SAQUAYAMYCINS, NEW AQUAYAMYCIN-GROUP ANTIBIOTICS

TAKESHI UCHIDA, MASAYA IMOTO, YOSHINORI WATANABE, KEIKO MIURA, TOSHIKO DOBASHI, NAOKO MATSUDA, TSUTOMU SAWA, HIROSHI NAGANAWA, MASA HAMADA, TOMIO TAKEUCHI and HAMAO UMEZAWA

Institute of Microbial Chemistry
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication May 23, 1985)

From the culture broth of *Streptomyces nodosus* MH190-16F3, four new antibiotics have been isolated, and named saquayamycins A, B, C and D. The compounds are glycosides of aquayamycin, and among aquayamycin-group antibiotics they are most closely related to P-1894B (vineomycin A_1). All saquayamycins act on Gram-positive bacteria and inhibit the growth of adriamycin-sensitive and adriamycin-resistant P388 leukemia cells.

In the course of our screening study for new antitumor antibiotics, which are effective on both adriamycin-sensitive (P388/S) and adriamycin-resistant (P388/ADR) sublines of P388 leukemia, new aquayamycin[1]-group antibiotics were isolated from the culture broth of a strain classified as *Streptomyces nodosus* MH190-16F3. All these antibiotics consisted of aquayamycin (an aglycone) and three sugar molecules, and we named them saquayamycins A (**1**), B (**2**), C (**3**) and D (**4**) (Fig. 1). This paper describes the isolation, physical properties, structure elucidation and biological activities of the new antibiotics.

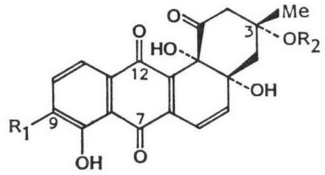
Results

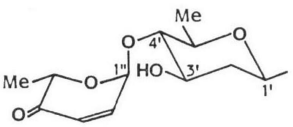
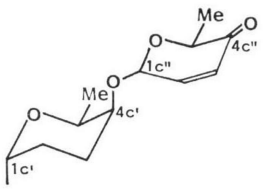
Fermentation and Isolation Procedures

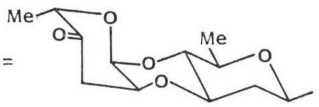
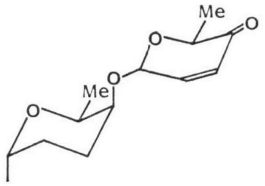
The producing strain was isolated from a soil sample collected in Tobata-ku, Kitakyushu, Japan. Spores obtained from a well-sporulated slant of this organism were inoculated to the medium which consisted of 2.0% galactose, 2.0% dextrin, 1.0% Bacto-soytone (Difco), 0.5% corn steep liquor and 0.1% $CaCO_3$ (pH 7.4), and shake-cultured for 48 hours in a 500-ml Erlenmeyer flask containing 110 ml of the medium described above. Thereafter 2 ml portions of the culture were inoculated to 500-ml Erlenmeyer flasks containing 110 ml of the same medium to produce saquayamycins. Fermentation was carried out at 27°C under agitation on a rotary shaker at 180 rpm. After 72 hours, fermentation broth of each flask was combined and centrifuged to separate filtrate and mycelia. The production of the antibiotics was assayed by a cylinder-plate method testing for the activity against *Micrococcus lysodeikticus* IFO 3333. Various saquayamycins were detected by silica gel thin-layer chromatography (TLC) (Kieselgel 60 F_{254}, Merck) developed with $CHCl_3$ - EtOAc - AcOH (10: 10: 0.1) and reverse-phase high performance liquid chromatography (Nucleosil $5C_{18}$, Macherey-Nagel) with a mobile phase consisting of acetonitrile - 0.02 M phosphate buffer (pH 3.0) (60: 40).

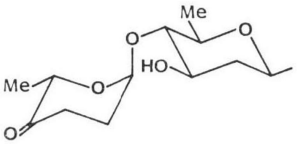
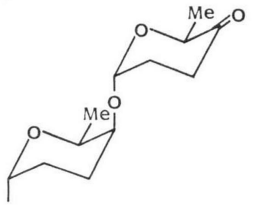
Active materials in the filtrate (8 liters) were extracted with BuOAc (8 liters) at pH 7.0, and concentrated *in vacuo*. From the crude dark brown powder (770 mg) thus obtained, saquayamycins A (32 mg) and B (45 mg) were isolated by silica gel column chromatography with $CHCl_3$ - EtOAc (100: 0 ~ 100: 4) followed by silica gel TLC with $CHCl_3$ - MeOH (100: 2). The antibiotics in the

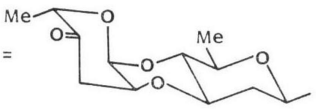
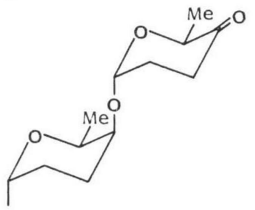
Fig. 1. Structures of saquayamycins.

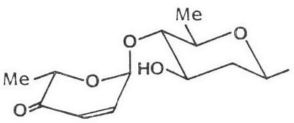


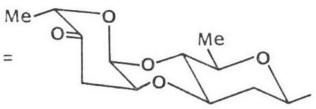
Saquayamycin A (**1**) R_1 =  R_2 = 

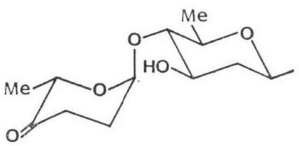
Saquayamycin B (**2**) R_1 =  R_2 = 

Saquayamycin C (**3**) R_1 =  R_2 = 

Saquayamycin D (**4**) R_1 =  R_2 = 

Saquayamycin A1 (**5**) R_1 =  R_2 = H

Saquayamycin B1 (**6**) R_1 =  R_2 = H

Saquayamycin C1 (**7**) R_1 =  R_2 = H

mycelia-cake were extracted with MeOH (1 liter) and concentrated to dryness. After washing with hexane, the residue was partitioned with BuOAc - H_2O (1 : 1) (1 liter), and the organic layer was concentrated *in vacuo* to give a dark brown crude powder (450 mg). From the curde powder, saquaya-

Table 1. Physico-chmical properties of saquayamycins.

	Saquayamycin						
	A (**1**)	B (**2**)	C (**3**)	D (**4**)	A1 (**5**)	B1 (**6**)	C1 (**7**)
MP (°C, dec)	149~152	164~166	142~145	152~155	166~168	180~182	158~160
$[\alpha]_D^{24}$ ($CHCl_3$)	+77°	+96°	−53.3°	+10.0°	+117°	+148°	+43°
	(*c* 0.4)	(*c* 0.2)	(*c* 0.3)	(*c* 0.2)	(*c* 0.2)	(*c* 0.2)	(*c* 0.2)
FD-MS *m/z*	596	596	598	596	596	596	598
	$(M-224)^+$	$(M-224)^+$	$(M-226)^+$	$(M-226)^+$	$(M)^+$	$(M)^+$	$(M)^+$
Molecular formula	$C_{43}H_{48}O_{16}$	$C_{43}H_{48}O_{16}$	$C_{43}H_{52}O_{16}$	$C_{43}H_{50}O_{16}$	$C_{31}H_{32}O_{12}$	$C_{31}H_{32}O_{12}$	$C_{31}H_{34}O_{12}$
UV λ_{max} nm ($E_{1cm}^{1\%}$)	218 (372)	218 (358)	218 (318)	218 (299)	218 (488)	218 (447)	220 (485)
in 0.1 N HCl -	317 (59)	317 (65)	316 (58)	318 (55)	316 (84)	316 (85)	318 (110)
90% MeOH	425 (64)	425 (68)	425 (65)	425 (63)	425 (89)	425 (92)	425 (95)
IR ν_{max} cm^{-1} (KBr)	1730	1735	1730	1730	1725	1730	1730
	1700	1700	1640	1640	1700	1640	1640
	1650	1640			1640		
Rf value*	0.51	0.64	0.44	0.59	0.31	0.40	0.30

* Solvent system: $CHCl_3$ - EtOAc - AcOH (10: 10: 0.1).

mycins C (5.9 mg) and D (6.6 mg) were isolated by silica gel TLC with $CHCl_3$ - EtOAc - AcOH (10: 10: 0.2). Saquayamycins A1 (**5**), B1 (**6**) and C1 (**7**) (Fig. 1) were obtained by mild acid hydrolysis of saquayamycins A, B and C with acetonitrile - 0.2% phosphoric acid (60: 40) at 37°C for 2 hours, respectively.

Physico-chemical Properties

Each compound was obtained as a orange powder, and was soluble in methanol, ethanol, acetone, $CHCl_3$, EtOAc and BuOAc, but insoluble in water, *n*-hexane and petroleum ether. These compounds are positive to ferric chloride, potassium permanganate and *p*-anisaldehyde-sulfuric acid reactions, but negative to ninhydrin and Ehrlich reactions. They contained no nitrogen and no halogen. The other physical properties and ^{13}C NMR data of saquayamycins A, B, C, D, A1, B1 and C1 are listed in Tables 1 and 2, respectively. The ^1H NMR data of saquayamycins A, B, C and D are shown in Table 3.

The UV spectra in 0.1 N HCl - 90% MeOH were very similar to that of aquayamycin, and the IR, ^1H NMR and ^{13}C NMR spectra suggested the presence of hexose moieties.

Structure Elucidation of Saquayamycins

The structures of these saquayamycins have been determined by their spectral analysis and chemical degradation studies, which are summarized in Fig. 2.

Mild methanolysis of **1** with 0.05 N HCl - MeOH at room temperature for 5 minutes yielded a pigment **5** and a methyl glycoside **8**, which were purified by silica gel TLC with benzene - EtOAc (3: 1). The glycoside **8** was shown by direct comparison of its $[\alpha]_D^{25}$, Rf value on TLC and ^1H NMR spectrum to be identical with a methyl disaccharide composed of L-rhodinose and L-aculose, which had been obtained from P-1894B[2] (vineomycin A_1[3]) and ditrisarubicin C[4]. Comparing the ^{13}C NMR spectra of **1** and **5**, a down-field shift was observed with the C-3 in **1**, but not with the C-4a and C-12b (Table 2). These data suggested that the disaccharide is attached to the C-3 in **1**. The mode of the glycosidic linkage was indicated to be α by the coupling constant (J=3, ~1 Hz) of the anomeric proton at the C-1c′ of **1**.

Table 2. ^{13}C Chemical shift assignments[a] of saquayamycins.

Carbon	Saquayamycin						
	A (1)	B (2)	C (3)	D (4)	A1 (5)	B1 (6)	C1 (7)
1	204.7	204.7	204.7	204.7	204.8	204.7	204.8
2	50.2	50.2	50.2	50.2	52.1	52.1	52.1
3	82.4	82.4	82.4	82.4	76.0[b]	75.99[b]	76.0[b]
4	44.5	44.5	44.5	44.5	43.3	43.3	43.3
4a	79.9	79.9	79.9	79.9	80.5	80.5	80.5
5	145.6	145.6	145.6	145.6	144.3	144.3	144.3
6	117.4	117.4	117.5	117.4	117.5	117.5	117.5
6a	138.8	138.8	138.8	138.8	138.8	138.8	138.8
7	188.2	188.3	188.2	188.3	188.0	188.0	187.9
7a	114.0	114.0	114.0	114.0	114.0	114.0	114.0
8	158.0	157.9	158.1	157.9	158.1	158.0	158.1
9	138.2	137.8	138.4	137.8	138.4	138.0	138.6
10	133.5	133.7	133.6	133.7	133.7	133.8	133.7
11	119.6	119.6	119.6	119.6	119.8	119.8	119.7
11a	130.5	130.6	130.5	130.6	130.4	130.4	130.4
12	182.2	182.2	182.2	182.2	182.0	182.0	182.0
12a	138.9	138.9	138.9	138.9	138.2	138.2	138.2
12b	77.4	77.4	77.5	77.5	76.1[b]	76.04[b]	76.1[b]
13	25.5	25.5	25.5	25.5	30.3	30.3	30.3
1′	71.1	71.5	71.11[b]	71.5	71.1	71.5	71.1[b]
2′	38.9	36.8	38.8	36.8	38.9	36.7	38.8
3′	71.3	76.8	71.21[b]	76.8	71.3	76.8	71.2[b]
4′	89.3	74.5	88.7	74.5	89.4	74.5	88.6
5′	74.4	74.6	74.5	74.6	74.4	74.6	74.5
6′	18.4	17.4	18.3	17.4	18.4	17.5	18.3
1″	95.2	91.4	99.6	91.4	95.2	91.4	99.6
2″	142.1	71.2	28.3	71.24[b]	142.1	71.2	28.2
3″	127.3	40.0	33.4	40.0	127.3	40.0	33.4
4″	195.1	207.6	209.2	207.6	195.1	207.6	209.2
5″	71.6	77.7	72.0	77.7	71.6	77.7	72.0
6″	15.2	16.2	14.8	16.2	15.2	16.2	14.8
1c′	92.4	92.5	92.6	92.6			
2c′	24.7	24.8	24.8[c]	24.8[c]			
3c′	24.5	24.6	24.7[c]	24.7[c]			
4c′	76.2	76.2	74.7	74.7			
5c′	67.0	67.0	67.2	67.2			
6c′	17.1	17.1	17.1	17.1			
1c″	95.3	95.3	99.0	99.0			
2c″	143.0	143.0	28.4	28.4			
3c″	127.3	127.3	33.5	33.5			
4c″	196.7	196.7	210.8	210.8			
5c″	70.7	70.7	71.16[b]	71.18[b]			
6c″	15.1	15.2	14.9	14.9			

[a] Measured in $CDCl_3$ at 100 MHz with TMS as an internal reference. Chemical shift assignments were made on the bases of C-H chemical shift correlation experiments and long-range proton decoupling experiments.

[b,c] Assignments may be interchangeable among the signals.

Table 3. ^{1}H NMR spectral data[a] of saquayamycins A, B, C and D.

Proton	Saquayamycin			
	A (1)	B (2)	C (3)	D (4)
2-CH_2 ax	2.51 d	2.51 d	2.51 d	2.51 d
eq	3.18 dd	3.19 dd	3.20 dd	3.20 dd
4-CH_2 ax	1.85 d	1.84 d	1.84 d	1.84 d
eq	2.29 dd	2.29 dd	2.29 dd	2.29 dd
5-H	6.46 d	6.45 d	6.45 d	6.45 d
6-H	6.91 d	6.91 d	6.91 d	6.91 d
8-OH	12.29 s	12.30 s	12.30 s	12.30 s
10-H	7.88	7.89 d	7.88 d	7.89 d
11-H	7.62	7.61 d	7.62 d	7.61 d
13-Me	1.41 s	1.41 s	1.41 s	1.41 s
1′-H	4.89 dd	4.97 dd	4.89 dd	4.97 dd
2′-CH_2 ax	1.38~1.48 m	1.43 ddd	1.35~1.50 m	1.35~1.45 m
eq	2.57 ddd	2.45 ddd	2.55 ddd	2.40~2.55 m
3′-H	3.91 dddd	3.81 ddd	3.86 dddd	3.81 ddd
3′-OH	4.26 d	—	4.52 d	—
4′-H	3.21 dd	3.48 t	3.15 dd	3.48 t
5′-H	3.58 dq	3.57 dq	3.56 dq	3.57 dq
6′-Me	1.39 d	1.40 d	1.38 d	1.40 d
1″-H	5.38 d (J=3.5 Hz)	5.19 d	5.18 t	5.19 d
2″-H	6.85 dd	4.34 ddd	—	4.34 ddd
2″-CH_2 ax	—	—	~2.1 m	—
eq	—	—	2.35~2.55 m	—
3″-H	6.15 d (J=10.2 Hz)	—	—	—
3″-CH_2	—	2.64 m	2.35~2.55 m	2.64 m
	—	2.68 m		2.67 m
5″-H	4.76 q	4.72 q	4.50 q	4.73 q
6″-Me	1.45 d	1.38 d	1.36 d	1.39 d
1c′-H	5.26 br d (J=3, ~1 Hz)	5.26 br d	5.27 br d	5.26 br d
2c′-CH_2	1.49 m	1.49 m	~1.46 m	~1.46 m
	2.04 m	2.03 m	2.03 m	2.03 m
3c′-CH_2	1.90 m	1.91 m	~1.87 m	~1.87 m
	1.92 m	1.92 m		
4c′-H	3.70 ddd	3.70 ddd	3.66 m	3.66 m
5c′-H	4.25 dq	4.25 dq	4.22 dq	4.22 dq
6c′-Me	1.29 d	1.29 d	1.27 d	1.27 d
1c″-H	5.27 d (J=3.5 Hz)	5.27 d	5.09 t	5.09 t
2c″-H	6.89 dd	6.89 dd	—	—
2c″-CH_2 ax	—	—	2.10 dddd	2.10 dddd
eq	—	—	2.36 dddd	2.36 dddd
3c″-H	6.09 d (J=10.2 Hz)	6.09 d	—	—
3c″-CH_2	—	—	2.35~2.55 m	2.35~2.55 m
5c″-H	4.55 q	4.55 q	4.29 q	4.29 q
6c″-Me	1.37 d	1.37 d	1.27 d	1.27 d
-OH	4.33 s	4.32 s	4.35 s	4.35 s
	4.59 s	4.59 s	4.59 s	4.59 s

[a] Measured in $CDCl_3$ at 400 MHz with TMS as an internal reference. Chemical shift assignments were made on the basis of decoupling experiments.

Fig. 2. Degradation of saquayamycins.

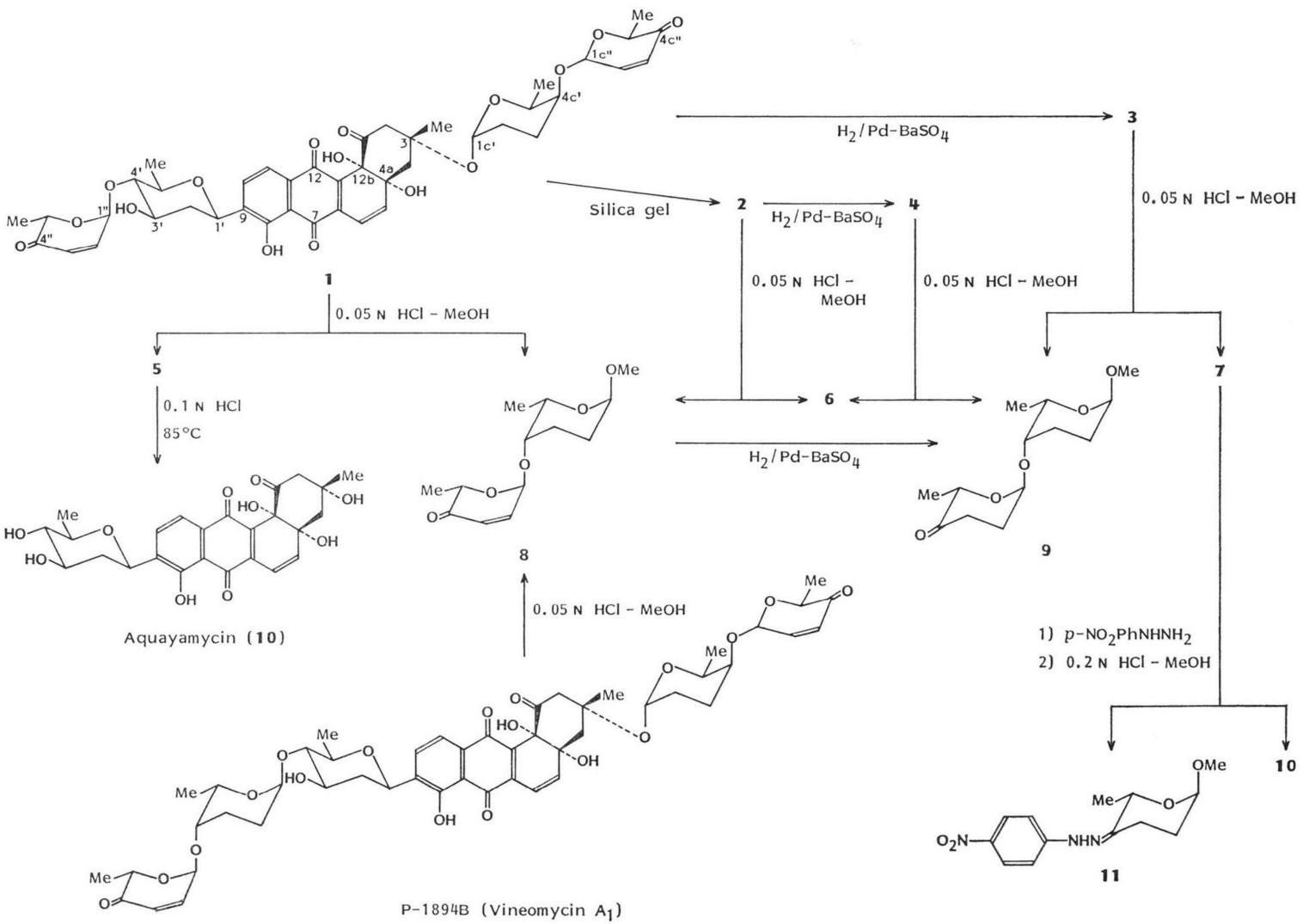


Table 4. Antimicrobial spectra of saquayamycins.

Test organism	MIC (μg/ml)						
	A (1)	B (2)	C (3)	D (4)	A1 (5)	B1 (6)	C1 (7)
Staphylococcus aureus FDA209P	3.12	1.56	6.25	12.5	6.25	1.56	6.25
S. aureus Smith	3.12	1.56	6.25	12.5	6.25	3.12	6.25
Micrococcus lysodeikticus IFO 3333	1.56	1.56	6.25	12.5	3.12	1.56	3.12
M. luteus PCI1001	3.12	3.12	6.25	6.25	3.12	1.56	3.12
Bacillus subtilis PCI 219	3.12	6.25	6.25	12.5	6.25	3.12	6.25
Escherichia coli NIHJ	50	50	50	50	50	50	25
Salmonella typhi T-63	>25	>25	>25	>25	>25	>25	>25
Serratia marcescens	25	25	25	25	25	25	25
Pseudomonas aeruginosa A3	25	25	25	25	25	25	25
Mycobacterium smegmatis ATCC 607	50	>50	>50	>50	>50	>50	50

Table 5. Effect of saquayamycins, aquayamycin and adriamycin on the growth of P388/S and P388/ADR leukemia cells.

Compound	IC_{50} (μg/ml)		Ratio R/S
	P388/S	P388/ADR	
Saquayamycin A (1)	0.06	0.06	1.0
〃 B (2)	0.078	0.08	1.0
〃 C (3)	0.14	0.15	1.1
〃 D (4)	0.15	0.15	1.0
〃 A1 (5)	0.17	0.13	0.8
〃 B1 (6)	0.15	0.11	0.7
〃 C1 (7)	0.27	0.22	0.8
Aquayamycin	2.0	2.2	1.1
Adriamycin	0.01	0.55	55

IC_{50} values were determined on day 2 culture.

On acid hydrolysis with 0.1 N HCl at 85°C, **5** gave an orange aglycone **10**. The $[\alpha]_D^{28}$ and ^1H NMR spectrum of **10** were coincided with those of aquayamycin[1,2], whose absolute configuration had been determined by the X-ray crystallographic analysis of P-1894B[5] and studies of the absolute configuration of its sugar components[2]. Moreover, the ^1H NMR, ^{13}C NMR and mass spectra indicated that **5** consists of **10** and aculose, and that the latter is combined to the C-4′ of the aglycone through an α-1,4 linkage.

Catalytic hydrogenation of **1** with 5% $Pd/BaSO_4$ at room temperature for 15 minutes gave saquayamycin C (**3**). Subsequent methanolysis of **3** with 0.05 N HCl - MeOH at room temperature for 5 minutes yielded **7** and **9**. The ^1H NMR spectrum and $[\alpha]_D^{30}$ of **9** showed good agreement with those of the hydrogenation product of **8**, that is a methyl disaccharide composed of L-rhodinose and L-cinerulose A. By the ^1H NMR, ^{13}C NMR and mass spectra, **7** was confirmed to be composed of **10** and cinerulose A. In order to determine the absolute configuration of the cinerulose A, **7** was treated with *p*-nitrophenylhydrazine, and subsequently methanolyzed with 0.2 N HCl - MeOH at room temperature for 30 minutes. From the resulting mixture, **10** and **11** were isolated by silica gel TLC with $CHCl_3$ - MeOH (20: 1). The $[\alpha]_D^{27}$, and ^1H NMR and mass spectra of **11** were identical with those of the *p*-nitrophenylhydrazone of methyl α-L-cinerulose A[2,6].

When treated with silica gel, **1** was converted to saquayamycin B (**2**), and catalytic hydrogenation of **2** with 5% $Pd/BaSO_4$ at room temperature for 15 minutes gave saquayamycin D (**4**). By methanolysis with 0.05 N HCl - MeOH, **2** and **4** were degraded into compounds **6** and **8**, and **6** and **9**, respectively. On the analysis of ^1H NMR, ^{13}C NMR and mass spectra of **6**, its sugar component was demonstrated to be cinerulose B[7,8], which is combined to the C-3′ and C-4′ of the aglycone part.

Based on these results, we propose the structures shown in Fig. 1 for saquayamycins A (**1**), B (**2**), C (**3**), D (**4**), A1 (**5**), B1 (**6**) and C1 (**7**).

Biological Activities

The antimicrobial activity of the antibiotics was examined by agar dilution method. As Table 4 shows, they act against Gram-positive bacteria, with MIC values between 1.56 and 6.25 μg/ml. Table 5 shows the 50% growth inhibition concentrations (IC_{50}) of saquayamycins, aquayamycin and adriamycin against adriamycin-sensitive (P388/S) and adriamycin-resistant (P388/ADR) sublines of P388 leukemia cells[9]. The IC_{50} values of saquayamycins and aquayamycin against P388/ADR cells were nearly equal to those against P388/S cells, respectively. The LD_{50} by intraperitoneal injection of saquayamycins A and B in mice were 6.25 ~ 12.5 mg/kg.

Discussion

As described above, saquayamycins A, B, C and D produced by *Streptomyces nodosus* MH190-16F3 are the antibiotics which belong to aquayamycin-group. Ayamycin A2[10] and TA-435 A[11] have been reported to have the same UV spectra. Therefore, we tested their Rf values on silica gel TLC, and confirmed that they should be different from saquayamycins.

Judging from the ^{13}C chemical shift assignments of saquayamycin A, the ^{13}C chemical shifts assigned to C-3, 4a, 12b, 2′, 4′, 6′, 4″, 4a′, 5″, 5a′, 5‴ and 5a″ in P-1894B[2] should be changed to C-12b, 3, 4a, 6′, 4″ or 4a′, 4a′, or 4″, 5a′, 5″, 5a″ or 5‴, 5‴ or 5a″, 2′ or 4′, and 4′ or 2′, respectively (This numbering follows that of P-1894B reported by OHTA *et al.*[2]).

Experimental

General

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were recorded with a Hitachi 260-10 spectrometer. UV spectra were taken on a Hitachi 220S spectrometer. ^1H and ^{13}C NMR spectra were recorded with a Jeol GX-400 spectrometer at 400 MHz (^1H) and 100 MHz (^{13}C), respectively. Chemical shifts are expressed in δ values (ppm) with TMS as an internal reference and coupling constants are given in Hz (J). The following abbreviations are used: s=singlet, d=doublet, dd=doublet of doublets, ddd=doublet of doublets of doublets, dddd=doublet of doublets of doublets of doublets, t=triplet, q=quartet, dq=doublet of quartets, br d=broad doublet and m=multiplet. ^{13}C Chemical shift assignments were made by carbon-proton chemical shift correlation experiments and long-range selective proton decoupling experiments. Mass spectra were determined with a Hitachi RMU-6M apparatus (EI-MS) or a Hitachi RMU-7M apparatus (FD-MS). TLC was carried out on silica gel plate (Kieselgel 60 F_{254}, Merck) and column chromatography was carried out with silica gel (Kieselgel 60, Merck).

Partial Hydrolysis of Saquayamycins A, B, C and D

Each saquayamycin solution in acetonitrile - 0.2% phosphoric acid (60: 40) was kept at 37°C for 2 hours, and extracted with $CHCl_3$. After washing with H_2O, the solvent layer was dried over Na_2SO_4, and concentrated to dryness. The pigment thus obtained was purified by silica gel plates with $CHCl_3$ -

MeOH (100:3). Thus, saquayamycin A (**1**) (20 mg), B (**2**) (20 mg), C (**3**) (40 mg) and D (**4**) (4 mg) gave saquayamycin A1 (**5**) (8 mg), B1 (**6**) (13 mg), C1 (**7**) (27 mg) and B1 (**6**) (2.5 mg), respectively.

Mild Methanolysis of Saquayamycin A (**1**)

1 (38 mg) in 0.05 N HCl - MeOH (10 ml) was allowed to stand at room temperature for 5 minutes. The reaction mixture was neutralized with Ag_2CO_3 (50 mg) and filtered. The filtrate was concentrated and the residue was separated into **5** (21 mg), compound **8** (5.0 mg) and β anomer of **8** (4.3 mg) by silica gel TLC with benzene - EtOAc (3:1).

8: Colorless needle; mp 67.0~68.0°C; $[\alpha]_D^{25}$ −20.0° (*c* 0.5, $CHCl_3$); FD-MS *m/z* 225 $(M-OMe)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (3H, d, *J*=6.4, 6-Me), 1.37 (3H, d, *J*=6.7, 6'-Me), 1.57 (1H, m, 2-H_B), 1.90 (1H, m, 3-H_B), 1.97 (1H, m, 2-H_A), 2.07 (1H, m, 3-H_A), 3.36 (3H, s, OMe), 3.61 (1H, br s, 4-H), 3.93 (1H, dq, *J*=~1.5, 6.4, 5-H), 4.58 (1H, q, *J*=6.7, 5'-H), 4.72 (1H, dd, *J*=3, ~1, 1-H), 5.23 (1H, d, *J*=3.5, 1'-H), 6.09 (1H, d, *J*=10.2, 3'-H), 6.88 (1H, dd, *J*=10.2, 3.5, 2'-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.1 (6'-C), 17.2 (6-C), 24.2 and 24.7 (2-C and/or 3-C), 54.7 (OMe), 65.7 (5-C), 70.6 (5'-C), 76.7 (4-C), 95.4 (1'-C), 98.1 (1-C), 127.3 (3'-C), 143.1 (2'-C), 196.8 (4'-C).

Hydrolysis of Saquayamycin A1 (**5**)

5 (10 mg) in 0.1 N HCl (10 ml) was treated at 85°C for 30 minutes. After cooling, the reaction mixture was extracted with EtOAc (10 ml), washed with H_2O, and concentrated to dryness. The residue was purified by silica gel TLC with $CHCl_3$ - MeOH (10:1) to obtain an aglycone **10** (3.2 mg).

10: Orange powder; $[\alpha]_D^{28}$ +151° (*c* 0.2, dioxane) (Ref[2]) $[\alpha]_D^{24}$ +149° (*c* 1.0, dioxane)); ^1H NMR (400 MHz, CD_3OD) δ 1.23 (3H, s, 13-Me), 1.36 (3H, d, *J*=6, 5'-Me), ~1.36 (1H, m, 2'-H_{ax}), 2.02 (2H, m, 4-CH_2), 2.41 (1H, ddd, *J*=13, 5, 2, 2'-H_{eq}), 2.66 (1H, dd, *J*=13, 2, 2-H_B), 2.80 (1H, d, *J*=13, 2-H_A), 3.02 (1H, t, *J*=9, 4'-H), 3.43 (1H, dq, *J*=9, 6, 5'-H), 3.68 (1H, ddd, *J*=11, 9, 5, 3'-H), 4.88 (1H, dd, *J*=11, 2, 1'-H), 6.39 (1H, d, *J*=9.8, 5-H), 6.86 (1H, d, *J*=9.8, 6-H), 7.58 (1H, d, *J*=8, 11-H), 7.86 (1H, d, *J*=8, 10-H).

Catalytic Hydrogenation of Saquayamycin A (**1**)

A solution of **1** (80 mg) in EtOAc (10 ml) and MeOH (10 ml) was hydrogenated with 5% Pd/$BaSO_4$ (100 mg) at room temperature for 15 minutes. The catalyst was filtered off and the fitrate was concentrated *in vacuo*. The yellow brown powder thus obtained were purified by silica gel TLC with $CHCl_3$ - MeOH (3:1) to yield saquayamycin C (**3**) (50 mg).

Mild Methanolysis of Saquayamycin C (**3**)

3 (40 mg) was dissolved in 0.05 N HCl - MeOH (10 ml), allowed to stand at room temperature for 5 minutes, and neutralized with Ag_2CO_3 (50 mg). After filtration, the filtrate was concentrated and chromatographed on silica gel plates with benzene - EtOAc (2:1) to give saquayamycin C1 (**7**) (11 mg) and compound **9** (3.5 mg).

9: Colorless oil; $[\alpha]_D^{20}$ −253° (*c* 0.21, $CHCl_3$); FD-MS *m/z* 227 $(M-OMe)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.19 (3H, d, *J*=6.4, 6-Me), 1.28 (3H, d, *J*=6.7, 6-Me), 1.56 (1H, m, 2-H_B), 1.85 (1H, m, 3-H_B), ~1.98 (1H, m, 2-H_A), ~2.03 (1H, m, 3-H_A), 2.13 (1H, m, 2'-H_B), 2.35 (1H, m, 2'-H_A), 2.39~2.56 (2H, m, 3'-CH_2), 3.36 (3H, s, OMe), 3.59 (1H, br s, 4-H), 3.91 (1H, dq, *J*=1, 6.5, 5-H), 4.33 (1H, q, *J*=6.7, 5'-H), 4.73 (1H, dd, *J*=~3, ~1.5, 1-H), 5.06 (1H, t, *J*=5.4, 1'-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.8 (6'-C), 17.2 (6-C), 24.3 and 24.8 (2-C and/or 3-C), 28.5 (2'-C), 33.6 (3'-C), 54.7 (OMe), 65.9 (5-C), 75.1 (4-C), 98.2 (1-C), 99.1 (1'-C), 210.9 (4'-C).

Catalytic Hydrogenation of Compound **8**

8 (4.0 mg) was dissolved in EtOAc (2 ml) and MeOH (2 ml), and hydrogenated over 5% Pd/$BaSO_4$ (10 mg) at room temperature and atmospheric pressure for 10 minutes. The reaction mixture was filtered, evaporated to dryness, and purified by silica gel TLC with benzene - EtOAc (2:1) to yield **9** (2.8 mg).

Identity of Compound **11** with *p*-Nitrophenylhydrazone of Methyl α-L-Cinerulose A

A solution of **7** (20 mg) in MeOH (8 ml) was mixed with *p*-nitrophenylhydrazine hydrochloride (8 mg) in H_2O (0.9 ml) and pyridine (0.05 ml). The resulting mixture was kept at 50°C for 20 minutes,

and extracted with $CHCl_3$ (20 ml). The solvent layer was washed with H_2O (20 ml) three times, and concentrated *in vacuo*. The residue was dissolved in 0.2 N HCl - MeOH (5 ml), and kept at room temperature for 30 minutes. After neutralizing with Ag_2CO_3 (20 mg), the reaction mixture was filtered and evaporated to 0.2~0.5 ml. The compounds in the solution were separated into aquayamycin (**10**) (8 mg) and compound **11** (3.5 mg) by silica gel TLC with $CHCl_3$ - EtOAc (1: 1).

11: Yellow powder; $[\alpha]_D^{27}$ −324° (*c* 0.1, $CHCl_3$), EI-MS *m/z* 279 $(M)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (3H, d, J=6, 6-Me), 1.95 (1H, m, 2-H_B), 2.10 (1H, m, 2-H_A), 2.47 (2H, m, 3-CH_2), 3.46 (3H, s, OMe), 4.54 (1H, q, J=6, 5-H), 4.84 (1H, t, J=4.5, 1-H), 7.06 (2H, d, J=9, Ar-2 and 6-H), 7.55 (1H, br s, NH), 8.16 (2H, d, J=9, Ar-3 and 5-H).

Conversion of Saquayamycin A (**1**) to Saquayamycin B (**2**)

1 (10 mg) absorbed on silica gel (1 g) was allowed to stand at room temperature overnight, and extracted with $CHCl_3$ - MeOH (10: 1). The extract was concentrated and purified by silica gel TLC with $CHCl_3$ - EtOAc (1: 1) to yield **2** (4.5 mg).

Catalytic Hydrogenation of Saquayamycin B (**2**)

A solution of **2** (20 mg) in EtOAc (5 ml) and MeOH (5 ml) was hydrogenated over 5% Pd/$BaSO_4$ (40 mg) at room temperature and atmospheric pressure for 15 minutes. The reaction mixture was filtered and evaporated to dryness. The residue was purified by silica gel TLC with $CHCl_3$ - EtOAc (1: 1) to give **4** (12 mg).

Mild Methanolysis of Saquayamycins B (**2**) and D (**4**)

2 (15 mg) and **4** (12 mg) were dissolved in 0.05 N HCl - MeOH (5 ml) and kept at room temperature for 5 minutes, respectively. The reaction mixtures were neutralized with Ag_2CO_3, filtered and evaporated to dryness. The products were purified by silica gel TLC with benzene - EtOAc (2: 1). Thus, **2** and **4** gave **6** (7 mg) and **8** (1.8 mg), and **6** (5 mg) and **9** (1.2 mg), respectively.

6: ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 (3H, s, 13-Me), 1.38 (3H, d, J=6.7, 6″-Me), 1.40 (3H, d, J=6, 6′-Me), 1.44 (1H, ddd, J=12.2, 11.8, 11.0, 2′-H_{ax}), 1.84 (1H, dd, J=15, 2, 4-H_{ax}), 2.28 (1H, dd, J=15, 3, 4-H_{eq}), 2.43 (1H, ddd, J=12.2, 4.5, 2, 2′-H_{eq}), 2.63 (1H, d, J=13, 2-H_{ax}), ~2.63 (1H, m, 3″-H_B), 2.67 (1H, m, 3″-H_A), 2.95 (1H, dd, J=13, 3, 2-H_{ex}), 3.49 (1H, t, J=9, 4′-H), 3.52 (1H, d, J=2, OH), 3.57 (1H, dq, J=9, 6, 5′-H), 3.81 (1H, ddd, J=11.8, 9, 4.5, 3′-H), 3.88 (1H, br s, OH), 4.34 (1H, ddd, J=3.5, ~3, ~3, 2″-H_{eq}), 4.72 (1H, q, J=6.7, 5″-H), 4.97 (1H, dd, J=11.0, ~2, 1′-H), 4.99 (1H, s, OH), 5.19 (1H, d, J=3, 1″-H), 6.40 (1H, d, J=10.0, 5-H), 6.90 (1H, d, J=10.0, 6-H), 7.62 (1H, d, J=8, 11-H), 7.90 (1H, d, J=8, 10-H), 12.28 (1H, s, 8-OH).

Determination of Antibacterial Activity

Minimum inhibitory concentration (MIC) was determined by agar dilution method on Mueller-Hinton agar after 18 hours incubation at 37°C, except for *Mycobacterium smegmatis*. *M. smegmatis* was cultivated for 40 hours at 37°C.

Determination of Cytotoxic Activity against P388/S and P388/ADR

Both sublines of P388 leukemia sensitive to adriamycin (P388/S) and resistant to adriamycin (P388/ADR) were supplied by Dr. M. INABA, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo. They were maintained by serial intraperitoneal transplantation in DBA mice. Ascitic cells 7 days after transplantation to CDF_1 mice were used for our experiments. They were diluted with RPMI 1640 medium supplemented with 10% fetal bovine serum, 10 μM 2-mercaptoethanol and 0.01% kanamycin sulfate to a cell density of approximately 5×10^4 cells/ml, and cultured at 37°C in a CO_2-incubator with a test compound. After 2-days cultivation, the cell growth was determined by counting cell number using a Coulter counter.

Acknowledgments

We are grateful to Dr. HISAYOSHI OKAZAKI, Takeda Chemical Industries, Ltd., for his generous gift of P-1894B, and Biochemical Research Laboratory of Tanabe Seiyaku Co., Ltd., for TA-435 A. We wish also thank Dr. MAKOTO INABA, Cancer Chemotherapy Center, for the supply of P388 cell lines.

This research was partially supported by a Contract No. NO1-CM-47593 with the Division of Cancer Treatment, National Cancer Institute, U.S.A. and by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan and from the Ministry of Health and Welfare, Japan.

References

1) SEZAKI, M.; S. KONDO, K. MAEDA, H. UMEZAWA & M. OHNO: The structure of aquayamycin. Tetrahedron 26: 5171~5190, 1970
2) OHTA, K.; E. MIZUTA, H. OKAZAKI & T. KISHI: The absolute configuration of P-1894B, a potent prolyl hydroxylase inhibitor. Chem. Pharm. Bull. 32: 4350~4359, 1984
3) IMAMURA, N.; K. KAKINUMA, N. IKEKAWA, H. TANAKA & S. ŌMURA: Identification of the aglycon part of vineomycin A_1 with aquayamycin. Chem. Pharm. Bull. 29: 1788~1790, 1981
4) UCHIDA, T.; M. IMOTO, T. MASUDA, K. IMAMURA, Y. HATORI, T. SAWA, H. NAGANAWA, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: New antitumor antibiotics, ditrisarubicins A, B and C. J. Antibiotics 36: 1080~1083, 1983
5) OHTA, K. & K. KAMIYA: X-Ray crystal structure of P-1894B, a collagen proline hydroxylase inhibitor produced by *Streptomyces albogriseolus* subsp. No. 1894. J. Chem. Soc. Chem. Commun. 1981: 154~155, 1981
6) ALBANO, E. L. & D. HORTON: A synthesis of 2,3,4,6-tetradeoxy-4-(dimethylamino)-D-*erythro*-hexose (forosamine) and its D-*threo* epimer. Carbohydr. Res. 11: 485~495, 1969
7) RICHLE, W.; E. K. WINKLER, D. M. HAWLEY, M. DOBLER & W. KELLER-SCHIERLEIN: Stoffwechselprodukte von Microorganismen. 104. Die Struktur des Cinerubin B. Helv. Chim. Acta 55: 467~480, 1972
8) UCHIDA, T.; M. IMOTO, T. MASUDA, H. YOSHIMOTO, K. IMAMURA, T. SAWA, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: New anthracycline antibiotics: Serirubicin and 1-hydroxyserirubicin. J. Antibiotics 38: 795~798, 1985
9) KUNIMOTO, S.; K. MIURA, K. UMEZAWA, C.-Z. XU, T. MASUDA, T. TAKEUCHI & H. UMEZAWA: Cellular uptake and efflux and cytostatic activity of 4′-*O*-tetrahydropyranyladriamycin in adriamycin-sensitive and resistant tumor cell lines. J. Antibiotics 37: 1697~1702, 1984
10) SATŌ, K.: Studies on a new antitumor antibiotic, ayamycin. II. On the isolation and physico-chemical properties of ayamycin A_2. J. Antibiotics, Ser. A 13: 321~326, 1960
11) NAGAHAMA, N.; T. FURUMAI, Y. ARAI & Y. ASHIKARI: Studies on a new antitumor antibiotic, TA-435 A. I. On the isolation and properties of TA 435 A. J. Antibiotics, Ser. B 17: 245~249, 1964