A NEW ANTITUMOR ANTIBIOTIC, GUANINE 7-*N*-OXIDE PRODUCED BY *STREPTOMYCES* SP.†

Sir:

In the screening of antitumor antibiotics, a new antibiotic, which has a weak anti-*Candida* activity and a strong antitumor activity both *in vitro* and *in vivo* was isolated from a culture filtrate of *Streptomyces* sp. No. 3780. The strain was isolated from a soil sample collected in Daimon-machi, Toyama Prefecture, Japan.

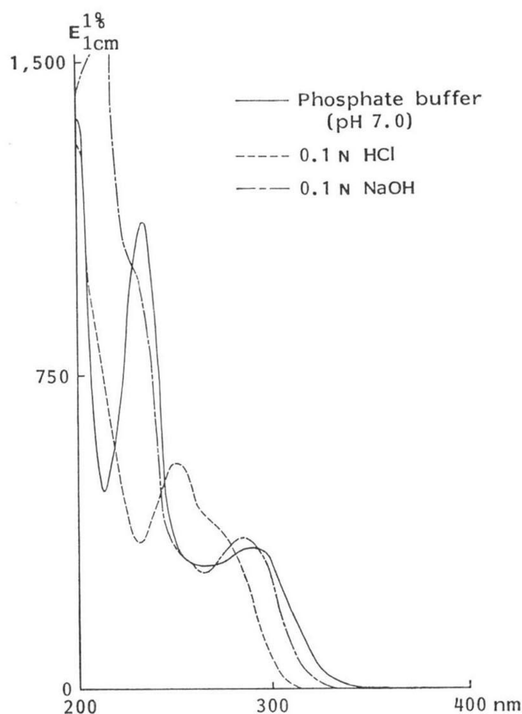
The strain was cultured at 28°C for 48 hours in a jar-fermentor containing 5 liters of a medium composed of glucose 2.5%, soybean flour 0.3%, yeast extract 0.3%, ammonium nitrate 0.4%, calcium carbonate 0.3%, sodium chloride 0.3% and magnesium phosphate (pH 6.6 before sterilization) 0.5%. The fermentation broth was centrifuged and the supernatant fluid (4.7 liters) was added to a column of Dowex-50WX4 (H^+). The column was washed with H_2O and then eluted with 3 liters of 2 N NH_4OH.

The eluate was concentrated to about 500 ml *in vacuo* and then adjusted to pH 5~6 with dilute HCl. The solution was added to an Amberlite IR-45 (OH^-) column. The column was washed with H_2O and then eluted with 2 liters of 1 N NH_4OH. The eluate was concentrated to about 15 ml *in vacuo* and then ice-cooled. The precipitate was filtered, washed with H_2O, and dried to give 268 mg of a crude powder. The powder was subjected to Sephadex G-10 equilibrated with 0.1 M ammonium bicarbonate. The column was eluted with 0.1 M ammonium bicarbonate and then with H_2O. Active fractions were collected, concentrated *in vacuo*, and lyophilized to give 184 mg of a purified powder. It was dissolved in 1 N NH_4OH. Acetic acid was added to the solution to precipitate the antibiotic, which was filtered, washed with H_2O, and dried to give the pure antibiotic.

The antibiotic is a slightly brownish crystalline powder and does not melt at 350°C. It is an amphoteric compound with *pKa'* of 2.6, 5.8 and 9.5. The molecular formula was determined to be $C_5H_5N_5O_2$ on the basis of EI-MS (M^+, *m/z* 167.0443, calcd 167.0443) and elementary analysis. *Anal* Calcd for $C_5H_5N_5O_2$: C 35.93, H 3.02, N 41.90. Found: C 35.50, H 3.15, N 41.81. It shows UV maxima at 234 and 290 nm in 0.1 M phosphate buffer (pH 7.0), 252 nm in 0.1 N HCl and 286 nm in 0.1 N NaOH (Fig. 1). It is soluble in alkaline water but insoluble in other organic solvents. It gives positive reactions to iodine vapor and permanganate but is negative to ninhydrin and aniline hydrogen phthalate reactions.

† An outline of this paper was presented at the 105th Annual Meeting of Pharmaceutical Society of Japan, Kanazawa, Apr. 5, 1985.

Fig. 1. UV spectrum of guanine 7-*N*-oxide.

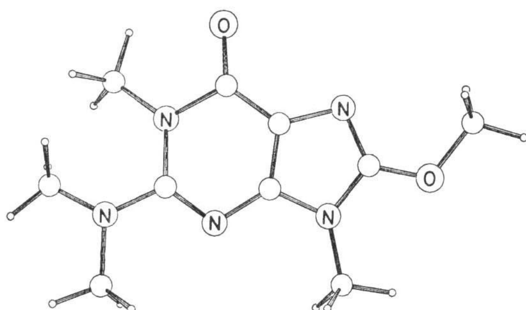


The EI-MS fragment ion *m/z* 151.0486, (M−oxygen)$^+$ (calcd 151.0494) is characteristic of *N*-oxides[1]. The collision-induced dissociation spectrum from *m/z* 151 obtained by tandem mass spectrometry (Kratos MS 50 triplo analyzor) was indistinguishable from that obtained from the molecular ion of guanine, indicating[2] the heterocyclic skeleton to be that of guanine. A proton on C-8 was observed in ^{1}H NMR (δ_H 7.53 ppm, s, in 1 N NaOD). These data indicate that the structure is guanine *N*-oxide. However, the UV spectrum is different from those of known guanine *N*-oxides, *i.e.*, 1-hydroxyguanine[3], guanine 3-*N*-oxide[4] and 9-hydroxyguanine[5]. Guanine 3-*N*-oxide was synthesized by oxidation of guanine[6] and compared with the antibiotic. They were different in IR spectra, re-

tention time of HPLC and in biological activity.

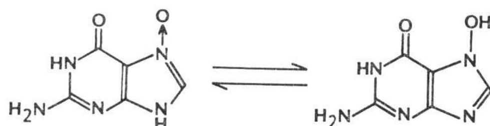
On refluxing the antibiotic in acetic acid, an inactive substance was obtained. This substance was also obtained with a similar treatment of guanine 3-*N*-oxide and identified as 8-hydroxyguanine by comparison with an authentic sample by UV, IR and HPLC analysis. Permethylation (NaH - DMSO-CH_3I) of the antibiotic gave several products. A main product was crystallized from methanol - ether. X-Ray crystal analysis has shown that the structure is the pentamethyl derivative of 8-hydroxyguanine (Fig. 2). The crystal belongs to orthorhombic space group $P2_12_12_1$ with $a=19.769(7)$, $b=9.845(4)$, $c=6.941(3)$ Å.

Fig. 2. Molecular structure of the permethyl derivative of guanine 7-*N*-oxide.



These results strongly support the conclusion that the structure of the antibiotic is guanine 7-*N*-oxide, which exists in tautomeric equilibrium with 7-hydroxyguanine in solution (Fig. 3). It is concluded that migration of the 7-*N*-oxygen to C-8 took place during acid treatment and the permethylation procedure.

Fig. 3. Tautomeric structure of guanine 7-*N*-oxide.



The antibiotic was inhibitory to *Candida albicans* but inactive against Gram-positive and Gram-negative bacteria and *Trichophyton* sp. (Table 1). It also inhibited Yoshida sarcoma and L-5178Y leukemia cells in culture at IC_{50} of 1.65 and 2.40 μg/ml, respectively.

Table 1. Antimicrobial activity of guanine 7-*N*-oxide.

Microorganism	MIC (μg/ml)
Staphylococcus aureus 209P*	>100
Escherichia coli NIHJ*	>100
*Pseudomonas aeruginosa**	>100
*Candida albicans***	6.3
C. albicans NHL 4019**	3.1
*Trichophyton mentagrophytes***	>100
*T. rubrum***	>100

Conventional agar dilution method was employed.
* Bouillon agar.
** Sabouraud's agar.

As shown in Table 2, the intraperitoneal administration of the antibiotic showed a life prolongation effect on mice bearing P388 leukemia. The activity at the dose of 6.0 mg/kg/day was almost comparable to that observed by mitomycin C at the dose of 1.0 mg/kg/day. It also showed a dose-dependent inhibition of the growth of Ehrlich solid carcinoma in mice by oral administration (Fig. 4). Further studies on antitumor activity are in progress.

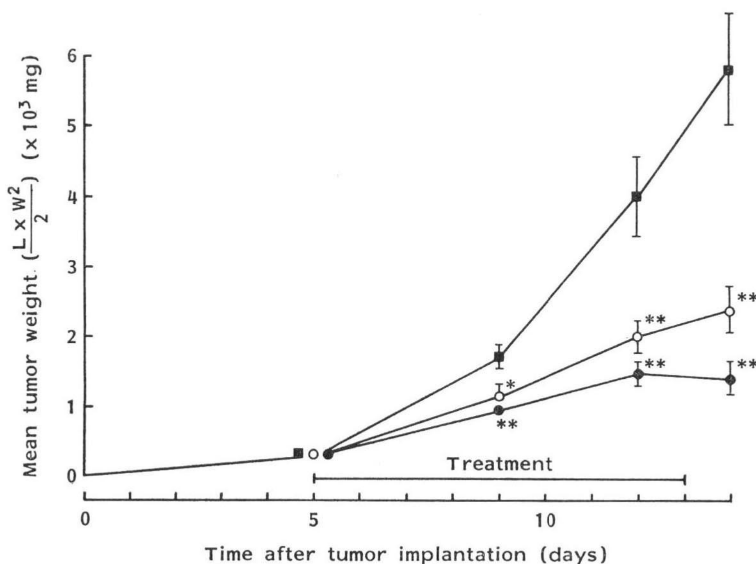
The LD_{50} of the antibiotic in mice is 53 mg/kg by intraperitoneal administration. It inhibited the replication of RNA virus (IHNV) at an ED_{50} of 10 μg/ml (Dr. M. SANEYOSHI, Hokkaido University, personal communication). It showed no mutagenicity against *Salmonella typhimurium* TA 100 and TA 98 in the AMES test.

Table 2. Antitumor activity of guanine 7-*N*-oxide and mitomycin C on BDF_1 mice bearing P388 leukemia.

Material	Treatment (ip)	Dose (mg/kg/day)	MST (days)	T/C (%)	Survivors/total treatments (day 30)
Control	qd 1→9	0.5% CMC	10.0	—	0/5
Guanine	qd 1→9	1.5	14.0	140	0/5
7-*N*-oxide		3.0	14.0	140	0/5
		6.0	17.0	170	1/5
Mitomycin C	qd 1→9	1.0	16.0	160	0/5

Tumor inoculum: 1×10^6 cells/mouse, ip, animal: 6-week-old BDF_1 male mice, MST: median survival time, T/C: MST of treatment/MST of control $\times100$.

Fig. 4. Antitumor activity of guanine 7-*N*-oxide on *dd*Y mice bearing Ehrlich solid carcinoma.
Tumor inoculum: 2×10^6 cells/mouse, sc.
Animal: 5-Week-old *dd*Y male mice.
■ Control: 0.5% CMC qd, po, ○ guanine 7-*N*-oxide: 30 mg/kg qd, po, ● guanine 7-*N*-oxide: 60 mg/kg qd, po.
* $P < 0.05$.
** $P < 0.01$.
$N=7$.



Addendum in Proof

After finishing this paper, a paper concerning the same antibiotic appeared in the May 25th issue of this journal (D. L. KERN *et al.*: J. Antibiotics 38: 572~574, 1985). After submission of this paper, the editor informed us that another paper reporting the same compound has also been received for publication (M. KITAHARA *et al.*: J. Antibiotics 38: 972~976, 977~980, 1985).

Our patents have been published (K. ISONO *et al.*: Japan Kokai 85-67,427, Apr. 17, 1985; 85-91,990, May 23, 1985).

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