2′-AMINO-2′-DEOXYADENOSINE PRODUCED BY A STRAIN OF *ACTINOMADURA*

Sir:

In the course of a screening program for antimycoplasmal antibiotics of actinomycete origin, *Actinomadura* sp. No. SA-4427 isolated from a soil sample collected at Nichinan City, Miyazaki Prefecture, Japan, was found to produce 2′-amino-2′-deoxyadenosine, which showed selective antimycoplasmal activity.

Fig. 1 shows an electronmicrograph of the spores of strain SA-4427. The long sporophores have about fifteen spores per chain. The end of the spore chains is curled. The spores, which have a warty surface, are cylindrical in shape and $0.6 \sim 0.7 \times 0.8 \sim 1.0\ \mu$ in dimensions. No sclerotia, zoospores, sporangia or pseudosporangia were observed. Strain SA-4427 showed good growth on various agar media. Vegetative mycelia were colorless or orange. Abundant aerial mycelia were observed on yeast extract-malt extract agar and inorganic salt-starch agar. Their colors were white or shell pink. Strain SA-4427 did not produce melanoid pigment, but produced soluble pigment with an apricot color on glucose-peptone agar. Analyses of cell walls (positive for *meso*-diaminopimelic acid; negative for arabinose and galactose) and whole cells (positive for madurose, ribose, mannose and glucose; negative for arabinose and galactose) placed strain SA-4427 in the LECHEVALIERS' grouping III B[1,2]. Of the actinomycete genera, such as *Actinomadura, Microbispora, Streptosporangium, Spirillospora, Planomonospora* and *Dermatophilus* which are known to be of type III B, *Actinomadura* accommodated strain SA-4427's morphological characteristics best.

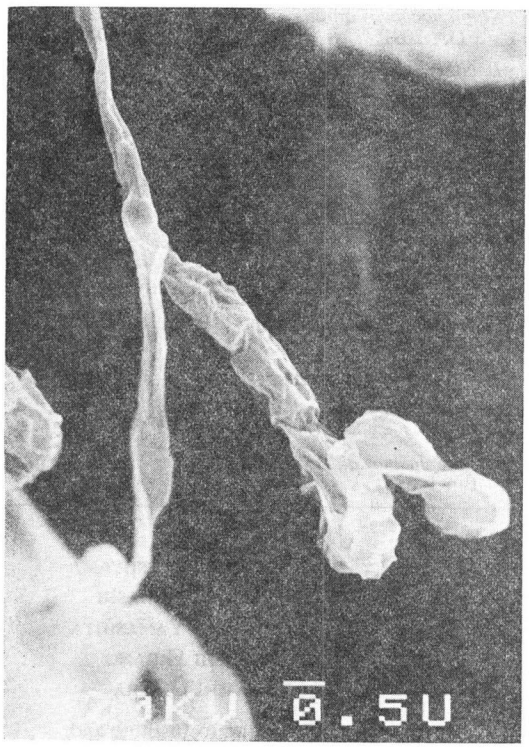

Fig. 1. Electronmicrograph of spore chain of strain SA-4427.

Seed medium (100 ml) in a 500-ml SAKAGUCHI flask was inoculated with strain SA-4427 and incubated at 27°C. A 72-hour culture was transferred into 20 liters of production medium and the fermentation was carried out at 27°C for 5 days. The seed medium contained 1% glucose, 2% starch, 0.5% yeast extract, 0.5% peptone and 0.4% $CaCO_3$ (pH 7 before sterilization), and the production medium was 2% dextrin, 0.2% glucose, 1.5% soybean meal, 0.3% yeast extract and 0.3% $CaCO_3$ (pH 7 before sterilization). Antibiotic SA-4427 was monitored by the paper disc method using *Mycoplasma gallisepticum* Kp-13[3] as a test organism and by thin-layer chromatography (TLC) on silica gel with the lower phase of $CHCl_3$ - isoPrOH - 17%NH_4OH (1: 1: 1, v/v) as developing solvent. Broth filtrate was adsorbed on Amberlite IRC-50 (H^+ form) cation-exchange resin and eluted with 0.5 N NH_4OH. The eluate was evaporated to dryness and the residue was chromatographed on a silica gel column. Active product was eluted with $CHCl_3$ - isoPrOH - 17% NH_4OH (3: 1: 1, v/v, the lower phase). Further purification of the crude product by preparative TLC on silica gel gave antibiotic SA-4427 as a white powder. The antibiotic was crystallized from methanol as needles, mp 193 ~ 195°C.

The molecular formula of antibiotic SA-4427 was determined to be $C_{10}H_{14}N_6O_3$ from molecular ion at *m/e* 266.115 (Calcd. for $C_{10}H_{14}N_6O_3$, 266.113) and elemental analysis (Calcd. for $C_{10}H_{14}N_6O_3$: C, 45.09%; H, 5.30%; N, 31.56%. Found: C, 45.17%; H, 5.30%; N, 31.65%).

The antibiotic was deduced to be a nucleoside consisting of adenine and aminoribose moieties from the following spectral data: $\lambda_{max}^{H_2O}$ 259 nm (ϵ 14,600); ν_{max}^{KBr} 3300 ~ 2600, 1680, 1600, 1560,

1460, 1415, 1290, 1030 cm^{-1}; fragment peaks *m/e* 236 (9%), 217 (4), 193 (5), 177 (20), 164 (100), 136 (68), 131 (67). From a comparison (in DMSO-d_6) of the ^1H- and ^{13}C-NMR spectra [δ 155.9 (C-6), 152.4 (C-2), 148.7 (C-4), 139.9 (C-8), 119.0 (C-5), 89.0 (C-1′), 86.8 (C-4′), 71.1 (C-3′), 62.0 (C-5′), 57.4 (C-2′)] with those of 2′-amino-2′-deoxyguanosine[4] and adenosine, antibiotic SA-4427 was concluded to be 2′-amino-2′-deoxyribofuranosyl adenine. The attachment[5] of an amino group to the 2′-position on ribose moiety was supported by the observation of characteristic mass fragmentation peaks at *m/e* 217, 193 and 177 (Chart 1). Crystallization of antibiotic SA-4427 in acetone gave its acetonide*, mp 187~188°C, $[\alpha]_D^{21}-65°$ (*c* 1, H_2O), $C_{13}H_{18}N_6O_3$.

The configuration of the anomeric center was deduced from the chemical shift value and coupling constant[4] of the anomeric proton at δ 5.68 ($J_{1',2'}=8.0$ Hz) in the ^1H–NMR spectrum (DMSO-d_6) and the specific optical rotation of $[\alpha]_D^{28}$ −65.3° (*c* 1, H_2O). This established the nucleoside to be 2′-amino-2′-deoxy-β-D-ribofuranosyl adenine: mp 194~196°; $[\alpha]_D^{22}-66\pm2°$ (*c* 0.98, methanol); $\lambda_{max}^{H_2O}$ 262 nm (ϵ 14,400); ν_{max}^{KBr} 3450~3225, 1690, 1615, 1560, 1465 cm^{-1}. It has been previously synthesized by M. L. WOLFROM and M. W. WINKLEY[6] but this is the first report of its occurrence in nature.

As shown in Table 1, various mycoplasma strains were sensitive to 2′-amino-2′-deoxyadenosine and other bacteria were resistant. Many antibiotics, such as those of the macrolide and polyether groups, have antimycoplasmal activity[7], and *Actinomadura* sp. are known to produce ansamycin, anthracycline and peptide antibiotics[8]. However, our findings that a nucleoside antibiotic has antimycoplasmal activity and that a strain of *Actinomadura* produces a nucleoside antibiotic, are new.

Acknowledgement

The authors wish to thank Mr. R. MASUMA and Miss Y. MAKINO for their assistance.

YUZURU IWAI
AKIRA NAKAGAWA
AKIKO NAGAI

* Details of the physicochemical and biological properties of the acetonide are reported in the abstracts (p. 460) of the 99th Meeting of Pharmaceutical Society of Japan (Sapporo City, August, 1979).

Chart 1.

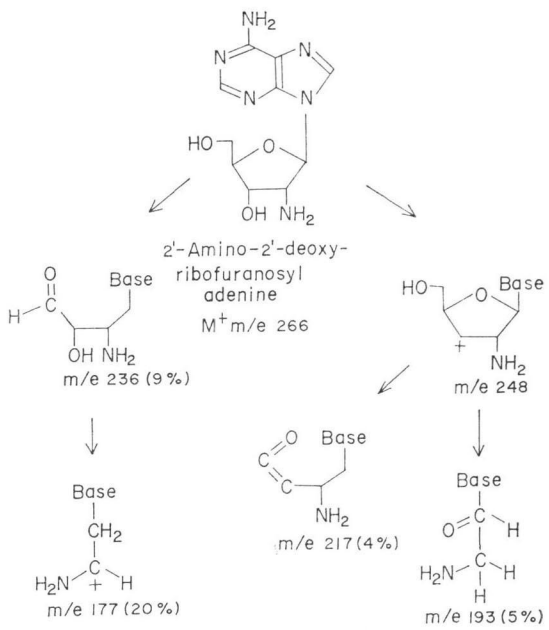

Table 1. Antimicrobial activity of 2′-amino-2′-deoxyadenosine

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	>100
Bacillus subtilis PCI 219	>100
Sarcina lutea PCI 1001	>100
Escherichia coli NIHJ	>100
Pseudomonas aeruginosa P-3	>100
Mycoplasma gallisepticum Kp-13	6.25
Mycoplasma gallisepticum S-6	6.25
Mycoplasma gallisepticum 333p	>100
Mycoplasma pneumoniae	6.25
Acholeplasma laidlawii (A) PG-8	6.25
Acholeplasma laidlawii (B) Bm-1	>100

KYŌKO MATSUYAMA
YŌKO TAKAHASHI
MARIKO YAMASHITA
ATSUSHI HIRANO
SATOSHI ŌMURA

The Kitasato Institute and

Kitasato University,
Shirokane
Minato-ku, Tokyo 108, Japan

(Received September 27, 1979)

References

1) BECKER, B.; M. P. LECHEVALIER & H. A. LECHEVALIER: Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl. Microbiol. 13: 236~243, 1965
2) LECHEVALIER, M. P. & H. LECHEVALIER: Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. 20: 435~443, 1970
3) TANAKA, H.; Y. KOYAMA, J. AWAYA, H. MARUMO, R. ŌIWA, M. KATAGIRI, T. NAGAI & S. ŌMURA: Nanaomycins, new antibiotics produced by a strain of *Streptomyces*. I. Taxonomy, isolation, characterization and biological properties. J. Antibiotics 28: 860~867, 1975
4) NAKANISHI, T.; T. IIDA, F. TOMITA & A. FURUYA: Identification of a novel aminonucleoside produced by *Enterobacter* sp. as 2′-amino-2′-deoxyguanosine. Chem. Pharm. Bull. 24: 2955~2960, 1976
5) SUHADOLNIK, R. J.; B. M. CHASSY & G. R. WALLER: Nucleoside antibiotics. III. Isolation, structural elucidation and biological properties of 3′-acetamide-3′-deoxyadenoside from *Helminthosporium* sp. 215. Biochim. Biophys. Acta 179: 258~267, 1969
6) WOLFROM, M. L. & M. W. WINKLEY: Anomeric purine nucleosides of the furanose form of 2-amino-2-deoxy-D-ribose. J. Org. Chem. 32: 1823~1825, 1967
7) UMEZAWA, H.: Index of antibiotics from actinomycetes. Vol. II, Japan Scientific Societies Press, Tokyo, 1978
8) NARA, T.; I. KAWAMOTO, R. OKACHI & T. OKA: Source of antibiotics other than *Streptomyces*. Jap. J. Antibiotics 30, Suppl.: S174~S189, 1977