CHEMICAL MODIFICATION OF SORBISTIN

I. N-ACYL ANALOGS OF SORBISTIN

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Sorbistin A_1 (**1b**) and sorbistin B (**1a**), bioactive components of a new type of aminoglycoside antibiotic produced by a strain of *Pseudomonas* species, have been converted into a key intermediate **3** by blocking of the 1- and 4-amino groups of sorbistins with dimedone and subsequent deacylation of the 4′-N-acyl group. Some 4′-N-acyl analogs of sorbistin (**1e**～**1t**) have been synthesized by 4′-N-acylation of **3** with an appropriate reactive derivative of carboxylic acids (mixed anhydride, acid chloride or activated ester) followed by deblocking of the protected group with bromine or sodium nitrite. Chemical interconversion of three natural sorbistins A_1 (**1b**), A_2 (**1c**) and B (**1a**) has been performed by this procedure. The 1-N-acyl (**4a**～**4c**) and the 1,4′-N,N-diacyl analogs (**6a**～**6c**) have been prepared by direct N-acylation of sorbistin D (**1d**) (the 4′-desacyl derivative) and sorbistin A_1, respectively. On the other hand, the 4-N-acyl (**5a** and **5b**) and the 4,4′-N,N-diacyl derivatives (**7a** and **7b**) have been prepared by acylation and subsequent hydrogenolysis of 1-N-Cbz-sorbistin D (**4b**) and 1-N-Cbz-sorbistin A_1 (**6b**), respectively.

Determination of *in vitro* antimicrobial activity showed that the 4′-N-propionyl (**1b**) and the 4′-N-cyclopropylcarbonyl (**1s**) derivatives are the most active members of the 4′-N-acyl derivatives. Elongation and shortening of the side chain and introduction of functional groups decreased the activity. N-Acylation of the amino group at C-1 or at C-4 gave virtually inactive products.

Sorbistin[1], a complex of new aminoglycoside antibiotics produced by a new *Pseudomonas* strain, has a unique structure consisting of 4-acylamino-4-deoxy-D-glucose and 1,4-diamino-1,4-dideoxy-D-sorbitol moieties which are joined together through 1→3 α-glycoside linkage. The complete structure determination has been described in a paper from this Institute[2]. The acyl groups on the 4-aminoglucose moiety were determined to be acetyl (sorbistin B, **1a**), propionyl (sorbistin A_1, **1b**) and butyryl (sorbistin A_2, **1c**), respectively. These components have broad spectrum activity against Gram-positive and Gram-negative bacteria including some resistant bacteria producing aminoglycoside-inactivating enzyme(s), but the activity is weak to moderate even in the most active component, sorbistin A_1. Therefore, this antibiotic is an interesting substrate for chemical modification. The acyl side chain can be cleaved by alkaline hydrolysis without affecting the structural integrity of the remainder of the molecule to give a deacylated product designated as sorbistin D (**1d**) which is totally inactive, indicating that the acyl side chain on the 4-aminoglucose moiety is essential for antibiotic activity. We have attempted to enhance the antibiotic activity of sorbistin by introduction of a new acyl group on the 4′-amino group and/or on the 1- or 4-amino group to afford sorbistin analogs (**1** and **4** through **7**).

Preparation of 4′-N-Acyl Analogs of Sorbistin

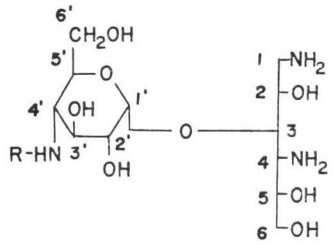
Blocking of the two primary amino groups of sorbistin with a suitable blocking group followed by cleavage of the acyl side chain on the 4′-amino group will give an intermediate for 4′-N-acylation. The protective group must be stable during the side chain cleavage with alkali and readily removed

Table 1. 4′-N-Acyl analogs of sorbistin

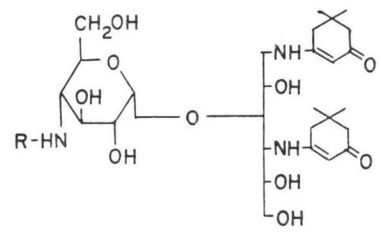
Compound	R	Method	Yield (%)	MP °C (dec.)	TLC (Rf)**		Molecular formula	Analysis					
					S-117	S-122		Calcd.			Found		
								C	H	N	C	H	N
1a	$-COCH_3$	B	15	156~158	0.36	0.27	$C_{14}H_{29}N_3O_9 \cdot {}^1/_2H_2CO_3$	42.02	7.30	10.14	42.36	7.39	9.83
1b	$-COCH_2CH_3$	A	39	139~141	0.46	0.45	$C_{15}H_{31}N_3O_9 \cdot {}^3/_2H_2CO_3$	40.41	6.99	8.57	40.24	7.23	8.51
1c	$-COCH_2CH_2CH_3$	C	10	115~117	0.55	0.55	$C_{16}H_{33}N_3O_9 \cdot {}^3/_2H_2CO_3$	41.66	7.19	8.33	41.53	7.43	8.13
1e	$-COCHCH_2CH_2NHCbz$ (L) OH	C	11	112~113.5	0.60	0.66	$C_{24}H_{40}N_4O_{12} \cdot {}^1/_2H_2CO_3 \cdot {}^1/_2H_2O$	48.19	6.93	9.18	47.86	6.92	8.63
1f	$-COCHCH_2CH_2NH_2$ (L) OH	—	76*	164~166	0.08	0.08	$C_{16}H_{34}N_4O_{10} \cdot {}^5/_2H_2CO_3$	37.19	6.58	9.38	37.51	6.93	9.41
1g	$-COC_6H_5$	B	8	132~135	0.58	0.59	$C_{19}H_{31}N_3O_9 \cdot {}^3/_2H_2CO_3$	45.72	6.36	7.80	45.30	6.51	7.81
1h	$-COCH(NHCbz)CH_3$(L)	C	13	126~128	0.66	0.66	$C_{23}H_{38}N_4O_{11} \cdot {}^3/_2H_2CO_3$	44.75	6.59	8.52	44.99	6.77	8.87
1i	$-COCH(NH_2)CH_3$(L)	—	62*	134~135	0.31	0.05	$C_{15}H_{32}N_4O_9 \cdot {}^3/_2H_2CO_3$	39.21	6.98	11.08	39.35	7.42	10.74
1j	$-COCH_2CH_2NHCbz$	C	9	110~111	0.61	0.65	$C_{23}H_{38}N_4O_{11} \cdot {}^3/_2H_2CO_3 \cdot H_2O$	44.75	6.59	8.52	45.01	6.51	8.41
1k	$-COCH_2CH_2NH_2$	—	34*	171~173	0.15	0.04	$C_{15}H_{32}N_4O_9 \cdot {}^5/_2H_2CO_3$	37.03	6.57	9.87	37.11	6.54	10.21
1l	$-COCH(CH_3)_2$	B	13	160~165	0.54	0.53	$C_{19}H_{37}N_3O_{10} \cdot {}^1/_2H_2CO_3 \cdot {}^1/_2H_2O$	46.33	7.78	8.31	46.19	7.77	8.11
1m	$-COCH(OH)CH_3$(DL)	D	14	165~168	0.33	0.33	$C_{15}H_{31}N_3O_{10} \cdot H_2CO_3$	40.42	7.00	8.84	40.42	6.89	8.51
1n	$-COCH(Cl)CH_3$(DL)	C	7	231~240	0.51	0.51	$C_{15}H_{30}N_3O_9Cl \cdot {}^3/_2H_2CO_3 \cdot H_2O$	36.50	6.50	7.74	36.56	6.30	8.04
1o	$-COCH_2NHCbz$	D	15	119~120	0.57	0.65	$C_{22}H_{36}N_4O_{11} \cdot {}^1/_2H_2CO_3$	47.95	6.62	9.94	47.70	6.63	9.99
1p	$-COCH_2NH_2$	—	87*	150~152	0.19	0.03	$C_{14}H_{30}N_4O_9 \cdot {}^3/_2H_2CO_3$	37.88	6.77	11.40	38.26	6.59	11.16
1q	$-COCHCl_2$	D	26	163~168	0.52	0.51	$C_{14}H_{27}N_3O_9Cl_2 \cdot 2H_2CO_3$	33.34	5.42	7.29	33.12	4.88	7.89
1r	$-COCH_2C_6H_5$	D	7	133~137	0.60	0.60	$C_{20}H_{33}N_3O_9 \cdot H_2CO_3 \cdot {}^1/_2H_2O$	47.54	6.84	7.92	47.44	6.67	7.96
1s	$-CO-\triangleleft$	D	23	155~157	0.48	0.47	$C_{16}H_{31}N_3O_9 \cdot {}^3/_2H_2CO_3$	41.83	6.82	8.36	42.37	6.75	7.71
1t	$-CO(CH_2)_3CH_3$	E	20	119~125	0.60	0.55	$C_{17}H_{35}N_3O_9 \cdot {}^1/_2H_2CO_3 \cdot {}^1/_2H_2O$	45.15	8.01	9.03	45.07	7.86	8.68

* deblocking yield

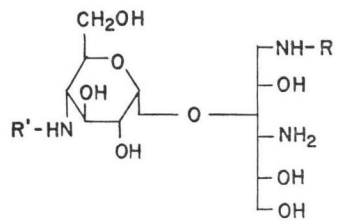
** S-117 = $CHCl_3$ - MeOH - 28%NH_4OH (1 : 3 : 2); S-122 = $CHCl_3$ - MeOH - AcOH - 2N NH_4OH (20 : 65 : 5 : 40).



1a R = $COCH_3$ (Sorbistin B)
1b R = COC_2H_5 (Sorbistin A_1)
1c R = CO-n-C_3H_7 (Sorbistin A_2)
1d R = H (Sorbistin D)
1e ~ 1t see Table I

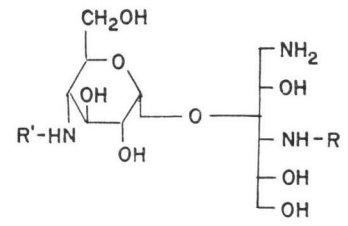


2a R = $COCH_3$
2b R = COC_2H_5
3 R = H



4a R' = H, R = COC_2H_5
4b R' = H, R = $COOCH_2C_6H_5$
4c R' = H, R = AHB

6a R' = COC_2H_5, R = AHB
6b R' = COC_2H_5, R = $COOCH_2C_6H_5$
6c R' = COC_2H_5, R = CO-i-C_3H_7



5a R' = H, R = COC_2H_5
5b R' = H, R = AHB

7a R' = COC_2H_5, R = AHB
7b R' = COC_2H_5, R = COC_2H_5

AHB = $COCHCH_2CH_2NH_2$ (OH)

after the 4′-N-acylation without injuring the newly formed amide bond and the remaining part of molecule. Dimedone was chosen as a protective group in this study, because it had been previously employed in modification studies on kasugamycin[3] and butirosins[4], and demonstrated to be stable to alkaline hydrolysis and readily removable with bromine or chlorine under mild conditions. Heating of sorbistin A_1 or B with dimedone in a sealed tube at 100°C for 17 hours gave the corresponding 1,4-di-N-dimedone derivative (**2**), which was hydrolyzed in 0.1 N NaOH at reflux for 8 hours to afford the key intermediate (**3**). The acylation of **3** was performed by the acid anhydride, the acid chloride or the activated ester method using N-hydroxysuccinimide. The resulting blocked 4′-N-acyl derivative was treated with either an aqueous bromine solution or sodium nitrite[5] to give the requisite sorbistin analogs (**1**). Thus sorbistin B (**1a**) was coverted into sorbistin A_1 (**1b**) and sorbistin A_2 (**1c**) *via* the key intermediate (**3**). Conversely sorbistin B (**1a**) was also obtained from sorbistin A_1 (**1b**). In addition to these three natural components, sixteen analogs (**1e** through **1t**) were prepared by acylation of **3** and subsequent deblocking of the acyl derivatives according to five typical procedures (Method A to Method

Table 2. 1- and 4-N-Monoacyl and 1,4′- and 4,4′-N,N-diacyl analogs of sorbistin

Compound	R	Yield (%)	MP (dec.)	TLC (Rf) S-117	Molecular formula	Analysis					
						Calcd.			Found		
						C	H	N	C	H	N
4a	$-COCH_2CH_3$	41	112~117	0.67	$C_{15}H_{31}N_3O_9 \cdot {}^1/_2H_2CO_3$	43.35	7.53	9.81	43.59	7.88	9.86
4b	$-COOCH_2C_6H_5$	45	89~91	0.63	$C_{20}H_{33}N_3O_{10} \cdot {}^1/_2H_2CO_3$	48.61	6.77	8.30	48.27	6.66	8.48
4c	$-COCHCH_2CH_2NH_2$ (L) OH	49	136~138	0.23	$C_{16}H_{34}N_4O_{10} \cdot {}^3/_2H_2CO_3$	39.25	6.96	10.46	39.38	7.07	10.40
5a	$-COCH_2CH_3$	31	129~134	0.66	$C_{15}H_{31}N_3O_9 \cdot {}^1/_2H_2CO_3 \cdot {}^1/_2H_2O$	42.56	7.60	9.61	42.75	7.44	9.44
5b	$-COCHCH_2CH_2NH_2$ (L) OH	13	149~152	0.13	$C_{16}H_{34}N_4O_{10} \cdot 2H_2CO_3$	38.16	6.76	9.89	38.03	6.45	9.78
6a	$-COCHCH_2CH_2NH_2$ (L) OH	55	142~145	0.40	$C_{19}H_{38}N_4O_{11} \cdot {}^3/_2H_2CO_3$	41.62	6.99	9.47	41.68	7.00	9.46
6b	$-COOCH_2C_6H_5$	56	102~105	0.83	$C_{23}H_{37}N_3O_{11} \cdot H_2CO_3 \cdot H_2O$	47.13	6.76	6.87	46.94	6.42	6.94
6c	$-COCH(CH_3)_2$	24	127~129	0.80	$C_{19}H_{37}N_3O_{10} \cdot {}^1/_2H_2CO_3 \cdot {}^1/_2H_2O$	46.33	7.78	8.31	46.19	7.77	8.11
7a	$-COCHCH_2CH_2NH_2$ (L) OH	56	155~160	0.31	$C_{19}H_{38}N_4O_{11} \cdot H_2CO_3 \cdot {}^1/_2H_2O$	42.18	7.26	9.84	42.29	7.49	9.80
7b	$-COCH_2CH_3$	19	129~131	0.92	$C_{18}H_{35}N_3O_{10} \cdot H_2CO_3$	44.27	7.23	8.15	44.35	7.31	8.62

Table 3. *In vitro* antimicrobial activity of several sorbistin analogs against resistant organisms producing aminoglycoside inactivating enzymes on nutrient agar

Compounds	Organisms	Minimum inhibitory concentrations (mcg/ml)							Relative Activity 100× RAm
		E. coli JR35/ C600	*E. coli* A20107	*E. coli* A20732	*Prov. stuartii* A20894	*Ps. aeruginosa* A20601	*Ps. aeruginosa* A20896	*Ps. aeruginosa* GN-315	
	Inactivating enzyme**	APH (3′)-1	APH (3′)-2	ANT(2″)	AAC(2′)	AAC (3)-1	AAC (3)-2	AAC (6′)-4	
1a (Sorbistin B)	4′-acyl= $COCH_3$	12.5	100	25	>100	100	>100	50	25
1b (Sorbistin A_1)	COC_2H_5	6.3	25	12.5	>100	50	50	50	100
1c (Sorbistin A_2)	$CO\text{-}nC_3H_7$	12.5	100	50	>100	>100	>100	100	30
1i	$COCH(NH_2)CH_3$	25	>100	50	>100	100	>100	100	26
1l	$CO\text{-}iC_3H_7$	12.5	25	25	>100	50	100	50	69
1m	$COCH(OH)CH_3$	25	>100	100	>100	>100	>100	>100	24
1n	$COCHClCH_3$	12.5	100	25	>100	>100	50	50	50
1q	$COCHCl_2$	6.3	50	25	>100	50	100	50	84
1s	CO-◁	12.5	50	12.5	>100	50	50	25	100
Kanamycin A		>100	12.5	25	1.6	100	>100	100	

* see in the text and reference 8.

** APH(3′)=Aminoglycoside 3′-phosphotransferase, ANT(2″)=Aminoglycoside 2″-nucleotidyltransferase AAC(2′)=Aminoglycoside 2′-N-acetyltransferase, AAC(3)=Aminoglycoside 3-N-acetyltransferase AAC(6′)=Aminoglycoside 6′-N-acetyltransferase.

Table 4. *In vitro* antimicrobial activity of **1b** and **1s** on nutrient agar

Organisms	MIC (mcg/ml)		
	1b (Sorbistin A_1)	**1s**	Kanamycin A
Escherichia coli NIHJ	12.5	12.5	0.2
Escherichia coli PO 1495	25	50	0.4
Escherichia coli ML 1630	25	25	100
Escherichia coli NR-79/W677	25	25	50
Escherichia coli JR35/C600	6.3	12.5	>100
Escherichia coli A20107	25	50	12.5
Escherichia coli JR66/W677	50	50	100
Escherichia coli R5	12.5	12.5	6.3
Escherichia coli JR88	12.5	12.5	0.8
Escherichia coli A20732	12.5	12.5	25
Klebsiella pneumoniae D11	3.1	3.1	0.2
Klebsiella pneumoniae 22-3038	50	50	>100
Enterobacter cloacae A20364	25	25	100
Enterobacter cloacae A21006	25	25	50
Proteus vulgaris A9436	12.5	12.5	0.2
Proteus mirabilis A9554	12.5	12.5	0.4
Providencia stuartii A20894	>100	>100	1.6
Pseudomonas aeruginosa A9930	12.5	12.5	6.3
Pseudomonas aeruginosa A20653	25	25	>100
Pseudomonas aeruginosa #130	12.5	12.5	12.5
Pseudomonas aeruginosa A20601	50	50	100
Pseudomonas aeruginosa A20896	50	50	>100
Pseudomonas aeruginosa GN-315	50	50	100
Pseudomonas aeruginosa GN-4925	25	25	50
Pseudomonas sp. A20621	>100	>100	12.5
Serratia marcescens A20019	>100	>100	0.8
Serratia marcescens A21247	100	100	>100
Staphylococcus aureus Smith	12.5	12.5	0.1
Staphylococcus aureus D133	25	25	0.8
Staphylococcus aureus D137	100	100	1.6
Staphylococcus aureus A20239	50	50	50
Bacillus subtilis PCI 219	25	25	0.05

E) given in the experimental part. The yields, analytical data and physicochemical properties of these sorbistin analogs are presented in Table 1.

1-N-Acylation

It was previously demonstrated[6,7] that the 6′-amino group bound to a methylene carbon in kanamycin was selectively acylated with moderately activated esters such as N-benzyloxycarbonyloxysuccinimide (Cbz-OSu) or N-(4-Cbz-amino-2-hydroxybutyryloxy) succinimide (Cbz-AHB-OSu). In sorbistin D (**1d**) the 1-amino group attached to a methylene carbon appears to be least hindered and most reactive. Therefore, **1d** was allowed to react with N-propionyloxysuccinimide (C_2H_5COOSu) to afford a position isomer (**4a**) of sorbistin A_1 as a sole product in which the NMR spectrum was diagnostic for the position of the acyl side chain. The peaks due to the C-1 methylene protons and the C-4 methine proton of non-acylated **1d** were observed at 2.5~3.1 ppm as over lapped multiplets. In the NMR of the 4′-N-propionyl derivative (**1b**), the C-1 methylene multiplet was observed at 2.9~

3.3 ppm, while the 4′-H peak was shifted downfield to 3.5~4.0 ppm and overlapped with other protons. In the isomer **4a** the multiplet of the C-1 methylene was shifted downfield to 3.39 ppm as a broad doublet (J=8 Hz), while the C-4′ proton remained at 2.5~3.1 ppm as a multiplet, indicating that the acylation took place on the 1-amino group.

Acylation of **1d** with N-benzyloxycarbonyloxy-5-norbornene-2,3-dicarboxyimide (CbzONB) gave 1-N-Cbz-sorbistin D (**4b**). In the NMR of **4b** the C-1 methylene peak was shifted to 3.29 ppm (br-d, J=7 Hz) and the 4′-H remained at 2.5~2.9 ppm, indicating the acylation also took place at the 1-amino group. In similar manner **1d** was acylated with Cbz-AHB-OSu and removal of the Cbz of the acylated product afforded 1-N-(4-amino-2-hydroxybutyryl) sorbistin D (**4c**).

Direct acylation of **1b** with Cbz-OSu gave 1-N-Cbz-sorbistin A_1 (**6b**) in 56% yield, showing a shift of the C-1 methylene doublet (J=8 Hz) to 3.70 ppm. In a similar manner **1b** was reacted with Cbz-AHB-OSu followed by removal of Cbz by catalytic reduction to give 1-N-(4-amino-2-hydroxybutyryl)sorbistin A_1 (**6a**) and a small amount of the 4-N-acyl derivative (**7a**). Acylation of **1b** with isobutyric anhydride afforded 1-N-isobutyrylsorbistin A_1 (**6c**).

4-N-Acylation

The preparation of another position isomer of sorbistin A_1 was accomplished by acylation of **4b** with N-hydroxysuccinimido ester of propionic acid and subsequent hydrogenolysis to remove the 1-N-benzyloxycarbonyl group. The reaction gave three products, which were assigned to 4,4′-N,N-dipropionylsorbistin D (**7b**, yield 19%), 4-N-propionylsorbistin D (**5a**, yield 31%) and sorbistin A_1 (**1b**, yield 9%) on the basis of NMR spectra and TLC. In addition to one propionyl peak, the C-1 methylene and the C-4′ methine protons were clearly visible as overlapped multiplets at 2.5~3.3 ppm in the NMR of **5a**, indicating that the acylation took place on the 4-amino group. The NMR spectrum of **7b** exhibited the C-1 methylene protons at 2.6~3.3 ppm, and two propionyl protons at 3.10 ppm (6H, t) and 2.30 ppm (4H, q), while the C-4′ proton overlapped with peaks below 3.3 ppm due to a downfield shift by 4′-N-acylation. Therefore **7b** was assigned to the 4,4′-di-N-propionyl derivative. Similarly acylation of **4b** with Cbz-AHB-OSu followed by hydrogenolysis gave 4-N-(4-amino-2-hydroxybutyryl)sorbistin D (**5b**) in 14% yield together with the 4′-N-acyl derivative (**1f**) in 3% yield. The reactions indicate that the 4-amino group is more reactive than the 4′-amino group towards acylating agents.

The 4-N-acyl derivative of sorbistin A_1 was prepared by acylation of **6b** with Cbz-AHB-OSu to give the 4-acyl intermediate, which was subjected to hydrogenolysis to afford 4-N-(4-amino-2-hydroxybutyryl)sorbistin A_1 (**7a**). This product was identical with the minor product obtained by direct acylation of **1b** with Cbz-AHB-OSu followed by reduction.

Antimicrobial Activity of Sorbistin Derivatives

Minimum inhibitory concentrations (MIC's) of sorbistin derivatives prepared in this study were determined against 32 Gram-negative and Gram-positive organisms by a two-fold agar dilution method on nutrient agar. Some of the derivatives retained *in vitro* activity comparable to that of the parent sorbistin A_1, but some lost most of the activity. Each of the derivatives was at first evaluated by "mean relative activity" (RAm)[8], which was indicated in terms of the geometric mean of the MIC ratio of the test compound to sorbistin A_1 against each test organism. Table 3 shows bioactive derivatives with

more than 20% mean relative activity (sorbistin A_1=100), the figure being shown in the last column of the table. The maximum activity was attained with the propionyl side chain (**1b**) carrying three carbons or the cyclopropylcarbonyl group (**1s**) of almost the same length and size as propionyl. The activity is greatly affected by the length of the side chain. Elongation or shortening of chain-length resulted in a marked decrease in the activity. The acetyl derivative (**1a**) and the butyryl derivative (**1c**) showed 25~30% the activity of **1b** and the valeryl derivative (**1t**) was virtually inactive. α-Substitution of the propionyl side-chain somewhat decreased the activity. The isobutyryl derivative (**1l**) and the α-chloropropionyl derivative (**1n**) showed 69% and 50% of the activity of **1b**, respectively. A slightly lower activity (84% of **1b**) was observed in the dichloroacetyl derivative (**1q**) in which bulkiness of the side chain was nearly equal to that of **1l** and **1n**. α-Substitution with a polar group such as an amino or hydroxy group (**1i** and **1m**) reduced the activity to a greater extent. No significant activity was observed in derivatives with a bulky group such as a phenyl (**1g** and **1r**) or NHCbz (**1e**, **1h** and **1j**) as well as with an ω-amino group (**1f**, **1k** and **1p**) including **1f** possessing the γ-amino-α-hydroxybutyryl side chain, which had a remarkable effect on 2-deoxystreptamine-containing aminoglycoside antibiotics as in amikacin[6,7]. Acylation of the amino group at C-1 (**4** and **6**) and at C-4 (**5** and **7**) resulted in a complete loss of activity.

Table 4 shows the MIC's of **1b** and **1s**, the most active compounds of this series, as compared with that of kanamycin. As can be seen, the intrinsic activity of both compounds is not high, but the antibacterial spectrum is broad with moderate activity against most kanamycin A-resistant organisms.

Table 3 also show the MIC's of nine compounds against some resistant bacteria producing aminoglycoside inactivating enzymes. These derivatives were moderately active (MIC=6.3~25 mcg/ml) against kanamycin A-resistant *Escherichia coli* JR35/C600 producing APH (3′)-1 (MIC of kanamycin A>100 mcg/ml) and showed nearly the same range of MIC's as kanamycin A (12.5~50 mcg/ml) against *E. coli* A 20732 producing ANT(2″), while they were quite inactive against *Providencia stuartii* A 20894 producing AAC(2′). Compounds **1b**, **1l**, **1n**, **1q** and **1s** were also moderately to weakly active (MIC=25~100 mcg/ml) against kanamycin A-resistant strains, *E. coli* A 20107, *Pseudomonas aeruginosa* A 20601, *P. aeruginosa* A 20896 and *P. aeruginosa* GN-315 which produce APH(3′)-2, AAC(3)-1, AAC(3)-2 and AAC(6′)-4, respectively.

Experimental

1,4-Bis-N-(5,5-dimethyl-3-oxo-1-cyclohexen-1-yl)sorbistin B (**2a**)

A solution of 5 g (13 m moles) of sorbistin B (**1a**) and 5 g (36.2 m moles) of dimedone in 50 ml of MeOH was heated in a sealed tube for 17 hours at 100 °C. The solvent was removed under reduced pressure. The residue was dissolved in a small amount of MeOH and adsorbed on a column of silica gel (60 g). The column was washed with $CHCl_3$ to remove dimedone and eluted with $CHCl_3$ - MeOH (1: 1). The anthrone-positive fractions were combined and evaporated under reduced pressure to give 6.8 g (85%) of **2a**. Mp 201~202 °C, $[\alpha]_D^{26}$+203° (*c* 1, H_2O), $\lambda_{max}^{H_2O}$ 290 nm (ε, 61,000). NMR (D_2O): δ 1.07 (12H, s, CH_3), 2.08 (3H, s, acetyl), 5.18 (1H, s, vinyl proton), 5.30 (1H, d, J=3Hz anomeric proton), 5.36 (1H, s, vinyl proton).

Anal. Calcd. for $C_{30}H_{49}N_3O_{11}\cdot 3H_2O$: C, 52.85; H, 8.13; N, 6.16.
Found: C, 53.05; H, 8.21; N, 6.01.

1,4-Bis-N-(5,5-dimethyl-3-oxo-1-cyclohexen-1-yl)sorbistin A_1 (**2b**)

A solution of 1 g (2.5 m moles) of sorbistin A_1 (**1b**) and 1 g (7.15 m moles) of dimedone in 20 ml of MeOH was heated in a sealed tube for 17 hours at 100°C. The solvent was removed under reduced pressure. The residue was dissolved in a small amount of water and adsorbed on a column of Diaion

HP-20 (20 ml). The column was eluted with 40% aqueous MeOH. Anthrone-positive fractions were collected and evaporated under reduced pressure to give 1.53 g (100%) of **2b**. An analytical sample was obtained by rechromatography on silica gel using the lower phase of $CHCl_3$ - MeOH - 28% NH_4OH (1 : 1 : 1) as an eluant. The eluted product was crystallized from MeOH - EtOAc, mp 157~160°C, $[\alpha]_D^{23}+207°$ (*c* 0.5, H_2O). $\lambda_{max}^{H_2O}$ 290 nm (ε 60,000). NMR (D_2O): δ 1.01 (12H, s, CH_3), 1.10 (3H, t, J=7.5 Hz, $COCH_2\underline{CH_3}$), 2.28 (2H, q, J=7.5 Hz, $CO\underline{CH_2}CH_3$), 5.08 (1H, s, vinyl proton), 5.17 (1H, d, J=3 Hz, anomeric proton), 5.26 (1H, s, vinyl proton).

Anal. Calcd. for $C_{31}H_{51}N_3O_{11}\cdot CH_3OH$: C, 57.07; H, 8.26; N, 6.24.
Found: C, 57.27; H, 8.59; N, 6.02.

1,4-Bis-N-(5,5-dimethyl-3-oxo-1-cyclohexen-1-yl)sorbistin D (3)

From **2a**: A solution of 6.8 g (10.9 m moles) of **2a** in 500 ml of 0.1 N NaOH was refluxed for 8 hours. The reaction mixture was cooled, adjusted to pH 8 with 2 N H_2SO_4 and evaporated to dryness under reduced pressure. The residue was dissolved in a small amount of MeOH and adsorbed on a column of silica gel (190 g). The column was eluted with $CHCl_3$ - MeOH (3 : 1). The anthrone-positive fractions were combined and concentrated under reduced pressure to give 4.39 g (69%) of **3**. Mp 182~184°C, $[\alpha]_D^{23}+196°$ (*c* 0.5, H_2O), $\lambda_{max}^{H_2O}$ 288 nm (ε 53,000). NMR (D_2O): δ 1.05 (12H, s, CH_3), 5.13 (1H, s, vinyl proton), 5.25 (1H, d, J=3.0 Hz, anomeric proton), 5.33 (1H, s, vinyl proton).

Anal Calcd. for $C_{28}H_{47}N_3O_{10}\cdot 2H_2O$: C, 54.09; H, 8.27; N, 6.76.
Found: C, 54.10; H, 8.36; N, 6.20.

From **2b**: A mixture of 642 mg (1 m mole) of **2b** in 50 ml of 0.1 N NaOH was heated at reflux during 8 hours. After cooling, the solution was neutralized with dil.HCl and evaporated to dryness *in vacuo*. The residue was dissolved in a small volume of water. The solution was passed through a HP-20 column (50 ml). The column was washed with water and eluted with 40% aqueous MeOH to give 373 mg (63%) of **3**, which was identical with **3** obtained from **2a** on the basis of mp, IR, TLC.

Preparation of the 4′-N-acyl derivatives (1)

The 4′-N-acyl derivatives (**1**) were prepared by five general procedures (Method A to Method E) consisting of acylation of **3** and subsequent deblocking as described below. Table 1 shows the results of synthesis of the 4′-acyl derivatives including the method employed, yield and physical properties. No attempt was made to determine the conditions necessary for optimum yields. For each of the five methods used there is given a representative preparation.

Method A. 4′-N-Propionylsorbistin D, Synthetic Sorbistin A_1 (1b)

To a stirred solution of 292 mg (0.5 m mole) of **3** in 20 ml of MeOH was added under cooling 130 mg (1 m mole) of propionic anhydride. The mixture was stirred overnight at ambient temperature and then evaporated *in vacuo*. The residue was dissolved in 20 ml of water. The solution was adjusted to pH 2 with dil.HCl, then with stirring treated dropwise with a solution of 69 mg (1 m mole) of $NaNO_2$ in 10 ml of water, stirred for 2 hours at room temperature and washed with $CHCl_3$ (30 ml × 3). The aqueous layer was neutralized with 1 N NH_4OH and evaporated *in vacuo*. The residue was purified by column chromatography on CG-50 (NH_4^+, 50 ml) using 0.05 N NH_4OH as an eluant. The ninhydrin positive and bioactive fractions were combined, evaporated *in vacuo* and freeze-dried to give 77.5 mg (39%) of **1b**, which is identical with natural sorbistin A_1 with respect to IR, mp, Rf value and antimicrobial spectrum.

Method B. 4′-N-Acetylsorbistin D, Synthetic Sorbistin B (1a)

To a stirred solution of 20 mg of **3** in 1 ml of MeOH was added 2 drops of acetic anhydride. The mixture was stirred overnight at room temperature and evaporated to dryness *in vacuo*. The residue was dissolved in a small amount of MeOH. The solution was treated with ether to give the dimedone derivative **2a**, which was collected by filtration. Yield 18.5 mg (87%). The product was identical with **2a** derived from natural **1a** with respect to IR and TLC.

A solution of 64.5 mg of **2a** in 5 ml of water was treated with an aqueous solution of bromine till a permanent yellow color persisted. The reaction mixture was chilled and the precipitated 2,2-dibromodimedone was removed by filtration. The filtrate was adjusted to pH 8 with 1 N NH_4OH and evaporated to

dryness *in vacuo*. The residue was chromatographed on a CG-50 (NH_4^+, 7 ml) column. After washing with water, the column was eluted with 0.05 N NH_4OH. The ninhydrin-positive fractions were combined, concentrated *in vacuo* and lyophilized to give 6 mg (15%) of **1a**, which was identical with natural sorbistin B with respect to IR, Rf value and its antibiotic spectrum.

Method C. 4'-N-Butyrylsorbistin D, Synthetic Sorbistin A_2 (**1c**)

To a chilled and stirred solution of 176 mg (2 m moles) of butyric acid and 230 mg (2 m moles) of N-hydroxysuccinimide (HOSu) in 20 ml of tetrahydrofuran (THF) was added 412 mg (2 m moles) of dicyclohexylcarbodiimide (DCC). The mixture was stirred for 3 hours at 5°C and filtered to remove the dicyclohexylurea. The filtrate was evaporated to dryness and the residue was triturated with a small amount of *n*-hexane to give the N-hydroxysuccinimide ester of butyric acid (258 mg), IR: $\nu_{C=O}$ 1810, 1780, 1730 cm^{-1}. The activated ester (92 mg) was reacted with 300 mg (0.5 m mole) of **3** in 4 ml of DMF. The mixture was stirred at ambient temperature overnight and evaporated to dryness *in vacuo*. The residue was dissolved in 15 ml of water, the blocking group was removed with bromine, and the isolation was performed by CG-50 (NH_4^+, 25 ml) column as described in Method B. Yield 21 mg (10%). The product was identical with natural sorbistin A_2 in respect to mp, Rf and antimicrobial spectrum.

Method D. 4'-N-DL-α-Hydroxypropionylsorbistin D (**1m**)

DL-Lactic acid was converted into the N-hydroxysuccinimido ester in the usual way, IR(liq.): $\nu_{C=O}$ 1820, 1780, 1730 cm^{-1}. The activated ester was condensed with 300 mg (0.51 m mole) of **3** as previously described. The crude product was adsorbed on a column of silica gel (25 g). The column was washed with 100 ml of $CHCl_3$ and eluted with $CHCl_3$ - MeOH (5: 1) to give 242 mg (71%) of the intermediate dimedone derivative. A 240-mg sample (0.356 m mole) of the intermediate was deblocked with aqueous $NaNO_2$. The crude product was purified by chromatography on CG-50 (NH_4^+, 30 ml). The product was eluted with 0.2 N NH_4OH; Yield 20.9 mg (13.5%). IR (KBr): 1660(sh), 1640, 1520, 1060, 1020 cm^{-1}.

Method E. 4'-N-Valerylsorbistin D (**1t**)

There was added 60.3 mg (0.5 m mole) of valeryl chloride to a stirred solution of 293 mg (0.5 m mole) of **3** and 0.14 ml (0.5 m mole) of triethylamine (Et_3N) in 20 ml of DMF. The reaction mixture was stirred overnight at room temperature and evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (30 g) with the lower phase of $CHCl_3$ - MeOH - 28% NH_4OH (1: 1: 1) as an eluant to give (53%) of the intermediate dimedone derivative. This intermediate was deblocked with aq. $NaNO_2$ in the usual manner. The crude product was purified by chromatography on CG-50 (NH_4^+, 30 ml) column, which was eluted with 0.05 N NH_4OH to afford 22.3 mg (20%) of **1t**.

1-N-Propionylsorbistin D (**4a**)

The N-hydroxysuccinimido ester prepared from 74 mg (1 m mole) of propionic acid was allowed to react with 341 mg (1 m mole) of sorbistin D (**1d**) in 20 ml of DMF overnight at room temperature. The reaction mixture was evaporated to dryness. The residue was dissolved in a small amount of water and adsorbed on a column of CG-50 (NH_4^+, 35 ml). The column was eluted with 0.05 N NH_4OH. The fractions containing **4a** were combined, evaporated to dryness *in vacuo* and freeze-dried to yield 164 mg (41%) of **4a**. IR (KBr): 1630, 1570, 1070, 1020 cm^{-1}, NMR(D_2O): δ 1.10 (3H, t, J=7.5 Hz), 2.26 (2H, q, J=7.5 Hz), 2.5~3.0 (1H, m), 3.39 (2H, broad d, J=7.0 Hz), 5.10 (1H, d, J=3.0 Hz).

1-N-Benzyloxycarbonylsorbistin D (**4b**)

To a chilled and stirred solution of 1.705 g (5 m moles) of sorbistin D (**1d**) in 100 ml of DMF was added 1.567 g (5 m moles) of N-(benzyloxycarbonyloxy)-5-norbornene-2,3-dicarboximide (Cbz-ONB). The reaction mixture was stirred for 3.5 hours at 5°C and evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml of water. The solution was adjusted to pH 7 with dil.HCl and adsorbed on a column of CG-50 (NH_4^+, 160 ml), which was eluted with 0.05 N NH_4OH. The ninhydrin-positive fractions containing **4b** were evaporated *in vacuo* and freeze-dried to give 1.07 g (45%) of **4b**. IR(KBr): 1705, 1070, 1010 cm^{-1}.

1-N-(L-4-Amino-2-hydroxybutyryl)sorbistin D (**4c**)

The product was prepared by acylation of sorbistin D (**1d**) with N-(L-4-Cbz-amino-2-hydroxybutyryloxy)succinimide (Cbz-AHB-OSu) similar to the preparation of **4a**. The intermediate Cbz-AHB derivative was subjected to hydrogenolysis with 10% Pd-C followed by column chromatography on CG-50 to give **4c**.

4-N-Propionylsorbistin D (**5a**)

To a stirred solution of 200 mg (0.42 m mole) of **4b** in 20 ml of DMF was added a slight excess of the succinimido ester of propionic acid. The reaction mixture was stirred overnight at room temperature and evaporated *in vacuo*. The residue was hydrogenated with 50 mg of 10% Pd-C in 50% aqueous THF. The reaction mixture was filtered. The filtrate was evaporated *in vacuo*. The residue was chromatographed on a CG-50 column (NH_4^+, 10 ml). The column was developed with 100 ml of water to give 36 mg (19%) of 4,4'-di-N-propionylsorbistin D (**7b**), and then eluted with 0.05 N NH_4OH, collecting each 10-ml fraction. Fractions from 9 to 13 were evaporated and lyophilized to give 53 mg (31%) of **5a**. IR(KBr): 1640, 1550, 1070, 1020 cm^{-1}. NMR(D_2O): δ 1.10 (3H, t, J=7.5 Hz), 2.30 (2H, q, J=7.5 Hz), 2.60~3.30 (3H, m), 5.03 (1H, d, J=3.0 Hz). Evaporation of the fractions 15~22 gave 14 mg (9%) of sorbistin A_1 (**1b**).

4-N-(L-4-Amino-2-hydroxybutyryl)sorbistin D (**5b**)

A sample of **4b** (475 mg, 1 mole) was reacted with 350 mg (1 m mole) of Cbz-AHB-OSu in 30 ml MeOH overnight. The reaction mixture was evaporated under reduced pressure. The residue was hydrogenated with 300 mg of 10% Pd-C in 50 ml of 50% aqueous THF. The reaction mixture was filtered. The filtrate was concentrated. The concentrate was passed through a CG-50 column (NH_4^+, 40 ml), which was washed with 200 ml of water and eluted with 0.1 N NH_4OH (900 ml), 0.2 N NH_4OH (900 ml), 0.3 N NH_4OH (900 ml), 0.5 N NH_4OH (500 ml) and 1 N NH_4OH (300 ml). The eluate was collected in 10-ml fractions. Fractions 50~75 were evaporated and freeze-dried to give 133 mg (30%) of the starting material **4b**. Evaporation of fractions 210 to 245 gave 62 mg (14%) of **5b**. IR(KBr): 1650, 1570, 1070, 1020 cm^{-1}. Fractions 321 to 331 gave 12 mg (3%) of **1f** by evaporation and lyophilization.

1-N-(L-4-Amino-2-hydroxybutyryl)sorbistin A_1 (**6a**)

To a chilled and stirred solution of 416.5 mg (1.05 m moles) of sorbistin A_1 (**1b**) in 30 ml of DMF was added 350 mg (1 m mole) of Cbz-AHB-OSu. The reaction mixture was stirred overnight at 5°C and evaporated to dryness. The residue was hydrogenated overnight with 100 mg of 10% Pd-C at room temperature in 40 ml of aqueous 50% THF. After filtration the filtrate was evaporated and the residue purified by chromatography on a column of CG-50 (NH_4^+, 23 ml) using 0.1 N NH_4OH as an eluant to give 281 mg (55%) of **6a**. IR(KBr): 1640, 1560, 1075, 1020 cm^{-1}. NMR(D_2O+DCl): δ 1.13 (3H, t, J=9 Hz), 1.8~2.6 (4H, m), 2.8~3.4 (2H, m), 5.3 (1H, d, J=3.0 Hz). Elution of the column with 0.3 N NH_4OH gave 15 mg of **7a** which was identical with **7a** derived from **6b**.

1-N-Benzyloxycarbonylsorbistin A_1 (**6b**)

To a chilled and stirred solution of 4.16 g (10.4 m moles) of sorbistin A_1 in 300 ml of MeOH was added 2.49 g (10 m moles) of Cbz-OSu. The reaction mixture was stirred for 20 minutes at 5°C, overnight at room temperature, and evaporated under reduced pressure. The residue was adsorbed on a column of silica gel (120 g), which was eluted with $CHCl_3$ - MeOH (2: 1) to yield 2.97 g (56%) of **6b**. IR(KBr): 1700, 1640, 1555, 1080, 1025 cm^{-1}. NMR(D_2O): δ 1.15 (3H, t, J=7.5 Hz), 2.35 (2H, q, J=7.5 Hz), 5.16 (2H, s), 5.20 (1H, d, J=3 Hz), 7.52 (5H, s).

1-N-Isobutyrylsorbistin A_1 (**6c**)

Sorbistin A_1 (416 mg, 1 m mole) was condensed with 158 mg (1 m mole) of isobutyric anhydride in a usual way in MeOH. The mixture was evaporated to dryness. The residue was chromatographed on a column of silica gel (30 g) with $CHCl_3$ - MeOH (2: 1) to afford 137 mg (24%) of **6c**. IR(KBr): 1640, 1560, 1080, 1025 cm^{-1}.

4-N-(L-4-Amino-2-hydroxybutyryl)sorbistin A_1 (**7a**)

To a stirred solution of 550 mg (1.03 m moles) of **6b** in 30 ml of DMF was added 350 mg (1 m mole) of Cbz-AHB-OSu. The mixture was stirred overnight at 5°C and evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (30 g) with $CHCl_3$ - MeOH (5: 1)

to give 467 mg (60%) of the intermediate 4-N-Cbz-AHB-sorbistin A_1. The starting material **6b** (88 mg, 21%) was recovered from the eluate with $CHCl_3$ - MeOH (1: 1). The intermediate was hydrogenated overnight with 100 mg of 10% Pd-C in 25 ml of 50% aqueous THF. The catalyst was filtered off and the filtrate was evaporated to remove the THF. The resulting aqueous concentrate was chromatographed on a column of CG-50 (NH_4^+, 25 ml) with 0.4 N NH_4OH as an eluant to afford 167 mg (56%) of **7a**. IR (KBr): 1640, 1555, 1075, 1020 cm^{-1}. NMR (D_2O+DCl): δ 1.12 (3H, t, J=9 Hz), 2.3 (2H, q, J=9 Hz), 5.2 (1H, d, J=3 Hz).

N-Benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide (Cbz-ONB)

To a stirred solution of 2.64 g (0.066 mole) of sodium hydroxide and 3.58 g (0.02 mole) of N-hydroxy-5-norbornene-2,3-dicarboximide in 50 ml of water was added dropwise 6.82 g (0.04 mole) of benzyloxycarbonyl chloride over a period of 40 minutes at 0~5°C and the mixture was stirred for 4 hours at room temperature. To the reaction mixture was added 20 ml of *n*-hexane to give a white precipitate which was collected by filtration and air-dried. The crude product was crystallized from benzene - *n*-hexane to give 5.1 g (81%) of colorless prisms, mp 121~122°C. IR(KBr): 1810, 1780, 1740, 1630 cm^{-1}. NMR (acetone-d_6): δ 1.66 (2H, broad s), 3.45 (4H, m), 5.37 (2H, s), 6.12 (2H, t, J=1.5 Hz), 7.48 (5H, s).

Anal. Calcd. for $C_{17}H_{15}NO_5$: C, 65.17; H, 4.83; N, 4.47.
Found: C, 65.50; H, 4.73; N, 4.44.

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