ENZYMATIC CONVERSION OF 7-HYDROXYGUANINE TO ITS NUCLEOSIDES

MIKIO KITAHARA, KIYOTO ISHII, TAKESHI OKAZAKI,
TAKAYOSHI HIDAKA and KIYOSHI WATANABE

Biochemical Research Laboratories, Kanegafuchi Chemical Industry Co., Ltd.,
Takasago, Hyogo, Japan

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7-Hydroxyguanine, an antitumor purine-analog, reacted with ribose (or deoxyribose)-1-phosphate in the presence of purine nucleoside phosphorylase. We prepared 7-hydroxyguanine riboside and deoxyriboside using purine nucleoside phosphorylase from bovine spleen. Each nucleoside exhibits antitumor activity against mouse leukemia L1210 cells. The therapeutic effect of 7-hydroxyguanine deoxyriboside is superior to those of 7-hydroxyguanine and 7-hydroxyguanine riboside.

In earlier publications we have described the isolation of 7-hydroxyguanine, an antitumor agent, from a strain of *Streptomyces purpurascens*[1,2]. In this study we have investigated the synthesis and antitumor activities of 7-hydroxyguanine nucleosides. This study have been influenced by the observation that, although 6-mercaptopurine is a useful purine-analog in cancer chemotherapy, 6-mercaptopurine riboside (thioinosine) inhibits growth of adenocarcinoma, over a wider non-lethal dosage range than 6-mercaptopurine[3].

Purine nucleoside phosphorylase (EC 2.4.2.1) which catalyzes the reaction:

$$\text{purine} + \text{pentose-1-phosphate} \rightleftharpoons \text{purine nucleoside} + \text{orthophosphate}$$

has been used for the enzymatic preparation of 7-hydroxyguanine nucleosides.

Materials and Methods

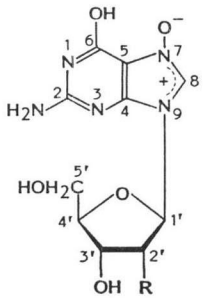
Materials

7-Hydroxyguanine was prepared as described previously[1]. α-D-Ribose-1-phosphate barium salt and α-D-2′-deoxyribose-1-phosphate barium salt were prepared according to the procedures described by PLESNER and KLENOW[4] and FRIEDKIN[5]. Purine nucleoside phosphorylase (PNPase) was purchased from Sigma. Chelating ion exchange resin, Chelex 100, was purchased from Bio-Rad Laboratories.

HPLC Analysis

The passage of the enzymatic reaction was monitored by HPLC with Nihonbunko V system and Partisil 10 SCX column (4.6×250 mm) developed with a mobile phase of 10 mM $NH_4H_2PO_4$ buffer (pH 3.0) at a flow rate of 1.0 ml/minute. 7-Hydroxyguanine riboside, 7-hydroxyguanine deoxyriboside and 7-hydroxyguanine were detected by UV absorption at 254 nm at 5.0, 6.8 and 8.2 minutes of retention time, respectively.

Fig. 1. Structure of 7-hydroxyguanine nucleosides.



7-Hydroxyguanine riboside R=OH
7-Hydroxyguanine deoxyriboside R=H

Table 1. Physico-chemical properties of 7-hydroxyguanine nucleosides.

	1	2
Appearance	Colorless needle	Colorless needle
MP (°C, dec)	138~139	136~137
$[\alpha]_D^{20}$ (*c* 0.5, H_2O)	−31.6°	−30.8°
FAB-MS (*m/z*)	300 $(M+1)^+$	284 $(M+1)^+$
Molecular formula	$C_{10}H_{13}N_5O_6$	$C_{10}H_{13}N_5O_5 \cdot H_2O$
Anal Found:	C 39.88, H 4.31, N 23.45.	C 40.03, H 5.12, N 23.40.
Calcd:	C 40.14, H 4.32, N 23.40.	C 39.87, H 5.02, N 23.25.
UV $\lambda_{max}^{H_2O}$ nm (ε)	236 (18,200), 268 (9,400)	234 (19,100), 267 (9,800), 282 (sh)
$\lambda_{max}^{0.1M\ HCl}$ nm (ε)	260 (10,600), 280 (sh)	254 (10,900), 272 (sh)
$\lambda_{max}^{0.1M\ NaOH}$ nm (ε)	234 (17,000), 276 (8,800)	234 (20,400), 278 (10,300)
IR (cm^{-1}, KBr)	3350, 1700, 1635, 1614, 1582, 1560, 1460, 1240, 1130, 870	3330, 1710, 1680, 1633, 1590, 1520, 1455, 1060, 925, 778
^1H NMR (ppm, D_2O)	5.85 (d, 1H), 4.59 (t, 1H), 4.34 (t, 1H), 4.18 (q, 1H), 3.82 (m, 2H)	6.27 (t, 1H), 4.54 (m, 1H), 4.06 (m, 1H), 3.73 (m, 2H), 2.4~2.7 (m, 2H)
Rf on TLC[a]	0.05	0.16

[a] Silica gel (Merck 5715) BuOH - EtOH - $CHCl_3$ - 17% NH_4OH, 4: 5: 2: 2.

Preparation of 7-Hydroxyguanine Riboside (1)

7-Hydroxyguanine (1.33 g) and ribose-1-phosphate barium salt (7.31 g) were dissolved in 800 ml of 50 mM sodium citrate buffer (pH 6.5) at 40°C. The reaction was started by the addition of 400 units of PNPase. After incubation for 2 hours at 40°C, 61% of 7-hydroxyguanine was converted to **1**. The reaction mixture was cooled with ice and centrifuged at 3,000 rpm for 10 minutes. The supernatant (2,073 μg/ml, 805 ml) was applied to a column of Chelex 100 (Ni^{2+}, 350 ml) and eluted with 0.3 M NH_4OH. The active eluate (1,832 μg/ml, 800 ml) was passed through a column of Amberlite IRC 50 (NH_4^+, 250 ml), to remove Ni^{2+}. The passage was concentrated to 15 ml and then 15 ml of ethanol was added. After 16 hours in the refrigerator, **1** formed as white precipitate was collected and dried *in vacuo* (1.44 g).

The white powder (1.44 g) was dissolved in 20 ml of hot water and placed in the refrigerator, yielding colorless needles of **1** (1.15 g).

Preparation of 7-Hydroxyguanine Deoxyriboside (2)

7-Hydroxyguanine (760 mg) and deoxyribose-1-phosphate barium salt (2.5 g) were dissolved in 1,150 ml of 50 mM sodium citrate buffer (pH 6.5). The reaction was started by the addition of 500 units of PNPase. After incubation for 2 hours at 40°C, 84% of 7-hydroxyguanine was converted to **2**. The reaction mixture was cooled with ice and centrifuged at 3,000 rpm for 10 minutes. The supernatant (941 μg/ml, 1,150 ml) was applied to a column of Chelex 100 (Ni^{2+}, 400 ml) and eluted with 0.2 M NH_4OH. The active eluate (796 μg/ml, 1,600 ml) was passed through a column of Amberlite IRC 50 (NH_4^+, 300 ml). The passage was evaporated *in vacuo* and dried, yielding white powder (2.3 g). The white powder (2.3 g) was dissolved in 20 ml of hot water and placed in the refrigerator. After 16 hours in the refrigerator **2** formed as colorless needles (868 mg).

Results and Discussion

Physico-chemical Properties

7-Hydroxyguanine riboside (**1**) and 7-hydroxyguanine deoxyriboside (**2**) are soluble in water, very slightly soluble in dimethyl sulfoxide, and almost insoluble in methanol, benzene, chloroform and hexane. **1** and **2** gave positive reactions with Folin-Ciocalteu reagent and orcinol-ferric chloride-sulfuric acid but showed a negative test with ninhydrin.

Physico-chemical properties of **1** and **2** are summarized in Table 1. Proton magnetic resonance

Table 2. ^{13}C NMR chemical shifts in 7-hydroxyguanine nucleosides*.

Position	1	2	Position	1	2
2	151.70	151.56	1′	85.49	87.32
4	151.14	151.53	2′	70.78	35.65
5	98.57	98.50	3′	69.75	71.38
6	153.16	153.34	4′	84.91	81.13
8	147.32	147.10	5′	62.30	62.40

* Spectra were recorded on a Varian XL-300 in DMSO-d_6.

study indicates that **1** and **2** have 8-CH signals at 8.42 and 8.32 ppm respectively in dimethyl sulfoxide solution, while in aqueous solution the 8-CH signals of **1** and **2** are not observed. The results in aqueous solution would due to the hydrogen exchange reaction of 8-CH with solvent as well known in the case of 7-methylguanosine[6]. In dimethyl sulfoxide solution the 2-NH_2 signals of **1** and **2** are observed at 6.78 and 6.88 ppm, but the 1-NH signals are not observed. So the hydrogen atoms may be located on 6-O. The ^{13}C NMR chemical shifts observed for **1** and **2** are given in Table 2.

Table 3. *In vivo* antitumor activity of 7-hydroxyguanine nucleosides against leukemia L1210.

Dose[a]	T/C (%)[b]		
	1	2	7-Hydroxyguanine[1]
0.5	108	107	124
1	118	116	135
2	135	112	145
4	148	146	151
8	157	144	119
16	152	161	Toxic
32	99	120	

[a] mg/kg/day, day 1～5 (ip). 6 mice in each group.
[b] T/C (%) means survival period of treated/mean survival period of controls.

Antitumor Activity

7-Hydroxyguanine nucleosides exhibited a prolongation effect in the survival period of mice inoculated with the mouse leukemia L1210 cells. Lymphoid leukemia L1210, 10^5 cells per mouse were inoculated intraperitoneally into female mice (BDF_1 strain). 7-Hydroxyguanine nucleosides were administered to mice intraperitoneally 24 hours after tumor inoculation. The treatments were given once daily for 5 days. The results are shown in Table 3. The therapeutic effect of the riboside (**1**) is equal to that of 7-hydroxyguanine and the deoxyriboside (**2**) is slightly superior. The acute toxicity of **1** and **2** were determined in male mice (ICR strain) by single intraperitoneal administration, the LD_{50}'s of **1** and **2** were in the range 148～248 mg/kg and 150～198 mg/kg, respectively.

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