GUANINE-7-OXIDE, A NOVEL ANTITUMOR ANTIBIOTIC

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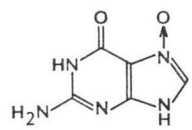
A new antibiotic, PD 113,876, was isolated from the culture broth of a *Streptomyces* sp. and was shown by X-ray diffraction analysis to be the N-7 oxide of guanine. This novel antimetabolite is very active *in vivo* against L1210 lymphocytic leukemia.

During the course of our screening for novel antitumor agents, a *Streptomyces* sp. (ATCC 39364) was found which produces an antimetabolite that is active against L1210 leukemia cells. Fractionation of beers obtained from this *Streptomyces* showed that the component responsible for this activity is an amphoteric compound (PD 113,876) which was identified as guanine-7-oxide (**1**). This compound possesses excellent *in vivo* activity against L1210 lymphocytic leukemia and good activity against P388 leukemia. The nature of the producing organism and the fermentation conditions used will be reported separately. The present report describes the isolation and characterization of guanine-7-oxide which, of the four possible monoheterocyclic-*N*-oxides of guanine, is the only one that has not been previously reported[1~3].



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Fermentation and Isolation

Fermentation beer (567 liters) was adjusted to pH 9, filtered, and the filtrate passed over 16 liters of Dowex-1X2 (Cl^-) resin. The resin was washed with 31 liters of water and then eluted with eight 19-liter portions of 0.5 M AcOH. Fractions 2 and 3, which contained most of the PD 113,876, were combined, concentrated *in vacuo* to 2.2 liters, and adjusted to pH 10 with 50% NaOH. The resulting solution was applied to a 15 cm (i.d.) column containing 7 kg of Sephadex G-10 packed in H_2O. Several 2-liter fractions were collected using H_2O as the eluent. The course of the chromatography was monitored by HPLC and bioactivity against *Streptococcus faecalis* grown on a synthetic medium devoid of purines. The HPLC procedure employed a C-18 μBondapak column and 0.05 M (pH 5.4) sodium phosphate buffer as the mobile phase. The retention time of guanine-7-oxide, detected by UV absorption at 254 nm, is approximately 4 minutes at a flow rate of 1.5 ml/minute. Fractions 5 and 6 from the Sephadex column contained the majority of guanine-7-oxide and were combined, adjusted to pH 5.5 with HCl, and allowed to stand overnight at 5°C. The solid that precipitated was washed with H_2O and dried to afford 5.35 g of nearly pure guanine-7-oxide. This product was further purified by crystallization of the hydrochloride salt, and converted to the water soluble sodium salt as described below.

Preparation of Guanine-7-oxide Hydrochloride

Guanine-7-oxide (5.1 g) was dissolved in 250 ml of warm 1 M HCl. The resulting solution was filtered and allowed to cool. The crystalline solid that precipitated was filtered off, washed with cold 1 M HCl, and dried *in vacuo* to yield 4.7 g of guanine-7-oxide hydrochloride. A portion of this product was recrystallized from 1 M HCl to afford colorless needles. MP >250°C.

Anal Calcd for $C_5H_5N_5O_2 \cdot HCl \cdot H_2O$: C 27.09, H 3.61, N 31.60, Cl 16.02.
Found: C 27.05, H 3.50, N 31.15, Cl 16.00.

Preparation of Guanine-7-oxide (1)

Guanine-7-oxide hydrochloride (4.7 g) was stirred with 120 ml of H_2O resulting in an immediate change in the physical appearance of the suspended solid. The mixture was adjusted to pH 4 with dilute NaOH and stirred for 15 minutes. The suspension was filtered and the solid was washed with H_2O until the filtrate was free of chloride ions. A portion of the very insoluble, microcrystalline solid prepared in this manner was dried to yield guanine-7-oxide.

Anal Calcd for $C_5H_5N_5O_2 \cdot 0.25H_2O$: C 34.99, H 3.21, N 40.81, O 20.99.
Found: C 34.97, H 3.41, N 40.76, O 20.55.

Preparation of Guanine-7-oxide, Sodium Salt

A suspension of guanine-7-oxide (3.5 g, 0.021 mol) in 300 ml of H_2O was treated with 25 ml of 1 M NaOH with stirring. The resulting solution was lyophilized to yield 4.2 g of guanine-7-oxide, sodium salt. UV $\lambda_{max}^{pH\ 10}$ nm (a) 234 (91.2), 290 (28.3); $\lambda_{max}^{pH\ 1}$ nm (a) 252 (46.4), 270 (sh); ^1H NMR (D_2O) δ 7.6 (s); ^{13}C NMR (D_2O) 109.7, 136.2, 155.3, 157.6, 161.6.

Anal Calcd for $C_5H_{3.8}N_5Na_{1.2}O_2 \cdot 1.6H_2O$: C 27.01, H 3.17, N 31.50, Na 12.41.
Found: C 27.04, H 2.91, N 31.09, Na 12.57.

This product is soluble in H_2O and was used to test the bioactivity of guanine-7-oxide.

Preparation of Guanine-7-oxide Hydrobromide

Guanine-7-oxide (50 mg) was dissolved in 5 ml of hot 1 M HBr. Upon cooling, 33 mg of the hydrobromide salt precipitated as colorless needles which were recrystallized from 1 M HBr to obtain material used to obtain X-ray crystallographic data.

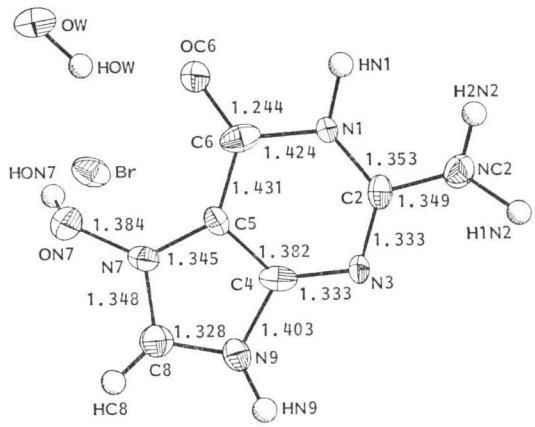
X-Ray Crystallography of Guanine-7-oxide Hydrobromide

A needle shaped crystal of guanine-7-oxide hydrobromide, diameter about 0.05 mm, was mounted on a Nicolet P3 autodiffractometer utilizing Mo K_α monochromated radiation. The material crystallized in the monoclinic space group $P2_1/c$ with $a=4.616(2)$ Å, $b=13.018(9)$ Å, $c=14.937(6)$ Å, and $\beta=92.22(3)°$ with $Z=4$. The lattice parameters were obtained using a least-squares procedure of 15 centered 2θ values. A total of 1,605 unique non-zero reflections were collected to a $\sin\theta/\lambda$ limit of 0.59. Of these, 1,130 were greater than 2σ (I) and were used in the structure refinement. The structure was solved using heavy atom methods and was refined using a full matrix least squares procedure to an R of 0.053 and a weighted R of 0.028. The weights were based on counting statistics. All hydrogen atoms with the exception of one of the water hydrogens were located in difference maps. Non-hydrogen atoms were refined anisotropically. The positional parameters of the hydrogen atoms were held constant during refinement but their isotropic temperature factors were varied. The structural results including bond lengths are shown in Fig. 1.

The difference map clearly showed a peak in the proper position for a hydrogen atom (HN9) bonded to N9. Unfortunately, in the refinement process the thermal parameter of HN9 became

unreasonably large causing some doubt of its presence. However, based on chemical and packing evidence, the HN9 assignment was made. Chemically the assignment is correct because the N9-HN9 bond distance is 1.00 Å, the angles about N9 involving HN9 are reasonable, and HN9 lies in the plane of the heterocycle. With regard to packing, HN9 is in a position which makes a reasonable hydrogen bond with OW, the oxygen of a water of hydration. The critical interatomic distance and angle are N9---OW, 2.72 Å and N9-HN9---OW, 165°. The difficulty in refining the thermal parameter of HN9 was likely caused by the dominating scattering power of the bromide ion. Tables of atomic parameters and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Center.

Fig. 1. Structure of guanine-7-oxide.



Discussion

Although the *N*-oxides, aspergillic acid and its analogs, and several *N*-oxides of phenazines are known microbial metabolites, to our knowledge, guanine-7-oxide is the first example of a purine-*N*-oxide being produced by a *Streptomyces*. Guanine-7-oxide exhibits excellent activity in mice that are inoculated either ip or sc with L1210 leukemia cells (Table 1). The mechanism of this anticancer activity is being studied and will be described in a future report.

Table 1. *In vivo* activity of guanine-7-oxide (administered ip, on days 1~9).

Dosage (mg/kg)	% T/C					
	L1210 leukemia (ip)			L1210 leukemia (sc)		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
25.0	134	164	—	—	—	171
12.5	168	234	162	163	268	276
6.25	168	172	195	148	164	133
3.12	138	144	176	118	278[a]	117

[a] 2/6 cures.

Acknowledgments

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