7	132.7	133.1	132.9	132.8	s, D, R-2
	$\beta=CH_2$	103.0	103.0	103.1	103.0	102.9	102.5	103.0	103.4	103.1	t, R-2
	CONH	162.5	162.2	162.5	162.5	162.3	162.0	162.3	162.2	162.1	s, D
Deala-2	α=C	134.9[c)]	134.9	135.0	134.9[c)]	134.9[c)]	134.9[c)]	—	—	—	s, C, D
	$\beta=CH_2$	100.9	100.6	100.9	100.6	100.6	100.2	—	—	—	t, C, R-2
	CONH	161.3	161.2	161.5	161.6	161.2	161.3	—	—	—	s, C, D
Ala-2	αNCH	—	—	—	—	—	—	49.9	50.0	49.9	d, C
	βCH_3	—	—	—	—	—	—	18.3	18.9	19.2	q, C
	CONH	—	—	—	—	—	—	169.3	169.5	169.4	s, C, D
Val	αNCH	68.3	68.4	68.5	68.7	68.5	68.6	—	—	68.3	d, C, J
	βCH	31.8	31.7	31.8	31.8	31.8	31.7	—	—	31.7	d, C
	γCH_3	17.1	16.9	17.0	16.9	16.9	16.9	—	—	17.0	q, C
	$\gamma' CH_3$	19.2[b)]	19.2[b)]	19.2[b)]	19.2[b)]	19.2[b)]	19.1[b)]	—	—	19.2[b)]	q, C
	CONH	174.0	173.7	174.1	173.7	173.9	173.1	—	—	173.5	s, C, D
Ile	αCH	—	—	—	—	—	—	66.6	66.1	—	d, C
	βCH	—	—	—	—	—	—	39.2	39.1	—	d, C
	γCH_3	—	—	—	—	—	—	15.9	16.1	—	q, C
	γCH_2	—	—	—	—	—	—	25.3	25.4	—	t, C
	δCH_3	—	—	—	—	—	—	11.6	11.8	—	q, C
	CONH	—	—	—	—	—	—	173.9	173.9	—	s, C
Q	2=C	144.4[d)]	144.2[d)]	144.4[d)]	144.2[d)]	144.2[d)]	143.9[d)]	144.1[d)]	144.1[d)]	144.0[d)]	s, A
	3=CH	123.0	123.0	123.3	125.1	123.1	122.6	122.9	122.9	122.9	d, A, J
	4=C	153.9	154.2	154.5	148.7	154.3	154.3	153.9	154.1	153.8	s
	5=CH	124.3	124.2	124.4	124.8	124.1	124.6	123.7	123.7	123.8	d, J
	6=CH	130.2	130.0	130.3	130.4	130.1	129.0	130.5	130.6	130.5	d, A, J
	7NCH	60.1	59.9	60.1	60.4	60.0	60.3	59.6	59.6	59.5	d
	8OCH	68.0	67.6	67.8	67.9	67.8	67.9	67.8	68.1	66.8	d
	9=C	155.2[d)]	155.0[d)]	155.3[d)]	155.4[d)]	155.1[d)]	155.6[d)]	155.2[d)]	155.2[d)]	155.2[d)]	s, A
	10=C	128.3	128.0	128.3	129.7	128.1	128.8	127.7	127.8	127.7	s, A
	11OCH	65.0	64.9	65.0	61.7	64.9	64.9	64.9	64.7	65.0[1)]	d, C
	$12CH_3$	23.0	23.1	23.1	—	23.2	23.3	23.0	23.1	23.1	q, C
	COO	170.6[e)]	170.3[e)]	170.6[e)]	170.7[e)]	170.7[e)]	170.6[e)]	170.4[e)]	170.3[e)]	170.6[e)]	s
Thr-2	αNCH	56.2	56.2	56.2	56.3	56.2	56.2	56.2	56.2	61.9	d, C, D, J
	βOCH	72.6	72.5	72.7	72.7	72.5	72.4	72.6	72.6	72.7	d, J
	γCH_3	19.5[b)]	19.4[b)]	19.5[b)]	19.6[b)]	19.6[b)]	19.4[b)]	19.3[b)]	19.4[b)]	19.3[b)]	q, C

Table 1. (Continued)

Carbons		SIM-A	SIM-B	SIM-C	SIM-D_1	SIM-A-Ac	SIM-A-Ac_2	TST	TST-B	TPT	Assignment Bases
Thstn	αNCH	53.7	53.7	53.7	53.8	52.6	53.0	53.7	53.6	54.0	d, A, D
	βOC	77.9	77.8	77.9	77.9	76.6	76.2	77.7	77.8	77.5	s, A, D
	γOCH	68.6	68.4	68.6	69.0	70.5	70.4	68.5	68.5	68.6	d, A, J
	γCH_3	18.8	19.1	19.0	19.2	20.2	20.0	19.0	19.3	19.0	q, A
	δCH_3	16.5	16.5	16.5	16.6	14.3	14.1	16.5	16.5	16.5	q, A
Thz-4	2SC=N	167.3	167.0	167.4	167.4	167.3	167.4	167.1	167.0	168.0	s
	4NC=	150.8	150.7	150.9	150.8	150.6	150.8	150.7	150.7	149.5	s
	5SCH=	126.0[f]	126.0[f]	126.2[f]	126.0[f]	126.0[f]	126.0[f]	125.7[f]	125.9[f]	124.7[f]	d, J
	CONH	162.7	162.4	162.7	162.5	162.5	162.6	162.6	162.3	191.1	s, D
Cys	αNCH	79.7	79.5	79.7	79.7	79.7	79.8	79.7	79.5	79.4	d, R-2
	βSCH_2	35.4	35.4	35.4	35.4	34.6	34.8	35.3	35.3	35.3	t, A, R-2
	CONH	172.7	172.4	172.7	172.4	170.4	171.0[h]	172.6	172.4	172.3	s, A, D
Debut	α=C	129.1	129.1	129.3	129.4	129.1	128.9	129.2	129.2	129.1	s
	β=CH	133.3	133.0	133.3	133.1	133.2	133.6	133.1	133.0	133.2	d, A, J
	γCH_3	15.7	15.7	15.7	15.7	15.6	15.5	15.6	15.7	15.6	q, R-1
	SC=N	171.0[e]	170.7[e]	171.0[e]	170.9[e]	171.2[e]	171.0[e]	170.8[e]	170.8[e]	170.9[e]	s
Thr-1	αNCH	56.4	56.5	56.5	56.6	56.5	54.6	56.4	56.4	56.4	d, A, C, R-1
	βOCH	67.1	67.1	67.2	67.3	67.0	69.0	67.1	67.1	66.8	d, A, C, J, R-1
	γCH_3	19.3	19.2	19.5	19.4	19.4	16.4	19.3	19.3	19.3	q, A, C
	CONH	166.3	166.0	166.3	166.2	166.2	165.1	166.2	166.1	166.1	s, A, D
ThstA	P2C=N	163.0	162.5	163.0	162.9	162.8	162.8	162.8	162.6	58.8[j]	s, C, D
	$3CH_2$	25.3	25.4	25.4	25.5	25.3	25.5	25.0	25.1	34.0	t, C
	$4CH_2$	29.6	29.7	29.7	30.3	29.7	30.2	29.7	29.9	29.2	t, C
	5NC	58.0	58.1	58.1	58.5	58.1	58.4	58.1	58.1	59.5	s, C, D
	6CH	65.0	64.9	65.0	65.0	65.0	65.2	64.9	65.0	62.6	d, C
Thz-1	2SC=N	168.9	168.6	168.7	169.0	168.9	169.0	169.0	168.8	168.8	s, D
	4NC=	157.7[g]	157.6[g]	158.0[g]	157.8[g]	157.8[g]	157.9[g]	157.8[g]	157.7[g]	156.7[g]	s
	5SCH=	125.6[f]	125.4[f]	125.7[f]	125.4[f]	125.5[f]	125.9[f]	125.4[f]	125.4[f]	125.4[f]	d, J
	CONH	162.7	163.6	162.7	162.5	162.5	162.6	162.6	163.5	162.1	s, C, D
Thz-2	2SC=N	171.0	170.7	171.0	170.8	171.0	171.0	170.8	170.8	173.1	s
	4NC=	147.3[g]	147.0[g]	147.3[g]	147.7[g]	147.1[g]	146.7[g]	147.2[g]	147.0[g]	146.4[g]	s, A
	5SCH=	128.3	128.1	128.3	128.1	128.1	128.0	128.4	128.1	128.2	d, J
	CONH	161.6[g]	161.4[g]	161.7[g]	161.7[g]	161.4[g]	161.8[g]	161.5[g]	161.4[g]	161.4[g]	s, D
Thz-3	2SC=N	174.3	173.8	174.1	174.0	173.9	174.0	174.1	174.1	174.1	s
	4NC=	150.8[g]	150.4[g]	150.9[g]	150.8[g]	150.6[g]	150.7[g]	150.6[g]	150.4[g]	155.6[g]	s
	5SCH=	118.9	118.9	119.1	119.4	118.8	119.1	118.8	118.8	118.8	d, J
Deala-S-1	α=C	135.1[c]	—	135.0	135.1[c]	135.0[c]	135.0[c]	135.0	—	135.0	s, C, D
	$\beta=CH_2$	104.0	—	104.1	104.0	103.9	103.9	104.0	—	103.5	t, C, R-2
	CONH	160.3	—	160.0	160.2	160.1	160.2	160.3	—	160.2	s, C, D

Table 1. (Continued)

Carbons		SIM-A	SIM-B	SIM-C	SIM-D_1	SIM-A-Ac	SIM-$A-Ac_2$	TST	TST-B	TPT	Assignment Bases
Deala-S-2	α=C	133.8	—	131.4	134.0	133.9	134.2	133.9	—	135.3	s, C, D
	β=CH_2	105.1	—	110.8	104.7	104.9	104.8	105.0	—	107.1	t, C, R-1
	CO	166.9	—	165.0	166.7	166.7	166.7	166.8	—	166.4	s, C, D
	OCH_3	—	—	53.4	—	—	—	—	—	—	q, C
Acetyl	CH_3	—	—	—	—	21.3	21.0	—	—	—	q, C
	CH_3	—	—	—	—	—	21.4	—	—	—	q, C
	CO	—	—	—	—	169.5	169.1	—	—	—	s, C
	CO	—	—	—	—	—	169.8[h]	—	—	—	s, C

a) ^{13}C NMR spectra were recorded on a JEOL FX 100 FT NMR spectrometer at 25.05 MHz in $CDCl_3$-CD_3OD (4 : 1) with TMS as an internal reference (δ_C 0).
FT measurement conditions were: spectral width, 5,000 Hz; pulse width, 5 μs (50°); repetition time, 1 s; number of data points, 8K; number of transients, 4K; 10-mm spherical cell; concentration, 0.085 mmol/ml; 60°C.
Abbreviations are as follows: Deala, dehydroalanine; Debut, dehydrobutyrine; P, piperidine ring; Q, quinaldic acid precursor; Thstn, thiostreptine residue; ThstA, thiostreptonic acid unit; Thz, thiazole ring. For assignment bases: s, singlet; d, doublet; t, triplet; q, quartet; A, acetylation shifts; C, chemical-shift comparisons; D, deuterium substitution isotope shifts; J, *Jr* values in SFORD spectra; R-1, reference 12; R-2, reference 13.

b~h) Assignments may be interchanged in each column.

i) Triplet.

j) Doublet.

^{13}C spectra between TST (**IV**) and TST-B (**V**). TST-B has no side chain, like SIM-B.

We also reinvestigated the ^{13}C signal assignments of SIM-A and TST which were very tentatively examined in a previous paper[4]. The assignments of signals were first done by single-frequency off-resonance decoupling (SFORD) and partially-relaxed FOURIER transform (PRFT) techniques to differentiate protonated and non-protonated carbon signals (see Fig. 1d).

The assignments of CH and CH_2 signals were easy from the chemical-shift comparisons of the compounds examined as well as those of TPT and SIM-D_1[6], and the residual *J*-values in their SFORD spectra, because the ^1H chemical shifts are known[3]. In a similar manner, CH_3 signals were assigned, but some could not be because of mutual signal overlappings.

Non-protonated carbon signals were assigned with the aid of deuterium substitution effects besides the chemical shift comparisons. Deuterium substitution isotope shifts arising from the deuterium exchange of the CONH groups[9] in the $CDCl_3$ - CD_3OD (4: 1) solvent system at various temperatures were observed. All COND and $-\overset{|}{C}ND$ signals were slightly shifted upfield from the corresponding CONH and $-\overset{|}{C}NH$ signals. In particular, the ThstA P-2 (N=C) signal was considerably shifted upfield due to the imine-enamine tautomerism which causes exchange of P-3-CH_2 hydrogens for deuteriums. In fact, the P-3-CH_2 signal gradually disappeared in $CDCl_3$ - CD_3OD (4:1).

Acetylation shifts[10], in the ^{13}C spectra of SIM-A monoacetate and diacetate[11] were useful for assigning the signals due to the Thstn and Thr-1 residues. Moreover, the acetylation caused a conformational change of residues in the vicinity. Thus, the signals of the Cys residue in both acetates and those of some of the Q residue in diacetate were affected.

Table 1 lists the ^{13}C signal assignments of SIM-A, -B, -C, TST, and TPT including SIM-D_1 and SIM-A mono- and diacetates.

Thus, we determined the chemical structures of TST-B, newly isolated from *S. azureus*, and of SIM-B and -C. The ^{13}C signals of SIM, TST, and TPT were reassigned as listed in Table 1.

KAZUO TORI*
KATSUYA TOKURA
YOHKO YOSHIMURA
YOSHIHIRO TERUI
KEI OKABE
HIDEO OTSUKA

Shionogi Research Laboratories,
Shionogi & Co., Ltd.,
Fukushima-ku, Osaka, 553 Japan

KAZUHIRO MATSUSHITA

Application Center,
Scientific Instrument Project,
JEOL Ltd.,
Nakagami, Akishima, Tokyo, 196
Japan

FUYUHIKO INAGAKI
TATSUO MIYAZAWA

Department of Biophysics and
Biochemistry, Faculty of Science,
The University of Tokyo,
Bunkyo-ku, Tokyo, 113 Japan

(Received October 22, 1980)

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