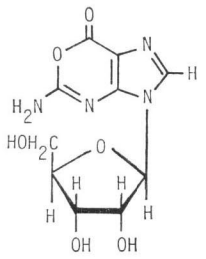
**Communications to the editor**

## OXANOSINE, A NOVEL NUCLEOSIDE FROM ACTINOMYCETES

Sir:

In the course of screening for new antibiotics, a novel nucleoside named oxanosine, which had antibacterial activity against *Escherichia coli* K-12 in a peptone medium, was isolated as crystals. The structure was determined to be 5-amino-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-d][1,3]oxazin-7-one (Fig. 1) by X-ray crystallographic analysis[1]. In this communication the production, isolation, and chemical and biological properties of oxanosine are reported.

Fig. 1. Structure of oxanosine.



5-Amino-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-d][1,3]oxazin-7-one

The oxanosine-producing strain (strain number in Institute of Microbial Chemistry, MG265-CF3) was isolated from a soil sample collected in our compound and classified as *Streptomyces capreolus* MG265-CF3 (unpublished). The strain was precultured in a 500 ml Sakaguchi flask containing 125 ml of medium [glucose 1.0%, glycerol 1.0%, sucrose 1.0%, oat meal 0.5%, soy bean meal (Prorich) 2.0%, pressed yeast (Oriental) 1.0%, Casamino acid (Difco) 0.5%, $CaCO_3$ 0.1%, pH 7.0 before sterilization] on a reciprocating shaking machine (120 strokes per minute) at 28°C for 2 days. The cultured broth was inoculated into 500 ml Erlenmeyer flask containing 120 ml of the following medium: Bacto-Soytone (Difco) 1.0%, galactose 2.0%, corn steep liquor (Ajinomoto) 0.5%, dextrin 2.0%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2%, antifoam 0.01%, pH 7.4 before sterilization. This was cultured on a rotary shaking machine (180 r.p.m.) at 28°C for 5 days.

The filtrate (11.6 liters) of the cultured broth thus obtained was passed through a carbon column (500 ml), and the adsorbed material was

Fig. 2. UV spectra of oxanosine.

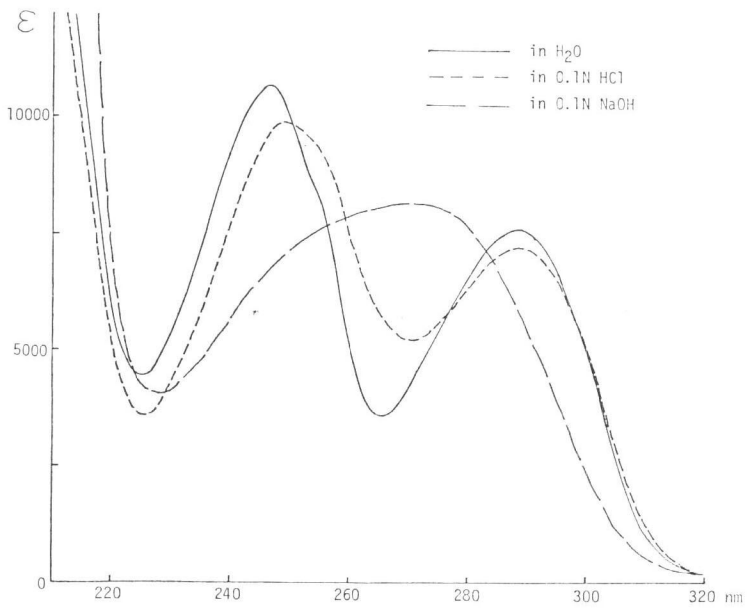
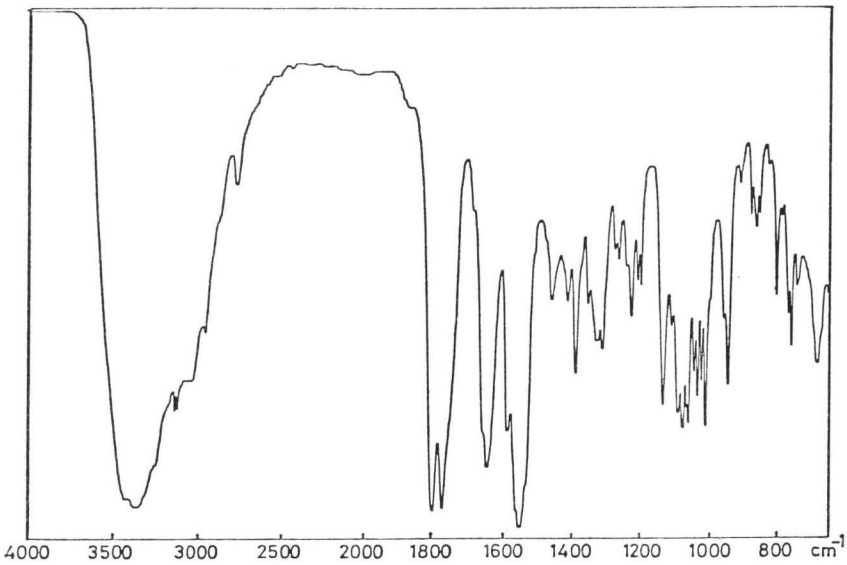


Fig. 3. IR spectrum of oxanosine (KBr).




eluted by a linear gradient of acetone. The eluate showing antibacterial activity was dried under reduced pressure to give 3.56 g of crude material. The bioactive component was extracted from the crude material with methanol. The methanol extract (1.29 g) was further purified by carbon chromatography (180 ml, linear gradient of acetone) followed by Avicel chromatography [600ml, EtOH - $H_2O$ (95: 5)→EtOH - $H_2O$ (90: 10)] to give 82.8 mg of pure oxanosine. It was crystallized from water (1st crop 48.8 mg, 2nd crop 16.1 mg), platy needles, 199°C (decomp.), $[\alpha]_D^{23}$ −36.7° (*c* 0.3, $H_2O$).

Table 1. Carbon-13 NMR chemical shifts of oxanosine and guanosine (in DMSO-$d_6$ at 25.2 MHz).

| Carbon | Oxanosine | Guanosine*** |
|---|---|---|
| 2 (8)* | 136.6 (d) | 136.1 |
| 3a (4) | 153.1 (s)** | 151.5 |
| 5 (2) | 153.9 (s)** | 153.8 |
| 7 (6) | 159.8 (s) | 157.1 |
| 7a (5) | 110.9 (s) | 116.7 |
| 1′ | 86.5 (d) | 86.7 |
| 2′ | 73.8 (d) | 73.9 |
| 3′ | 70.1 (d) | 70.6 |
| 4′ | 85.3 (d) | 85.5 |
| 5′ | 61.1 (t) | 61.6 |

Chemical shifts are expressed by $\delta$-value (internal TMS reference).

* Numbering of guanine base.

** May be exchanged.

*** From reference 2.

The molecular formula of oxanosine was established as $C_{10}H_{12}N_4O_6$ (MW 284.23) by field desorption mass spectrometry and elemental analysis ($M^+$ *m/z* 284. Calcd: C, 42.25; H, 4.26; N, 19.71. Found: C, 42.55; H, 4.41; N, 19.84). The UV spectra are shown in Fig. 2. $\lambda_{max}^{H_2O}$ (log $\varepsilon$) 247 nm (4.08), 288 (3.93). $\lambda_{max}^{0.1N\ HCl}$ 249 (4.05), 288 (3.91). $\lambda_{max}^{0.1N\ NaOH}$ 272 (3.96). The IR spectrum is shown in Fig. 3. It suggested the presence of sugar moiety (1,000 ~ 1,100 $cm^{-1}$) and an unusual chromophore (1,795 and 1,770 $cm^{-1}$). The $^1H$ NMR spectrum measured in $D_2O$ at 100 MHz (external TMS reference) suggested a new kind of *N*-ribonucleoside structure. After the structure determination by X-ray crystallographic analysis[1], the definite assignment was made as follows: 2-H $\delta$8.45 (s), 1′-H 6.33 (d, 6 Hz), 2′-H 5.17 (dd, 6 and 6), 3′-H 4.86 (dd, 6 and 4), 4′-H 4.68 (m), 5′-H 4.34 (m). The $^{13}C$ NMR chemical shifts measured in DMSO-$d_6$ at 25.2 MHz (internal TMS reference) were also assigned in comparison with those of guanosine (Table 1)[2].

Oxanosine showed weak antibacterial activity against the following bacteria on peptone agar: *Escherichia coli* K-12 (MIC 12.5 mcg/ml), *E. coli* ML 1629 (25), *Shigella flexneri* 4b JS 11811 (6.25), *S. dysenteriae* JS 11910 (25), *S. sonnei* JS

11746 (25), *Proteus mirabilis* IFM OM-9 (12.5), *P. rettgeri* GN 466 (12.5) and *P. vulgaris* OX-19 (25). Other bacteria so far tested were not inhibited at 100 mcg/ml. On nutrient agar, however, oxanosine did not show any antibacterial activity at 100 mcg/ml. Guanine, guanosine and 5′-guanylic acid showed an antagonistic effect to oxanosine. For example, in a cylinder plate assay using *E. coli* K-12 on peptone agar, oxanosine at 10 mcg/ml produced an inhibition zone of 21 mm in diameter, while by addition of 2.5 mcg/ml of guanosine to the medium the diameter decreased to 10 mm and the addition of 5 mcg/ml completely eliminated the antibacterial activity of oxanosine. Oxanosine inhibited the growth of HeLa cells *in vitro* ($IC_{50}$ 32 mcg/ml) and suppressed the growth of L-1210 leukemia in mice. Intravenous injection of 4 mg of oxanosine to mice (*ca.* 200 mg/kg) did not show any sign of toxicity.

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2) Johnson, L. F. & W. C. Jankowski: Carbon-13 NMR Spectra. Spectrum No. 379, Wiley-Interscience, New York, 1972