STUDIES ON SIOMYCIN. I

PHYSICOCHEMICAL PROPERTIES OF SIOMYCINS A, B AND C

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Siomycin, a sulfur-containing peptide antibiotic, has been found to consist of one major (A) and two minor components (B and C). Siomycins A, B and C were isolated as single components from crude siomycin preparations and characterized in detail. Physicochemical properties of the three antibiotics are similar. Siomycin B is derived from siomycin A during storage, while siomycin C is a natural product of *Streptomyces sioyaensis*.

Siomycin is a sulfur-containing peptide antibiotic, active against gram-positive bacteria and mycobacteria. This antibiotic has been isolated from cultures of *Streptomyces sioyaensis*, and in a preliminary characterization, its properties were compared with those of thiostrepton.[1~3] Further purification of siomycin preparations has shown that siomycin consists of one major (siomycin A) and two minor (siomycins B and C) compounds. The present paper, therefore, deals in more detail with the physicochemical properties of siomycins A, B and C, although some results might be presented here doubly.

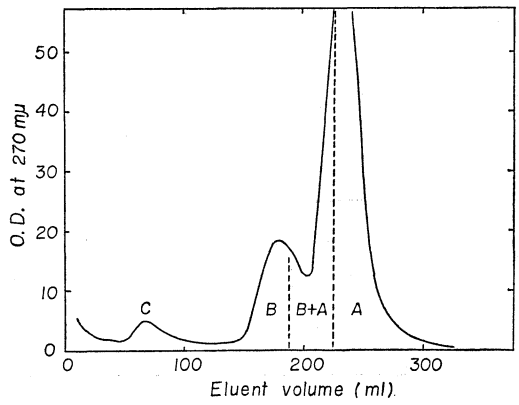
Materials and Methods

Siomycins. Some siomycin preparations were supplied from the pilot plant of our company. Another, consisting of pure siomycin A, was isolated from cultures of *Streptomyces sioyaensis* (unpublished data).

Analytical methods. The molecular weight of siomycin A was measured by the osmotic method. Ultraviolet absorption spectra were measured in methanol, with a Perkin-Elmer type 202 Spectrophotometer. Infrared spectra were taken in chloroform with a Nihon Bunko DS-201B Spectrophotometer. Analyses for amino acid and ninhydrin-positive substances were performed by an automatic amino acid analyzer (Hitachi KLA modified type 2). Optical rotations were measured in dioxane, with a Perkin-Elmer Polarimeter, type 141.

Separation of siomycins A, B and C. A solution of 1 g of crude siomycin preparation was dissolved in 10 ml of a mixture of chloroform and methanol (95 : 5), and applied to a column (18×400 mm) of silica gel G (for thin-layer chromatography use), which was equilibrated with the same solvent. Fractions (5 g) were collected, and the siomycin peaks located by their absorption at 270 mμ. The elution diagram is presented in Fig. 1. Fractions containing each type of siomycin were combined and dried *in vacuo*. Siomycin C (8 mg), siomycin B (28 mg), siomycin A+B (260 mg) and siomycin A (700 mg) were obtained. By rechromatography of the siomycin A+B fraction using the same procedure except that the column size (10×320 mm) was smaller, siomycin B (50 mg), siomycin A (150 mg) and siomycin A+B (60 mg) were also obtained. Siomycins were

Fig. 1. Chromatography of a crude siomycin preparation. Sample, one gram crude siomycin preparation; Column, silica gel column 18×400 mm; Solvent, a mixture of chloroform and methanol (95 : 5); Fractions, 5 g. A, siomycin A; B, siomycin B; and C, siomycin C.



recrystallized from a mixture of chloroform and methanol (1 : 1). The content of each component in usual crude siomycin preparations was approximately 98 % (A), 1.2~2 % (B) and 0.4~1 % (C).

A picture of typical thin-layer chromatogram of these antibiotics is shown in Fig. 2.

Results

Some of the physicochemical properties of siomycins A, B and C are listed in Table 1. Amino acid analysis gave 1,740 as a minimum molecular weight, and the osmotic method gave 1,114. Values of 1,741 and 1,713 were obtained from the chlorine content of mono-chloroacetyl- and di-chloroacetyl

Fig. 2. Thin-layer chromatogram of siomycins A, B and C. Plate, silica gel G; solvent, chloroform and methanol (90 : 10).

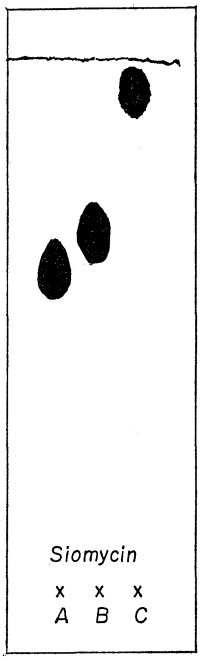


Table 1. Some physicochemical properties of siomycins A, B and C.

	Siomycin A	Siomycin B	Siomycin C
Appearance	colorless crystalline	colorless~slightly yellow crystalline	colorless crystalline
m. p.	darkens at about 200° 255~260°C (dec.)	darkens at about 200°C, 255~260°C (dec.)	darkens at about 200°C, 255~260°C (dec.)
Elementary analysis (%)	C 51.55 H 5.36 N 15.81 S 9.28	C 43.83 H 4.71 N 13.11 S 8.53	C 47.97 H 5.11 N 11.17 S 8.00
Molecular weight with amino acid analysis	1,740	1,879	1,700
Formula	$C_{74}H_{92}O_{19}N_{19}S_5$	$C_{63}H_{80}O_{32}N_{16}S_5$ (tentative)	$C_{69}H_{87}O_{31}N_{14}S_{4-5}$ (tentative)
$[\alpha]_D^{23}$ (c 0.5, dioxane)	−95.3	−102.9	−84.6
Rf value for:			
Mixtures of chloroform and methanol			
(95 : 5) (TLC)*	0.14	0.21	0.42
(90 : 10) (TLC)	0.60	0.67	0.94
A mixture of methanol-acetic acid-water (25 : 3 : 72) (PPC)	0.08~0.10	0.30~0.31	0.00

* Silica gel G.

siomycins[4], respectively. Based upon these molecular weights, the formula $C_{74}H_{92}O_{19}N_{19}S_5$ (molecular weight, 1,712) comes closest to the values of elemental analysis of siomycin A. A final formula for the antibiotic, however, can be expected only after complete structural elucidation. Formulas for the B and C components are tentative,

since there is still a question about their purity.

Siomycins gave a positive DRAGENDORFF's and hydroxamic acid-Fe tests, while they are negative to ninhydrin, ferric chloride and EHRLICH reagents.

The ultraviolet absorption spectra are shown in Fig. 3. Molar extinction coefficients (ε) were calculated by assuming the same molecular weights (1,712) for siomycins A, B and C.

Fig. 3. Ultraviolet absorption spectra of siomycins A, B and C in methanol. Values of ε are expressed on a semi-log scale. A, siomycin A; B, siomycin B; and C, siomycin C.

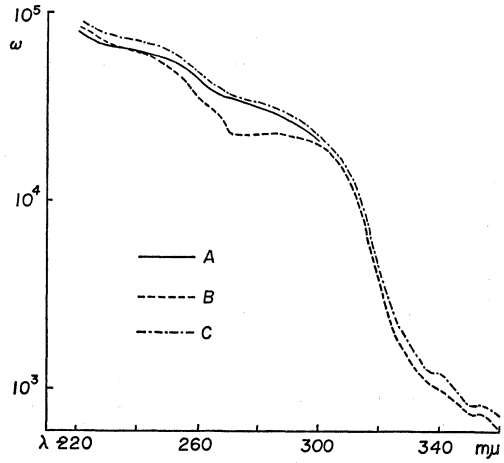


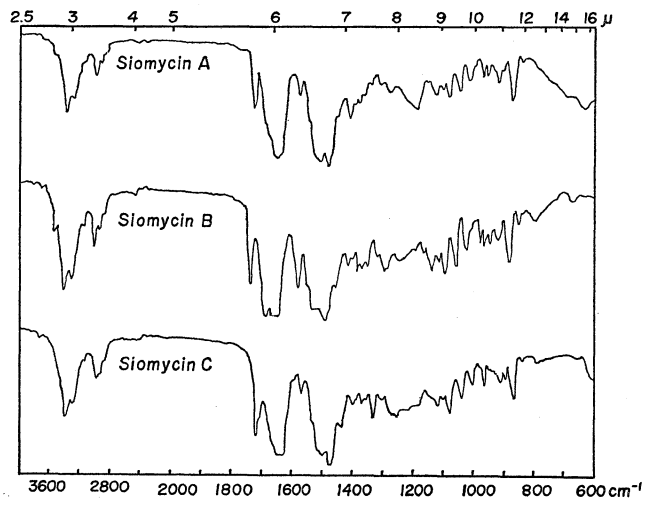
Fig. 4 shows infrared absorption spectra of siomycins A, B and C. In the spectrum of siomycin C a medium intensity band at 1345 cm^{-1} may be attributed to CH_3- of lower alkyl groups. A weak band at 930 cm^{-1} probably due to a vinyl group appeared in the spectra of siomycins A and C but not siomycin B.

Siomycins are soluble in chloroform, dimethylformamide and dioxane, and quite insoluble in water as shown in Table 2. Siomycins are stable in neutral or weakly acidic solution, but not in alkaline solution.

Amino acid analysis of 20-hour acid hydrolysates (5.9 N HCl, 110°C) of siomycins A, B and C are summarized in Table 3. There are no meaningful differences. The four amino acids, threonine, alanine, cysteine and valine occupy only 22 % by weight in siomycin A molecule.

Antimicrobial activities of the siomycins are listed in Table 4. The three siomycins are highly active against Gram-positive bacteria and mycobacteria, with little or no activity against Gram-negative bacteria. The antibacterial spectra of siomycins B and C are almost identical with that of siomycin A.

Fig. 4. Infrared spectra of siomycins A, B and C in chloroform.



Siomycins A and C have been found in all fresh dried mycelia of *S. sioyaensis*, though the C component was always minor one (less than 1 %). On the other hand, siomycin B has never been isolated from the fresh mycelia. It can be prepared only from stored siomycin

Table 2. Solubility of siomycins A, B and C (g/100 ml) at 20°C.

Solvent	Siomycin A	Siomycin B	Siomycin C
Methanol	0.004	0.04	0.04
Acetone	0.07	0.92	>1
Water	0.00	0.00	0.00
dil. NaOH	—	—	—
dil. HCl	—	—	—
Chloroform	+	+	++
Dimethyl-formamide	++	++	++
Dioxane	++	++	++
Ether	—	—	—
Petroleum ether	—	—	—

++, very soluble; +, poorly soluble; —, insoluble.

Table 3. Composition of siomycins A, B and C.

Component	Siomycin A	Siomycin B	Siomycin C
Threonine	0.88	0.92	0.94
Glycine	trace	trace	trace
Alanine	0.98	1.00	1.00
Valine	0.95	0.98	1.03
Cysteine	1.18	1.10	1.11
Thiostreptine	0.58	0.44	0.54
2-Aminomethyl-thiazole-4-carboxylic acid	0.15	0.35	0.12
Ammonia	6.10	4.56	4.95

Data for siomycin A are expressed as molar ratio based on weight.

Data for siomycins B and C are also expressed as molar ratio but assuming alanine =1.00.

Cysteine was determined as cysteic acid after oxidation by performic acid.

Compounds with underlines would be expected as the component.

Table 4. Antimicrobial activity of siomycins A, B and C.

Test organisms	MIC (mcg/ml) of siomycins		
	A	B	C
Shigella dysenteriae	>20	>20	>20
Shigella paradysenteriae, Ohara	>20	>20	>20
Salmonella typhosa	>20	>20	>20
Salmonella paratyphi A	>20	>20	>20
Escherichia coli, Umezawa	>20	>20	>20
Pseudomonas aeruginosa	>20	>20	>20
Klebsiella pneumoniae	>20	>20	>20
Bacillus subtilis PCI-219	0.05	0.1	0.02
Bacillus anthracis	0.2	0.5	0.2
Staphylococcus aureus FDA 209P	0.1	0.1	0.05
Sarcina lutea	0.1	0.1	0.05
Diplococcus pneumoniae, type I	0.005	0.005	0.05
〃 type I-V	0.005	0.005	0.005
〃 type II	0.05	0.02	0.02
〃 type III	0.01	0.005	0.005
Streptococcus hemolyticus, D	0.05	0.05	0.05
〃 HA	0.02	0.02	0.05
Corynebacterium diphtheriae, S	0.05	0.01	0.05
〃 T	0.02	0.02	0.05
Mycobacterium tuberculosis var. *hominis*, H37Rv	1.0	0.5	2.0

Table 5. Differences between siomycin A and thiostrepton.

	Siomycin A	Thiostrepton
Formula	$C_{74}H_{92}O_{19}N_{19}S_5$	$C_{72}H_{83}O_{19}N_{19}S_5$ [13]
Amino acid	valine	isoleucine[13]
Difference	alanine	2 alanines
$[\alpha]_D$ (dioxane)	−95.3	−61.0[1]
Rf-value for PPC	0.12~0.14	0.38~0.40[1]
EHRLICH test	negative	deep violet[13]

preparations or old mycelia of the organism. This indicates that siomycin B would be derived from siomycin A during storage, while siomycins A and C are natural products of this streptomyces.

Discussion

Since no meaningful difference was found in the amino acid compositions of siomycins A, B and C, it was suggested that some structural difference is present in the "ninhydrin negative part" of molecules, such as a quinaldic acid derivative[4] or very labile residues.

Thiostrepton, which is similar to siomycin, has been isolated from *Streptomyces azureus*.[5~8] This antibiotic has been object of structural investigation in some labora-

tories for several years,[9~14] and its physicochemical properties can be compared with those of the siomycins. Some differences are observed as shown in Table 5. A big difference is the amino acid composition. Siomycin contains valine and alanine, while thiostrepton has isoleucine and two alanines.

Studies on the structure and derivatives of siomycin A are now in progress in our laboratory. New structural features and some derivatives of the antibiotics will be reported in this series.

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