SAPHENAMYCIN, A NOVEL ANTIBIOTIC FROM A STRAIN OF *STREPTOMYCES*

Sir:

A new antibiotic which we named saphenamycin has been isolated from the cultured broth of a *Streptomyces* designated as MG314-hF8. The strain was classified as *Streptomyces canarius* by taxonomic study and direct comparison with a type culture[1]. In this paper, we report on the production, isolation, physical properties, structure, and biological activity of saphenamycin.

Streptomyces canarius MG314-hF8 was cultured at 27°C under agitation (250 rpm) and aeration (15 liters/minute) for 48 hours in 15 liters of a medium placed in 30-liter jar fermentor. The medium consisted of glycerol 1.5%, soluble starch 1.5%, soy bean meal 0.5%, fish meal (Hokuyo Meal®) 1.5%, and $CaCO_3$ 0.2% (pH 7.4 before sterilization). The amount of saphenamycin was determined by the paper disc-plate method using *Bacillus subtilis* PCI 219 and pure saphenamycin (1,000 μg/ml) as the standard.

Saphenamycin mainly contained in the filtered cake of the cultured broth. The filtered cake (1.5 kg) obtained from the cultured broth (30 liters) was extracted with methanol to elute 3.36 g of saphenamycin, which was purified by silica gel column chromatography (methanol - benzene, 1: 49) followed by recrystallization from chloroform - *n*-hexane (1: 1), yielding yellow prisms of saphenamycin (320 mg).

Saphenamycin has mp 200~202°C, $[\alpha]_D^{24}$ 0° (*c* 1.0, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{18}N_2O_5$: C 68.65, H 4.51, N 6.96, O 19.88. Found: C 68.30, H 4.54, N 6.61, O 19.75. The molecular formula was shown by FD-MS of saphenamycin (M^+ 402). UV_{max}: 254 nm (ε 106,000) and 366 nm (ε 15,600) in methanol. The IR spectrum is shown in Fig. 1. The PMR chemical shifts are shown as Table 1.

Table 1. Chemical shifts of PMR spectra.

Proton	δ ppm (*J* Hz)
2′-H_3	1.98 d (7)
6″-CH_3	2.72 s
3″ or 5″-H	6.75 d (8)
5″ or 3″-H	6.82 d (8)
4″-H	7.29 t (9)
1′-H	7.47 q (7)
3, 7, 8, 9-H	8.06~8.24 m
4-H	8.59 dd (10, 3)
2-H	8.99 dd (8, 3)
2″-OH or 1-COOH	11.10 s
1-COOH or 2″-OH	15.38 s

Yellow prismatic crystals were grown in chloroform - *n*-hexane (1: 1) solutions to determine the molecular structure by X-ray diffraction. The crystal data are as follows: saphenamycin, $C_{23}H_{18}N_2O_5$, space group $P2_1/a$, a=19.605(10), b=12.850(7), c=15.799(8) Å and β=99.54(5)°, Z=8. The structure was shown by direct methods and shown to be 6-[1-(2-hydroxy-6-methyl)benzoyloxy]ethyl-phenazine-1-carboxylic acid. Refinement by block-diagonal least-squares methods yielded an R factor 0.083 for 4423 reflections without hydrogen atom contributions. The structures of the two molecules, A and B, contained in the same asymmetric unit differ only slightly; the most significant differences are about 10° for the torsion angles around C(8)-C(18) and C(18)-O(20) which connect the benzoyl group to the phenazine ring. A perspective view of the

Fig. 1. Infrared spectrum of saphenamycin (KBr).

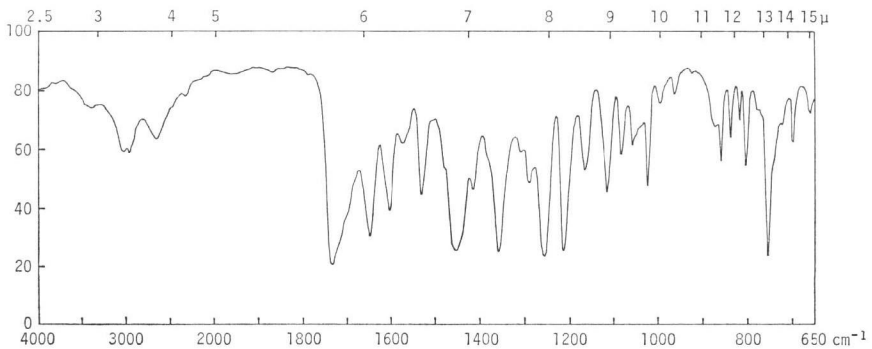


Fig. 2. A perspective view of the molecule of saphenamycin (molecule A).

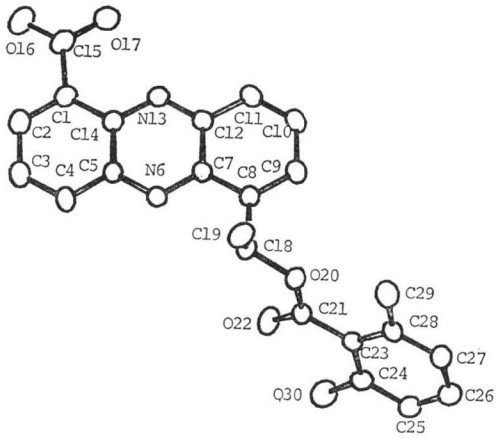
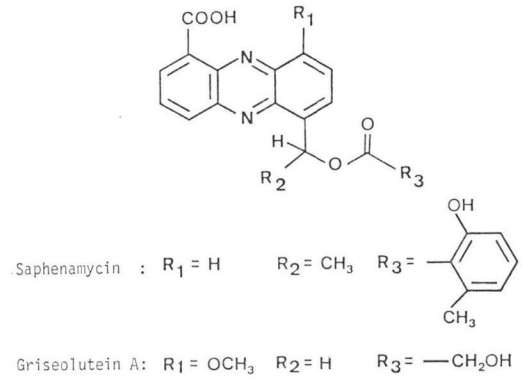


Fig. 3. Structure of saphenamycin and griseolutein A.



molecule A is shown in Fig. 2.

Saphenamycin inhibits growth of Gram-positive bacteria. Especially it inhibited *Bacillus subtilis* strongly as shown in Table 2. The minimum inhibitory concentrations of saphenamycin were compared with those of griseoluteins A[2] and B[3] as shown in Table 3. Saphenamycin exhibited a strong inhibition against *rec* B2 strain of *B. subtilis*. Furthermore, it exhibited a

Table 2. Antibacterial spectra of saphenamycin.

Test organisms	Medium	MIC (μg/ml)
Staphylococcus aureus FDA209P	1	0.39
Staphylococcus aureus Smith	1	0.20
Micrococcus flavus FDA16	1	0.10
Micrococcus luteus PCI1001	1	0.39
Bacillus subtilis PCI219	1	<0.05
Bacillus subtilis NRRL B-558	1	<0.05
Bacillus cereus ATCC10702	1	0.39
Corynebacterium bovis 1810	1	0.20
Escherichia coli NIHJ	1	50
Escherichia coli K12	1	100
Shigella dysenteriae JS11910	1	6.25
Shigella sonnei JS11746	1	>100
Proteus vulgaris OX19	1	>100
Mycobacterium smegmatis ATCC607	2	3.12
Mycobacterium phlei	2	1.56
Aeromonas salmonicida ATCC14174	1*	0.78
Vibrio anguillarum NCMB-6	1*	0.78

Medium 1: Nutrient agar
2: Nutrient agar +1% Glycerine
37°C, 17 hours. *27°C, 17 hours.

Table 3. Antibacterial spectra of saphenamycin, griseolutein A and griseolutein B.

Test organisms		MIC (μg/ml)		
		Saphenamycin	Griseolutein A	Griseolutein B
B. subtilis	PCI219	0.025	0.1	0.1
	GSY908 (*rec* E4)	0.025	6.25	0.1
	GSY1025 (*rec* A1)	0.05	0.39	0.1
	GSY1028 (*rec* B2)	0.006	0.003	0.0015
E. coli	K12	100	0.39	0.39
	AR20 (*rec* A1, *rec* B21)	25	3.12	3.12
	AB1885 (*uvr* B5)	100	3.12	6.25
	AB1886 (*uvr* A6)	25	0.78	0.78
	YK344 (*pol* A1)	100	6.25	6.25
	DM344 (*lex*-1)	100	6.25	25

Medium: Nutrient agar, 37°C, 17 hours.

strong action against *rec*⁻ strain of *E. coli* K12. ID_{50} of saphenamycin against mouse leukemia L5178Y cells and L1210 *in vitro* were 0.15 and 0.6~2.5 μg/ml, respectively. However, saphenamycin (250 μg/mouse/day×10 days) exhibited only a weak effect in prolonging the survival period of mice into which mouse leukemia L1210 cells or Ehrlich ascites carcinoma were implanted: (T/C; 119% against L1210; 120% against Ehrlich ascites carcinoma).

The structure of saphenamycin, a member of phenazine group of antibiotics such as griseolutein A[2], is shown in Fig. 3.

Acknowledgments

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The final atomic parameters will be deposited at the Cambridge Crystallographic Data Center and the list of Fo and Fc may be obtained from the author (Y.I. or H.N.).

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References

1) VAVRA, J. J. & A. DIETZ: U-13,714, a new antiviral agent. I. Discovery and biological properties. Antimicrob. Agents & Chemother. -1964: 75~79, 1965
2) NAKAMURA, S.; E. L. WANG, M. MURASE, K. MAEDA & H. UMEZAWA: Structure of griseolutein A. J. Antibiotics, Ser. A, 12: 55~58, 1959
3) NAKAMURA, S.; K. MAEDA & H. UMEZAWA: The structure of griseolutein B. J. Antibiotics, Ser. A, 17: 33~36, 1964