NOVEL ANTITUMOR ANTIBIOTICS, SAPTOMYCINS

II. ISOLATION, PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION

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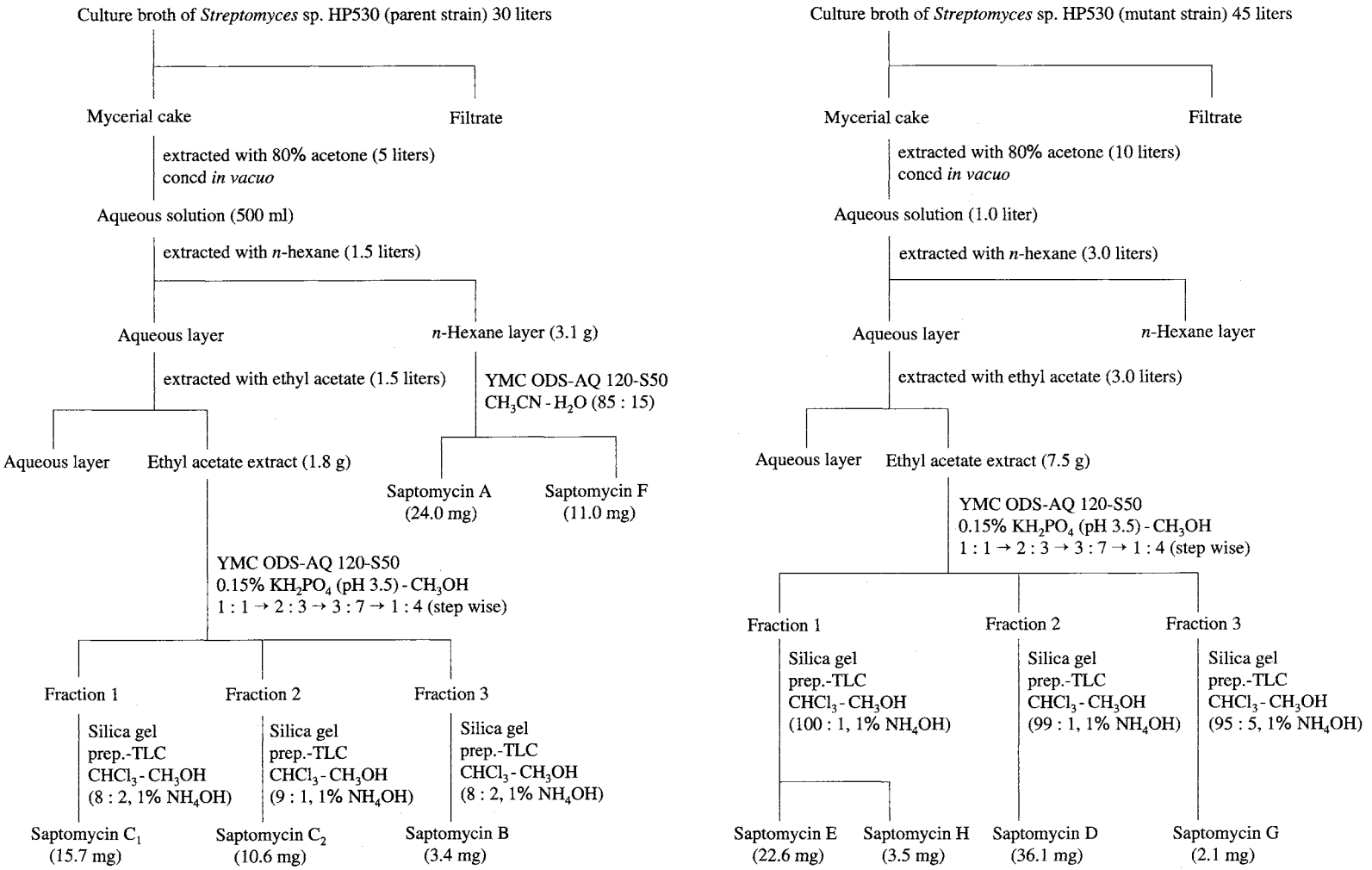
A complex of novel antitumor antibiotics related to the pluramycin-group was isolated from the fermentation of actinomycete, named *Streptomyces* sp. HP530. The producing strain mutated frequently. The products isolated from the parent strain were designated saptomycins A, B, C_1, C_2 and F, while those of the mutant were named saptomycins D, E, G and H. These structures were elucidated by several NMR spectral analyses and other spectroscopic experiments.

The saptomycins, novel antitumor antibiotics, were detected and isolated from the culture of an actinomycete strain HP530 in the screening of soil microorganisms for novel antitumor antibiotics. The producing strain was named *Streptomyces* sp. HP530 on the basis of taxonomic studies[1]. Further, the strain was separated into two strains besed on their differences in products as determined by HPLC analysis. The parent strain, the dominant one, yielded saptomycins A, B, C_1, C_2 and F, while the spontaneous mutant strain gave saptomycins A, D, E, F, G and H as products. The UV and IR data determined for the saptomycins were similar to those of the pluramycin-group of anthraquinone type antibiotics. The pluramycin-group included pluramycin A[2], neopluramycin[2], hedamycin[3], kidamycin[4], rubiflavins[5], ankinomycin[6] and altromycins[7]. The FAB-MS spectra and the NMR studies of the saptomycins indicated that saptomycins A, B, C_1, C_2, D, E, F, G and H were novel members of the family. In this paper, we describe the isolation, physico-chemical properties and structure elucidation of saptomycins A, B, C_1, C_2 and F from the parent strain and those of saptomycins D, E, G and H from the mutant.

Isolation

The isolation procedure from the cultured whole broth of the cultured *Streptomyces* sp. HP530 parent strain[1] is shown in Fig. 1. Saptomycins A, B, C_1, C_2 and F displayed lipophilic properties mainly associated with the mycelia. The mycelial cake was separated from 30 liters of the cultured broth by centrifuging and was then extracted with 5 liters of 80% acetone. The acetone extract was concentrated *in vacuo* to 500 ml of aqueous solution. The residual solution was extracted with *n*-hexane (500 ml × 3) and then ethyl acetate (500 ml × 3). The *n*-hexane extract (3.1 g) evaporated under reduced pressure was applied to a column packed with ODS-AQ 120-S50 (Yamamura Kagaku: YMC), and saptomycins A and F were separately eluted with acetonitile - H_2O (85 : 15). Two yellowish fractions were concentrated to dryness, yielding pure saptomycins A (24.0 mg) and F (11.0 mg) as yellowish powders. On the other hand, the ethyl acetate extract was concentrated and dissolved in a small volume of methanol. The methanol solution was charged on the column shielded against light with ODS-AQ 120-S50 and was then developed stepwise with a solvent system of 0.15% KH_2PO_4 (pH 3.5) - methanol, 1 : 1 to 1 : 4. Three yellowish active fractions, including

Fig. 1. The isolation procedure of saptomycins A, B, C_1, C_2, D, E, F, G and H.



saptomycins C_1, C_2 and B, respectively, were successively eluted, and then each fraction evaporated *in vacuo* was further purified by preparative TLC. Two fractions with saptomycins C_1 and B were developed using the solvent system of chloroform-methanol (8:2, 1% NH_4OH), respectively, and gave pure saptomycins C_1 (15.7 mg) are B (3.4 mg), while the fraction containing saptomycin C_2 was developed with the same solvents using a 9:1 (1% NH_4OH) ratio and yielded pure saptomycin C_2 (10.6 mg).

Saptomycins D, E, G and H were isolated by a similar procedure from the mycelia cultured from the mutant strain. 7.5 g of the concentrated ethyl acetate extract from 45 liters of the fermentation broth was dissolved in a small volume of methanol and charged on the column packed with ODS-AQ 120-S50. This mixture was further purified stepwise by chromatography using a solvent system of 0.15% KH_2PO_4-methanol, 1:1 to 1:4, and separated into three active fractions. Fraction 1 was separated by preparative TLC (chloroform-methanol, 100:1, 1% NH_4OH) to give saptomycins E (22.6 mg) and H (3.5 mg) as yellow amorphous powders. Fractions 2 and 3 were also purified by preparative TLC, developed with the same solvent system using different ratios (chloroform-methanol, 99:1, 1% NH_4OH and 95:5, 1% NH_4OH), and yielded saptomycin D (36.1 mg) as a yellowish red powder and saptomycin G (2.1 mg) as a yellow powder, respectively.

Physico-chemical Properties

The physico-chemical properties of saptomycins A, B, C_1, C_2, D, E, F, G and H are summarized in Table 1. These compounds were yellow needles, yellow powder or yellowish red powder. Saptomycins A and F were soluble in chloroform, methylene chloride and ethyl acetate, while saptomycins B, C_1 and C_2

Table 1. Physico-chemical properties of saptomycins A, B, C_1, C_2, D, E, F, G and H.

	A	B	C_1	C_2	D
Appearance	Yellow needles	Yellowish red powder	Yellowish red powder	Yellow powder	Yellowish red powder
$[\alpha]_D^{20}$ (c 0.1, $CHCl_3$)	−12.5°	+106.0°	+196.0°	+202.7°	+152.0°
Molecular formula	$C_{24}H_{20}O_6$	$C_{41}H_{52}N_2O_9$	$C_{41}H_{52}N_2O_9$	$C_{43}H_{54}N_2O_{10}$	$C_{35}H_{37}NO_9$
HRFAB-MS (m/z) $(M+H)^+$					
Calcd:	404.1260 $(M)^{+a}$	717.3751	717.3751	759.3857	616.2546
Found:	404.1219	717.3747	717.3743	759.3818	616.2556
UV λ_{max} nm (ε)	244 (35,600), 270 (21,800), 418 (8,600)	247 (53,800), 268 (41,200), 427 (13,700)	245 (52,000), 265 (sh 38,400), 425 (11,500)	243 (44,400), 264 (sh 26,400), 426 (8,900)	246 (51,600), 265 (sh 29,000), 418 (9,800)
IR (KBr) cm^{-1}	3480, 1670, 1600	3460, 1670, 1650, 1600	3430, 1665, 1590	3480, 1740, 1660, 1635, 1590	3470, 1745, 1642, 1590

	E	F	G	H
Appearance	Yellow powder	Yellow needles	Yellow powder	Yellow powder
$[\alpha]_D^{20}$ (c 0.1, $CHCl_3$)	+147.0°	+106.9°	+118.0°	−105.0°
Molecular formula	$C_{33}H_{35}NO_9$	$C_{24}H_{18}O_6$	$C_{33}H_{35}NO_8$	$C_{33}H_{35}NO_9$
HRFAB-MS (m/z) $(M+H)^+$				
Calcd:	590.2390	402.1103 $(M)^{+a}$	574.2441	590.2390
Found:	590.2398	402.1133	574.2362	590.2412
UV λ_{max} nm (ε)	244 (47,200), 265 (sh 28,000), 425 (10,800)	244 (49,000), 270 (30,600), 415 (11,900)	242 (46,800), 270 (29,800), 415 (9,600)	245 (40,100), 263 (sh 26,400), 415 (9,200)
IR (KBr) cm^{-1}	3470, 1665, 1642, 1590	3480, 1670, 1650, 1595	3460, 1740, 1650	3450, 1740, 1660, 1585

[a] HREI-MS, M^+.

Table 2. ^1H NMR chemical shifts of saptomycins A, B, C_1, C_2, D, E, F, G and H.

Proton	A[a]	B[a]	C_1[a]	C_2[a]	D[b]
3-H	6.28 s	6.27 s	6.25 s	6.24 s	6.58 s
13-H_3	3.02 s	3.03 s	3.01 s	3.00 s	2.90 s
6-H	8.09 s	7.99 s	7.96 s	7.96 s	7.98 s
8-H	7.83 d (7.5)				7.99 d (7.9)
9-H	7.69 dd (7.5, 8.4)	8.43 s	8.31 s	8.25 s	8.02 d (7.9)
10-H	7.36 d (8.4)				
14-H	2.99 dq (3.8, 7.0)	2.88 ddq (6.3, 7.1, 6.5)	2.86 ddq (7.2, 7.0, 6.8)	2.85 ddq (6.9, 7.5, 6.0)	
15-H_3	1.45 d (7.0)	1.52 (6.5)	1.44 (6.8)	1.46 d (6.0)	1.68 s
16-H_a	5.01 dd (8.5, 3.8)	2.50 ddd (7.1, 8.0, 14.1)	2.53 ddd (7.0, 7.3, 14.2)	2.51 ddd (7.5, 6.8, 14.1)	4.06 dd (7.6, 1.2)
16-H_b		2.74 ddd (6.3, 7.3, 14.1)	2.70 ddd (7.0, 7.2, 14.2)	2.70 ddd (7.2, 6.9, 14.1)	
17-H	5.50 dd (8.5, 10.5)	5.42 ddd (8.0, 7.3, 10.9)	5.41 m	5.41 m	5.32 ddq (7.6, 11.2, 1.8)
18-H	5.65 dd (10.5, 7.0)	5.58 dq (6.8, 10.9)	5.55 dq (10.9, 6.7)	5.55 m	5.70 ddq (1.2, 11.2, 7.1)
19-H_3	1.70 d (7.0)	1.61 (6.8)	1.59 d (6.7)	1.59 d (6.8)	1.63 (1.8, 7.1)
2′-H		3.87 dq (6.0, 1.0)	4.04 br q (6.4)	4.32 dq (4.5, 6.8)	4.40 dq (5.2, 6.8)
2′-CH_3		1.52 d (6.0)	1.50 d (6.4)	1.51 d (6.8)	1.63 d (6.8)
3′-H		3.33 br s	3.36 br s	5.15 d (4.5)	5.36 d (5.2)
3′-OAc				2.20 s	1.80 s
4′-CH_3		1.23 s	0.72 s	0.93 s	0.89 s
4′-$N(CH_3)_2$		2.26 s	2.25 s	2.36 s	2.37 s
5′-H_a		2.10 dd (12.5, 11.5)	2.01 dd (6.7, 13.5)	1.75 dd (8.7, 13.1)	1.42 dd (10.1, 14.0)
5′-H_b		2.33 dd (12.5, 3.0)	2.61 dd (3.1, 13.5)	2.45 dd (3.1, 13.1)	2.60 dd (3.1, 14.0)
6′-H		4.95 dd (11.5, 3.0)	5.43 overlap	5.55 dd (3.1, 8.7)	5.79 dd (3.1, 10.1)
2″-H		3.54 dq (8.8, 6.5)	3.56 dq (8.5, 6.0)	3.55 dq (6.8, 9.3)	
2″-CH_3		1.42 d (6.5)	1.43 d (6.0)	1.44 d (6.8)	
3″-H		3.40 dd (9.4, 8.8)	3.24 dd (9.2, 8.5)	3.28 dd (9.5, 9.3)	
4″-H		3.01 ddd (9.4, 8.9, 1.5)	2.99 m	2.92 m	
4″-$N(CH_3)_2$		2.47 s	2.38 s	2.36 s	
5″-H_a		1.3 overlap	1.31 m	1.40 m	
5″-H_b		2.2 overlap	2.28 m	2.24 m	
6″-H		5.35 br d (9.6)	5.44 br d (8.9)	5.41 br d (9.0)	

[a] Measured at 400 MHz in $CDCl_3$; ppm from TMS.
[b] Measured at 400 MHz in C_6D_6; ppm from TMS.
Coupling constants (Hz) are in parentheses.

Table 2. (Continued)

Proton	E[b]	F[a]	G[b]	H[b]
3-H	6.47 s	6.53 s	6.22 s	6.47 s
13-H_3	2.86 s	3.02 s	2.90 s	2.85 s
6-H	7.97 s	8.08 s	7.90 s	7.90 s
8-H	7.95 d (7.9)	7.82 d (7.4)	7.94 s[c]	7.89 d (8.0)
9-H	7.98 d (7.9)	7.67 dd (7.4, 8.4)	7.94 s[c]	8.10 d (8.0)
10-H		7.36 d (8.4)		
14-H				
15-H_3	1.56 s	1.83 s	1.52 s	1.56 s
16-H_a	2.99 q (5.4)	4.24 d (8.0)	7.38 q (7.6)	3.07 q (5.4)
16-H_b				
17[d]	0.99 d (5.4)	5.42 ddq (8.0, 11.0, 1.6)	1.57 d (7.6)	1.01 d (5.4)
18-H		6.05 dq (11.0, 7.0)		
19-H_3		1.89 dd (1.6, 7.0)		
2′-H	4.34 dq (5.1, 6.6)		4.36 dq (5.2, 6.8)	3.65 br q (6.3)
2′-CH_3	1.58 d (6.6)		1.60 d (6.8)	1.29 d (6.3)
3′-H	5.30 d (5.1)		5.32 d (5.2)	4.92 br s
3′-OAc	1.76 s		1.77 s	1.96 br s
4′-CH_3	0.84 s		0.86 s	0.94 br s
4′-$N(CH_3)_2$	2.32 s		2.33 s	2.09 s
5′-H_a	1.40 dd (10.0, 14.0)		1.42 dd (10.0, 14.1)	1.87 dd (10.6, 12.4)
5′-H_b	2.58 dd (2.5, 14.0)		2.59 dd (3.2, 14.1)	2.17 br d (12.4)
6′-H	5.74 dd (2.5, 10.0)		5.73 dd (3.2, 10.0)	5.14 br d (10.6)
2″-H				
2″-CH_3				
3″-H				
4″-H				
4″-$N(CH_3)_2$				
5″-H_a				
5″-H_b				
6″-H				

[a] Measured at 400 MHz in $CDCl_3$; ppm from TMS.
[b] Measured at 400 MHz in C_6D_6; ppm from TMS.
[c] Coupling between 8-H and 9-H was not observed for the two protons to resonate at the same value of chemical shift.
[d] At C-17, CH in saptomycin F and CH_3 in saptomycins E, G and H.
Coupling constants (Hz) are in parentheses.

were soluble in acidic water, methanol, chloroform and acetone. Further, saptomycins D, E, G and H were soluble in acidic water, methanol, chloroform and benzene. All the compounds were insoluble or slightly soluble in *n*-hexane. The molecular formulae were determined to be $C_{24}H_{20}O_6$ for saptomycin A, $C_{41}H_{52}N_2O_9$ for B, $C_{41}H_{52}N_2O_9$ for C_1, $C_{43}H_{54}N_2O_{10}$ for C_2, $C_{35}H_{37}NO_9$ for D, $C_{33}H_{35}NO_9$ for E, $C_{24}H_{18}O_6$ for F, $C_{33}H_{35}NO_8$ for G and $C_{33}H_{35}NO_9$ for H by high resolution fast atom bombardment mass (HRFAB-MS) high resolution electron impact mass (HREI-MS) analysis. The UV spectra of the saptomycins showed characteristic absorption maxima near 245, 265 and 415 nm in methanol. The IR spectrum of saptomycins C_2, D, E, G and H in KBr showed an absorption band at 1740~1745 cm^{-1}, suggesting the existence of a carbonyl group of acetate, whereas no acetyl carbonyl absorption was observed

Table 3. ^{13}C NMR data of saptomycins A, B, C_1, C_2, D, E, F, G and H.

Position	A[a]	B[a]	C_1[a]	C_2[a]	D[b]	E[b]	F[a]	G[b]	H[b]
2	170.7 s	172.3 s	172.4 s	172.3 s	167.1 s	167.8 s	167.4 s	163.4 s	167.7 s
3	112.2 d	111.3 d	111.3 d	111.3 d	109.9 d	109.5 d	110.1 d	109.0 d	109.7 d
4	178.8 s	179.3 s	179.3 s	179.3 s	178.4 s	178.3 s	178.9 s	178.9 s	178.3 s
4_a	126.6 s	126.1 s	126.0 s	126.3 s	126.6 s	126.7 s	126.5 s	126.6 s	126.6 s
5	150.3 s	149.7 s	149.7 s	149.6 s	149.7 s	149.8 s	149.8 s	149.7 s	149.8 s
6	125.6 d	126.6 d	126.6 d	126.7 d	125.8 d	125.9 d	125.3 d	125.5 d	125.8 d
6_a	136.0 s	137.1 s	137.0 s	137.1 s	136.2 s	136.2 s	136.0 s	136.2 s	136.2 s
7	181.7 s	183.6 s	183.5 s	183.6 s	181.3 s	181.3 s	181.6 s	181.2 s	181.2 s
7_a	132.2 s	126.4 s	126.1 s	126.1 s	131.1 s	131.1 s	132.1 s	131.1 s	131.3 s
8	119.5 d	140.0 s	139.7 s	140.5 s	119.4 d	119.4 d	119.4 d	119.3 d	119.3 d
9	136.7 d	132.4 d	132.9 d	132.3 d	133.6 d	133.6 d	136.4 d	133.5 d	133.8 d
10	125.9 d	138.4 s	138.1 s	138.3 s	140.7 s	140.7 s	125.9 d	140.6 s	139.9 s
11	162.8 s	158.8 s	159.7 s	159.3 s	159.4 s	159.4 s	162.6 s	159.4 s	158.9 s
11_a	116.7 s	115.6 s	116.0 s	115.9 s	116.3 s	116.3 s	116.7 s	116.3 s	116.4 s
12	187.7 s	188.0 s	188.1 s	188.0 s	188.0 s	188.1 s	187.1 s	188.3 s	188.0 s
12_a	119.6 s	119.2 s	119.2 s	119.2 s	119.9 s	119.9 s	119.8 s	119.7 s	119.9 s
12_b	156.5 s	156.4 s	156.7 s	156.4 s	156.3 s	156.3 s	156.5 s	156.2 s	156.3 s
13	24.3 q	24.2 q	24.3 q	24.2 q	24.0 q	24.0 q	24.2 q	24.0 q	24.0 q
14	44.9 d	39.0 d	39.0 d	39.0 d	59.1 s	57.4 s	59.0 s	126.4 s	57.3 s
15	13.5 q	17.6 q	17.6 q	17.6 q	14.4 q	13.6 q	14.3 q	11.7 q	13.5 q
16	68.9 d	31.6 t	31.6 t	31.6 t	61.6 d	61.7 d	61.4 d	133.5 d	61.5 d
17	128.7 d	125.4 d	125.4 d	125.4 d	124.1 d	13.8 q	123.2 d	14.5 q	13.7 q
18	130.1 d	126.7 d	126.7 d	126.6 d	133.4 d		134.1 d		
19	12.9 q	12.9 q	12.8 q	12.9 q	13.7 q		13.9 q		
2′		67.7 d	67.0 d	69.8 d	70.8 d	70.8 d		70.8 d	71.7 d
2′-CH_3		18.0 q	17.7 q	14.9 q	14.7 q	14.7 q		14.8 q	18.2 q
3′		70.3 d	70.8 d	76.9 d	76.9 d	76.9 d		76.8 d	72.0 d
3′-$OCOCH_3$				170.6 s	169.7 s	169.6 s		169.6 s	170.3 s
3′-$OCOCH_3$				21.3 q	20.7 q	20.6 q		20.7 q	20.9 q
4′		58.9 s	57.3 s	57.8 s	58.0 s	58.0 s		58.0 s	63.6 s
4′-$N(CH_3)_2$		36.7 q	36.7 q	39.3 q	39.7 q	39.7 q		39.7 q	37.5 q
4′-CH_3		11.0 q	12.2 q	13.7 q	13.8 q	13.8 q		13.8 q	11.7 q
5′		36.9 t	33.1 t	41.1 t	43.0 t	43.0 t		42.9 t	38.2 t
6′		72.5 d	69.9 d	64.9 d	64.1 d	64.1 d		64.2 d	70.4 d
2″		77.7 d	77.4 d	77.7 d					
2″-CH_3		18.6 q	18.9 q	18.9 q					
3″		71.2 d	71.8 d	71.7 d					
4″		67.7 d	67.3 d	67.5 d					
4″-$N(CH_3)_2$		40.4 q	40.3 q	40.4 q					
5″		28.6 t	28.4 t	28.7 t					
6″		75.4 d	75.1 d	75.3 d					

[a,b] Measured at 100 MHz in (a) $CDCl_3$ or (b) C_6D_6; ppm from TMS.

in the spectra of the other compounds. The ^1H and ^{13}C NMR spectra data in chloroform-*d* or benzene-d_6 are summarized in Tables 2 and 3.

Structure Elucidation

The UV spectra of saptomycins A, B, C_1, C_2, D, E, F, G and H indicated the presence of a 11-hydroxy-4H-anthraceno[1,2-b]pyran-4,7,12-trione chromophore moiety in common, suggesting that the compounds were closely related to the pluramycin-group antibiotics[2]. The ^1H and ^{13}C NMR spectral data (Tables 2 and 3) and other spectral data for each saptomycin, however, showed different characteristics from known pluramycin-group antibiotics.

Saptomycins A and F

The HREI-MS data of saptomycins A (as $C_{24}H_{20}O_6$) and F (as $C_{24}H_{18}O_6$) suggested the absence of the two amino sugar moieties at C-8 and C-10, which are well-known in the pluramycin-group antibiotics. The ^1H NMR spectra of saptomycins A and F, illustrating simple shapes, supported this suggestion. In the ^1H NMR of saptomycins A and F, characteristic signals were detected at 3-H (δ 6.28 and δ 6.53), 6-H (δ 8.09 and δ 8.08), 8-H (δ 7.83 and δ 7.82), 9-H (δ 7.69 and δ 7.67), 10-H (δ 7.36) and 13-H_3 (δ 3.02) in common. These properties are similar to those of α-indomycinone[8], SF2330[9], SS43405D[10] and E[11], β-indomycinone, rubiflavinone C-1 and C-2[5] and hydramycin[12]. The side chains at C-2 of saptomycins A and F were, however, different from those of the known compounds without sugar moieties.

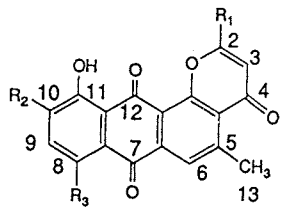
The spectrum of saptomycin A indicated that the side chain of the compound was constituted of 2 × CH_3 groups (δ 1.45 and δ 1.70), 2 × olefin protons (δ 5.50 and δ 5.65), 1 × O–CH group (δ 5.01) and 1 × CH group (δ 2.99). Examination of the ^1H-^1H COSY spectrum revealed the presence of a 2-hydroxy-1-methyl-3-pentenyl moiety at C-2, and the coupling constant for 17-H and 18-H was 10.5 Hz, indicating the (*Z*)-configuration of an olefin system. This alkyl side chain had the same substructure as SS21020C[13]; hence the comparison of ^1H NMR spectra for both compounds supported the common structure. In addition, the ^{13}C NMR data for the C-2 side chain described for SS21020C (δ 130.4 d; δ 125.6 d; δ 68.8 d; δ 44.9 d; δ 13.3 q; δ 12.7 q) was identical to those of saptomycin A (δ 130.1 d; δ 128.7 d; δ 68.9 d; δ 44.9 d; δ 13.5 q; δ12.9 q).

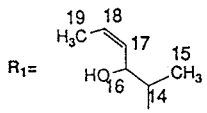
For determination of the structure of the side chain at C-2 of saptomycin F, the ^1H and ^{13}C NMR spectral data were also available; therefore, structural elucidation was mainly based on these data. Examination of the ^1H NMR and ^1H-^1H COSY spectra of saptomycin F suggested that the side chain was 1,2-dihydroxy-1-methyl-3-pentenyl moiety or a 1,2-epoxy-1-methyl-3-pentenyl moiety with a (*Z*)-configuration ($J_{17,18}$ = 11.0 Hz). The chemical shifts at 16-H in the ^1H and ^{13}C NMR data (δ_H 4.24/δ_C 61.4) were obviously different from that of the 14,16-dihydroxyl compound PD121,222[14] (δ_H 4.86/δ_C 71.7) but identical to those of the 14,16-epoxy compound saptomycin D[15] and pluramycin A[2] (δ_H 4.06/δ_C 61.6 and δ_H 4.15/δ_C 61.7, respectively). The structures of saptomycins A and F were determined to be 11-hydroxy-5-methyl-2-(2-hydroxy-1-methyl-3-(*Z*)-pentenyl)-4H-anthraceno[1,2-b]pyran-4,7,12-trione and 11-hydroxy-5-methyl-2-(1,2-epoxy-1-methyl-3-(*Z*)-pentenyl)-4H-anthraceno[1,2-b]pyran-4,7,12-trione, respectively (Fig. 2).

Saptomycins B, C_1 and C_2

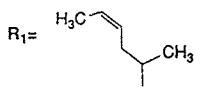
The HRFAB-MS data of saptomycins B, C_1 and C_2 indicated $C_{41}H_{52}N_2O_9$, $C_{41}H_{52}N_2O_9$ and

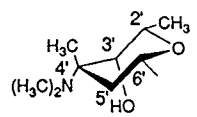
Fig. 2. The structure of saptomycins A (**1**), B (**2**), C_1 (**3**), C_2 (**4**), D (**5**), E (**6**), F (**7**), G (**8**), H (**9**), ankinomycin (**10**) and pluramycin A (**11**).

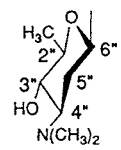




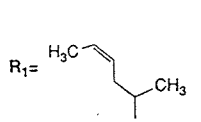
R_1= R_2= H R_3= H Saptomycin A (**1**)

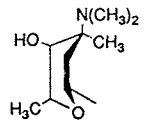


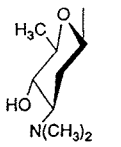




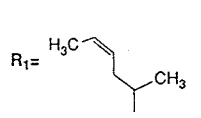
R_1= R_2= R_3= Saptomycin B (**2**)

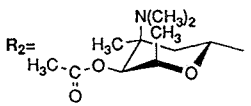


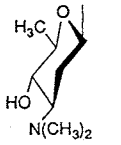




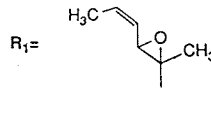
R_1= R_2= R_3= Saptomycin C_1 (**3**)

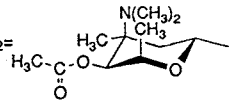




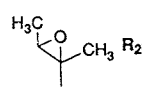


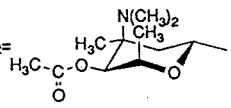
R_1= R_2= R_3= Saptomycin C_2 (**4**)



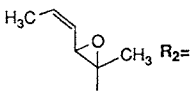


R_1= R_2= R_3= H Saptomycin D (**5**)

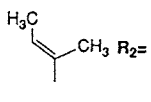


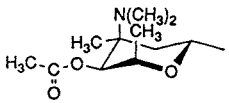


R_1= R_2= R_3= H Saptomycin E (**6**)

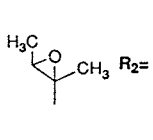


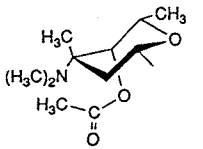
R_1= R_2= H R_3= H Saptomycin F (**7**)



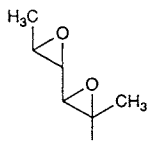


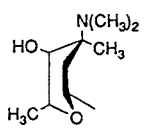
R_1= R_2= R_3= H Saptomycin G (**8**)



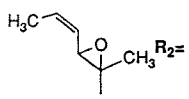


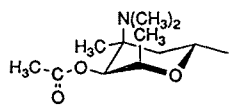
R_1= R_2= R_3= H Saptomycin H (**9**)

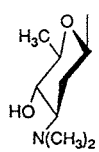




R_1= R_2= R_3= H Ankinomycin (**10**)







R_1= R_2= R_3= Pluramycin A (**11**)

$C_{43}H_{54}N_2O_{10}$, respectively. Further, the ^{13}C NMR spectra of saptomycins B, C_1 and C_2 indicated 41, 41, and 43 carbons, including in common $6\times C-CH_3$, $4\times N-CH_3$, $3\times$ methylenes, $8\times$ methines, $5\times -CH=$, $11\times C=$, $3\times C=O$ and $1\times$ quaternary carbon (total 41 carbons), and that of saptomycin C_2 showed two additional resonances from acetate ($C-CH_3$ and $C=O$). These results showed that the structures of these three compounds were closely related.

The ^1H NMR spectra of saptomycins B, C_1 and C_2 are shown in Figs. 3~5. The spectrum of saptomycin C_1 (Fig. 3) was very similar to those of kidamycin[4], hedamycin[16], and rubiflavins[5] with the exception of the signals due to the side chain moiety. On the other hand, the proton resonances for saptomycin B (Fig. 4) correspond very closely to that of isokidamycin[4], which was the configurational isomer at C-6′ of kidamycin, with the exception of resonances from the C-2 side chain. The ^1H NMR

Fig. 3. ^1H NMR spectrum of saptomycin C_1 in $CDCl_3$ at 400 MHz.

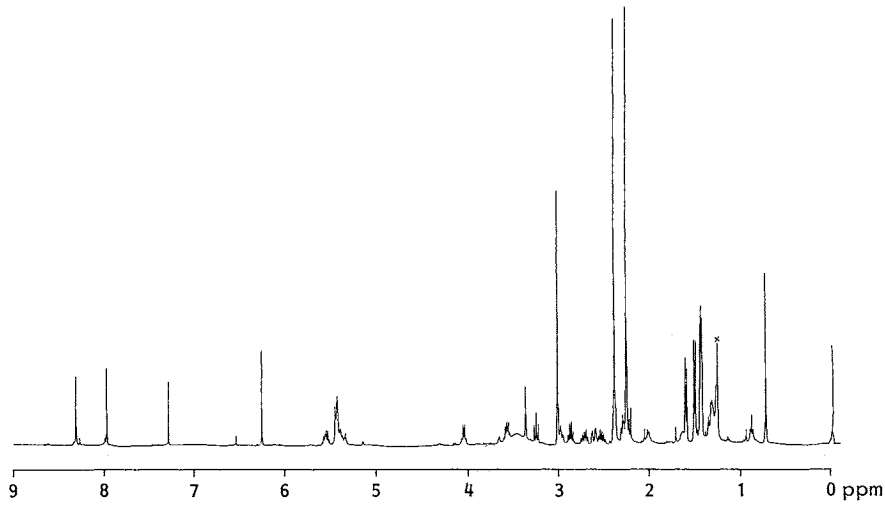


Fig. 4. ^1H NMR spectrum of saptomycin B in $CDCl_3$ at 400 MHz.

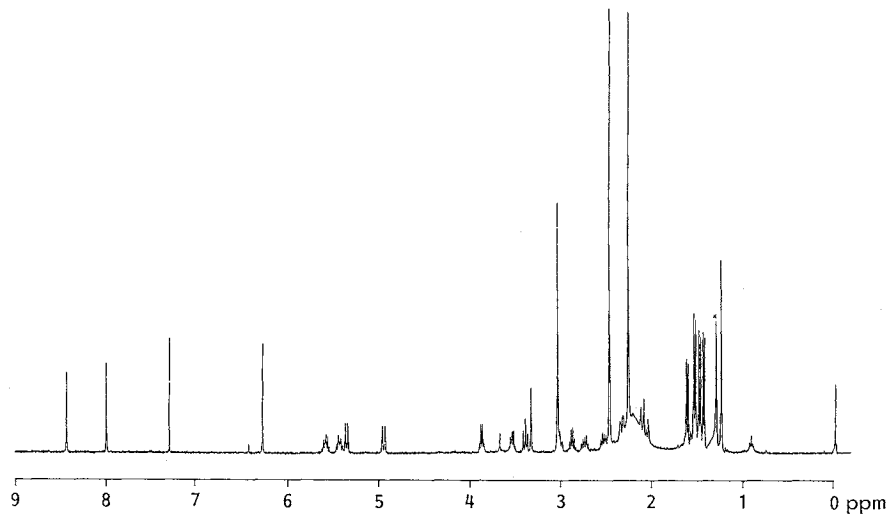
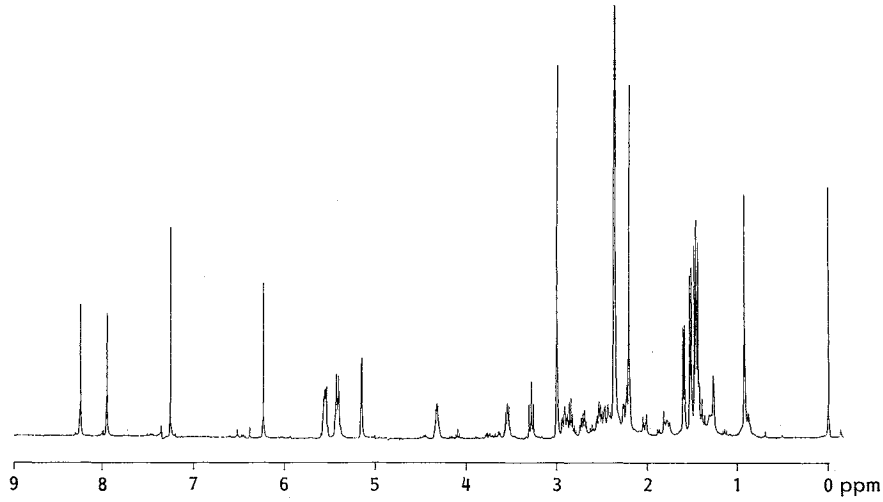
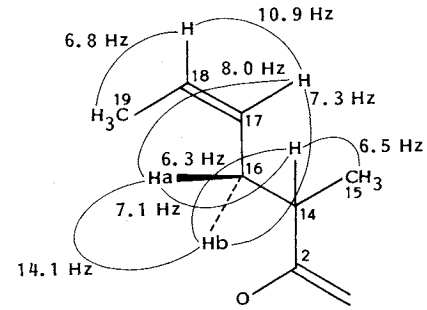


Fig. 5. ^1H NMR spectrum of saptomycin C_2 in $CDCl_3$ at 400 MHz.



data for saptomycin C_2 (Fig. 5) also showed that the compound was the C-2 side chain analog of pluramycin A and neopluramycin[2]. The three types of compounds described above were distiguishable in terms of the structural difference of each *N,N*-dimethylvancosamine moiety. In addition, the ^1H and ^{13}C NMR spectra of saptomycins B, C_1 and C_2 revealed the existence of an unknown side chain in common.

Fig. 6. The structure of side chain at C-2 of saptomycins B, C_1 and C_2.



Chromophore

Hz = ^1H-^1H coupling constants (Saptomycin B)

The structural determination of the side chain was mainly carried out by decoupling experiments with saptomycin C_1. The proton at δ 2.86 (14-H) was coupled to the protons at δ 2.53 (16-H_a) and δ 2.70 (16-H_b) and a methyl group at δ 1.44 (15-H_3). Two olefin protons at δ 5.41 (17-H) and δ 5.55 (18-H) were coupled to each other; further, 17-H was coupled to two protons at δ 2.53 (16-H_a) and δ 2.70 (16-H_b), and 18-H was connected to a methyl group at δ 1.59 (19-H_3). The coupling constant between the proton at δ 2.53 (16-H_a) and at δ 2.70 (16-H_b) was 14.2 Hz, indicating geminal protons. The result of the experiment proved the 1-methyl-3-pentenyl constitution of the side chain from C-14 to C-19. The coupling constant between the olefinic protons of 17-H and 18-H was, however, not measured exactly because the former was overlapped with the proton at C-6′ of the *N,N*-dimethylvancosamine ring (Fig. 3). In contrast, the ^1H NMR of saptomycin B indicated two isolated vinyl protons at δ 5.42 (17-H) and δ 5.58 (18-H), and the coupling constant between these two protons was 10.9 Hz, pointing to the (*Z*)-configuration (Fig. 4); hence the structure of the side chain at C-2 was determined as shown in Fig. 6.

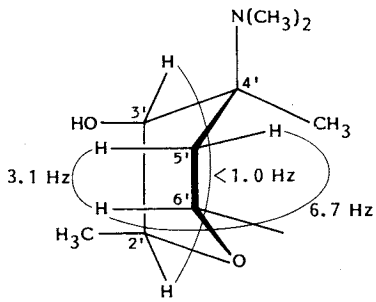
The UV spectra of saptomycins B, C_1 and C_2 were similar to those of saptomycins A and F,

characterizing the chromophore of these compounds as an 11-hydroxy-4H-anthraceno[1,2-b]pyran-4,7,12-trione system, which is well-known as the chromophore of pluramycin group compounds. The ^{1}H NMR spectra of saptomycins B, C_1 and C_2 confirmed this suggestion by the three characteristic signals at δ 6.27, δ 6.25 and δ 6.24 (3-H), δ 7.99, δ 7.96 and δ 7.96 (6-H) and δ 8.43, δ 8.31 and δ 8.25 (9-H). This behavior suggested that two aminosugars were substituted at C-8 and C-10 in such a way as the previously described pluramycins carried two *C*-glycosidically bound aminosugars at C-8 and C-10.

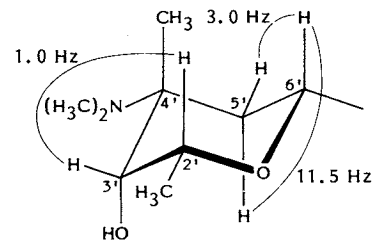
The aminosugar ring attached to C-8, angolosamine, of saptomycins B, C_1 and C_2 showed similar behavior in the ^{1}H and ^{13}C NMR spectra (Figs. 3~5 and Table 3). This ring was investigated in detail in the case of hedamycin[16]. The identity of conformation and configuration of the aminosugar moiety was established by comparison of the chemical shifts assigned in the ^{1}H and ^{13}C NMR data (Tables 2 and 3) and the ^{1}H-^{1}H coupling constants, except for a small number of overlapped signals.

As previously described, another aminosugar ring, *N*,*N*-dimethylvancosamine, was recognized to be of a different form in the cases of saptomycins B, C_1 and C_2, respectively, by the NMR studies of these compounds. In particular, in the ^{1}H NMR spectra (Fig. 3~5), the sugar moiety possessed different coupling constants between 5′-H_a, 5′-H_b and 6′-H and between 2′-H and 3′-H ($J_{2'-3'}=1.0$ Hz, $J_{5'a-6'}=11.5$ Hz, $J_{5'b-6'}=3.0$ Hz for saptomycin B; $J_{2'-3'}<1.0$ Hz, $J_{5'a-6'}=6.7$ Hz, $J_{5'b-6'}=3.1$ Hz for saptomycin C_1; $J_{2'-3'}=4.5$ Hz, $J_{5'a-6'}=8.7$ Hz, $J_{5'b-6'}=3.1$ Hz for saptomycin C_2, shown in Fig. 7). In our previous papers[15,17], we reported that the acetylation at the 3′-OH of saptomycin D caused a conformational change in the *N*,*N*-dimethylvancosamine ring. The different coupling constants between saptomycins C_1 and C_2 suggested that this conformational change had occurred. Further, deacetylation at 3′-OAc of

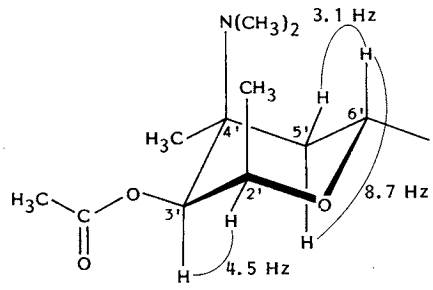
Fig. 7. Summary of ^{1}H-^{1}H coupling constants *N*,*N*-dimethylvancosamine moieties in saptomycins B, C_1 and C_2.



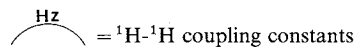
Saptomycin C_1



Saptomycin B



Saptomycin C_2



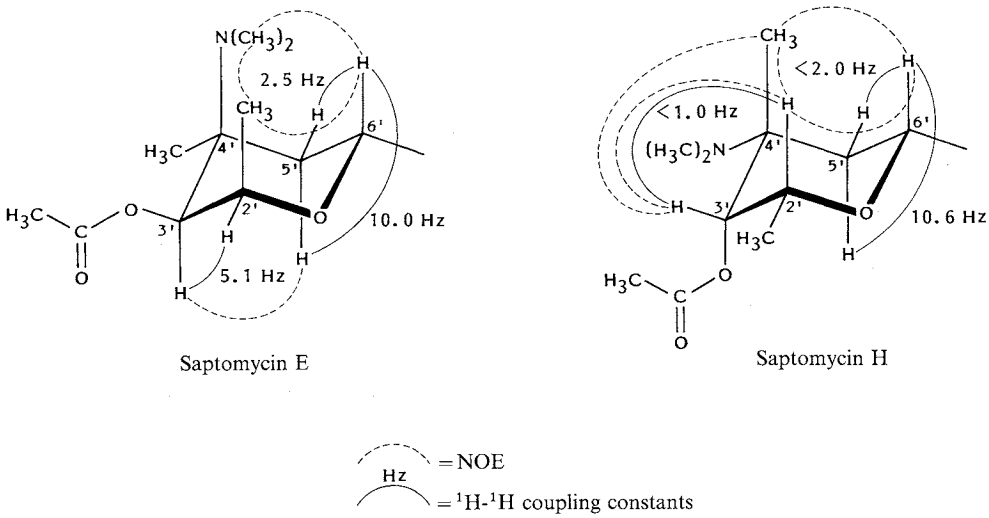
saptomycin C_2 in methanol at room temperature for 48 hours yielded saptomycin C_1, proving that the observation was correct. It was proven that saptomycin B was a configurational isomer at the C-6′ of saptomycin C_1 from the detailed comparison of ^1H and ^{13}C NMR data with those of isokidamycin (Tables 2 and 3). The ^1H NMR data of kidamycin and isokidamycin[4] clearly showed the different values of chemical shifts at 6′-H, 4′-CH_3 and 2′-H of the *N*,*N*-dimethylvancosamine moiety (δ 5.40, δ 0.77 and δ 4.07 in kidamycin and δ 4.83, δ 1.21 and δ 3.83 in isokidamycin, respectively), while those of saptomycins C_1 and B were observed the similar results in the case of kidamycin and isokidamycin (δ 5.43, δ 0.72 and δ 4.04 in saptomycin C_1 and δ 4.95, δ 1.23 and δ 3.87 in saptomycin B, respectively). In the ^{13}C NMR spectra of kidamycin and isokidamycin[16], the chemical shifts at C-6′, 4′-CH_3 and C-5′ gave the characteristic values depending of the configurational distinction (δ 69.5, δ 12.3 and δ 33.6 in kidamycin and δ 72.3, δ 10.9 and δ 37.0 in isokidamycin, respectively). Saptomycins C_1 and B also revealed the characteristic values of the chemical shifts at C-6′, 4′-CH_3 and C-5′ in the ^{13}C NMR spectra (δ 69.9, δ 12.2 and δ 33.1 in saptomycin C_1 and δ 72.5, δ 11.0 and δ 36.9 in saptomycin B, respectively). The structures of saptomycins B, C_1 and C_2 were elucidated as shown in Fig. 2.

Saptomycins D, E, G and H

The molecular formulae of saptomycins D, E, G and H were determined to be $C_{35}H_{37}NO_9$, $C_{33}H_{35}NO_9$, $C_{33}H_{35}NO_8$ and $C_{33}H_{35}NO_9$, respectively, according to the data of the HRFAB-MS spectra. These profiles suggested that saptomycins D, E, G and H were mono-*C*-aminoglycosyl compounds as is ankinomycin[6]. As previously reported[15], the structural characteristics of saptomycins D and E were supported by the ^1H and ^{13}C NMR spectra studies. A characteristic AB type spin system 8-H and 9-H ($J_{8-9}=7.9$ Hz) in the ^1H NMR and the chemical shift at C-8 (δ 119.4) in the ^{13}C NMR indicated the common structure of saptomycins D and E, lacking the angolosamine substituent at C-8 of typical pluramycin-group compounds. The other signals in the ^1H and ^{13}C NMR spectra from the chromophore were also assigned on the basis of the results of ^1H-^1H COSY, ^{13}C-^1H HETCOR and long range ^{13}C-^1H HETCOR experiments (Tables 2 and 3). In addition, these experiments showed the structures of the side chains at C-2 of saptomycins D and E to be a 1,2-epoxy-1-methyl-3-pentenyl moiety with a (*Z*)-configuration and a 1,2-epoxy-1-methylpropyl moiety, respectively. The corresponding resonances in the ^1H and ^{13}C NMR spectra of the side chains at C-2 in pluramycin A and epoxykidamycin[18] supported the partial structures in saptomycins D and E. The configuration of the epoxides of both compounds was determined to have *cis* geometry by the δ_C value (δ 14.4 and δ 13.6) of the methyl group at C-15: *cis* geometry such as in pluramycin A, hedamycin and ankinomycin had a value of *ca.* 14 ppm, while *trans* geometry indicated a value of *ca.* 19 ppm in the case of altromycins[7]. Therefore, the relative configurations at C-2 in saptomycins D and E were determine to be (14*R**,16*S**,17*Z*) and (14*R**,16*S**), respectively.

The ^1H and ^{13}C NMR spectra of saptomycin G showed a difference only in the side chain at C-2 from saptomycins D and E. The side chain in saptomycin G was composed of four varbon units: a quaternary carbon (C-14, δ_C 126.4), two CH_3 carbons (C-15, δ_C 11.7; δ_H 1.52 s, C-17, δ_C 14.5; δ_H 1.57 d) and a CH carbon (C-16, δ_C 133.5; δ_H 7.38 q). The values of the chemical shifts and coupling constants of the side chain at C-2 were nearly identical to those of the resonances in kidamycin and neopluramycin[2,4]. As a result, the structure of the substituent was determined to be a 1-methyl-1-propenyl group with an (*E*)-configuration, as confirmed by the observation of an NOE between C-15 and C-17 in the NOESY experiment.

Fig. 8. Summary of ^{1}H-^{1}H coupling constants and NOESY data of the *N*,*N*-dimethylvancosamine moieties in saptomycins E and H.



Saptomycins D, E and G carried a *C*-glycosidically bound 3′-*O*-acetyl-*N*,*N*-dimethylvancosamine moiety at C-10 as pluramycin A and neopluramycin did, supported by the comparison of their ^{1}H and ^{13}C NMR data. The conformation of the aminosugar moiety was confirmed to be a chair form by comparison of the coupling constants between 5′-H and 6′-H ($J_{5'a-6'}=ca.$ 10.0 Hz and $J_{5'b-6'}=ca.$ 3.2 Hz) and 2′-H and 3′-H ($J_{2'-3'}=ca.$ 5.2 Hz). As a result of all the experiments, the relative structures of saptomycins D, E and G were determined to be as shown in Fig. 2.

Saptomycin H appeared to be a configurational isomer at C-6′ of the 3′-*O*-acetyl-*N*,*N*-dimethyvancosamine moiety on saptomycin E, based on the ^{1}H and ^{13}C NMR data (Tables 2 and 3). The comparison of the values of the chemical shift of 6′-H and the ^{1}H-^{1}H coupling constants in the ^{1}H NMR of the sugar in saptomycin H with those of saptomycin B indicated that saptomycins B and H had a characteristic configuration in common ($\delta_{6'-H}=4.95$, $J_{2'-3'}=1.0$ Hz, $J_{5'a-6'}=11.5$ Hz and $J_{5'b-6'}=3.0$ Hz in saptomycin B; $\delta_{6'-H}=5.14$, $J_{2'-3'}<1.0$ Hz, $J_{5'a-6'}=10.6$ Hz and $J_{5'b-6'}<2.0$ Hz in saptomycin H). Further, saptomycin H displayed different NOESY data from that of saptomycin E on their sugar moieties, summarized as Fig. 8. Therefore, the structure of saptomycin H was determined as shown in Fig. 2.

The relative structure of the novel pluramycin-group antibiotics, saptomycins A, B, C_1, C_2, D, E, F, G and H were determined to be those of structure **1**~**9** based on analyses of all physico-chemical and spectroscopic studies (Fig. 2). The absolute configuration of these compounds were not determined, but it seemed that saptomycins were biologically related to other pluramycin-group antibiotics; hence we assumed that the absolute configuration of saptomycins were the same as those of kidamycin[19] and hedamycin[20], previously studied in detail.

Experimental

EI-MS and FAB-MS were measured on a JEOL JMS-SX102 spectrometer. IR spectra were recorded on a Hitachi 270-30 spectrometer. Optical rotations were taken on a Horiba SEPA-200 spectrometer. ^{1}H and ^{13}C NMR measurements were performed on a Varian VXR400 spectrometer.

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