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Synthetic Nucleosides and Nucleotides. III. On the Syntheses of Several 2,6,8-Trisubstituted Purine Ribonucleosides[1]

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The syntheses of nucleoside analogues as potential antineoplastic agent and their effects on experimental tumor have been investigated in this laboratory in recent year.[3-5]

In a previous report of this series, Saneyoshi and Chihara have reported the syntheses of 8-mercaptoxanthosine, 8-thiocyanatoadenosine, 8-thiocyanatoxanthosine and 8-thiocyanatoguanosine.[4]

Now, an attempt was made on the preparation of several 2,6,8-trisubstituted purine ribonucleosides along the line of our carcinostatic programs.

In this paper, the synthesis of 8-hydrazino, 8-azido, 8-amino and 8-hydroxy derivatives of guanosine and xanthosine are reported.

Thus, 8-bromo derivatives of guanosine[6,7] or xanthosine[4] were prepared by the procedures originally reported. The treatment of 8-bromoguanosine (Ia) or xanthosine (Ib) with 60% aqueous or methylcellosolve solution of hydrazine hydrate at 90—100° gave 8-hydrazino derivatives (IIa) or (IIb) in 55—75% yield.[8] When they were heated in a sealed tube with 80% hydrazine hydrate at 160° for 6 hr, only a dark colored materal was obtained.

The rection conditions and the yield of hydrazination studied were summerized in Table I.

Table I. Hydrazination of 8-Bromoguanosine with Hydrazine Hydrate

Hydrazine concentration	Condition	Reaction temperature and time	Yield of 8-hydrazinoguanosine
80%	sealed tube	150—160° 6 hr	decompsoition
80%	reflux	boiling point 6 hr	14%
80%	boiling water bath	90—100° 6 hr	26%
60%	boiling water bath	90—100° 6 hr	73%
80% + $CH_3OCH_2CH_2OH$(60%)	boiling water bath	90—100° 6 hr	70%
40%	reflux	boiling point 6 hr	25%
40%	boiling water bath	90—100° 6 hr	13%
20%	reflux	boiling point 6 hr	recovered
10%	reflux	boiling point 6 hr	recovered

Diazotization of the hydrazino derivatives, (IIa) or (IIb), to convert them to the corresponding azido derivatives with sodium nitrite in acetic acid, was attempted but failed. When

1) Part of this work was presented at the Annual Meeting of the Pharmaceutical Society of Japan, Tokushima, Oct. 1965; Part II of this series: *Chem. Pharm. Bull.* (Tokyo), **16**, 509 (1968).
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8) Holmes and Robins reported the direct amination of 8-bromoguanosine by refluxing with very diluted hydrazine for a long period.[6] We obtained the hydrazino derivative, instead of 8-amino derivative, under the reaction conditions employed in our experiment.

IIa or IIb was treated with sodium nitrite in 5% hydrochloric acid at 0°, the 8–azido derivative, (IIIa) or (IIIb) was obtained in a satisfactory yield. Next, IIa or IIb was heated with Raney Ni under reflux in aqueous or methylcellosolve solution and the 8–amino derivative, (IVa) or (IVb), was obtained in an excellent yield. Compound IIIa or IIIb was converted to the corresponding IVa or IVb by catalytic hydrogenation over palladium on charcoal. Since one mole of nitrogen was evolved in this reaction, the apparent volume of the gas in the eudiometer remained almost constant during the reaction. It was necessary to replace the gas in the reaction flask with fresh hydrogen every 10 minutes. The reduction was completed in 30 minutes, and IVa or IVb was obtained in a good yield. Compound IVa or IVb thus obtained was identical with that synthesized by refluxing the corresponding IIa or IIb with Raney Ni, in respect to its behavior in paper chromatography and in ultraviolet (UV) and infrared (IR) spectroscopies.

As an azido group was located adjacent to nitrogen atoms of purine rings in IIIa or IIIb molecule, it was thought that tetrazole rings might be formed.[9,10] But, both of their infrared absorption spectra have a strong and sharp absorption band near 2140 cm^{-1}, characteristic of an azido group. From this fact together with the results in the hydrogenation of IIIa or IIIb, it could be concluded that there existed a –NNN group as a substituent without the tetrazole ring formation.

Then, conversion of IVa to the corresponding 8–hydroxy derivative was unsuccessful. But, IVb was successfully converted to 8–hydroxyxanthosine (Vb) with sodium nitrite in the presence of 5% hydrochloric acid. This is a convenient synthetic method of 9–β–D–ribofuranosyluric acid.[7,11,12] Antitumor activities of these compounds will be reported elsewhere.

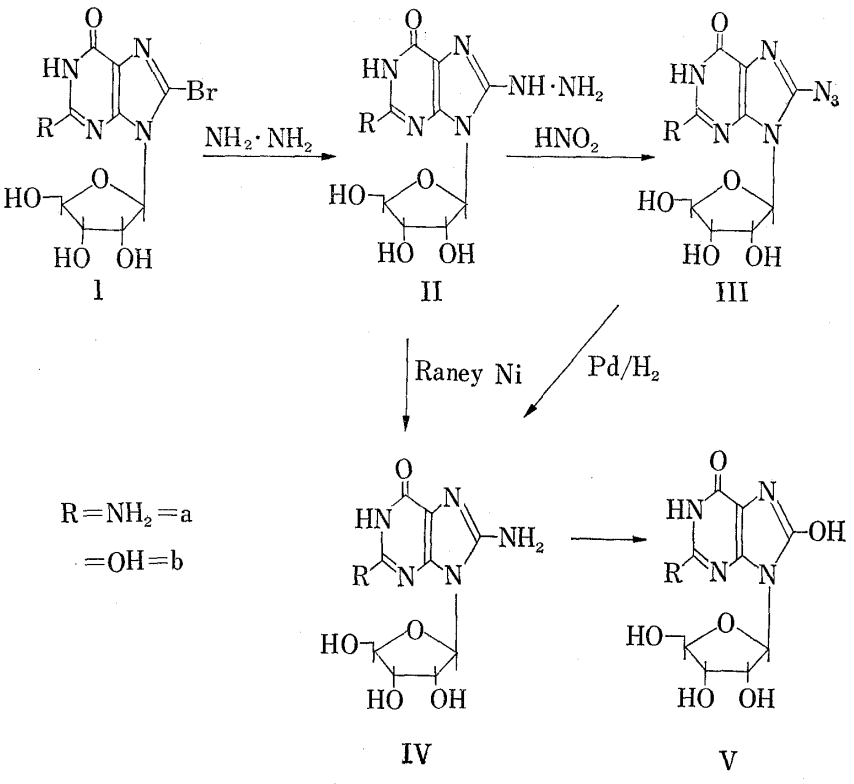

Chart 1

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Experimental

Paper chromatography was accomplished by the ascending technique on Toyo Roshi No. 51A with the two solvent systems; (A) *n*-butyl alcohol: water 86:14 and (B) water adjusted to pH 10 with ammonium hydroxide.

8-Hydrazinoguanosine (IIa)——8-Bromoguanosine (1.51 g) was added to an aqueous solution of 60% hydrazine hydrate (10 ml). Then the resulting clear solution was heated on boiling water bath. After 2—3 hr from the start of the reaction, it was observed that a light yellow precipitate began to separate. The reaction was continued for 6 hr in total. On cooling the reaction mixture to room temperature, the precipitate which resulted was filtered. The yield was 0.4 g. When the filtrate was concentrated to *ca.* 2 ml, further precipitation took place (0.6 g). This was filtered and dried. Total yield was 1 g (72.6%). Recrystallization from boiling water gave white fine needles. mp >240° (decomp.) *Anal.* Calcd. for $C_{10}H_{15}O_5N_7 \cdot H_2O$: C, 36.23; H, 5.13; N, 29.59. Found; C, 36.33; H, 4.99; N, 29.68. UV absorption: λ_{max}^{pH1} 248 mμ, 275 mμ; λ_{min}^{pH1} 229 mμ, 267 mμ; $\lambda_{max}^{H_2O}$ 245 mμ, 275 mμ; $\lambda_{min}^{H_2O}$ 232 mμ, 262 mμ; λ_{max}^{pH11} 275 mμ; λ_{min}^{pH11} 242 mμ. Paper chromatography: Rf=0.12 (A), Rf=0.54 (B).

8-Hydrazinoxanthosine (IIb)——8-Bromoxanthosine monohydrate (1 g) was added to 6 ml of a mixed solution of 80% hydrazine hydrate and freshly distilled methylcellosolve (4.5:1.5 v/v) at room temperature. Then, the resulting homogeneous solution was heated on boiling water bath for 6 hr. When the light yellow clear solution was concentrated to a half volume, a white solid separated on cooling to room temperature. This was collected on a glass filter, washed with ice-water and dried. 0.65 g (69.7%). Recrystallization from large amount of boiling water gave a cream-colored crystal. mp 240° (decomp.) *Anal.* Calcd. for $C_{10}H_{14}O_6N_6$: C, 38.22; H, 4.46; N, 26.75. Found: C, 38.42; H, 4.55; N, 26.73. UV absorption: λ_{max}^{pH1} 238 mμ, 274 mμ; λ_{min}^{pH1} 227 mμ, 245 mμ; $\lambda_{max}^{H_2O}$ 240 mμ, 227.5 mμ; $\lambda_{min}^{H_2O}$ 230 mμ, 255 mμ; λ_{max}^{pH11} 250 mμ (shoulder), 289 mμ; λ_{min}^{pH11} 262 mμ. Rf=0.09 (A), 0.68 (B).

8-Azidoguanosine (IIIa)——To a solution of (IIa) (626 mg) in 10 ml of 5% hydrochloric acid which was cooled in an ice-bath, was added a chilled solution of 166 mg of sodium nitrite in 8 ml of water. After 3 hr stirring at 0°, precipitates began to separate. The reaction mixture was kept cold for another 1 hr, and the solid was collected by filtration. Yield, 510 mg (79.7%) mp >200°(decomp.). This product slowly darkens on exposure to air. *Anal.* Calcd. for $C_{10}H_{11}O_5N_8$: C, 37.15; H, 3.40; N, 34.74. Found: C, 36.88; H, 3.72; N, 34.56. UV absorption: λ_{max}^{pH1} 251 mμ, 276 mμ; λ_{min}^{pH1} 227 mμ, 245 mμ; $\lambda_{max}^{H_2O}$ 245 mμ, 270 mμ; $\lambda_{min}^{H_2O}$ 230 mμ, 255 mμ; λ_{max}^{pH11} 282 mμ; λ_{min}^{pH11} 256 mμ. IR absorption: λ_{max}^{KBr} 2140 cm^{-1} ($-N_3$). Rf=0.08 (A), 0.46 (B).

8-Azidoxanthosine (IIIb)——Compound (IIb) (120 mg) was suspended in 8 ml of 5% hydrochloric acid which was cooled to 0°. Then, a cooled solution of sodium nitrite (48 mg) in 2 ml of water was added to this suspension. After 3 hr stirring at 0°, the solid material was collected by centrifugation and recrystallized from boiling water to give 98 mg of an amorphous powder. Yield, 75% mp >240°. *Anal.* Calcd. for $C_{10}H_{11}O_6N_7$: C, 36.81; H, 3.31; N, 30.06. Found: C, 36.64; H, 3.26; N, 30.48. UV absorption: λ_{max}^{pH1} 269 mμ; λ_{min}^{pH1} 229 mμ; $\lambda_{max}^{H_2O}$ 268 mμ; $\lambda_{min}^{H_2O}$ 230 mμ; λ_{max}^{pH11} 285.5 mμ; λ_{min}^{pH11} 252 mμ. Rf=0.09 (A), 0.76 (B).

8-Aminoguanosine (IVa)——Method A: A mixture of 1.56 g of (IIa) and 5 g of wet Raney Ni in 80 ml of water containing 5 ml of concentrated ammonium hydroxide was refluxed for 3 hr. The reaction mixture was filtered, and the catalyst washed several times with boiling water containing ammonium hydroxide. The filtrate and washings were combined and evaporated under reduced pressure to dryness. The residue was recrystallized from boiling water to a colorless powder. 1.29 g (81%) mp >240°(decomp.) *Anal.* Calcd. for $C_{10}H_{14}O_5N_6$: C, 40.24; H, 4.69; N, 28.17. Found: C, 40.16; H, 4.57; N, 28.22. UV absorption: λ_{max}^{pH1} 249 mμ, 288 mμ; λ_{min}^{pH1} 218 mμ, 269 mμ; $\lambda_{max}^{H_2O}$ 280 mμ; $\lambda_{min}^{H_2O}$ 240 mμ; λ_{max}^{pH11}256 mμ (shoulder), 280 mμ; λ_{min}^{pH11} 241 mμ. Rf=0.11 (A), 0.42 (B).

Method B: Compound (IIIa) (328 mg) was dissolved in 250 ml of hot water, and the solution was added to 300 mg of 5% palladium on charcoal in 50 ml of water. The resulting solution was hydrogenated for 4 hr. The catalyst was filtered and washed with 100 ml of 5% aqueous ammonium hydroxide, then the filtrate and washings were combined and concentrated to a small volume (*ca.* 1 ml), which was kept in ice-box. The solid separated was collected by filtration and recrystallized from boiling water to yield 262 mg (80.9%). This product was identified with the specimen provided by the method A.

8-Aminoxanthosine (IVb)——Method A: Compound (IIb) (630 mg) was dissolved in 0.5 N ammonium hydroxide (50 ml) and then 2.4 g of wet Raney Ni which was suspended in 10 ml of water was added. The resulting mixture was refluxed for 3 hr. The reaction mixture was filtered, the catalyst was removed by centrifugation and the filter cake was washed with hot water containing ammonium hydroxide. The light green filtrate and washings were combined and concentrated to a small volume (*ca.* 4 ml) and cooled to 4°, a precipitate gradually being formed and filtered (200 mg). The mother liquid was evaporated to dryness to yield 240 mg of residue. The solid was recrystallized from boiling water to give a white amorphous powder: 410 mg (66.2%) mp >240°(decomp.) *Anal.* Calcd. for $C_{10}H_{13}O_6N_5 \cdot H_2O$: C, 37.85; H, 4.73; N, 22.08. Found: C, 37.85; H, 4.73; N, 22.11. UV absorption: λ_{max}^{pH1} 230 mμ, 270 mμ; λ_{min}^{pH1} 245 mμ; $\lambda_{max}^{H_2O}$ 243 mμ, 273 mμ; $\lambda_{min}^{H_2O}$ 219 mμ, 250 mμ; λ_{max}^{pH11} 248 mμ (shoulder), 286 mμ; λ_{min}^{pH11} 255 mμ. Rf=0.07 (A), 0.46 (B).

Method B: Compound (IIIb) (122 mg) was dissolved in 200 ml of hot water. The resulting solution was added to 150 mg of palladium on charcoal in 50 ml of water and hydrogenated for 6 hr at room temperature. The catalyst was removed by filtration and washed with 100 ml of hot water. The aqueous solution was evaporated to dryness and the residue was recrystallized from 200 ml of boiling water to give a white powder. 79 mg (70%). This sample was identified with the specimen obtained by the method A.

8-Hydroxyxanthosine (9-β-D-Ribofuranosyluric Acid) (Vb)——To a solution of (IVb) (150 mg) in 5 ml of hydrochloric acid which was cooled in ice–salt mixture, was added a cooled solution of 42 mg of sodium nitrite in 2 ml of water. After 5 hr stirring at 0°, precipitate began to separate; the reaction mixture was kept cold in an ice-box, and solid was collected by filtration; 132 mg was obtained. Yield 84%. Analytical sample was recrystallized from hot water to give white needles. mp >240° *Anal.* Calcd. for $C_{10}H_{12}O_7N_4 \cdot H_2O$: C, 37.74; H, 4.40; N, 17.61. Found: C, 37.82; H, 4.42; N, 17.75; UV absorption: λ_{max}^{pH1} 238 mμ, 288 mμ; λ_{min}^{pH1} 258 mμ; $\lambda_{max}^{H_2O}$ 241 mμ, 293 mμ; $\lambda_{min}^{H_2O}$ 225 mμ, 270 mμ; λ_{max}^{pH11} 251 mμ, 302 mμ; λ_{min}^{pH11} 280 mμ. Rf=0.10 (A), 0.59 (B).

This product was also identical with specimen by the Holmes's procedure[12] by paper chromatography and IR spectrophotometry.

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Studies on Synthetic Sweetening Agents. XII.[1] The Binding of Sodium Cyclamate with Bovine Serum Albumin

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It has been reported by some workers[3–7] that the distribution, metabolism, and elimination of a drug are affected by various kinds of proteins in plasma. Davis[3] has pointed out that the albumin fraction of plasma is primarily responsible for the properties of binding of a drug with plasma proteins.

In the previous paper,[8] the authors reported that albumin and casein were precipitated by the addition of sodium cyclamate (CHS–Na) from the aqueous solutions. However, nothing has been known about the interaction between CHS–Na and serum albumin.

The present report deals with physicochemical studies on the mode of binding of CHS–Na with bovine serum albumin (BSA) using the equilibrium dialysis method, and it was suggested that the binding of CHS–Na with BSA is predominantly a reversible one based on electrostatic force, by which an anonic form of CHS–Na may be bound with the positively–charged residues in a BSA molecule. Furthermore, it was found that the strength of binding of CHS–Na to BSA is considerably weak.

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