NEW ANTHRACYCLINE ANTIBIOTICS, AURAMYCINS AND SULFURMYCINS

II. ISOLATION AND CHARACTERIZATION OF 10 MINOR COMPONENTS (C~G)

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Following the discovery of new anthracycline antibiotics, auramycins A and B and sulfurmycins A and B, we found 10 minor analogues of auramycins and sulfurmycins, C, D, E, F and G, from the culture broth of a mutant strain of *Streptomyces galilaeus* OBB-111 and prepared 2 analogues as the chemical derivatives from auramycin G and sulfurmycin G. All analogues have a sugar moiety at C-7 position of the aglycones. These analogues exhibit activities against Gram-positive bacteria and P388 leukemia.

In the previous paper, we reported the discovery of new anthracycline antibiotics from the fermentation broth of *Streptomyces galilaeus* OBB-111 (ATCC 31533); auramycins A and B and sulfurmycins A and B and their aglycones, auramycinone, sulfurmycinone, 7-deoxyauramycinone and 7-deoxysulfurmycinone[1,2]. As the strain OBB-111 co-produced aclacinomycin A and its analogues[3,4], it was difficult to isolate other components of auramycins or sulfurmycins which were produced in minor quantities. Therefore, we tried to select a strain which produced only auramycins and sulfurmycins by mutation using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) as a mutagen. As a result, we were able to obtain a mutant strain named OBB-111-610, which lacked the productivity of aclacinomycins. From the fermentation broth of the strain OBB-111-610, we isolated 14 anthracycline glycosides; auramycins A, B, C, D, E, F and G, and sulfurmycins A, B, C, D, E, F and G. We also obtained auramycin H and sulfurmycin H by hydrolysis of auramycin G and sulfurmycin G, respectively.

In this paper, we describe fermentation, isolation, physicochemical properties, structural elucidation and biological activities of these antibiotics.

Results

Physicochemical Properties

All the components of auramycins and sulfurmycins (C~G) are soluble in dimethyl sulfoxide, methanol, ethanol, acetone, ethyl acetate, chloroform, benzene and acidic water, but insoluble in *n*-hexane and water. These components are yellow in hydrochloric acid - methanol and red to dark red in concentrated sulfuric acid. The yellow color in methanol turned to pinkish red by addition of magnesium acetate to the solution. All the components are negative in ninhydrin and FEHLING's reactions. The physicochemical properties of the components are summarized in Table 1. The ultraviolet and visible light absorption spectra of auramycins and sulfurmycins in methanol solution are very similar to each other and the λ_{max}'s are found around 228, 258, 288 and 433 nm. In alkaline solution, however, some differences in absorption spectra ranging 300 to 315 nm were noted between auramycins

Table 1. Physicochemical properties of

	Auramycin C		Auramycin D		Auramycin E		Auramycin F		Auramycin G	
Molecular formula	$C_{35}H_{48}O_{18}N$		$C_{29}H_{33}O_{10}N$		$C_{41}H_{53}O_{15}N$		$C_{41}H_{53}O_{15}N$		$C_{41}H_{53}O_{14}N$	
Elemental analysis (%)	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
C:	61.18	61.29	62.09	62.68	61.45	61.56	61.50	61.56	63.01	62.82
H:	6.29	6.32	5.90	5.99	6.50	6.68	6.73	6.68	6.85	6.81
O:	30.39	30.35	29.31	28.81	30.37	30.01	30.01	30.01	28.46	28.58
N:	2.14	2.04	2.70	2.52	1.68	1.75	1.76	1.75	1.68	1.79
Melting point (°C)	151.0		139.5		157.0		160.0		148.5	
$[\alpha]_D^{20}$ (c 0.1, $CHCl_3$)	+78.8°		+189.3°		+32.3°		+26.7°		+38.5°	
λ_{max} nm ($E_{1cm}^{1\%}$)										
in MeOH	228	(640)	228	(715)	228	(515)	228	(615)	228	(570)
	258	(405)	258	(450)	258	(330)	258	(390)	258	(375)
	288	(155)	288	(170)	288	(125)	288	(150)	288	(150)
	433	(190)	433	(225)	433	(155)	433	(175)	433	(160)
in 0.1 N NaOH/MeOH	219(S)	(355)	219(S)	(400)	219(S)	(280)	239	(640)	239	(585)
	237	(590)	237	(675)	237	(500)	288	(195)	288	(160)
	252(S)	(415)	252(S)	(480)	252(S)	(350)	315	(115)	315	(80)
	288	(155)	288	(170)	288	(125)	525	(120)	525	(175)
	315	(85)	315	(95)	315	(65)				
	525	(190)	525	(210)	525	(155)				
IR spectrum (cm^{-1})	3,450		3,450		3,450		3,450		3,450	
	2,975		2,975		2,980		2,980		2,950	
	2,940		2,925		2,940		2,940		2,920	
	2,825		2,825		2,880		2,880		2,800	
	2,775		2,775		2,820		2,820		2,750	
	1,745		1,740		2,770		2,760		1,740	
	1,685		1,680		1,740		1,740		1,680	
	1,630		1,625		1,680		1,680		1,620	
	1,580		1,570		1,625		1,630		1,610	
					1,610		1,610		1,570	
					1,580		1,580			
Rf value on silica gel TLC										
$CHCl_3$ - MeOH, 7: 1	0.20		0.08		0.44		0.39		0.38	
Benzene - MeOH, 5: 1	0.30		0.14		0.41		0.38		0.40	

and sulfurmycins as shown in Table 1.

The infrared absorption spectra (KBr) of auramycins and sulfurmycins indicate the presence of ester carbonyl (1,740~1,745 cm^{-1}), non-chelated carbonyl (1,680~1,685 cm^{-1}) and chelated carbonyl (1,620~1,630 cm^{-1}), as shown in Table 1.

Structural Elucidation

The structural determination of these components was carried out based on their physicochemical properties and analyses of sugars liberated by hydrolysis of each component. Sugars were analyzed on TLC (silica gel plate, solvent system: 1-butanol - acetic acid - water, 4: 1: 1, visualized by *p*-anisaldehyde). The configuration of each sugar obtained by methanolysis or hydrolysis was determined

auramycins and sulfurmycins.

Sulfurmycin C		Sulfurmycin D		Sulfurmycin E		Sulfurmycin F		Sulfurmycin G	
$C_{37}H_{45}O_{14}N$		$C_{31}H_{35}O_{11}N$		$C_{43}H_{55}O_{16}N$		$C_{43}H_{55}O_{16}N$		$C_{43}H_{55}O_{15}N$	
Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
60.54	61.05	61.97	62.29	61.55	61.34	61.08	61.34	62.30	62.53
6.22	6.24	5.50	5.91	6.60	6.58	6.62	6.58	6.81	6.71
31.29	30.78	30.16	29.46	30.19	30.42	30.60	30.42	29.16	29.06
1.95	1.93	2.37	2.34	1.66	1.66	1.70	1.66	1.73	1.70
146.0		128.0		151.0		151.5		139.0	
+55.8°		+167.6°		+19.6°		+7.9°		+25.6°	
228	(575)	228	(680)	228	(530)	228	(475)	228	(560)
257	(370)	257	(425)	257	(340)	258	(300)	257	(360)
288	(140)	288	(160)	288	(125)	288	(115)	288	(135)
433	(175)	433	(210)	433	(155)	433	(120)	433	(170)
219(S)	(375)	219(S)	(410)	219(S)	(330)	219(S)	(325)	219(S)	(340)
237	(460)	237	(505)	237	(405)	238	(515)	237	(430)
252(S)	(385)	252(S)	(410)	252(S)	(330)	268	(180)	252(S)	(355)
268(S)	(305)	268(S)	(340)	268(S)	(280)	290(S)	(190)	268(S)	(325)
290(S)	(245)	290(S)	(285)	290(S)	(225)	302(S)	(145)	290(S)	(255)
302(S)	(240)	302(S)	(285)	302(S)	(225)	525	(165)	302(S)	(255)
525	(185)	525	(215)	525	(165)			525	(175)
3,450		3,425		3,450		3,450		3,450	
2,975		2,975		2,970		2,975		2,970	
2,925		2,925		2,930		2,940		2,945	
2,800		2,825		2,875		2,880		2,930	
2,750		2,775		2,770		2,825		2,900	
1,740		1,740		1,740		2,770		2,820	
1,680		1,680		1,720		1,740		2,770	
1,620		1,630		1,680		1,720		1,740	
1,570		1,580		1,625		1,680		1,715	
				1,610		1,630		1,680	
				1,575		1,610		1,625	
						1,580		1,610	
								1,580	
0.20		0.08		0.38		0.34		0.33	
0.30		0.14		0.37		0.36		0.36	

by comparison of its PMR and α_D value with the reported data for authentic compounds[4,5].

Because sugar moiety of component C, D, E, F or G was the same in sulfurmycins and auramycins, we only deal in this report with the structures of sulfurmycin C, D, E, F and G.

All components released sulfurmycinone as an aglycone by total hydrolysis (0.1 N hydrochloric acid, 90°C, 60 minutes). The sugar terminal of these components were proved to attach to C-7 position of sulfurmycinone, which was confirmed by the difference of the CMR chemical shift of C-7 of sulfurmycinone and its glycosides as shown in Table 2.

Sulfurmycin D: Sulfurmycin D gave sulfurmycinone and L-rhodosamine by hydrolysis (0.1 N hydrochloric acid, 90°C, 60 minutes). The structure of sulfurmycin D was estimated to be L-rhodo-

Table 2. The CMR chemical shift assignments.

Carbon No.	Sulfurmycin C	Sulfurmycin D	Sulfurmycin E	Sulfurmycin F	Sulfurmycin G	Sulfur-mycinone
5	192.6	192.6	192.6	192.6	192.6	192.6
12	181.1	181.2	181.2	181.2	181.2	181.1
16	170.8	170.6	170.8	170.8	170.8	170.7
4	162.5	162.6	162.5	162.5	162.5	162.4
6	162.0	162.2	162.0	162.0	162.1	161.6
10a	141.7	141.7	141.7	141.7	141.6	140.8
2	137.3	137.3	137.3	137.3	137.3	137.3
12a	133.4	133.4	133.4	133.4	133.4	133.4
6a	132.7	132.8	132.7	132.7	132.7	133.3
11a	131.2	131.1	131.3	131.3	131.3	132.4
3	124.8	124.8	124.7	124.7	124.7	124.8
11	121.0	121.0	121.0	121.0	121.0	121.0
1	120.1	120.1	120.1	120.1	120.1	120.1
4a	115.8	115.8	115.8	115.8	115.8	115.7
5a	114.7	114.7	114.7	114.7	114.6	114.6
9	71.0	70.7	71.0	71.0	71.0	71.9
7	69.9	70.6	69.9	69.9	69.9	61.7
10	56.7	56.2	56.7	56.7	56.8	55.4
17	52.7	52.7	52.6	52.6	52.6	52.7
8	34.7	35.2	34.8	34.8	34.7	36.8
13	51.0	50.7	51.0	51.0	51.0	50.5
14	207.0	207.7	205.9	206.8	206.8	209.6
15	32.5	32.2	32.5	32.5	32.5	31.7
1′	101.4	100.9	101.5	101.5	101.5	
2′	29.2	27.6	29.2	29.3	29.2	
3′	61.6	61.1	61.5	61.5	61.7	
4′	74.2	65.6	74.0	74.1	73.9	
5′	66.2	66.9	66.9	66.9	66.9	
6′	16.7	16.9	17.0	17.0	17.1	
N-Me_2	43.2	41.8	43.2	43.2	43.2	
1″	99.1		99.4	100.3	99.5	
2″	33.0		34.3	34.4	23.7	
3″	65.9		65.6	65.6	24.9	
4″	71.5		83.7	83.7	75.4	
5″	68.5		68.5	68.5	66.7	
6″	18.0		17.9	17.9	18.0	
1‴			99.4	99.5	98.6	
2‴			27.2	24.0	24.6	
3‴			29.5	25.7	26.0	
4‴			71.5	67.5	67.5	
5‴			71.5	68.3	68.8	
6‴			17.7	17.0	17.1	

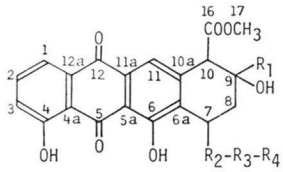
saminylsulfurmycinone. The CMR spectrum of the sugar moiety of sulfurmycin D coincided with that of 1-deoxypyrromycin[4]. Sulfurmycin D was also obtained by partial hydrolysis of sulfurmycins A, B, C, E, F, G and H.

Sulfurmycin C: Sulfurmycin C gave sulfurmycin D and methyl 2-deoxy-L-fucoside by partial methanolysis. 2-Deoxy-L-fucose was proved to attach at C-4 position of L-rhodosamine from the observation of the change in the chemical shift of C-4′ position (Table 2). The CMR spectra of the sugar moieties of sulfurmycin C and MA-144S1[4] were identical to each other. It was estimated sulfurmycin C had a sugar sequence of L-rhodosaminyl-2-deoxy-L-fucoside.

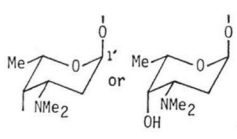
Sulfurmycins E and F: Sulfurmycins E and F were appeared to correspond to the two compounds which were obtained by reduction of sulfurmycin A with $NaBH_4$. These two compounds were stereoisomers of hydroxyl group at C-4‴ position generated by the reduction of carbonyl group of L-cinerulose. It was estimated that sulfurmycin E had L-amicetose and sulfurmycin F had L-rhodinose as the third sugar by the comparison of their CMR spectra with MA-144M1 and MA-144N1[4].

Sulfurmycins G and H: Sulfurmycin G had three sugars in it, which was confirmed by the carbon number of 43 in the CMR spectrum. By acid hydrolysis (0.1 N hydrochloric acid, 90°C, 60 minutes), only two kinds of sugar were noted on TLC, namely, L-rhodosamine and L-rhodinose. When sulfurmycin G was hydrolyzed under mild condition (0.1 N hydrochloric acid at room temperature for

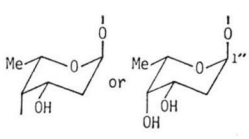
Table 3. Structures of auramycins and sulfurmycins.



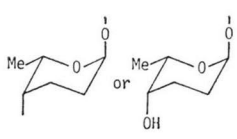
Compound	R_1	R_2	R_3	R_4
Auramycin				
C	$-\overset{13}{C}H_3$	L-Rhodosamine	2-Deoxy-L-fucose	
D	〃	〃		
E	〃	〃	2-Deoxy-L-fucose	L-Amicetose
F	〃	〃	〃	L-Rhodinose
G	〃	〃	L-Rhodinose	〃
H	〃	〃	〃	
Sulfurmycin				
C	$-\overset{13}{C}H_2\overset{14}{C}O\overset{15}{C}H_3$	L-Rhodosamine	2-Deoxy-L-fucose	
D	〃	〃		
E	〃	〃	2-Deoxy-L-fucose	L-Amicetose
F	〃	〃	〃	L-Rhodinose
G	〃	〃	L-Rhodinose	〃
H	〃	〃	〃	



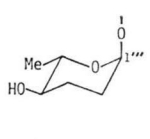
L-Rhodosamine



2-Deoxy-L-fucose



L-Rhodinose



L-Amicetose

30 minutes), a new component, designated sulfurmycin H, and L-rhodinose were obtained. Sulfurmycin H gave sulfurmycin D and L-rhodinose by partial hydrolysis (0.1 N hydrochloric acid, 25°C, 30 minutes). The structure of sulfurmycin H was determined to be L-rhodinosyl-L-rhodosaminylsulfurmycinone. Thus the structure of sulfurmycin G was estimated to be L-rhodinosyl-L-rhodinosyl-L-rhodosaminylsulfurmycinone.

Structures of auramycins C, D, E, F, G and H were determined by the same methods as described above. The structures which we proposed were confirmed by the comparison of their physicochemical properties with those of aclacinomycins reported by T. OKI *et al.*[3,4]. All the structures of these antibiotics are summarized in Table 3.

Biological Activities

Antimicrobial activities of auramycins and sulfurmycins are shown in Table 4. Minimum inhibitory concentrations (MIC) of antibiotics were determined by agar dilution method. All the components showed marked antibacterial activity against Gram-positive bacteria.

Antitumor activities of auramycins and sulfurmycins were evaluated by prolongation of the median survival time of mice bearing P388 leukemia. Antibiotics were administered intraperitoneally on day 1, 5 and 9. All the components showed antitumor activity at a dose range of 1.5~15 mg/kg/day. Detailed report on antitumor activities of auramycins and sulfurmycins will be reported elsewhere.

Table 4. Antimicrobial activities of auramycins and sulfurmycins.

Strain	Medium	MIC (μg/ml)									
		AM-C**	AM-D	AM-E	AM-F	AM-G	SM-C***	SM-D	SM-E	SM-F	SM-G
Staphylococcus aureus 209P IAM 1011	(1)*	6.25	12.5	1.56	3.12	6.25	6.25	25.0	3.12	3.12	6.25
Micrococcus luteus IAM 1009	(1)	6.25	6.25	3.12	3.12	6.25	6.25	25.0	3.12	1.56	12.5
Micrococcus flavus ATCC 10240	(1)	6.25	12.5	3.12	3.12	6.25	6.25	25.0	3.12	1.56	12.5
Bacillus subtilis IAM 1027	(1)	6.25	12.5	3.12	3.12	12.5	12.5	12.5	3.12	1.56	6.25
Pseudomonas aeruginosa IFO 12689	(1)	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Escherichia coli K-12 IAM 1264	(1)	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Escherichia coli NIHJ IFO 12734	(1)	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Candida albicans ATCC 10231	(2)	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Candida tropicalis ATCC 13803	(2)	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

* (1); Heart infusion agar. (2); Sabouraud dextrose agar.
** AM; auramycin.
*** SM; sulfurmycin.

Discussions

We obtained 14 anthracycline glycosides in total (auramycins A, B, C, D, E, F and G, and sulfurmycins A, B, C, D, E, F and G) from the culture broth of *Streptomyces galilaeus* OBB-111-610. The sugar moieties of components A, B, C, D, E and F correspond to those of the components A1, B1, S1,

T1, M1 and N1 of aclacinomycins reported by T. OKI *et al.*[4] The sugar moiety of component G was determined to be rhodinosyl-rhodinosyl-rhodosamine, which was first reported in rhodirubin B[6]. When we compared the antimicrobial activity of auramycins F and G (or sulfurmycins F and G) as shown in Table 4, component G showed a lower activity than F. The structural difference between the two is the presence or absence of OH group at C-3'' position of the second sugar.

From these results, it is suggested that OH group at C-3'' position plays an important role in the antimicrobial activity of these anthracycline antibiotics.

Experimental

General

Melting points were determined with a Yanagimoto micro melting point apparatus, type MP-S3, and were not corrected. Infrared absorption spectra were measured with a Hitachi EPI-G3 spectrophotometer (KBr pellets) and mass spectra with a Hitachi RMU-6M spectrometer and JEOL JMS-D300 mass spectrometer. NMR spectra were recorded on a JEOL FX-100 spectrometer. Chemical shifts are given in δ values with tetramethylsilane as an internal standard. Abbreviation: s=singlet, d=doublet, t=triplet, bs=broad singlet, q=quartet and m=multiplet. Optical rotations were determined using a Perkin-Elmer polarimeter.

For thin-layer chromatography, silica gel F_{254} plates (Merck Co.) were used. For sugar analysis, the plates were sprayed with 5% *p*-anisaldehyde and 5% sulfuric acid in ethanol and heated at 90°C for color development. Column chromatography was carried out with silica gel (Merck Co.), 0.063 to 0.2 mm, and Sephadex LH-20 (Pharmacia Fine Chemicals Co.). Preparative HPLC was carried out with a Prep PAK-500/SILICA (Waters Associates, Inc.) column and Prep LC/System 500 (Waters).

Fermentation

The spores of the agar slant of *Streptomyces galilaeus* OBB-111-610 were used to inoculate the medium containing glucose 20 g, soluble starch 20 g, S-3 meat (Ajinomoto Co., Ltd.) 5 g, yeast extract (Daigo Eiyo Kagaku Co., Ltd.) 2.5 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 1 g, NaCl 3 g and $CaCO_3$ 3 g in 1 liter of tap water. The seed culture was incubated at 27°C on a rotary shaker (180 rpm) for 72 hours. Six hundred ml of the seed culture was transferred into a 50-liter jar fermentor containing 30 liters of sterile medium consisting of glucose 600 g, soluble starch 600 g, Pharmamedia (Traders Oil Mill Co., U.S.A.) 300 g, K_2HPO_4 30 g, $MgSO_4 \cdot 7H_2O$ 30 g, NaCl 90 g, $CaCO_3$ 90 g and Nissan Disfoam (Nippon Yushi Co., Ltd.) 30 g. The fermentation was carried out at 27°C with agitation of 350 rpm and aeration of 30 liters/minute for 90 hours.

Extraction and Isolation of Antibiotics

The culture (240 liters) was centrifuged to separate cells from the culture broth. The cells were extracted with 120 liters of methanol, to which 240 liters each of chloroform and water were added and stirred. After standing, chloroform layer was separated. The supernatant, on the other hand, was extracted with 480 liters of the solvent mixture of chloroform and methanol (1: 1, v/v) and chloroform layer was separated.

Both chloroform extracts were combined and evaporated to a small volume (400 ~ 500 ml), to which *n*-hexane was added to precipitate yellow solid. After drying *in vacuo*, 37 g of yellow powder was obtained which contained auramycins, sulfurmycins and their aglycones. Sephadex LH-20 soaked for 15 hours in a solvent mixture of chloroform and methanol (2: 1, v/v) was packed into a column of 8 cm in diameter and 80 cm in length. The powder of the antibiotic complex (37 g) was dissolved in 50 ml of a solvent mixture of chloroform and methanol (2: 1, v/v) and applied to the column. On eluting with the same solvent mixture, two distinct yellow bands were detected. The fractions of the first band were combined and the solvent was evaporated under reduced pressure to obtain 16.8 g of yellow powder. Analysis on TLC (toluene - methanol, 9: 1) indicated that these fractions contained anthracycline glycosides. The fractions of the second band, which contained aglycones, were collected and concentrated *in vacuo* to obtain 9.13 g of yellow powder.

The yellow powder (16.8 g) containing anthracycline glycosides was dissolved in 20 ml of chloro-

form and applied to a column of 5 cm in diameter and 50 cm in length packed with silica gel. By gradient elution with chloroform containing increasing amount of methanol, 14 components were fractionated. Each component was further purified by preparative silica gel thin-layer chromatography and/or preparative high performance liquid chromatography (Prep. PAK-500/Silica, Waters Assoc. Inc., solvent system; dichloromethane - methanol, 99: 1 ~ 90: 10). Antibiotics were recovered as yellow powder: Auramycin A, 432 mg; sulfurmycin A, 525 mg; auramycin B, 784 mg; sulfurmycin B, 979 mg; auramycin C, 72 mg; sulfurmycin C, 230 mg; auramycin D, 7 mg; sulfurmycin D, 11 mg; auramycin E, 12 mg; sulfurmycin E, 13 mg; auramycin F, 13 mg; sulfurmycin F, 18 mg; auramycin G, 45 mg and sulfurmycin G, 167 mg.

Total Hydrolysis of Sulfurmycin D and Other Components

A solution of sulfurmycin D (100 mg) in 20 ml of 0.1 N hydrochloric acid was heated at 90°C for 60 minutes. A yellow precipitate (50 mg) was recovered and crystallized from a *n*-hexane - chloroform mixture to give sulfurmycinone (35 mg) as yellow needles, mp 153°C. The hydrochloric acid solution was neutralized and its sugar composition was examined by TLC (silica gel plate, solvent system: 1-butanol - acetic acid - water, 4: 1: 1). One sugar corresponding to rhodosamine was detected at Rf 0.12 with sky-blue color. Rhodosamine, mp 137°C, $[\alpha]_D^{20}$ −46.5°, was isolated by preparative TLC and crystallized from methanol [*cf.* L-rhodosamine[4], mp 138.5 ~ 139.5°C, $[\alpha]_D^{20}$ −46.7° (*c* 0.1, H_2O)].

Other components were also hydrolyzed and analyzed by the same method as in sulfurmycin D. As a result all the components liberated sulfurmycinone as an aglycone. Sulfurmycin C released rhodosamine and 2-deoxyfucose, and sulfurmycin E released rhodosamine, 2-deoxyfucose and amicetose, and sulfurmycin F released rhodosamine, 2-deoxyfucose and rhodinose, and sulfurmycin G released rhodosamine and two moles of rhodinose as a sugar moiety.

Partial Methanolysis

Sulfurmycin C: To a solution of 100 mg of sulfurmycin C in 20 ml of dry acetone and 1 ml of methanol was added 1 ml of 0.2 N methanolic hydrogen chloride with stirring and reacted at room temperature for 30 minutes. The reaction mixture was neutralized with silver carbonate, filtered and evaporated. The residue was extracted with ethyl ether. The insoluble material was chromatographed on a column packed with silica gel (chloroform - methanol, 195: 5) to yield 43 mg of sulfurmycin D, mp 128°C. The ether extract was purified by preparative TLC with ethyl acetate to give 4 mg of methyl 2-deoxy-α-L-fucoside of colorless oil, $[\alpha]_D$ −110° [Reference 4: $[\alpha]_D$ −119° ($CHCl_3$)].

Sulfurmycin E: To a solution of 200 mg of sulfurmycin E in 40 ml of dry acetone was added 10 ml of 0.1 N methanolic hydrogen chloride. The reaction mixture was allowed to stand at 10°C for 30 minutes and neutralized with silver carbonate, filtered and evaporated. The residue was extracted with ethyl ether. From the ether-insoluble material, there was obtained 75 mg of sulfurmycin D, mp 128°C.

The ether extract was purified by preparative TLC to give 3.5 mg of colorless powder, methyl 4-*O*-(α-L-amicetosyl)-2-deoxy-α-L-fucoside, mp 101 ~ 102°C, $[\alpha]_D$ −165° ($CHCl_3$) [Reference 4: $[\alpha]_D$ −176° ($CHCl_3$)].

Chemical Conversion of Sulfurmycin A to E and F

To a solution of 500 mg of sulfurmycin A in 20 ml of ethyl acetate was added 50 mg of $NaBH_4$ in 20 ml of water with stirring. The reaction was carried out at room temperature for 20 minutes with stirring. The reaction mixture was washed with 20 ml of water twice, dehydrated over sodium sulfate and concentrated *in vacuo* to dryness. The residue was purified by silica gel TLC with chloroform - methanol - NH_4OH (400: 30: 3). The product was separated into two bands; the faster was identified as sulfurmycin E and the slower was F. Each band was scraped off and extracted, concentrated and precipitated with *n*-hexane to give yellow powder; properties of sulfurmycin E (162.5 mg) and F (30.5 mg) are shown in Table 1.

Partial Hydrolysis of sulfurmycin F

A solution of 100 mg of sulfurmycin F in 25 ml of 0.5% hydrochloric acid was allowed to stand at room temperature for 25 minutes. The reaction mixture was neutralized by diluted sodium hy-

droxide solution and extracted with 50 ml of chloroform twice. The extract was concentrated and purified by preparative TLC with chloroform - methanol (5: 1) to yield 45 mg of sulfurmyin C.

The neutralized aqueous layer was concentrated and purified by preparative TLC with chloroform - methanol (1: 2) to yield 2 mg of a colorless oil; L-rhodinose, $[\alpha]_D -10°$ ($CHCl_3$) [Reference 4: $[\alpha]_D -10°$ ($CHCl_3$)].

Partial Hydrolysis of Sulfurmycin G

A solution of 100 mg of sulfurmycin G in 4 ml of 0.1 N hydrochloric acid was allowed to stand at room temperature for 30 minutes. The reaction mixture was neutralized by diluted sodium hydroxide solution and extracted with 4 ml of ethyl acetate twice and concentrated to dryness. The residue was purified by preparative TLC with chloroform - methanol (5: 1). The extract from TLC plate was concentrated and precipitated with *n*-hexane to yield yellow powder; sulfurmycin H (25 mg), mp 98.5°C, ν_{max}^{KBr} cm^{-1}: 3425, 2930, 1740, 1720, 1680, 1630, 1610, molecular formula: $C_{37}H_{45}O_{13}N$, M.W. 711.3, PMR ($CDCl_3$) δ in ppm: 1.1 ~ 3.07 (m, overlap), 2.24(s), 2.26(s), 3,59(m), 3.69(s), 4.0(m), 4.27(s), 4.54(q), 4.68(s), 4.95(s), 5.18(bs), 5.20(bs), 5.53(s), 7.30(d), 7.67(t), 7.70(s), 7.83(d).

The neutralized aqueous layer was concentrated and purified by preparative TLC with chloroform - methanol (1: 2) to yield L-rhodinose, $[\alpha]_D -10°$ ($CHCl_3$).

Partial Hydrolysis of Sulfurmycin H

A solution of 100 mg of sulfurmycin H in 4 ml of 0.1 N hydrochloric acid was allowed to stand at 25°C for 30 minutes. The reaction mixture was neutralized by diluted sodium hydroxide solution, extracted with 4 ml of ethyl acetate twice, concentrated and precipitated with *n*-hexane, to yield 45 mg of sulfurmycin D.

The neutralized aqueous layer was concentrated and purified by preparative TLC with chloroform - methanol (1: 2) to yield L-rhodinose, $[\alpha]_D -10°$ ($CHCl_3$).

Partial Hydrolysis of Auramycin G

Auramycin G was also hydrolyzed and analyzed by the same method as in sulfurmycin G. Starting from 100 mg of auramycin G, 20 mg of auramycin H was obtained as a yellow powder, mp 119.5°C, ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2920, 2800, 2750, 1740, 1680, 1620. Molecular formula: $C_{35}H_{43}O_{12}N$, M.W. 669.3.

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