WATER-SOLUBLE SIOMYCIN-A DERIVATIVES

PREPARATION, CHEMICAL STRUCTURES AND BIOLOGICAL PROPERTIES OF HALF-ESTERS OF THE PEPTIDE ANTIBIOTIC

KATSUYA TOKURA,* KUNIO HAYASHI, KEI OKABE, KAZUO TORI,
YOHKO YOSHIMURA, MIKAO MAYAMA, SHINZO MATSUURA
and HIDEO OTSUKA

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

(Received for publication March 9, 1981)

The peptide antibiotic siomycin-A was transformed into half-esters with dicarboxylic acids with the intention of making siomycin-A soluble in water. Sodium salts of the half-esters were also prepared. Some of the salts showed antibacterial activities comparable to siomycin-A against Gram-positive bacteria *in vitro* and exhibited better therapeutic effects in infected mice than siomycin-A. The chemical structures of siomycin-A hemiadipate-II and -III were elucidated by comparing their ^{13}C and ^{1}H NMR spectra with those of siomycin-A. Their physicochemical properties are described.

Siomycin, a sulfur-containing peptide antibiotic, has been isolated from a culture broth of *Streptomyces sioyaensis*[1] and has been shown to consist of one major component (siomycin-A) and three minor components (siomycin-B, -C, and -D_1).[2,3] The chemical structure of siomycin-A (**1**) (Fig. 1) has been proposed on the basis of the X-ray analysis[4] of thiostrepton (**2**), isolated from *Streptomyces azureus,* and 15-MHz ^{13}C NMR spectroscopy,[5] and confirmed by 36.5-MHz ^{15}N NMR spectroscopy.[6] Based on detailed analysis of the 270-MHz ^{1}H NMR spectra, the molecular conformations of **1** and **2** in solution have also been proposed.[7] The structures of siomycin-B, -C, and -D_1 were confirmed by analysis of ^{13}C and ^{1}H NMR spectra.[3,8]

Siomycin shows high antibiotic activity *in vitro* against Gram-positive bacteria and little or no activity against Gram-negative bacteria. It exhibits marked activity against experimental pneumococcal infection in mice and relatively low toxicity (LD_{50} for mice: 100 mg/kg). However, its low solubility in water poses a number of serious limitations to its use as a therapeutic agent. Although its solubilization may be possible to some extent by pharmaceutical methods, we tried to find water-soluble derivatives of **1** having the original or enhanced activity.

There are several possible ways to prepare water-soluble derivatives of **1**: 1) salt formation with an acid, 2) introduction of an amino group, 3) formation of a phosphate, 4) introduction of a sulfonic acid group, 5) introduction of a carboxyl group by ring opening of the lactone or by the formation of a half-ester with a dicarboxylic acid. Here we report the preparation of half-esters of **1**, the structures of which are elucidated by ^{13}C and ^{1}H NMR spectroscopy, and their biological activities.

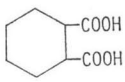
Preparation

Reactions were carried out by warming **1** and acid anhydrides in pyridine. In each case, the acylation reaction gave a similar product which was separated into half-ester-I (assumed to be a mix-

ture of polyacylated products) and two monoacylated products, half-ester-II (the less polar of the two) and half-ester-III, by column chromatography on silica gel or preparative thin-layer chromatography. Half-ester-III could not be isolated in some cases. Siomycin-A hemiadipate-I was further separated into four components (**18**, **19**, **20**, and **21**) to examine the biological activities. Water-soluble sodium salts of the half-esters were prepared by dissolving the half-esters in 0.1% aqueous sodium bicarbonate solution and lyophilizing the solutions.

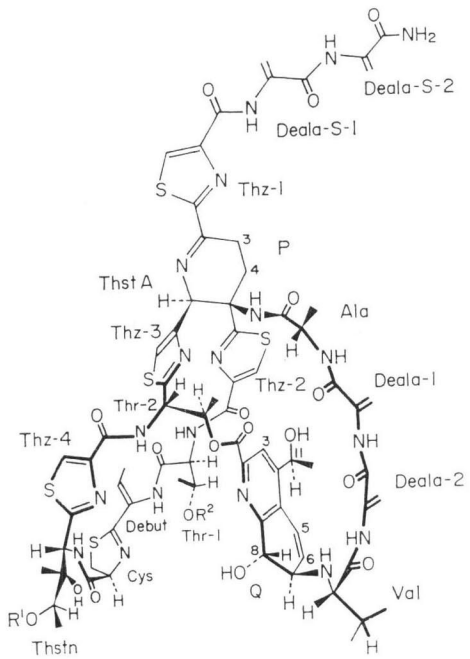
Hemiadipates (**22** and **23**) of **2** were also prepared in a similar manner as above. Compound **1** was acetylated to obtain reference compounds for structure determination and biological tests, and its monoacetate (**3**) and diacetate (**4**) were isolated from the reaction mixture.

Table 1. Esters derived from **1** and **2**.

Compound	Acid	TLC Rf values $CHCl_3$ - CH_3OH, 8 : 2	Formula	Analyses[e]
1		0.43[b]	$C_{71}H_{81}O_{18}N_{19}S_5 \cdot 12H_2O$	C, H, N, S
3	CH_3COOH	0.50[b]	$C_{73}H_{83}O_{19}N_{19}S_5 \cdot 8H_2O$	C, H, N, S
4	CH_3COOH	0.48[b]	$C_{75}H_{85}O_{20}N_{19}S_5 \cdot 8H_2O$	C, H,[f] N
5	$HOOC(CH_2)_2COOH$	0.30	$C_{75}H_{85}O_{21}N_{19}S_5 \cdot 12H_2O$	C, H, N, S
6	$HOOC(CH_2)_3COOH$	0.49	$C_{76}H_{87}O_{21}N_{19}S_5 \cdot 5H_2O$	C, H, N, S
7	$HOOC(CH_2)_4COOH$	0.55	$C_{77}H_{89}O_{21}N_{19}S_5 \cdot 7H_2O$	C, H, N, S
8	$HOOC(CH_2)_4COOH$	0.50	$C_{77}H_{89}O_{21}N_{19}S_5 \cdot 6H_2O$	C, H, N, S
9	$HOOC(CH_2)_5COOH$	0.50	$C_{78}H_{91}O_{21}N_{19}S_5 \cdot 8H_2O$	C, H, N, S
10	$HOOC(CH_2)_5COOH$	0.42	$C_{78}H_{91}O_{21}N_{19}S_5 \cdot 4H_2O$	C, H, N,[g] S
11	$HOOC(CH_2)_6COOH$	0.53	$C_{79}H_{93}O_{21}N_{19}S_5 \cdot 6H_2O$	C, H, N
12	$HOOC(CH_2)_6COOH$	0.49	$C_{79}H_{93}O_{21}N_{19}S_5 \cdot 6H_2O$	C, H, N
13	$HOOC(CH_2)_{10}COOH$	0.58	$C_{83}H_{101}O_{21}N_{19}S_5 \cdot 9H_2O$	C, H, N
14	$HOOCCH=CHCOOH$	0.24	$C_{75}H_{83}O_{21}N_{19}S_5 \cdot 12H_2O$	C, H, N
15	$HOOCC(CH_3)=CHCOOH$	0.37	$C_{76}H_{83}O_{21}N_{19}S_5 \cdot 13H_2O$	C, H, N
16	$HOOCC(CH_3)=CHCOOH$	0.31	$C_{76}H_{85}O_{21}N_{19}S_5 \cdot 11H_2O$	C, H, N
17		0.52	$C_{79}H_{91}O_{21}N_{19}S_5 \cdot 11H_2O$	C, H, N
18	$HOOC(CH_2)_4COOH$	0.28[c]	[d]	[h]
19	$HOOC(CH_2)_4COOH$	0.24[c]	[d]	[h]
20	$HOOC(CH_2)_4COOH$	0.18[c]	[d]	[h]
21	$HOOC(CH_2)_4COOH$	0.10[c]	[d]	[h]
22[a]	$HOOC(CH_2)_4COOH$	0.55	$C_{78}H_{93}O_{21}N_{19}S_5 \cdot 9H_2O$	C, H, N
23[a]	$HOOC(CH_2)_4COOH$	0.50	$C_{78}H_{93}O_{21}N_{19}S_5 \cdot 10H_2O$	C, H, N

[a] Esters of **2**, others are esters of **1**.
[b] $CHCl_3$ - CH_3OH, 9 : 1.
[c] $CHCl_3$ - CH_3OH - H_2O, 87 : 13 : 1.
[d] Not known.
[e] Microanalyses were carried out after the sample had been exposed to air overnight.
[f] H: Calcd. 5.42; found 4.71.
[g] N: Calcd. 14.29; found 13.48.
[h] Not analyzed.

Fig. 1. Chemical structure of siomycin-A (**1**) and related substances.



1 $R^1=R^2=H$
2 $R^1=R^2=H$ Ile-Ala instead of Val-Deala
3 $R^1=Ac$, $R^2=H$
4 $R^1=R^2=Ac$
7 $R^1=Adip$, $R^2=H$
8 $R^1=H$, $R^2=Adip$

Abbreviations: Deala, dehydroalanine; Debut, dehydrobutyrine; P, piperidine ring; Q, quinaldic acid precursor; Thstn, thiostreptine residue; ThstA, thiostreptonic acid unit; Thz, thiazole ring; Ac, acetyl group; Adip, adipic acid residue.

Fig. 2. Ultraviolet absorption spectra of siomycin-A hemiadipate-II (**7**).

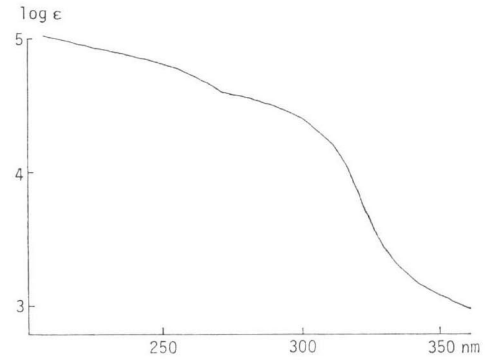


Table 2. Amino acid composition (molar ratios).

	Amm	Thr	Ala	Val
1	5.75	0.84	1.00	0.98
7	6.15	0.99	1.00	0.99

Physicochemical Properties

Table 1 lists the half-esters-II and -III and the acetates prepared. Half-esters-I except hemiadipate-I were omitted because they were mixtures and showed only weak antibacterial activities in the preliminary paper disc assay. Hemiadipate-II (**7**), hemipimelate-II (**9**), and hemisuberate-II (**11**) showed equally strong antibacterial activities as described below. Among these, **7** was considered the best because of the ready availability of adipic acid; the physicochemical properties and chemical structures of **7** and **8** were investigated in detail. Both showed plain curves ascending to 205 nm in the UV absorption spectra (Fig. 2). The IR spectrum of **7** is shown in Fig. 3.

Fig. 3. Infrared spectra of siomycin-A hemiadipate-II (**7**).

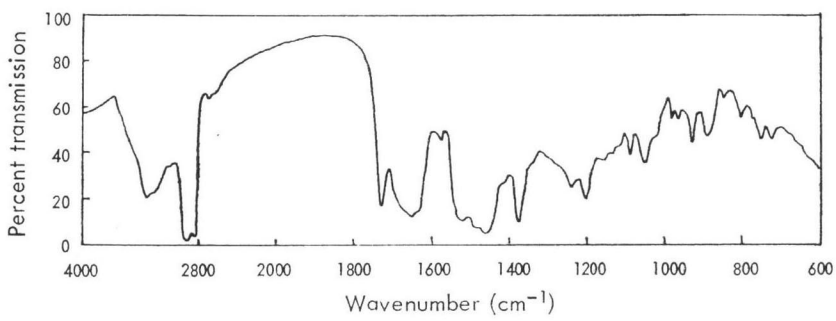


Table 3. ^{13}C Chemical shift data,[a] δ_C.

		1	3	4	7	8
Q	12CH_3	23.2	23.2	23.3	23.1	23.3
	7NCH	60.1	60.0	60.3	59.9	60.2
	11OCH	65.1	64.9	64.9	64.8	65.0
	8OCH	67.8	67.8	67.9	67.7	67.8
	3=CH	123.3	123.1	122.6	123.1	122.6
	5=CH	124.4	124.1	124.6	124.1	124.9
	10=C	128.1	128.1	128.8	128.1	128.9
	6=CH	130.2	130.1	129.0	130.1	128.9
	2=C	144.4[b]	144.2[b]	143.9[b]	144.1[b]	143.8[b]
	4=C	154.4	154.3	154.3	154.3	154.3
	9=C	155.3[b]	155.1[b]	155.6[b]	155.0[b]	155.8[b]
	COO	170.6[c]	170.7[c]	170.6[c]	170.7[c]	170.8[c]
Thstn	δCH_3	16.6	14.3	14.1	14.3	16.6
	γCH_3	19.4	20.2	20.0	20.3	19.2
	αNCH	53.9	52.6	53.0	52.6	53.8
	γOCH	68.7	70.5	70.4	70.3	68.8
	βOC	78.0	76.6	76.2	76.6	77.6
	Thz-4					
	SCH=	126.1[c]	126.0[c]	126.0[c]	126.1[c]	126.0[c]
	NC=	150.9	150.6	150.8	150.6	150.8
	CO	162.6	162.5	162.6	162.4	162.6
	S-C=N	167.3	167.3	167.4	167.3	167.4
Cys	βSCH_2	35.5	34.6	34.8	34.5	35.4
	αNCH	79.7	79.7	79.8	79.6	79.7
	CO	172.5	170.4	171.0[d]	170.3	172.4
Debut	γCH_3	15.7	15.6	15.5	15.6	15.6
	α=C	129.4	129.1	128.9	129.1	128.9
	β=C	133.2	133.2	133.6	133.1	133.9
	S-C=N	170.9[c]	171.2[c]	171.0[c]	171.1[c]	171.1[c]
Thr-1	γCH_3	19.5[c]	19.4[c]	16.4	19.4[c]	16.1
	αNCH	56.7	56.5	54.6	56.4	54.9
	βOCH	67.3	67.0	69.0	67.0	68.9
	CO	166.2	166.2	165.1	166.1	165.2
Adip	βCH_2			169.8[d]	24.4	24.2
					24.7	25.0
	αCH_2				34.1	34.5
					34.3	34.5
	CO				169.4	170.5
					173.1	173.9

[a] Signals which shifted more than 0.4 ppm from the corresponding ones of **1** are underlined. The data on the other residues, the signal shifts of which were within experimental errors, are not represented here (see Ref. 8).

[b~d] Assignments may be interchanged in each column.

[e] Assignments may be interchanged with those for signals not included here (see Ref. 8).

Table 4. ^1H Chemical shift data,[a] δ_H.

		1	3	7	8
Q	3=CH	7.31s	7.31s	7.33s	7.34s
	5=CH	6.94d	6.98d	6.97d	6.88d
	6=CH	6.44dd	6.44dd	6.48dd	6.31dd
	7CH	3.60d	3.60d	3.57d	3.49d
	8CH	4.42s	4.46s	4.39s	4.25s
	11CH	5.34q	5.36q	5.35q	5.46q
	$12CH_3$	1.40d	1.39d	1.40d	1.40d
Thstn	δCH_3	1.32d	1.37d	1.35d	1.30d
	γCH	3.82q	5.07q	5.09q	3.76q
	γCH_3	1.18s	1.29s	1.29s	1.14s
	αCH	5.78d	5.69d	5.68d	5.78d
	NHCO	7.58bd	7.62bd	7.60bd	7.52bd
	Thz-4=CH	8.31s	8.33s	8.32s	8.32s
Cys	βCH	3.18dd	3.22dd	3.24d	3.27dd
	β'CH	3.66dd	3.61dd	3.61dd	3.72dd
	αCH	5.00dd	4.94dd	4.94dd	5.12dd
Debut	γCH_3	1.64d	1.61d	1.61d	1.63d
	βCH	6.23q	6.24q	6.23q	6.30q
	NHCO	8.55bs	8.57bs	8.56bs	8.59bs[b]
Thr-1	γCH_3	0.86d	0.87d	0.83d	0.92d
	βCH	1.57dq	1.50dq	1.55dq	2.80dq
	αCH	4.43dd	4.42dd	4.46dd	4.46dd
	NHCO	7.09bd	7.06d	7.07bd	7.19d
Adip	βCH_2	—	—	1.69	1.58
	αCH_2	—	—	2.37	2.22

[a] Abbreviations for signal multiplicities: s, singlet; d, doublet; q, quartet; m, multiplet; b, broad signal. Signals which shifted more than 0.05 ppm from the corresponding ones of **1** are underlined. The data on the other residue, the signals of which showed only slight shifts, are not represented here (see Ref. 7).

[b] Signal intensities are reduced due to partial deuterium exchange.

There was no meaningful difference between the amino acid compositions of **1** and **7** (Table 2). Compound **1** and its derivatives are hygroscopic and easily hold water and other solvents inside the globular molecules. Part of the water once held in the molecules is easily lost while the other part is tightly retained. Thus microanalyses of these compounds showed variable water content (Table 1). The sodium salt of **7** was much more soluble in water (5%) than **1** (0.00015%).

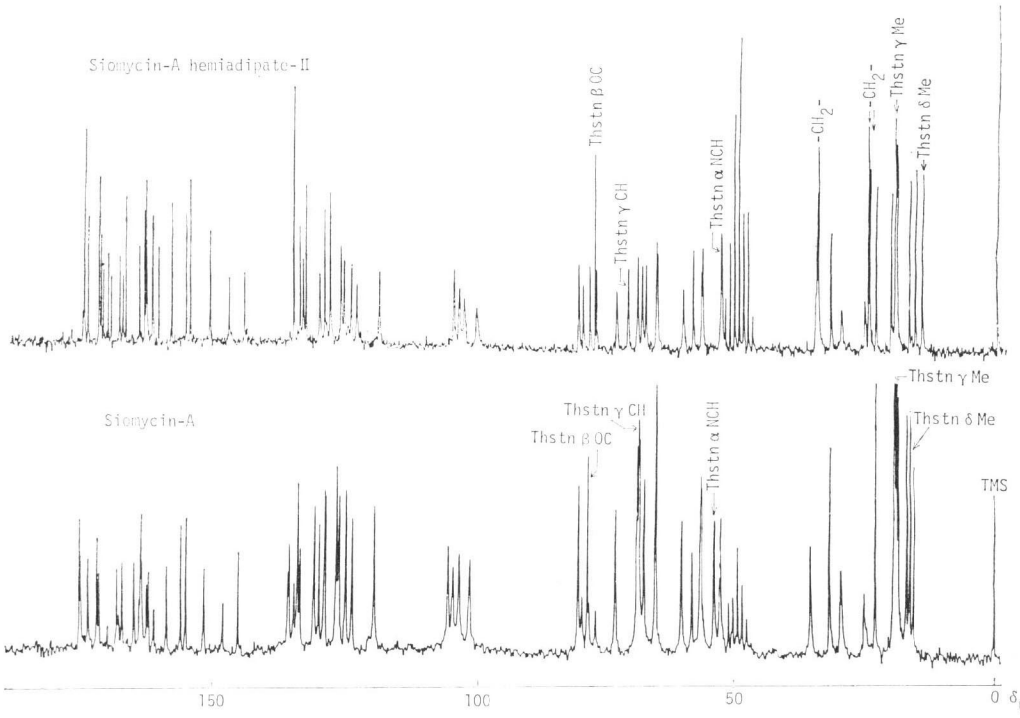
Structure Elucidation

^{13}C and ^1H NMR data are summarized in Tables 3 and 4, respectively. The 25-MHz ^{13}C and 270-MHz ^1H NMR spectra of **1** and **7** are shown in Figs. 4 and 5, respectively. Signals were assigned according to the descriptions in our earlier reports.[5,7,8]

The ^1H NMR spectrum of **7** in $CDCl_3$ - CD_3OD (4: 1) shows downfield shifts of the γCH_3 (+0.11 ppm) and γCH (+1.27 ppm) signals and an upfield shift of the αCH signal (−0.10 ppm) of the Thstn

Fig. 4. 25-MHz ^{1}H complete-decoupled ^{13}C NMR spectra of siomycin A (**1**) (lower trace) and siomycin-A hemiadipate-II (**7**) (upper trace) in $CDCl_3$ - CD_3OD.

FT measurement conditions: spectral width, 5000 Hz; pulse width, 10 μs (70°); acquisition time, 0.8 s; number of data points, 8K; number of transients, 20K; 10-mm spherical cell; concentration, 120 mg/ml; 30°C.



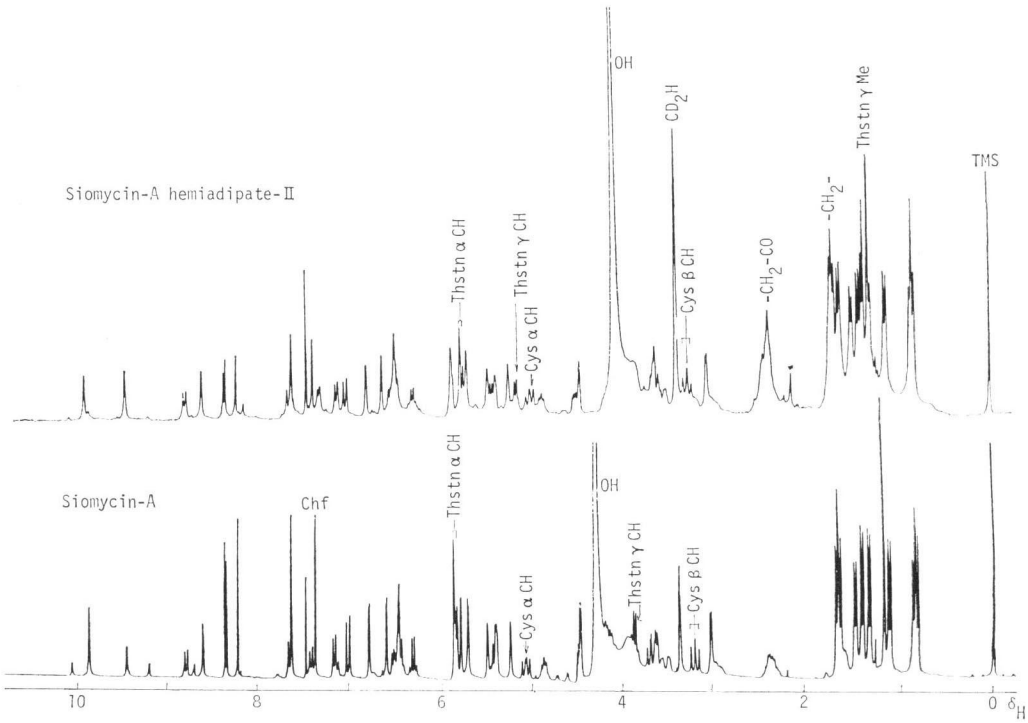
residue from the corresponding signal positions in **1**. In the ^{13}C NMR spectrum of **7** in $CDCl_3$ - CD_3OD (4: 1), downfield shifts of the γCH (+1.6 ppm) and γCH_3 (+0.9 ppm) signals and upfield shifts of the δCH_3 (−2.3 ppm), βC (−1.4 ppm), and αCH (−1.3 ppm) signals of the Thstn residue from the corresponding signals of **1** were observed. Similar signal shifts were also seen in the ^{13}C and ^{1}H NMR spectra on going from **1** to **3** in $CDCl_3$ and $CDCl_3$ - CD_3OD. The ^{13}C and ^{1}H NMR spectra of **3** were superimposable on those of **7** except for the signals due to an Ac residue in **3** and an Adip residue in **7**. These signal shifts observed in the ^{1}H and ^{13}C NMR spectra of **3** and **7** led us to the conclusion that **3** and **7** were acylated at the terminal hydroxyl group of the Thstn residue according to the known acylation shifts in ^{1}H NMR[9] and ^{13}C NMR spectroscopy.[10]

In the 270-MHz ^{1}H NMR spectrum of **8** in $CDCl_3$ - CD_3OD, downfield shifts of the γCH_3 (+0.06 ppm) and βCH (+1.23 ppm) signals of the Thr-1 residue were observed as well as slight shifts of the signals due to the Q and Cys residues compared with those of **1**. These observations indicated that the Adip residue becomes attached to the hydroxyl group of the Thr-1 residue. This was also confirmed by comparison of the ^{13}C NMR spectrum of **8** with that of **1**.

The ^{13}C NMR spectrum of **4** showed signal shifts of both Thstn and Thr-1 residue carbons; thus the hydroxyl groups of the Thstn and Thr-1 residues were concluded to be acetylated in **4**. Half-esters-II and -III other than hemiadipates were assumed to be acylated at the hydroxyl groups of the Thstn and

Fig. 5. 270-MHz ^1H NMR spectra of siomycin-A (**1**) (lower trace) and siomycin-A-hemiadipate-II (**7**) (upper trace) in $CDCl_3$ - CD_3OD.

FT measurement conditions: spectral width, 4000 Hz; pulse width, 12 μs (45°); acquisition time, 2 s; number of data points, 16 K; number of transients, 256; 5-mm spinning tube; concentration, 40 mg/ml; 23°C.



Thr-1 residues, respectively, by analogy with the case of the hemiadipates, although their structures were not studied by NMR spectroscopy.

Biological Activity

The *in vitro* antibacterial activities of acetates and half-esters of **1** are shown in Tables 5 and 6. The activities of half-esters were tested using their sodium salts. Acetates and half-esters-II and -III were active against Gram-positive bacteria but not against Gram-negative bacteria. The activity of **1** was little affected by acetylating one of the hydroxyl groups, while it was considerably reduced by blocking two hydroxyl groups with acetyl groups (Table 5). The half-esters-II were more active than

Table 5. Antibacterial spectra of **1** and its acetates against Gram-positive bacteria.

Compound	Minimum inhibitory concentration (μg/ml), Test organisms[a]				
	B. s.	*S. a.*	*S. a.* (R)	*S. pn.*	*S. py.*
1	0.1	0.2	0.1	0.05	0.1
3	0.2	0.2	0.2	0.1	0.2
4	1.56	3.13	1.56	0.39	1.56

[a] Abbreviations: see Table 6.

Table 6. Antibacterial spectra of half-esters of **1** against Gram-positive and Gram-negative bacteria.

Compound	Minimum inhibitory concentration (μg/ml) Test organisms[a]									
	B. s.	*B. a.*	*S. a.*	*S. a.* (R)	*S. pn.*	*S. py.*	*E. c.*	*K. p.*	*S. t.*	*P. a.*
1	0.39	0.78	0.39	0.39	0.2	0.2	>50	>50	>50	>50
5	6.25	25	6.25	6.25	1.56	3.13	—	—	—	—
6	3.13	6.25	1.56	1.56	0.39	0.39	>50	>50	>50	>50
7	0.78	1.56	1.56	0.78	0.1	0.2	>50	>50	>50	>50
8	3.13	1.56	1.56	1.56	0.78	1.56	>50	>50	>50	>50
9	0.78	1.56	1.56	0.78	—	0.1	>50	>50	>50	>50
10	3.13	6.25	3.13	3.13	0.2	0.78	>50	>50	>50	>50
11	0.39	0.78	1.56	0.78	0.025	0.05	>50	>50	>50	>50
12	1.56	1.56	3.13	1.56	0.1	0.2	>50	>50	>50	>50
13	>50	>50	>50	>50	25	25	>50	>50	>50	>50
14	6.25	12.5	3.13	3.13	0.78	1.56	—	—	—	—
15	1.56	12.5	3.13	3.13	0.39	0.78	>50	>50	>50	>50
16	1.56	6.25	3.13	1.56	0.39	0.78	>50	>50	>50	>50
17	6.25	12.5	12.5	6.25	0.78	1.56	>50	>50	>50	>50
18	6.25	12.5	6.25	6.25	1.56	3.13	>50	>50	>50	>50
19	25	50	25	12.5	6.25	6.25	>50	>50	>50	>50
20	50	50	>50	50	12.5	25	>50	>50	>50	>50
21	>50	>50	>50	>50	25	50	>50	>50	>50	>50
22	0.78	3.13	0.78	0.78	0.39	0.39	>50	>50	>50	>50
23	6.25	25	12.5	6.25	1.56	3.13	>50	>50	>50	>50

[a] Abbreviations: *B. s., Bacillus subtilis* PCI 219; *B. a., Bacillus anthracis*; *S. a., Staphylococcus aureus* FDA 209P JC-1; *S. a.* (R), *Staphylococcus aureus* 80257 (R); *S. pn., Streptococcus pneumoniae* type I; *S. py., Streptococcus pyogenes* C-203; *E. c., Escherichia coli* NIHJ JC-2; *K. p., Klebsiella pneumoniae*; *S. t., Salmonella typhimurium*; *P. a., Pseudomonas aeruginosa*.

the corresponding half-esters-III. In a series of half-esters with saturated straight-chain dicarboxylic acids, their activities varied depending on the chain length. Compounds **7**, **9**, and **11** among the half-esters of **1** retained a substantial part of the activity shown by **1**. The activity of **22** was comparable to that of **7**, but **23** was less active than **8** (Table 6). Although the half-esters of **2** have been shown to possess the full antibacterial activity of **2** in patent literature,[11] the hemisuccinate of **2** prepared according to the patent showed considerably reduced activity in a preliminary paper disc assay.

Table 7. Therapeutic activities against experimental bacterial infections in mice.

Compound	ED_{50} mg/kg/dose			
	Streptococcus pneumoniae type I		*Streptococcus pyogenes* C-203	
	Intraperitoneal	Subcutaneous	Intraperitoneal	Subcutaneous
1	0.031	23.2	0.039	75.2
5	0.24	16.5	—	—
6	0.031	4.38	0.028	3.54
7	0.045	2.47	0.011	2.08
9	0.070	2.50	0.007	3.06

The *in vivo* activities with some of the half-esters (as sodium salts) are shown in Table 7. Compounds **6**, **7**, and **9** showed activities comparable to that of **1** by intraperitoneal injection. In contrast, when administered subcutaneously, they exhibited enhanced therapeutic activities compared with that

of **1**. This was probably due to the improved solubility in water.

Intraperitoneal and intravenous acute toxicities of **7** in mice (LD_{50}) were estimated to be approximately 200 and 50 mg/kg, respectively. Subcutaneous or oral administration of 500 mg/kg of **7** into mice did not cause any acute toxicity for 14 days.

Experimental

Melting points (uncorrected) were determined with a Yanagimoto micro melting point apparatus. Thin-layer chromatography was run on silica gel GF plates (E. Merck) and components were visualized using short-wavelength UV fluorescence or iodine vapor. For column chromatography, silica gel 60 (less than 0.08 mm, E. Merck) was used. Analytical values were within $\pm 0.4\%$ of calculated values unless otherwise indicated. Amino acid analyses were performed with an automatic amino acid analyzer, JEOL JLC-3B. Ultraviolet spectra were measured with a Hitachi EPS-3T spectrophotometer. Infrared spectra were obtained with a JASCO DS-403G spectrophotometer.

The 270-MHz and 100-MHz ^1H FT NMR spectra were respectively measured with a Bruker WH-270 spectrometer equipped with a Nicolet 1180 computer system and a Varian XL-100-12A FT NMR spectrometer in $CDCl_3$ and $CDCl_3$ - CD_3OD (4: 1) at ordinary temperatures. The 25.16-MHz ^1H complete-decoupled ^{13}C FT NMR spectra were determined in $CDCl_3$ - CD_3OD (4: 1) and $CDCl_3$ - CD_3OH (4: 1) at 70°C with a Varian XL-100 FT NMR or a JEOL FX-100 FT NMR spectrometer. Chemical shifts were expressed as δ (ppm downfield from the internal tetramethylsilane signal). Typical FT measurement conditions are described in Figs. 4 and 5.

Siomycin

The siomycin complex was supplied by the pilot plant division of our company. Siomycin was used after chromatographic separation or without further purification.

Adipic Anhydride

Adipic anhydride was prepared according to the method described by HILL.[12] Apidic acid (10 g) was refluxed in 30 ml of acetic anhydride for 4 hours. The reaction mixture was evaporated *in vacuo* to dryness and the residual oil was crystallized from benzene. The crystalline mass was dried over paraffin, KOH, and P_2O_5, giving 5.0 g of adipic-α-anhydride (polymeric): mp 76~78°C; IR (Nujol) 1810, 1760 cm^{-1}. Adipic-α-anhydride was distilled *in vacuo* to give oily adipic-β-anhydride (monomeric). Because the reaction of both anhydrides with **1** gave similar reaction mixtures, polymeric anhydride was used in this study. Succinic anhydride and maleic anhydride were commercially available. Other anhydrides were prepared by heating dicarboxylic acids with acetic anhydride in ways similar to that described for adipic anhydride.

Siomycin-A Hemiadipates (**7**, **8**, **18**, **19**, **20** and **21**)

Compound **1** (10 g) and adipic anhydride (10 g) were stirred in 30 ml of pyridine at 39~40°C for 2 hours. The reaction mixture was poured over ice and the precipitate was collected by filtration. The crude product was washed with water and dried, then chromatographed on silica gel using a mixture of $CHCl_3$ and CH_3OH (100: 5) as an eluant. This procedure gave 0.61 g (6.0%) of **3**, 1.17 g (11.7%) of recovered **1**, 2.12 g (19.7%) of **7**, 0.71 g (6.6%) of **8**, and 4.62 g hemiadipate-I. Part of the hemiadipate-I was separated into **18**, **19**, **20**, and **21** by preparative thin-layer chromatography on silica gel plates. Reactions of **1** with other acid anhydrides were carried out similarly.

Siomycin-A Acetates (**3** and **4**)

Compound **1** (1.30 g) was allowed to react with 13 ml of acetic anhydride in 3.9 ml of pyridine at 39~40°C for 2 hours. The reaction mixture was poured onto icewater and the precipitate was chromatographed on silica gel with $CHCl_3$ - CH_3OH (100: 2~100: 5) to give 1.07 g of a mixture of **3** and **4** (**3** was predominant). Purification of part of the mixture by repeated preparative thin-layer chromatography on silica gel plates with $CHCl_3$ - CH_3OH (95: 5) and $CHCl_3$ - CH_3OH - Et_2O (6: 1: 3) afforded 285 mg of **3** and 86 mg of **4**.

Preparation of Sodium Salts

Method A: Compound **6** (800 mg) was stirred with 70.8 ml of 0.1% aqueous $NaHCO_3$ solution (2 mol. equiv.) at room temperature. The insoluble substance was filtered off and the filtrate was lyophilized yielding 815 mg of white powder.

Method B: To a solution of **7** (5.0 g) in 200 ml of *t*-BuOH, 42.1 ml of 1% aqueous $NaHCO_3$ solution was added, and the resultant solution was filtered and concentrated *in vacuo*. Next, 50 ml of H_2O was added then the solution was lyophilized. The powder obtained was redissolved in 500 ml of H_2O, filtered, and lyophilized giving 5.17 g of white powder.

Biological Tests

The antibacterial spectra *in vitro* were obtained by the agar dilution method. MICs were determined in accordance with the standard method of the Japan Society of Chemotherapy. Inoculum size: 10^8 CFU/ml.

The chemotherapeutic test was done with mice. ICR strain female mice aged 5 weeks and weighing 19~22 g were infected intraperitoneally with a sufficient number of organisms to kill all nontreated mice within 3 days. Compound **1** was suspended in 5% gum arabic and the sodium salts of half-esters were dissolved in distilled water. Three doses at 0, 4, and 8 hours after infection were administered to the infected mice by the intraperitoneal or subcutaneous route. Ten mice were used for each dose level. The survival rate of the treated mice was recorded at 7 days after infection and the amount of the single dosage required to protect 50% of the treated mice (ED_{50}) was calculated by the Probit method.

To determine acute toxicity, aqueous solution of **7** (sodium salt) was administered to groups of five mice and toxic symptoms were observed for 14 days.

Acknowledgments

We would like to thank Prof. T. MIYAZAWA and Dr. F. INAGAKI (The University of Tokyo) for the 270-MHz ^1H NMR spectra, and Mr. K. MATSUSHITA (JEOL Ltd., Japan) for the ^{13}C NMR spectra recorded on the JEOL FX-100 spectrometer. We are indebted to Dr. K. TAKEDA, the former director of these laboratories for encouragement during this work. We thank the pilot plant division of this company for the supply of siomycin complex. We also thank Messrs. H. NAGATA and K. KANAZAWA for the biological data.

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