7-HYDROXYGUANINE, A NOVEL ANTIMETABOLITE FROM A STRAIN OF *STREPTOMYCES PURPURASCENS*

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE DETERMINATION

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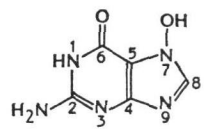
The molecular formula of a new antimetabolite produced by *Streptomyces purpurascens* was determined to be $C_5H_5N_5O_2$ by elemental analysis, FD-MS and ^{13}C NMR. Reduction of this antimetabolite with Raney nickel yielded guanine. The antimetabolite was distinguishable from known *N*-hydroxyguanines by comparison of their UV spectra. The structure of the antimetabolite was finally established to be 7-hydroxyguanine by X-ray crystallography.

A new antimetabolite (**1**) was isolated from the culture broth of *Streptomyces purpurascens* A-347. This antimetabolite exhibited antitumor activity against mouse leukemia L1210 cells.

Taxonomy of the producing organism, fermentation, isolation and biological properties of the antimetabolite are reported in the preceding paper[1]. In the present paper, physico-chemical properties and the structure determination of the antimetabolite are presented.



7-Hydroxyguanine

Physico-chemical Properties

The antimetabolite (**1**) is soluble in acidic and alkaline water, very slightly soluble in water and dimethyl sulfoxide, practically insoluble in methanol, benzene, chloroform and hexane. The antimetabolite (**1**) gave positive reactions with Folin-Ciocalteu reagent and cupric chloride but a negative test with ninhydrin. The molecular formula of **1** was determined to be $C_5H_5N_5O_2$ based on the elemental analysis, ^{13}C NMR and FD-MS.

Physico-chemical properties of **1** are summarized in Table 1. The IR spectrum of **1** is shown in Fig. 1. The ^{13}C NMR (22.5 MHz, 0.5 M NaOD) spectrum showed five signals at 110.0 (s), 134.8 (d), 155.4 (s), 161.1 (s) and 165.7 (s).

Fig. 1. IR spectrum of the antimetabolite (**1**).

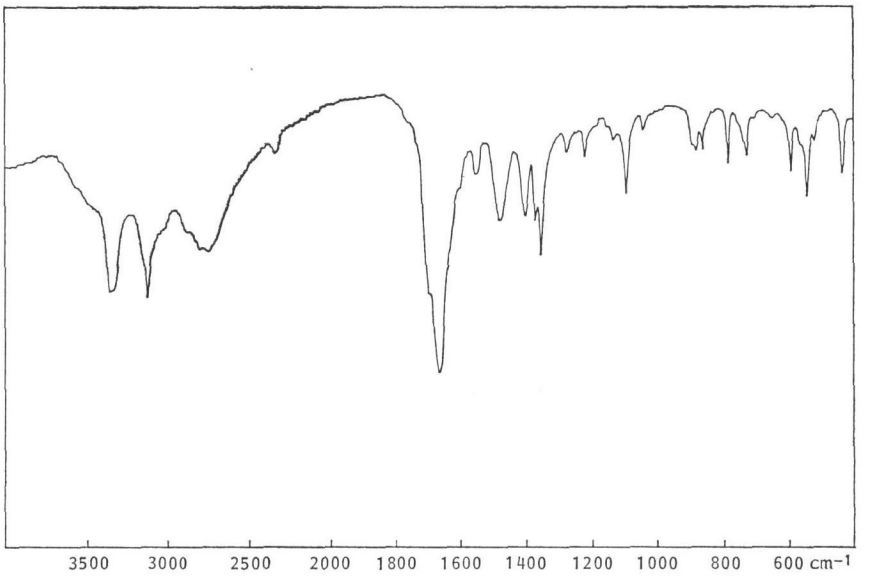


Table 1. Physico-chemical properties of the antimetabolite (**1**).

Appearance	Colorless needle
MP	>300°C
Anal Calcd for $C_5H_5N_5O_2$:	C 35.93, H 3.02, N 41.90, O 19.15.
Found:	C 35.08, H 3.10, N 40.92, O 20.18.
FD-MS	*m/z* 167 (M^+, $C_5H_5N_5O_2$)
UV $\lambda_{max}^{0.04N\ NaOH}$ nm (ε)	233 (20,800), 288 (6,300)
^1H NMR (90 MHz, 0.5 M NaOD)	δ 7.52 (s)
Rf on TLC[a]	0.16

[a] Silica gel (Merck Art. 5715).
$CHCl_3$ - MeOH - 17% NH_4OH, 20: 30: 1.

Structure Determination

The antimetabolite (**1**) produced by a strain A-347 competed with purine bases and appeared to be a purine analog. The UV spectrum, ^{13}C NMR and molecular formula suggested that this antimetabolite was a *N*-hydroxyguanine or a guanine-*N*-oxide. The antimetabolite was reduced by Raney nickel, yielding guanine quantitatively.

The *N*-hydroxyl derivatives of guanine except 7-hydroxyguanine were synthesized by WATSON *et al.*[2~4]; the UV spectral data of the *N*-hydroxyguanines are distinguishable from that of **1** (Table 2). The pH's were adjusted with NaOH and HCl for **1** and with KOH and HCl (or H_2SO_4) for others.

The structure of **1** was finally established by X-ray crystallography. The crystal used was about 0.7×0.5×0.15 mm in size, grown in 15% tetrahydrofuran - 2 M NH_4OH. The lattice constants and the intensity data were obtained on a Syntex R3 four circle diffractometer using Mo-Kα radiation monochromated by a graphite plate. Cell dimensions were determined by least-squares calculations from 2θ values of 18 well-centered, resolved diffraction peaks. The crystal data are given in Table 3.

Table 2. UV spectral data of *N*-hydroxyguanines.

Antimetabolite (**1**)			1-Hydroxyguanine[2]			3-Hydroxyguanine[3]			9-Hydroxyguanine[4]		
pH	nm	ε	pH	nm	ε	pH	nm	ε	pH	nm	ε
0.02	250	6,850				−2.0	244	10,600	0	252	10,200
	275	4,340					260	—		278	6,600
1.0	204	16,500	1.0	208	16,100	1.0	213	12,500			
	250	9,600		248	9,800		245	7,800			
	270	7,100		275	7,200		267	9,500			
4.18	230	7,210	5.25	247	9,470	4.8	217	23,000	4.18	207	17,000
	255	3,190		273	7,290		270	8,800		238	9,200
	280	3,270								253	9,500
8.0	235	12,300				8.0	224	31,000	8.0	234	24,200
	285	4,040					254	5,200		274	6,800
9.0	233	18,580	9.0	227	30,400		292	6,600			
	287	5,680		257	7,510						
				287	5,960						
12.0	223	17,030	14.0	225	31,500	12~15	226	31,000	13.0	228	14,900
	254	5,430		267	7,200		283	9,700		272	8,600
	285	6,260		278	7,400						

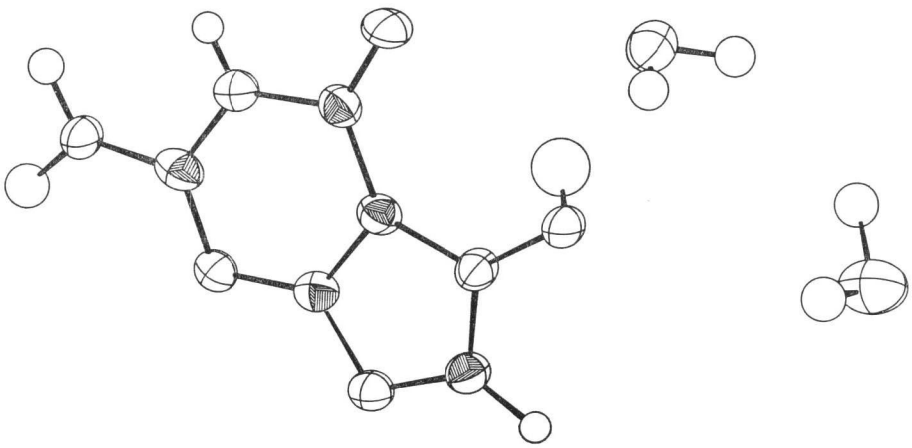
Table 3. Crystal data of the antimetabolite (**1**).

$C_5H_5N_5O_2 \cdot 2H_2O$, MW=203.16
Orthorhombic, space group Pcab
a=6.723(2), b=9.552(3), c=25.935(12) Å
Z=8; Dc=1.62 g/cm^3; U=1665.5 Å^3

A total of 1,456 reflections was measured by the ω-scan method within a 2θ range of 0~50°; the 1,216 reflections with intensities above 1.96 σ (Io) level were used in the structure determination.

The phases of 109 strong reflections with $|E|<1.7$ were determined by the direct method using MULTAN[5]. The E map for the best solution yielded positions for all the non-hydrogen atoms. The position for hydrogen atoms were determined by difference-Fourier synthesis. Least-squares refinements using anisotropic temperature factors for oxygen, nitrogen and carbon atoms and isotropic ones for hydrogen atoms reduced the R index to 0.059;[1] an Ortep[6] drawing of the molecule of **1** is shown in Fig. 2. The structure of **1** was thus established to be 7-hydroxyguanine.

Fig. 2. An Ortep drawing[6] of the molecule of the antimetabolite (**1**).



DELIA and BROWN[7] had reported the synthesis of 7-hydroxyguanine, but corrected the location of hydroxyl group from 7 to 3 position in a later report[3].

Addendum in Proof

After submitting this paper, the compound 3780 (M. NISHII, *et al.*, Isolation and biological activity of 3780, a new antitumor compound. Abstracts Papers of 105th Annual Meeting of Pharmaceutical Society of Japan, p. 438, Kanazawa, Apr. 3~5, 1985) was identified with 7-hydroxyguanine by a direct comparison of the two substances, and also the editor of this journal informed the authors by providing a copy of the galley proof that the paper reporting the same new antibiotic guanine-7-oxide as above would be described in this journal, 38: 572~574, 1985, by D. L. KERN *et al.*

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