NOVEL ANTITUMOR ANTIBIOTICS, SAPTOMYCINS D AND E

Sir:

In the course of our search for novel antitumor antibiotics from microorganisms, *Streptomyces* sp. HP530 was found to produce two potent antitumor antibiotics, saptomycins D and E. Chemical structures of these compounds are closely related to those of the pluramycin-group antibiotics (Fig. 1), which have remarkable antitumor activities. In this communication, we describe the production, purification, physico-chemical characterization, structure determination and biological properties of saptomycins D and E.

The producing organism named strain HP530 was isolated from a soil sample collected in Ichikawa City, Chiba Prefecture, Japan. The strain was cultured at 30°C for 3 or 4 days in 45 liters of a production medium composed of glucose 0.5%, oatmeal 3.0%, Pharmamedia 1.0%, $MgSO_4 \cdot 7H_2O$ 0.5%, $CoCl_2$ 0.002% and $CaCO_3$ 0.3% (pH 7.0). The fermentation broth was filtered and the obtained mycelial cake was soaked in 15 liters of 80% aqueous acetone for 24 hours. The aqueous solution after removal of acetone was extracted with 3 liters of ethyl acetate. The organic layer was evaporated *in vacuo*, and then washed with *n*-hexane. The residue was charged on a column packed with ODS-AQ120-S50 (Yamamura Kagaku: YMC), developed with the solvent system of 0.15% KH_2PO_4 (pH 3.5)-MeOH (4 : 1), and after being washed with the same solvent, it was developed with the same solvents using a 7 : 3 ratio. The eluate was separated into two active fractions, which were further purified by preparative TLC. The first fraction, which eluted rapidly from the above column was developed by the solvent system of $CHCl_3$-MeOH (19 : 1), and yielded a yellow powder (13.5 mg) of saptomycin E. The second fraction was rechromatographed under the same condition and yielded saptomycin D (27.8 mg) as a yellowish red powder.

Physico-chemical properties of these compounds are summarized in Table 1. The UV spectra of saptomycins D and E showed similar profiles, which suggested the presence of a 11-hydroxy-4*H*-anthraceno[1,2-*b*]pyran-4,7,12-trione skeleton known as the chromophore of the pluramycin-group antitumor antibiotics, pluramycin A[1,2], rubiflavins[3~5], kidamycin[6], hedamycin[7], ankinomycin[8], altromycin[9], SF-2330[10], A51493A[11] and DC92-B[12].

The HRFAB-MS spectra of saptomycins D and E established the molecular formulas as $C_{35}H_{37}NO_9$ and $C_{33}H_{35}NO_9$, respectively. The strong absorption band at 1745 cm^{-1} (saptomycin D) and 1740 cm^{-1} (saptomycin E) shown in the IR spectra suggested the existence of a carbonyl group of acetate.

The analysis of saptomycins D and E on TLC and HPLC, showed that their polarities were lower than those of known pluramycin-group antibiotics possessing two *C*-glycosyl moieties. These chromatographic behaviors suggested that saptomycins D and E had mono-*C*-glycosyl structures as does ankinomycin.

Further study of the ^1H NMR (Table 2) and ^{13}C NMR (Table 3) supported this characteristic structure lacking the angolosamine at C-8, and indicated that both antibiotics saptomycins D and

Table 1. Physico-chemical properties of saptomycins D and E.

	Saptomycin D	Saptomycin E
Appearance	Yellowish red powder	Yellow powder
$[\alpha]_D^{20}$ (*c* 0.1, $CHCl_3$)	+152°	+147°
Molecular formula	$C_{35}H_{37}NO_9$	$C_{33}H_{35}NO_9$
HRFAB-MS (*m/z*) $(M+H)^+$		
Calcd:	616.2546	590.2390
Found:	616.2556	590.2398
UV λ_{max} nm (ε)	246 (51,600), 265 (sh, 29,000), 418 (9,800)	244 (47,200), 265 (sh, 28,000), 425 (10,800)
IR ν_{max} (KBr) cm^{-1}	1745, 1665, 1642, 1590	1740, 1665, 1642, 1590
Rf value[a]		
$CHCl_3$-CH_3OH (20 : 1)	0.69	0.66
$CHCl_3$-EtOAc (1 : 1)	0.21	0.17
$CHCl_3$-Me_2CO (1 : 1)	0.61	0.60

[a] Silica gel TLC (Merck Art. No. 5715).

Table 2. ^{1}H NMR chemical shifts[a] of saptomycins D and E.

Proton	Chemical shifts (δ) in ppm	
	Saptomycin D	Saptomycin E
3-H	6.58 (1H, s)	6.47 (1H, s)
6-H	7.98 (1H, s)	7.97 (1H, s)
8-H	7.99 (1H, d, J=7.9)	7.95 (1H, d, J=7.9)
9-H	8.02 (1H, d, J=7.9)	7.98 (1H, d, J=7.9)
11-OH	13.60 (1H, s)	13.73 (1H, s)
13-H_3	2.90 (3H, s)	2.86 (3H, s)
15-H_3	1.68 (3H, s)	1.56 (3H, s)
16-H	4.06 (1H, dd, J=1.2, 7.6)	2.99 (1H, q, J=5.4)
17[b]	5.32 (1H, ddq, J=7.6, 11.2, 1.8)	0.99 (3H, d, J=5.4)
18-H	5.70 (1H, ddq, J=1.2, 11.2, 7.1)	—
19-H_3	1.63 (3H, dd, J=1.8, 7.1)	—
2′-H	4.40 (1H, dq, J=5.2, 6.8)	4.34 (1H, dq, J=5.1, 6.6)
2′-CH_3	1.63 (3H, d, J=6.8)	1.58 (3H, d, J=6.6)
3′-H	5.36 (1H, d, J=5.2)	5.30 (1H, d, J=5.1)
3′-OAc	1.80 (3H, s)	1.76 (3H, s)
4′-CH_3	0.89 (3H, s)	0.84 (3H, s)
4′-$N(CH_3)_2$	2.37 (6H, s)	2.32 (6H, s)
5′-H_a	1.42 (1H, dd, J=10.1, 14.0)	1.40 (1H, dd, J=10.0, 14.0)
5′-H_b	2.60 (1H, dd, J=3.1, 14.0)	2.58 (1H, dd, J=2.5, 14.0)
6′-H	5.79 (1H, dd, J=3.1, 10.1)	5.74 (1H, dd, J=2.5, 10.0)

[a] δ from TMS in C_6D_6, 400 MHz.
[b] At C-17, CH in saptomycin D and CH_3 in saptomycin E.
J=Hz.

Table 3. ^{13}C NMR chemical shifts[a] of saptomycins D and E.

Carbon	Chemical shifts (δ) in ppm		Carbon	Chemical shifts (δ) in ppm	
	Saptomycin D	Saptomycin E		Saptomycin D	Saptomycin E
C-2	167.1 s	167.8 s	C-13	24.0 q	24.0 q
C-3	109.9 d	109.5 d	C-14	59.1 s	57.4 s
C-4	178.4 s	178.3 s	C-15	14.4 q	13.6 q
C-4a	126.6 s	126.7 s	C-16	61.6 d	61.7 d
C-5	149.7 s	149.8 s	C-17	124.1 d	13.8 q
C-6	125.8 d	125.9 d	C-18	133.4 d	—
C-6a	136.2 s	136.2 s	C-19	13.7 q	—
C-7	181.3 s	181.3 s	C-2′	70.8 d	70.8 d
C-7a	131.1 s	131.1 s	C-3′	76.9 d	76.9 d
C-8	119.4 d	119.4 d	C-4′	58.0 s	58.0 s
C-9	133.6 d	133.6 d	C-5′	43.0 t	43.0 t
C-10	140.7 s	140.7 s	C-6′	64.1 d	64.1 d
C-11	159.4 s	159.4 s	2′-CH_3	14.7 q	14.7 q
C-11a	116.3 s	116.3 s	4′-CH_3	13.8 q	13.8 q
C-12	188.0 s	188.1 s	4′-$N(CH_3)_2$	39.7 q	39.7 q
C-12a	119.9 s	119.9 s	3′-$OCO-$	169.7 s	169.6 s
C-12b	156.3 s	156.3 s	3′-$OCO-CH_3$	20.7 q	20.6 q

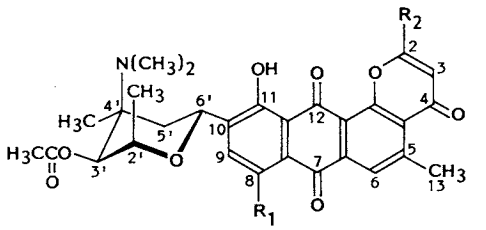
[a] δ from TMS in C_6D_6, 100 MHz.

E had very similar structures. The ^{1}H NMR spectra of saptomycins D and E showed differences only in the side chain at C-2 and a characteristic AB type spin system at 8-H and 9-H ($J_{8\text{-H}/9\text{-H}}$=7.9 Hz) indicated an ankinomycin-type substituent. In addition, the chemical shifts at C-8 (119.4 ppm) in the ^{13}C NMR spectra supported this suggestion[8].

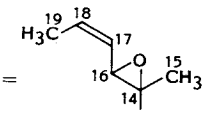
As a common structural unit, saptomycins D and

E had 3-*O*-acetyl-*N*,*N*-dimethylvancosamine attached to the chromophore. This type of amino sugar was identical with those of pluramycin A and neopluramycin[2]. The conformation of the amino sugar was determined to be a chair form by the comparison of coupling constants between C-5′ and C-6′: saptomycins D and E ($J_{5'\text{-}H_a/6'\text{-}H}$=10.0 or 10.1 Hz, $J_{5'\text{-}H_b/6'\text{-}H}$=3.1 or 2.5 Hz), ankinomycin ($J_{5'\text{-}H_a/6'\text{-}H}$=5.8 Hz, $J_{5'\text{-}H_b/6'\text{-}H}$=6.3 Hz)[8] and altromycins ($J_{5'\text{-}H_a/6'\text{-}H}$=10.7 Hz)[9]. NOESY experiments showed the existence of 1,3,5-triaxial cross peaks between 2′-CH_3, 4′-$N(CH_3)_2$ and 6′-H and a 1,3-diaxial cross peak between 5′-H_a and 3′-H.

Fig. 1. The structures of saptomycins D, E, pluramycin A and rubiflavin E.



Saptomycin D R_1=H R_2=



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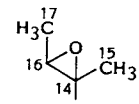
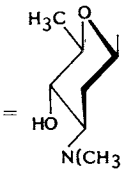
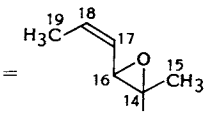


Pluramycin A

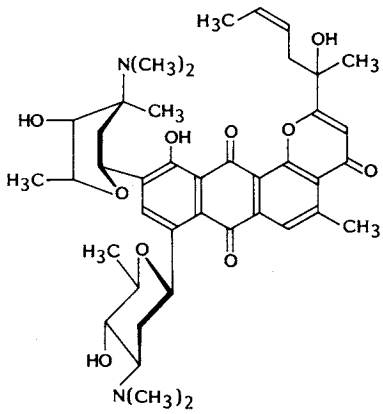
R_1=



R_2=



Angolosamine



Rubiflavin E

Details of structural elucidation will be reported in a separate paper.

As shown in the spectra of ^1H and ^{13}C NMR, the side chains at C-2 of saptomycins D and E closely resembled to those of pluramycin A and epoxykidamycin[13], respectively. The side chain of saptomycin D has a 1,2-epoxy-1-methyl-3-pentenyl moiety with (*Z*)-configuration ($J_{17\text{-}H/18\text{-}H}$=11.2 Hz) and saptomycin E has a 1,2-epoxy-1-methylpropyl moiety. The configuration of epoxides in their side chains was assumed to be *cis* geometry. The value of the methyl group at C-15 in the ^{13}C NMR data of pluramycin A, hedamycin, ankinomycin and saptomycins D and E showed *ca.* 14 ppm, while its shift in *trans* geometry indicated *ca.* 19 ppm in the case of altromycins[8,9]. Consequently, the relative configurations in the side chains at C-2 of saptomycins D and E were determined to be (14*R**,16*S**,17*Z*) and (14*R**,16*S**), respectively[14]. All the assignments of chemical shifts on ^1H and ^{13}C NMR spectra of saptomycins D and E were further confirmed by the ^1H-^1H COSY, ^{13}C-^1H HETCOR and long range ^{13}C-^1H HETCOR experiments. The relative structures of saptomycins D and E were determined and shown in Fig. 1 as the result of observation of all the experiments.

Antimicrobial activities of saptomycins D and E are shown in Table 4. Both compounds strongly inhibited the growth of Gram-positive bacteria and

Table 4. Antimicrobial activities of saptomycins D and E.

Test organisms	MIC (μg/ml)	
	Saptomycin D	Saptomycin E
Bacillus subtilis M45 (Rec⁻)[a]	0.4	0.2
B. subtilis H17	3.2	12.5
Staphylococcus aureus JCM2151	1.6	6.3
S. epidermidis JCM2414	3.2	>100
Micrococcus luteus JCM1464	1.6	0.8
Escherichia coli JCM1649	>100	>100
Klebsiella pneumoniae JCM1662	>100	>100
Proteus vulgaris JCM1668	>100	>100
Xanthomonas maltophilia JCM1975	3.2	>100
Salmonella typhimurium TA1535	1.6	0.8
Candida albicans JCM1542	100	>100
Saccharomyces cerevisiae JCM1499	6.3	>100

[a] Recombination deficient.

Table 5. Antitumor activities of saptomycins D and E and rubiflavin E.

Dose (mg/kg/day)	T/C (%)	Dose (mg/kg/day)	T/C (%)	
	Saptomycin D		Saptomycin E	Rubiflavin E
0.5	>215	5.0	>192	—
0.25	192	2.5	>196	192
0.125	123	1.25	127	108
0.0625	108	0.625	104	92

Six mice (CDF_1, female) were inoculated ip with 1×10^6 Meth-A cells on day 0. Each sample dissolved in CH_3OH-PBS(−) (1:9) was administered ip on days 1~4. The increase in life span was indicated as T/C (%).

weakly inhibited the growth of Gram-negative bacteria. Especially, saptomycin D was weakly active against a certain kind of yeast. Saptomycins D and E were effective against mouse fibrosarcoma Meth-A *in vivo*, as shown in Table 5. The antitumor activities of saptomycins D and E were remarkable for their clear increases in life span. In fact the activity of saptomycin D was over 10-fold higher than that of rubiflavin E, which was prepared for us to isolate from the strain producing rubiflavins. The LD_{50} value of saptomycin D in CDF_1 mouse was about 45 mg/kg at a single ip injection. Further investigations, against human cancer cell lines *in vitro* and their xenografts *in vivo* in nude mice, are under way.

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