Communications to the editor

ANTIBIOTIC H 537 SY2, A NEW ANTIYEAST ANTIBIOTIC

Sir:

During the course of our screening for anti-cholesterol substances produced by microbes[1], a non-polyene antibiotic active against *Candida* was shown in the culture broth of a *Streptomyces* strain designated as *Str.* H 537 SY2. *Str.* H 537 SY2, classified to belong to *Streptomyces yokosukanensis*[2], was isolated from a soil sample collected at Misumi-Cho, Shimane Prefecture, Japan. The antibiotic was recovered from the broth filtrate by adsorption on activated carbon following elution with aqueous alcohol, evaporation and lyophilization. Purification was carried out by adsorption column chromatography on carbon and partition chromatography on a cellulose powder column. The purified antibiotic inhibits growth of yeasts, but not that of bacteria. The infrared absorption spectrum of the antibiotic suggests existence of an unusual carbonyl group (1780 cm^{-1}). Examples of groups with such absorption bands include the carbonyl group of an α,β-unsaturated γ-lactone, a β-lactam fused to another ring, *etc.* The antibiotic appears to be composed of three amino sugars based upon Avicel thin-layer chromatography of an acid hydrolyzate of the antibiotic. Validamycins[3~5] are composed of three kinds of sugars and are effective against fungi but lack the band at 1780 cm^{-1}. Thus, the antibiotic is considered to be new and tentatively named as antibiotic H 537 SY2.

Chart 1. Isolation and purification of antibiotic H 537 SY2.

Broth filtrate (18.2 liters, pH 7.4)
- stirred with activated carbon (182 g) for 60 minutes and filtered

Carbon cake
- eluted with 60% aqueous acetone

First eluate (4,900 ml, total activity 25.7%)
- concentrated *in vacuo* to 2,800 ml

Carbon column (45 cm × 3.5 cm diam., carbon 100g)
1. washed with H_2O (2,500 ml)
2. eluted with a linear gradient concentration of aqueous EtOH using H_2O (3,000 ml)—50% aqueous EtOH (3,000 ml) and collected in 18-ml fractions each

Fractions 1~50, 1,416 mg (total activity 7.86%)
Fractions 51~100, 1,793 mg (total activity 7.86%)
Fractions 101~162, 7,999 mg (total activity 16.75%)

Fractions 51~100 (600 mg)
- Cellulose powder column (80 cm × 1.8 cm diam.)
  - developed with *n*-BuOH - EtOH - H_2O (5:4:2) and collected in 11-ml fractions each

Fractions 26~30
- concentrated *in vacuo* and lyophilized

Pure antibiotic H 537 SY2 (30.5 mg)

Filtrate (total activity 30%)
- treated with active carbon (60g) for 60 minutes and filtered

Carbon cake
- eluted with 60% aqueous acetone

Second eluate (3,840 ml, total activity 8%)

Str. H 537 SY2 was cultured to prepare an inoculum seed in shaking flasks containing 100 ml of inoculation medium composed of 1.0% maltose and 0.4% yeast extract (pH 7.0) at 27°C for 42 hours on a reciprocal shaker (amplitude 7 cm, 130 strokes per minute). The inoculum was used to inoculate shaking flasks each containing 100 ml of a production medium composed of 1.5% soluble starch, 1.0% glucose, 2.0% soyameal, 0.5% yeast extract, 0.25% NaCl, 0.3% $CaCO_3$, 0.0008% $MnCl_2 \cdot 4H_2O$, 0.0007% $CuSO_4 \cdot 5H_2O$, 0.0002% $ZnSO_4 \cdot 7H_2O$ and 0.0001% $FeSO_4 7 \cdot H_2O$

Fig. 1. Ultraviolet absorption spectrum of antibiotic H 537 SY2 in H_2O

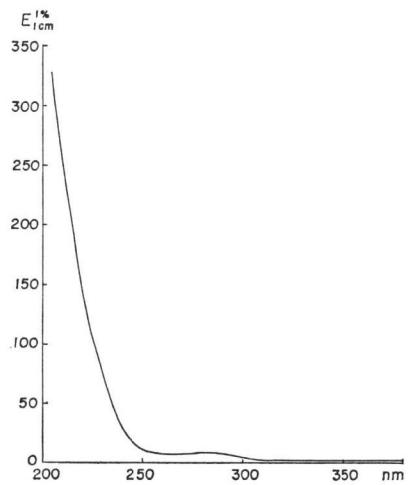

Fig. 2. Infrared absorption spectrum of antibiotic H 537 SY2 in KBr

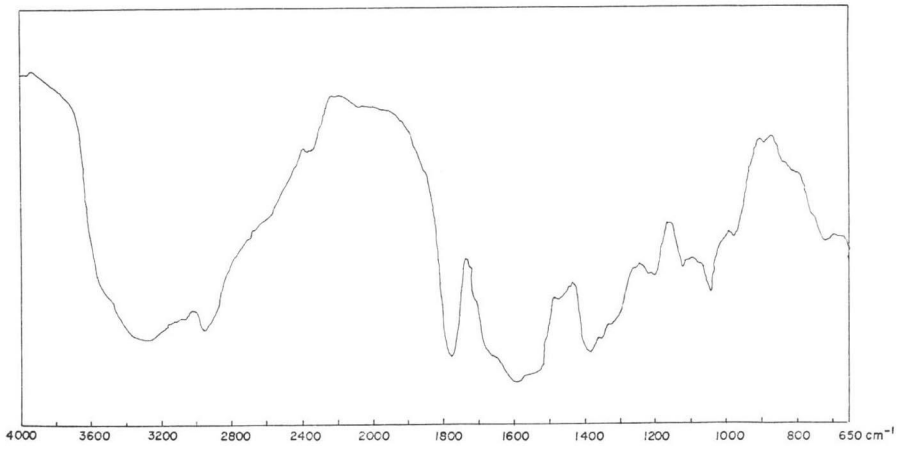

Table 1. Avicel thin-layer chromatography of the acid hydrolyzates of antibiotic H 537 SY2.

Hydrolysis periods (hours)	Mobility (cm)/Developing distance (cm)					
	Antibiotic H 537 SY2	Compound I	Compound II	Compound III	Compound IV	Compound V
	2.6/17.5[a)]	2.2/17.5[a)]	3.4/17.5[b)]	4.6/17.5[a)]	5.7/17.5[a)]	8.1/17.5[a)]
0	++	−	−	−	−	−
4	++	±	±	+	±	±
8	++	+	±	+	+	±
12	+	+	±	+	+	+
20	+	++	±	+	++	++

Developed with *n*-BuOH - pyridine - AcOH - H_2O (6: 4: 1: 3) by two ascents method.

a) Ninhydrin and ELSON-MORGAN reactions are positive.

b) Only ninhydrin reaction is positive.

++; strongly positive, +; positive, ±; weakly positive, −; negative

(pH 7.8 before sterilization). The culture was grown at 27°C for 4 days on the shaker. The mycelial cake was removed by filtration and 18.2 liters of the filtrate was recovered. The procedures of extraction and purification of the antibiotic are summarized in Chart 1. The purified antibiotic was obtained as white amorphous powder, shrank at 163°C and decomposed at 175~179°C. $[\alpha]_D^{20}$ 17° (*c* 0.177, H_2O). Determination of molecular weight of the antibiotic by mass spectroscopy failed and a tentative molecular formula, $C_{19}H_{33}N_5O_{11}$, is proposed by elementary analysis; Calculated for $C_{19}H_{33}N_5O_{11}$, C, 44.95; H, 6.56; N, 13.80. Found, C, 45.18; H, 6.67; N, 13.56. Ultraviolet and infrared absorption spectra of the antibiotic are shown in Fig. 1 and Fig. 2, respectively.

The antibiotic was shown to be homogeneous by paper chromatography developed with several kinds of solvent systems and Rf values were as follows; 0.21 with *n*-BuOH - acetone - H_2O (2 : 4 : 1); 0.24 with *n*-BuOH - MeOH - H_2O (4 : 2 : 1); 0.34 with *n*-BuOH - EtOH - H_2O (5: 4: 2). The antibiotic was detected by bioautography and by α-naphthol-phosphate or ninhydrin reaction. The antibiotic gives positive α-naphthol-phosphate, ELSON-MORGAN and ninhydrin reactions but negative SAKAGUCHI reaction. The antibiotic is soluble in water, sparingly soluble in methanol and insoluble in ethanol, *n*-butanol, acetone or ethyl acetate. Antibiotic H 537 SY2 is stable when kept at 100°C for 5 minutes at pH 4~8, but loses almost of the antibiotic activity immediately at room temperature at pH 2. Half of the activity is also lost after heating at 60°C for 30 minutes in 90% aqueous

acetone and 16% of the activity is lost under the same condition in 90% aqueous ethanol. The band at 1780 cm^{-1} in the infrared absorption spectrum disappeared after loss of the activity by treatment at pH 2.0 at room temperature.

The antibiotic dissolved in 4 N HCl (10 mg/ml) was hydrolyzed in sealed capillary tubes for various periods at 80°C and hydrolyzates were examined on Avicel thin-layer plates developed with *n*-BuOH - pyridine - AcOH - H_2O (6: 4: 1: 3) by two ascents method. The hydrolysis products were detected by ninhydrin and ELSON-MORGAN reactions and mobilities of the products are shown in Table 1. The products were designated as Compounds I, II, III, IV and V respectively in order of low mobility on the thin-layer plate. Amounts of Compounds I, IV and V in the hydrolyzate increased as the hydrolysis period increased, while that of Compounds II and III decreased. Thus, compounds II and III may be intermediate products of the hydrolysis and the antibiotic is suggested to contain three amino sugar components, namely Compounds I, IV and V.

Minimum inhibitory concentrations of the antibiotic against various microbes are listed in Table 2 and three times intraperitoneal administrations (25 mcg/mouse/day) caused death, but no toxic diagnosis was observed by seven times intraperitoneal administrations of 12.5 mcg/mouse/day.

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Table 2. Antimicrobial spectrum of antibiotic H 537 SY2

Test organisms	MIC (mcg/ml)
Staphylococcus aureus FDA 209P	>100
Bacillus subtilis PCI 219	>100
Corynebacterium bovis	>100
Micrococcus flavus	>100
Pseudomonas aeruginosa Ishii 14	>100
Escherichia coli NIHJ	>100
Shigella sonnei	>100
Klebsiella pneumoniae	>100
Mycobacterium smegmatis ATCC 607	>100
Candida tropicalis NI 7495	50
Candida pseudotropicalis NI 7494	0.78
Candida albicans 3147	1.56
Candida YU-1200	3.12
Candida krusei	100
Saccharomyces cerevisiae	6.25

Agar dilution method on glucose nutrient agar.

References

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