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Synthesis of 9-(4′-Hydroxybutyl)-2-amino-6-hydroxypurine 4′-Phosphate[1]

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Numerous purine derivatives bearing alkyl or hydroxyalkyl group at 9-position have been prepared, and interestingly, some of them are known to show biological activeties. For example, 6-amino-9-(3′-hydroxypropyl)purine[3] is reported to be a good inhibitor of adenosine deaminase. It is also of interest that 9-ethyl- and 9-butyl-6-purinethiols[4] and 2-amino-9-propyl-6-purinethiol[5] are highly active against Adenocarcinoma 755. Recently, as an analog of adenosine 5′-triphosphate, 9-(4′-hydroxybutyl)-6-aminopurine 4′-phosphate was prepared by Ikehara and Ohtsuka,[6] who reported that it was hydrolyzed by myosin.

The present investigation was undertaken to prepare 9-(2′-hydroxyethyl)-2-amino-6-hydroxypurine 2′-phosphate and 9-(4′-hydroxybutyl)-2-amino-6-hydroxypurine 4′-phosphate, the analogs of 5′-guanylic acid, and to examine their flavoring activities. These compounds were also desired for biochemical studies.

The preparation of VIa and VIb was achieved by a modification of previously reported procedure of Robins, *et al.*,[5] starting from 2-amino-4-chloro-6-hydroxypyrimidine (I) as shown in Chart 1. When compound I was heated with monoethanolamine in methyl cellosolve, 2-amino-4-(2′-hydroxyethylamino)-6-hydroxypyrimidine (IIa) was readily obtained. Nitrosation of IIa with nitrous acid gave the nitroso derivative (IIIa), in quantitative yield, which was reduced with sodium dithionite and then formylated to afford 2-amino-4-(2′-hydroxyethylamino)-5-formamido-6-hydroxypyrimidine (IVa). Ring closure of IVa in refluxing formamide yielded 9-(2′-hydroxyethyl)-2-amino-6-hydroxypurine (Va). In a similar fashion, reaction of I with 4-amino-1-butanol afforded the hydroxybutyl derivative (IIb), which was nitrosated and reduced in the presence of formic acid to give the formamido derivative (IVb). On heating in formamide, IVb was cyclized to 9-(4′-hydroxybutyl)-2-amino-6-hydroxypurine (Vb). The structures of both Va and Vb was supported by their elemetary analyses and ultraviolet absorption spectra similar to those of guanosine. Phosphorylation of Va and Vb with polyphosphoric acid[7] followed by heating for 30 min on a steam-bath gave VIa and VIb, respectively. These compounds were purified by column chromatography on decolorizing resin[8] and isolated as barium salts.

1) A part of this paper was presented at the 85th Annual Meeting of the Pharmaceutical Society of Japan, Tokushima, October, 1965.
2) Location: *Suzuki-cho, Kawasaki.*
3) H.J. Schaeffer and R. Vince, *J. Med. Chem.*, **8**, 33 (1965).
4) H.E. Skipper, J.A. Montgomery, J.R. Thomson, and F.M. Schabel, Jr., *Cancer Res.*, **19**, 425 (1959).
5) C.W. Noell and R.K. Robins, *J. Med. Chem.*, **5**, 558 (1962).
6) M. Ikehara, E. Ohtsuka, S. Kitagawa, K. Yagi, and Y. Tonomura, *J. Am. Chem. Soc.*, **83**, 2679 (1961).
7) R.H. Hall and H.G. Khorana, *J. Am. Chem. Soc.*, **77**, 1971 (1955).
8) This decolorizing resin was prepared in our Laboratories by copolymerization of metaphenylenediamine, resorcin and formalin.[9]
9) Y. Tsuchiya, I. Hayashi, T. Kato, M. Yoshikawa, T. Mori, and S. Miyasaka, Japan. Patent 12343 (1964).

The compound, VIa and VIb, in which the ribose moiety of guanosine 5′-phosphate was replaced by hydroxyethyl or hydroxybutyl group, were found to have no flavoring activity.[10]

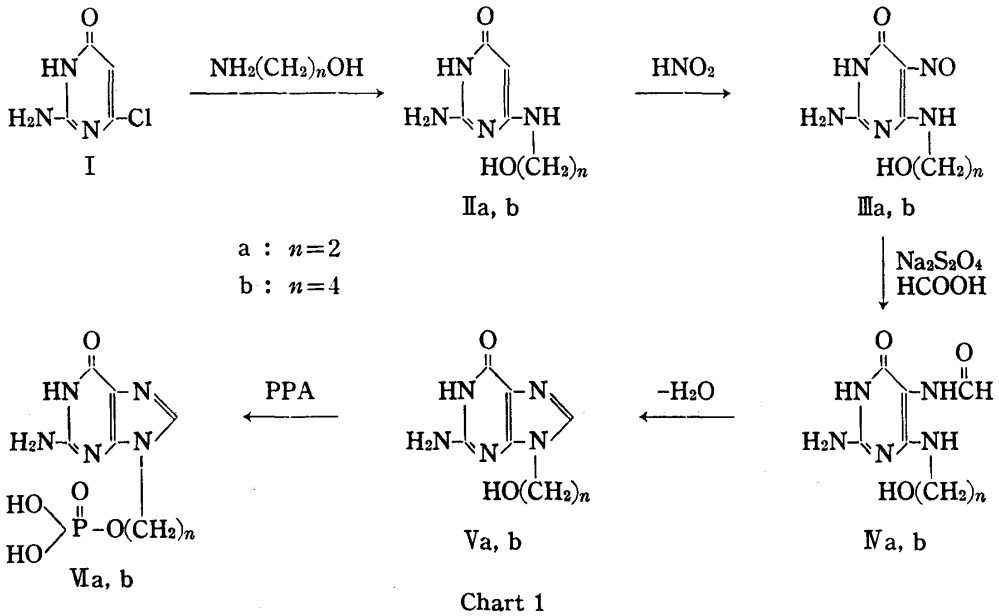

Chart 1

Experimental[12]

2-Amino-4-(2′-hydroxyethyl)amino-6-hydroxypyrimidine (IIa)——A solution of I[13] (7 g, 4.84 mmoles) and monoethanolamine (8.9 g, 14.6 mmoles) in 50 ml of methyl cellosolve was refluxed for 2 hr. After removal of the solvent under reduced pressure, the residue was dissolved in a small amount of H_2O and the solution was allowed to stand at room temperature. The resulting crystals were recrystallized from H_2O to give a pure sample; yield 3.7 g (44.8%); mp 233° (decomp.). *Anal.* Calcd. for $C_6H_{10}O_2N_4$: C, 42.35; H, 5.88; N, 32.93. Found: C, 42.31; H, 6.00; N, 32.61.

2-Amino-4-(2′-hydroxyethyl)amino-5-nitroso-6-hydroxypyrimidine (IIIa)——To a stirred solution of IIa (15 g) in a mixture of H_2O (180 ml) and AcOH (60 ml) was added portionwise a solution of sodium nitrite (12 g) in 50 ml of H_2O at 25—30°. An orange-colored nitroso derivative precipitated. This was filtered, washed with H_2O, and dried at 60° *in vacuo*, affording 17 g (97%) of a product, which was suitable for use in the next reaction. An analytically pure sample was obtained by recrystallization from cellosolve. *Anal.* Calcd. for $C_6H_9O_3N_5$: C, 36.18; H, 4.52; N, 35.18. Found: C, 36.66; H, 4.78; N, 35.09.

9-(2′-Hydroxyethyl)-2-amino-6-hydroxypurine (Va)——The compound IIIa (10 g) was dissolved in a mixture of $HCONH_2$ (60 ml) and HCOOH (30 ml), and $Na_2S_2O_4$ (8 g) was added portionwise at 70°. When the addition ended, the color of the solution changed from red to light yellow. The mixture was refluxed for 15 min, diluted with 150 ml of H_2O, and allowed to stand in a refrigerator, giving white crystals (IVa). After being collected by filtration and dried, the crystals were added to a mixture of $HCONH_2$ (28 ml) and HCOOH (2 ml), and the solution was refluxed for 3.5 hr. After cooling, ice water (100 ml) was added, and the resulting precipitate was filtered and recrystallized from H_2O to give a pure sample, mp 308—309.5°. Yield 4.5 g (45.9%). UV $\lambda_{max}^{PH\,1}$ mμ (ε): 254.5 (9700), 280 (6100); $\lambda_{max}^{PH\,13}$ mμ (ε): 255 (inf.), 268.5 (9600). *Anal.* Calcd. for $C_7H_9O_2N_5$: C, 43.07; H, 4.62; N, 35.89. Found: C, 43.07; H, 4.92; N, 35.59.

2-Amino-4-(4′-hydroxybutyl)amino-6-hydroxypyrimidine (IIa)——The compound I (28 g) was treated with 4-amino-1-butanol (44 g) in a manner similar to that described for IIa. A crude product was recrystallized from H_2O to afford 16.5 g (42.5%) of pale yellow crystals, mp 205—206°. *Anal.* Calcd. for $C_8H_{14}O_2N_4$: C, 48.49; H, 7.07; N, 28.28. Found: C, 48.53; H, 7.73; N, 28.99.

10) 9-(4′-Hydroxybutyl)-6-hydroxypurine 4′-phosphate was also shown by Honjo, *et al.*[11] to be tasteless.
11) Honjo, *et al.*, Abstract of Papers, the Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, April, 1963, p.40.
12) All melting points are uncorrected. Ultraviolet absorption spectra were taken with a Hitachi Type EPS-3T automatic recording spectrophotometer.
13) H.S. Forrest, R. Hull, H.J. Rodda, and A.R. Todd, *J. Chem. Soc.*, **1951**, 3.

2-Amino-4-(4′-hydroxybutyl)amino-5-nitroso-6-hydroxypyrimidine (IIIb)——This compound was prepared by the same procedure as described for IIIa; mp 228° (decomp.); Yield 96%. *Anal.* Calcd. for $C_8H_{13}O_3N_5$: C, 42.29; H, 5.73; N, 30.84. Found: C, 42.19; H, 5.83; N, 30.76.

2-Amino-4-(4′-hydroxybutyl)amino-5-formamido-6-hydroxypyrimidine (IVb)——To a stirred solution of IIIb (5 g) in a mixture of $HCONH_2$ (30 ml) and HCOOH (15 ml), $Na_2S_2O_4$ (4 g) was added portionwise at 70°. Gradually, the color of the solution became yellow. The reaction mixture was refluxed for 15 min and then allowed to stand in a refrigerator. The crystals that precipitated were collected by filtration and recrystallized from H_2O to afford a pure sample, mp 244—245° (decomp.). Yield 3.2 g (59.3%). *Anal.* Calcd. for $C_9H_{15}O_3N_5 \cdot \frac{1}{4}H_2O$: C, 43.99; H, 6.31; N, 28.51. Found: C, 44.30; H, 6.59; N, 28.10.

9-(4′-Hydroxybutyl)-2-amino-6-hydroxypurine (Vb)——The foregoing IVb was refluxed for 3.5 hr in $HCONH_2$ (14 ml) containing HCOOH (1 ml). The reaction solution was then worked up as described for Va, giving 1 g (37.1%) of a pure product, mp 220—222° (decomp.). UV $\lambda_{max}^{PH\,1}$ mμ (ε): 254 (9700), 280 (6400); $\lambda_{max}^{PH\,13}$ mμ (ε): 255 (inf.), 269.5 (9800). *Anal.* Calcd. for $C_9H_{13}O_2N_5 \cdot \frac{1}{2}H_2O$: C, 46.55; H, 6.04; N, 30.17. Found: C, 47.00; H, 5.74; N, 30.54.

9-(2′-Hydroxyethyl)-2-amino-6-hydroxypurine 2′-Phosphate (VIa)——The compound Va (1.2 g) was phosphorylated described for the preparation of VIb and the product was purified by column chromatography on decolorizing resin. The resulting ammonium salt of VIa was converted with $Ba(OAc)_2$ to the barium salt. Yield 490 mg (18.6%). UV $\lambda_{max}^{PH\,1}$ mμ (ε): 254.5 (6100), 280 (inf.); $\lambda_{max}^{PH\,13}$ mμ (ε): 255 (inf.), 269 (9500). *Anal.* Calcd. for $C_7H_8O_5N_5PBa \cdot H_2O$: C, 19.63; H, 2.34; N, 16.36. Found: C, 19.23; H, 2.57; N, 16.26.

9-(4′-Hydroxybutyl)-2-amino-6-hydroxypurine 4′-Phosphate (VIb)——To a mixture of 85% H_3PO_4 (13 g) and P_2O_5 (10 g) was added Vb (1 g), and the mixture was stirred for 3 hr at 60—70°. Gradually, it became clear. After the reaction, 100 ml of H_2O was added and the solution was heated on a steam-bath to hydrolyze the resulting polyphosphates. An aliquot from the solution showed a single spot on a paper chromatogram. The solution was brought to pH 2 and passed through a column (2 × 60 cm) of decolorizing resin.[9] The column was washed with H_2O, and VIb was eluted with 0.5 N NH_4OH. The effluent was concentrated *in vacuo* to *ca.* 5 ml, to which one volume of EtOH was added to precipitate the ammonium salt of VIb. This was collected by filtration and taken up in 100 ml of H_2O. The pH of the solution was adjusted to 8.5, and to this was added a solution of $Ba(OAc)_2$ (909 mg) in 20 ml of H_2O. The resulting precipitate, mainly consisted of $Ba_3(PO_4)_2$, was removed. After the filtrate was concentrated to *ca.* 50 ml, addition of EtOH (50 ml) afforded a white precipitate, which was collected by centrifugation. Washing with EtOH and ether and drying *in vacuo* at 80° gave 320 mg (14.5%) of VIb. The above ammonium salt of VIb was found to be tasteless. *Rf*: 0.22, *n*-PrOH–NH_4OH(28%)–H_2O (20:12:3, v/v); 0.51, iso-PrOH–sat. $(NH_4)_2SO_4$–H_2O (2:79:19). UV $\lambda_{max}^{PH\,1}$ mμ (ε): 254 (10600), 281 (6500); $\lambda_{max}^{PH\,13}$ mμ (ε): 270 (10300). *Anal.* Calcd. for $C_9H_{12}O_5N_5PBa \cdot 2H_2O$: C, 22.79; H, 3.38; N, 14.77; P, 6.54. Found: C, 22.68; H, 3.38; N, 14.39; P, 6.90.

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Bis(guanidinoethyl)amine Derivatives

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Recently, we have synthesized derivatives of 2-guanidinoethylamine[2] (I), 3-guanidinopropylamine[3] (II) and bis(3-guanidinopropyl)amine[4] (III), in order to find pharmacolo-

1) Location: *Shirokane-Sankocho, Shiba, Minatoku, Tokyo.*
2) T. Ueda and S. Akihama, unpublished.
3) T. Ueda and K. Ishizaki, *Chem. Pharm. Bull.* (Tokyo) **15**, 228 (1967).
4) T. Ueda and S. Watanabe, Japan. Patent 470947 (1966).