Short Communication

Microbial Preparation of Guanosine 7-N-Oxide

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Recently, we reported a new antibiotic, guanine 7-N-oxide, produced by Streptomyces sp., which is inhibitory to Candida albicans and showed antitumor activity both in vitro and in vivo.1) It also showed antiviral activity against DNA and RNA viruses derived from salmonid.2) It is suggested that the mechanism of its action is related to the maturation of messenger RNA including capping.2) In order to improve the solubility and the activity, we attempted the preparation of guanosine 7-N-oxide utilizing bacterial purine nucleoside phosphorylase of Bacillus subtilis PCI 219 (ATCC 6633), which catalyzes the reaction;

\[ \text{Purine nucleoside + Phosphate } \rightarrow \text{Purine + Pentose-1-phosphate}. \]

Thus, we have succeeded in the synthesis of guanosine 7-N-oxide from guanine 7-N-oxide and ribose-1-phosphate. In this paper, we report on the preparation and the properties of guanosine 7-N-oxide (Fig. 1).

![Guanosine 7-N-Oxide](image)

**Fig. 1.** Guanosine 7-N-Oxide.

Bacillus subtilis PCI 219 was cultured at 30°C for 24 hr in a medium containing 1% meat extract, 1% polypepton, 0.5% yeast extract and 0.5% NaCl in distilled water adjusted to pH 7.0 with 5 N KOH. Wet cell paste was obtained by centrifugation, after washing with 50 mm Tris–HCl buffer, pH 7.2. Ribose-1-phosphate was prepared by incubation of inosine and KH₂PO₄ with the cells and purification according to the method of Kammen *et al.*4) with a slight modification.

To prepare guanosine 7-N-oxide, the reaction mixture containing 10 mm guanine 7-N-oxide, 30 mm ribose-1-phosphate, 50 mm Tris–HCl buffer (pH 7.2) and the cell paste of Bacillus subtilis PCI 219 (15 g) in a total volume of 300 ml was incubated at 60°C for 3.5 hr with gentle shaking. After incubation, the reaction mixture was centrifuged at 10,000 rpm for 10 min and the supernatant was applied onto a column of Dowex 50W×4 (H⁺). Immediately after the column was washed with water, it was eluted with 1 N NH₄OH. The eluate was concentrated in vacuo and subjected to a column of Dowex 1×4 (OH⁻). The column was washed with water and then eluted with 0.2 M NH₄HCO₃.

The fractions containing guanosine 7-N-oxide were concentrated in vacuo and applied onto a carbon column. After washing with water and 20% aq. acetone, the column was eluted with 60% aq. acetone. The guanosine 7-N-oxide fractions were concentrated in vacuo and applied onto a Sephadex G-10 column. Elution was carried out with water and the guanosine 7-N-oxide fractions were collected, concentrated in vacuo, and then lyophilized to give 70 mg of the purified product.

Guanosine 7-N-oxide was obtained as a colorless crystalline powder which is easily soluble in water. The physico-chemical properties are as follows: mp > 300°C (dec.); S I MS m/z 300 (M + H)⁺; color reactions: positive to HIO₄-benzidine, negative to ninhydrin; UV λₘₐₓ nm (ε): 236 (19,290) and 270 (9820) in 0.1 M phosphate buffer (pH 7.0), 260 (10,980) and 280 (sh. 7680) in 0.1 N HCl, 231 (18,460) and 279 (9160) in 0.1 N NaOH; IR νₘₐₓ (KBr)
Table I. Antitumor Activity of Guanosine 7-N-Oxide and 5FU on BDF1 Mice Bearing P388 Leukemia

<table>
<thead>
<tr>
<th>Material</th>
<th>Treatment</th>
<th>Dose (mg/kg/day)</th>
<th>MST (days)</th>
<th>T/C (%)</th>
<th>Survivors/Total treatments (day 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>qd 1→9</td>
<td>0.5% CMC</td>
<td>9.0</td>
<td>122</td>
<td>0/5</td>
</tr>
<tr>
<td>Guanosine 1-N-oxide</td>
<td>qd 1→9</td>
<td>1.5</td>
<td>11.0</td>
<td>144</td>
<td>0/5</td>
</tr>
<tr>
<td>7-N-oxide</td>
<td>qd 1→9</td>
<td>3.0</td>
<td>13.0</td>
<td>233</td>
<td>0/5</td>
</tr>
<tr>
<td>5FU</td>
<td>qd 1→9</td>
<td>20.0</td>
<td>21.0</td>
<td>233</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Tumor inoculum, 1 × 10⁶ cells/mouse, i.p.; animal, 6-week-old BDF1 male mice; MST, median survival time; T/C, MST of treatment/MST of control × 100.

For hydrolysis, a small amount of the powder was dissolved in water and applied onto a column of Dowex 50W × 8 (H⁺). After heating at 65°C for 2 hr, the column was eluted with water and then eluted with 1 M NH₄OH. An aqueous eluate contained D-ribose, which was identified by comparison with an authentic sample by silica gel TLC (Merck Kieselgel 60 F₂₅₄ Art. 5715; BuOH–AcOH–H₂O = 4:1:2; Rf 0.38). An eluate with 1 M NH₄OH contained guanine 7-N-oxide, which was identified by silica gel TLC (Rf 0.30) and reverse phase HPLC analysis (column: Finepak SIL C₁₈ 4.6 i.d. × 150 mm; mobile phase: 10 mM KH₂PO₄; flow rate: 1.0 ml/min; temperature: 50°C; detection: UV absorbance at 280 nm; tR: 3.95 min). These results strongly support the finding that the structure is guanosine 7-N-oxide.

To prove the anomeric configuration, the powder was heated at 65°C for 2 hr in acetic acid. After removal of acetic acid, a new substance was obtained almost quantitatively, which was compared with 2-amino-9-β-D-ribofuranosyl-6,8-purinedione (8-hydroxyguanosine) synthesized from 8-bromoguanosine. They were superimposable in UV and IR spectra, Rf of silica gel TLC (Rf 0.44) and retention time of reverse phase HPLC (tR 12.1 min). It is considered that the migration of the 7-N-oxygen to C-8 took place during acid treatment as in the case of guanine 7-N-oxide. The optical rotation of the substance is [α]D₂⁰ = −26.4° (c = 0.25, H₂O), which is in agreement with the value of the synthetic sample, [α]D₂⁰ = −30.8° (c = 0.25, H₂O). The results confirm the β configuration of the nucleoside bond.

The antimicrobial activity of guanosine 7-N-oxide was very weak but it inhibited L-5178Y mouse leukemia cells in culture at IC₅₀ of 0.60 μg/ml (2.01 × 10⁻⁶ M).

As shown in Table I, intraperitoneal administration of guanosine 7-N-oxide showed a life prolongation effect on mice bearing P388 leukemia. It also showed a dose-dependent inhibition of the growth of Ehrlich solid carcinoma in mice (data not shown). Further studies on its antitumor activity are in progress. Mouse tolerated it at 50 mg/kg by intraperitoneal administration.
assistance.

REFERENCES


