parallel with the colorimetric determination. One characteristic feature in the present experiment is that a large amount of 4HAQO accumulated in the course of reduction. Although many investigations have revealed that hydroxyamino compounds are intermediates in the reduction of arylnitro compounds, the survey of literatures did not uncover the accumulation such a large amount of hydroxyamino compounds as indicated in this experiment. As discussed earlier,[4] the biological activities of nitroquinoline 1-oxides are rather peculiar among diverse organic nitro compounds. This peculiarity may, if not all but at least partly, be explained by the accumulation of large amount of hydroxyamino intermediates in the course of metabolism.

We wish to thank Prof. Emeritus Eiji Ochiai, University of Tokyo for his encouragement. This work was partially supported from the Ministry of Education of Japan.

Summary

Colorimetric methods for the estimation of 4-hydroxyaminoquinoline 1-oxide and 4-aminoquinoline 1-oxide have been developed. The former was estimated by a modification of Zucker and Nason's method (Zucker and Nason, 1955). The method for the estimation of 4-aminoquinoline 1-oxide is based on the formation of a stable hydroxyazo dye which is formed after coupling of diazotized 4-aminoquinoline 1-oxide with 2-hydroxy-6,8-naphthalenedisulfonic acid in alkaline condition.

The formation of 4-hydroxyaminoquinoline 1-oxide and 4-aminoquinoline 1-oxide in the course of microbial reduction of 4-nitroquinoline 1-oxide was studied by using the present methods.

(Received October 7, 1963)

[Chem. Pharm. Bull. 12 (3) 267 ~ 271]

UDC 612.398.145

38. Morio Ikehara, Hitoshi Uno, and Fumiyoshi Ishikawa : Studies of Nucleosides and Nucleotides. XXIII.*1 A Versatile Method for Replacement of 6-Hydroxyl Group of Purine Nucleoside.

(*Faculty of Pharmaceutical Sciences, School of Medicine, Hokkaido University**2)

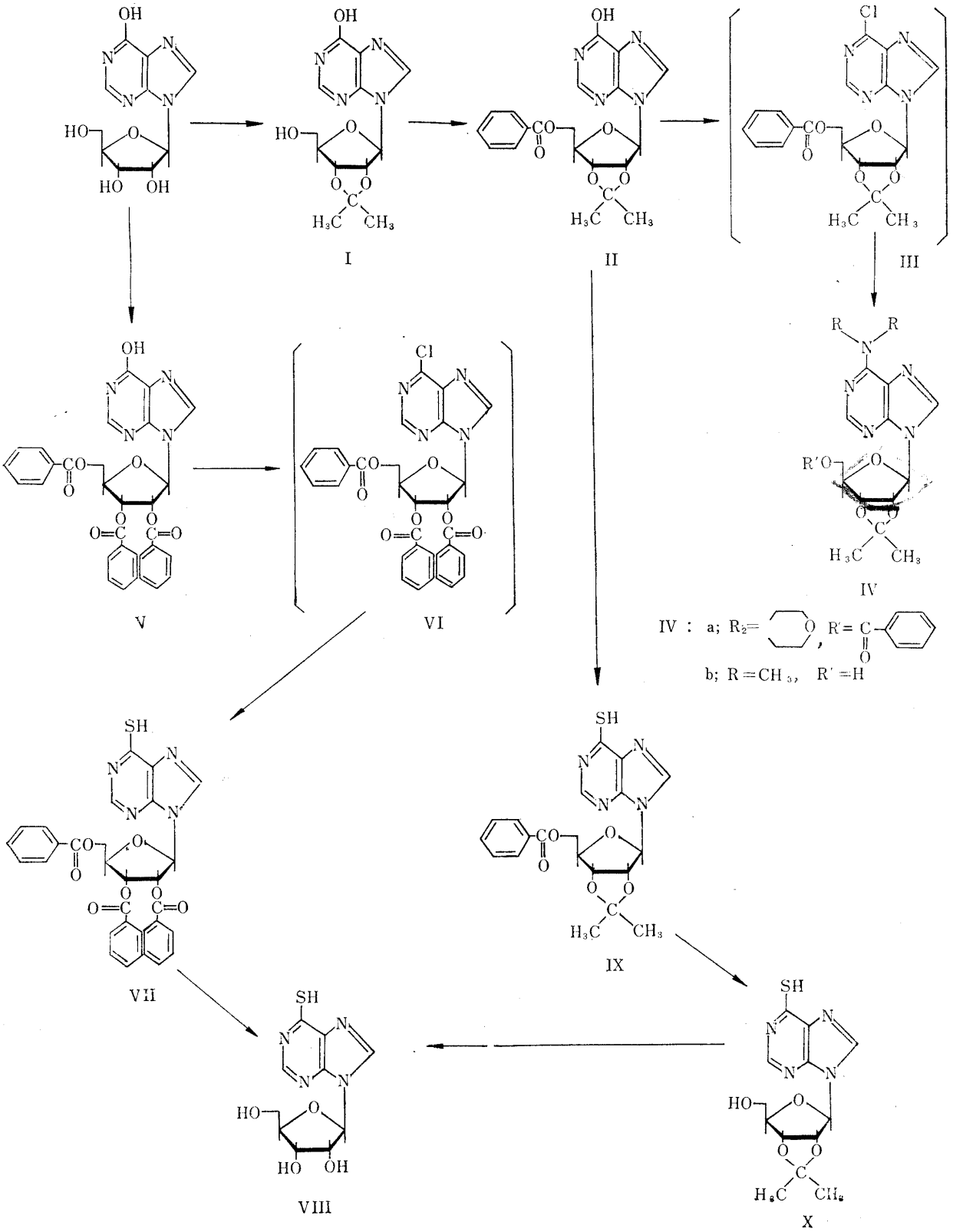
In the previous papers from our laboratory[1~3] the synthesis of purine nucleoside and nucleotide bearing various substituents in 6-position has been reported. In the main, 6-hydroxyl group was converted to mercapto group by the reaction with phosphorus pentasulfide in refluxing pyridine[4] and then replaced by the attack of various nucleophiles. Another course investigated so far by several researchers[5~8] involved

---

*1 Part XXII. M. Ikehara, E. Ohtsuka, Y. Kodama : This Bulletin, **12**, 145 (1964).

*2 Kita 12-jo, Nishi-5-chome, Sapporo (池原森男, 宇野 準, 石川文義).

1) M. Ikehara : This Bulletin, 8, 367 (1960).
2) M. Ikehara, E. Ohtsuka, F. Ishikawa : *Ibid.*, **9**, 173 (1961).
3) M. Ikehara, T. Ueda, S. Horikawa, A. Yamazaki : *Ibid.*, **10**, 665 (1962).
4) J. J. Fox, I. Wempen, A. Hampton, I. L. Doerr : J. Am. Chem. Soc., **80**, 1669 (1958).
5) J. F. Gerster, J. W. Jones, R. K. Robins : J. Org. Chem., **28**, 945 (1963).
6) G. H. Hitchings, I. Goodman : U. S. P. 3,074,929; Chem. Abs., **59**, 739 (1963).
7) R. K. Robins, *et al.* : J. Am. Chem. Soc., **82**, 2654 (1960); J. Org. Chem., **26**, 477 (1961); *Ibid.*, **27**, 986 (1962).
8) H. Lettlré, H. Bullweg : Ann., **656**, 158 (1962).



direct or indirect chlorination of inosine. Quite recently, Zemlicka, *et al.*[9] briefly reported the direct chlorination of 2′,3′,5′-tri-O-benzoyl-6-azauridine by Vilsmeier complex[10] derived from DMF*[3] and thionyl chloride. The fact that in the low temperature range phosphorus oxychloride would not cleave the nucleoside linkage of protected inosine,[5] encouraged us to attempt the utilization of DMF-thionyl chloride method for the chlorination of 6-hydroxyl group of inosine.

The chlorination of 2′,3′-O-isopropylidene-5′-O-benzoylinosine was first investigated. The compound can form a suitable intermediate for phosphorylation as reported in the case of cytidylic acid analogs[11] and could be used for the synthesis of analogs of isopropylidene-ATP.[12] 2′,3′-O-Isopropylideneinosine (I), which was synthesized from inosine by a slightly modified method of Hampton,[13] was benzoylated in pyridine to afford 2′,3′-O-isopropylidene-5′-O-benzoylinosine (II), m.p. 206～207° in a yield of 88%. Compound (II) was dissolved in chloroform and refluxed for 4 hours with 3 equivalents of thionyl chloride and a slight excess of DMF under exclusion of moisture. At the end of the reaction a yellow solution was obtained. Although ultraviolet absorption spectrum of this solution closely resembled to that of 6-chloroinosine, attempt to isolate isopropylidene-benzoyl-6-chloroinosine (III) in the crystalline form was difficult. This fact may be ascribed to the presence of a complex of DMF and 6-chloropurine riboside, in which the chlorine atom may be activated. Compound (III) was reacted with morpholine without further purification at 100° for 3 hours. 6-Morpholino-9-(2′,3′-O-isopropylidene-5′-O-benzoyl-β-D-ribofuranosyl)purine (IVa), m.p. 114～115°, was obtained as colorless crystal. The yield calculated from II was 62.4%. The structure of IVa was confirmed by the ultraviolet absorption spectrum and the elementary analyses. The reaction of compound (II) with aqueous dimethylamine gave crystalline 6-dimethylamino-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (IVb), m.p. 173～175°, in the overall yield of 52.4%. This was identical with an authentic sample[14] in its physical and optical behaviors.

The chlorination of 2′,3′,5′-tri-O-benzoylinosine[4] (V) was then investigated. Compound (V) was reacted with thionyl chloride-DMF in chloroform solution as described above. The resulting solution was examined by paper chromatography, Rf 0.93 (solvent, butanol-water 86:14), and ultraviolet absorption spectra, λ_{max} 264 mμ. Although these values indicated the complete chlorination, the attempt for isolation of compound (VI) in the crystalline form was not carried out. However, the reaction of VI with thiourea to afford 6-mercapto-9-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)purine (VII), m.p. 210～216°, showed the conversion of hydroxyl to chlorine in compound (VI). Physical properties of VII was coincided with those of Fox's description.[4] 6-Mercapto-9-β-D-ribofuranosylpurine (VIII) was obtained by the debenzoylation of VII with sodium methoxide in methanol. In order to obtain a sample of 6-mercaptopurine riboside by the known thiolation procedure, 2′,3′-O-isopropylidene-5′-O-benzoylinosine (II) was treated according to Fox.[4] Reflux of II with phosphorus pentasulfide in pyridine for 6 hours gave 6-mercapto-9-(2′,3′-O-isopropylidene-5′-O-benzoyl-β-D-ribofuranosyl)purine (IX), m.p. 233～235°. The structure of this compound was elucidated from the data of elementary analysis and optical behaviors. Compound (IX) was debenzoylated with sodium methoxide in methanol and resulting 6-mercapto-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (X). m.p. 250°, was deprotected with dilute acetic acid to afford compound (VIII), m.p. 206～209° (decomp.).

*[3] Abbreviations : DMF, N,N-dimethylformamide; ATP, adenosine 5′-triphosphate.

9) J. Zemlicka, J. Smrt, F. Sorm : Tetrahedron Letters, **1962**, 379.
10) A. Vilsmeier, E. Haack : Ber., **60B**, 119 (1927).
11) M. Ikehara, T. Ueda, K. Ikeda : This Bulletin, **10**, 767 (1962).
12) M. Ikehara, E. Ohtsuka : *Ibid.*, **11**, 1353 (1963).
13) A. Hampton, D. I. Magrath : J. Am. Chem. Soc., **79**, 3250 (1957).
14) J. M. Andrews, W. E. Barber : J. Chem. Soc., **1958**, 2768.

Both samples of Ⅷ, obtained by DMF-thionyl chloride chlorination and phosphorus pentasulfide thiolation, were compared to each other and with the data given by Fox and colaborators.[4] From the comparison it was concluded that these samples were the same substances.

As judged from the results of the experiments described above, it was proved that thionyl chloride-DMF complex was a powerful and suitable reagent for the replacement of 6-hydroxyl group of inosine to various groups, which were required for the synthesis of substrates of enzymatic studies.[15,16]

Experimental

2′,3′-O-Isopropylideneinosine——Into a solution of 10 g. of *p*-toluene sulfonic acid in 150 ml. of Me_2CO was added 1 g. of inosine. After 10 min. of stirring, the reaction mixture became transparent. Solid $NaHCO_3$ (10 g.) was added to the reaction mixture and stirred further for 1.5～2 hr. When the test with bromthymolblue paper treated with NH_3 gave blue color, the whole was refluxed for 5 min. on a steam bath. Solid salt was filtered while hot and the residue was extracted twice with hot Me_2CO. Filtrate and washings were combined, concentrated *in vacuo*, and crystalline isopropylideneinosine, which separated from the solution, was collected in a filter, m.p. 247～248° (yield, 913 mg., 80%). *Anal.* Calcd. for $C_{13}H_{16}O_5N_4$: C, 50.64; H, 5.23; N, 18.17. Found : C, 50.34; H, 5.18; N, 18.03. UV : λ_{max}^{EtOH} 249 mμ.

2′,3′-O-Isopropylidene-5′-O-benzoylinosine——620 mg. of above isopropylideneinosine was dissolved in 10 ml. of dry pyridine and 560 mg. (2 mol. equivalent) of BzCl dissolved in 2 ml. of pyridine was added at 60～65.° Reaction was continued for 3 hr. at 40～45° (total 5 hr.). After standing overnight at room temperature, pyridine was evaporated *in vacuo* and residual syrup was poured in ice H_2O containing 1 g. of $NaHCO_3$. Trituration of resinous substance gave a solid, which was powderized, collected on a filter, and dried in a desiccator. Recrystallization from 35 ml. of EtOH gave needles, m.p. 206～207°. Yield was 715 mg. (88%). *Anal.* Calcd. for $C_{20}H_{20}O_6N_4$: C, 58.49; H, 4.89; N, 13.58. Found : C, 58.51; H, 4.82; N, 13.00.

6-Morpholino-9-(2′,3′-O-isopropylidene-5′-O-benzoyl-β-D-ribofuranosyl)purine——$SOCl_2$ (1.8 g., 0.015 mole) was dissolved in 30 ml. of $CHCl_3$. Into this solution 0.5 ml. (0.006 mole) of DMF was added and shaken for 10 min. under exclusion of moisture. Into this, 2.06 g. (0.005 mole) of 2′,3′-O-isopropylidene-5′-O-benzoylinosine was added. After reflux for 3 hr., reaction mixture was kept in standing at room temperature overnight. Paper chromatography (BuOH-H_2O=86:14) gave a single spot having Rf 0.90. UV absorption spectrum of the EtOH extract of this spot cut out from the paper showed λ_{max} 263～264 mμ. $CHCl_3$ was evaporated *in vacuo* and the residue was codistilled with abs. EtOH (20 ml.×3) under reduced pressure in order to remove $SOCl_2$. Into the residue 10 g. of morpholine was added and heated at 100° for 3 hr. in a sealed tube. Excess morpholine was removed by vacuum distillation and the residue was evaporated several times with H_2O until the odor of amine was diminished. The residue was taken up in hot EtOH, and added with H_2O until slight turbidity appeared. Slow cooling of this solution gave pale yellow crystal, m.p. 107～110° (yield, 1.72 g., 62.4%). Further recrystallization from EtOH gave m.p. 114～115°. *Anal.* Calcd. for $C_{24}H_{27}O_6N_5$: C, 59.86; H, 5.65; N, 14.55. Found: C, 59.56; H, 5.88; N, 14.70. UV $\lambda_{max}^{H_2O}$ mμ (log ε) : 279 (1.609×10^4), 237 (0.907×10^4). Paper chromatography (solvent BuOH-H_2O=86:14) Rf 0.91. Metaperiodate test[17] : negative.

6-Dimethylamino-9-(2′,3′-O-isopropylidene)-β-D-ribofuranosyl)purine——$SOCl_2$ (1.8 g., 0.015 mole) was dissolved in 15 ml. of $CHCl_3$, 0.5 ml. (0.006 mole) of DMF was added and shaken for 10 min. at room temperature under exclusion of moisture. Into this solution 2.06 g. (0.005 mole) of 2′,3′-O-isopropylidene-5′-O-benzoylinosine was added and refluxed for 4 hr. $CHCl_3$ was evaporated under reduced pressure, EtOH (20 ml.) was added and evaporated *in vacuo*. This treatment was repeated until no odor of $SOCl_2$ remained in the residue. The residue was taken up in EtOH (10 ml.), 40% aqueous solution of dimethylamine (40 ml.) was added and heated at 100° (in a boiling water bath) for 3 hr. in a sealed tube. Reaction mixture was decolorized with Norit A, charcoal was filtered off and the filtrate was concentrated to its half volume by vacuum distillation. After cooling white crystal, m.p. 170～173°, appeared. Yield was 1.0 g. (52.3%). Further recrystallization from MeOH gave a sample having m.p. 173～175°. *Anal.* Calcd. for $C_{15}H_{21}N_5O_4$: C, 53.78; H, 6.32; N, 20.90. Found : C, 53.65; H, 6.50; N, 20.97. UV: λ_{max}^{EtOH} 273 mμ.

15) M. Ikehara, E. Ohtsuka, S. Kitagawa, K. Yagi, Y. Tonomura : J. Am. Chem. Soc., **83**, 2679 (1961).
16) N. Azuma, M. Ikehara, E. Ohtsuka, Y. Tonomura : Biochim. Biophys. Acta, **60**,104 (1962).
17) M. Viscontini, O. Hoch, P. Karrer : Helv. Chim. Acta, **38**, 642 (1955).

6-Mercapto-9-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)purine——$SOCl_2$ (1.5 g., 0.013 mole) was dissolved in 50 ml. of $CHCl_3$, DMF (0.51 ml., 0.007 mole) was added and shaken for 10 min. at room temperature under exclusion of moisture. Into this solution 2′,3′,5′-tri-O-benzoylinosine (3.5 g., 0.007 mole) was added and refluxed for 3 hr. Paper chromatography (solvent BuOH-H_2O=86:14) of an aliquot showed Rf 0.93. UV absorption of the EtOH-extract of this spot cut out from the paper showed λ_{max} 264 mμ. $CHCl_3$ was evaporated *in vacuo* and the residue was codistilled twice with 10 ml. of EtOH. The residue after this treatment was taken up in 40 ml. of abs. EtOH, thiourea (1.14 g., 2.5 equivalent) was added and refluxed for 1 hr. After cooling white precipitates, which began to appear during reflux, was collected by filtration and washed well with hot H_2O. This crystal has m.p. 210~216° (yield 2.8 g., 77.8%) and λ_{max}^{EtOH} 321 mμ.

6-Mercapto-9-(2′,3′-O-isopropylidene-5′-O-benzoyl-β-D-ribofuranosyl)purine——Isopropylidenebenzoylinosine (412 mg.) obtained as above was dissolved in 20 ml. of pyridine and 890 mg. of P_2S_5 was added. After the addition of 0.18 ml. of H_2O under cooling, the whole was refluxed for 6 hr. under vigorous stirring. After the reaction 2 layers were observed. From the upper layer pyridine was evaporated *in vacuo*, poured into ice H_2O and extracted with $CHCl_3$. Drying over Na_2SO_4 and evaporation of the solvent *in vacuo* gave a glassy material, which was recrystallized from a large amount of EtOH, m.p. 233~235°. Yield was 170 mg. (42%). *Anal.* Calcd. for $C_{20}H_{20}O_5N_4S$: C, 56.07; H, 4.71; N, 13.07. Found: C, 55.73; H, 4.53; N, 12.63. UV λ_{max}^{EtOH} mμ: 322, 228.

6-Mercapto-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine——Isopropylidene-benzoyl-6-mercaptopurine riboside (150 mg.) obtained as above was suspended in 10 ml. of dry MeOH previously saturated with dry NH_3 at 0°. All of the solid material disappeared after a while. The whole was set aside under exclusion of moisture for 2 days at room temperature. MeOH and NH_3 were removed under reduced pressure and the residue was washed well with $CHCl_3$ in order to remove benzoic methylester and amide. Insoluble material was collected and recrystallized from EtOH, m.p. 250°. Yield was 110 mg. *Anal.* Calcd. for $C_{13}H_{16}O_4N_4S$: C, 48.13; H, 4.97; N, 17.28. Found: C, 48.19; H, 4.77; N, 16.96. UV: λ_{max}^{EtOH} 322 mμ.

6-Mercapto-9-β-D-ribofuranosylpurine——i) 6-Mercapto-9-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-purine (2.5 g., 0.0042 mole) obtained as above was suspended in 180 ml. of abs. MeOH and 4 ml of 2*N* MeONa was added dropwise during reflux. During the reaction pH was maintained at 9.0. After 4.5 hr's reflux MeOH was removed *in vacuo* and the residue was taken up in 40 ml. of H_2O. pH of the solution was adjusted to 8.0 and extracted with $CHCl_3$ (30 ml. × 2). The H_2O-layer was filtered, adjusted to pH 4.0 with AcOH and set aside at room temperature. Precipitate, thus obtained, was collected by filtration, suspended in 30 ml. of H_2O and NH_3-aq. was added until a clear solution was obtained. Acidification with AcOH to pH 4.0 gave a crystallineous precipitate, m.p. 209~211° (decomp.). Yield was 765 mg., 64.3%. *Anal.* Calcd. for $C_{10}H_{12}N_4O_4S \cdot \frac{1}{2}H_2O$: C, 40.99; H, 4.47; N, 19.12. Found: C, 40.76; H, 4.95; N, 18.91. UV $\lambda_{max}^{H_2O}$ 319, $\lambda_{max}^{1N\ HCl}$ 324, $\lambda_{max}^{1N\ NaOH}$ 309 mμ.

ii) 6-Mercapto-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (50 mg.) obtained as above dissolved in 20% AcOH (10 ml.) was heated at 100° for 1.5 hr. AcOH was removed under reduced pressure and the residue was dissolved in NH_3-aq. Precipitate appeared by acidification with AcOH to pH 4 was collected by filtration and dried, m.p. 206~209° (decomp.). UV: $\lambda_{max}^{H_2O}$ 319 mμ. Mixed melting point test with sample obtained in i) showed no depression.

Authors wish to thank Mrs. T. Tohma and Miss A. Maeda of this faculty for the performance of elementary analyses.

Summary

2′,3′-O-Isopropylidene-5′-O-benzoylinosine and 2′,3′,5′-tri-O-benzoylinosine were reacted with N,N-dimethylformamide-thionyl chloride complex in chloroform solution. Resulting 6-chloro compound was further reacted with morpholine, dimethylamine and thiourea to afford 6-morpholino-, 6-dimethylamino- and 6-mercapto-derivative of 9-β-D-ribofuranosylpurine in a good overall yield.

(Received October 21, 1963)