[Chem. Pharm. Bull. 11 (9) 1102 ~ 1107]

UDC 577.15 : 612.498.145

182. Morio Ikehara and Hiroshi Tada : Studies on Coenzyme Analogs. XVII.*1 A New Synthesis of Purine Nucleoside by the Condensation of Chloropyrimidine with Glucosylamine.

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

As has been reported by several investigators, nucleosides were synthesized by the procedure classified essentially by three groups : 1) condensation of protected halogenosugar with chloromercury salt of purine,[1] 2) cyclization of base moiety after the Schiff's base formation between aminopyrimidine and aldehydesugar,[2] and 3) cyclization of base after amide formation.[3]

In the series of experiments investigated earlier,[4,5] we established a general procedure for synthesis of 9-alkanoladenines. If alkanolamines used in these experiments were replaced by glycosylamines, adenine glycoside would be obtained by an analogous method.

In order to avoid bifunctional reactions,[4~6] which would be caused by the high reactivity of chlorine atom of 4,6-dichloro-5-nitropyrimidine (I), 2,6-dichloro-5-aminopyrimidine (II) was taken as the starting material. The condensation reaction of II with tetra-O-acetyl-D-glucosylamine (III) was not shown against to our expectation and a crystalline substance, m.p. 200~201°, was obtained. The elementary analytical data and an analogous reaction[7] suggest that this compound could be octa-O-acetyl-di-D-glucopyranosylamine.

As described in the synthesis of furfuryladenine,[8] compound (I) could be used for monosubstitution reaction at low temperature, 10~20°. The condensation of (I) with glucosylamine (II) was investigated at relatively low temperature. Both were reacted in dioxane solution at 40~50°, at which temperature amine hydrochloride began to appear as a white precipitate. By this reaction two crystalline product (A), m.p. 170° (decomp.), and B, m.p. 150~155°, were obtained in the ratio of 2:3. Compound (A) showed ultraviolet absorption maxima at 234 and 320 mμ and Rf 0.90 by paper chromatography. After the reaction with ammonia, the absorption maxima of the compound (A) converted to a single peak at 319 mμ. Compound (B), having λ_{max} 230 and 322 mμ, also converted to a substance having λ_{max} 310 mμ by the reaction with ammonia. These facts, together with elementary analytical data, showed the structure 4,6-bis-(2,3,4,6-tetra-O-acetyl-D-glucopyranosylamino)-5-nitropyrimidine (IV) corresponded to compound (A) and 4-chloro-5-nitro-6-(2,3,4,6-tetra-O-acetyl-D-glucopyranosylamino)pyrimidine corresponded to B. After the treatment with zinc dust in boiling water, compound (A) showed λ_{max} 278 mμ, which was identical with that of 4,5-diamino-6-(2-hydroxyethylamino)pyrimidine[4] having λ_{max} 278.5 mμ. When B was treated with zinc dust,

*1 Part XVI. M. Ikehara, E. Ohtsuka : This Bulletin, **11**, 1095 (1963).
*2 Kita 12-jo, Nishi-5-chome, Sapporo (池原森男, 多田 宏).
1) J. Davoll, B. Lowy : J. Am. Chem. Soc., **73**, 1650 (1951).
2) G. W. Kenner, C. W. Tailor, A. R. Todd : J. Chem. Soc., **1949**, 1620.
3) G. Shaw, D. V. Wilson : *Ibid.*, **1962**, 2937.
4) M. Ikehara, E. Ohtsuka : This Bulletin, **9**, 27 (1961).
5) M. Ikehara, E. Ohtsuka, S. Kitagawa, K. Yagi, Y. Tonomura : J. Am. Chem. Soc., **83**, 2679 (1961).
6) D. J. Brown : J. Appl. Chem., **4**, 72 (1954).
7) R. S. Tipson : J. Org. Chem., **26**, 2462 (1961).
8) R. Hull : J. Chem. Soc., **1958**, 2746.

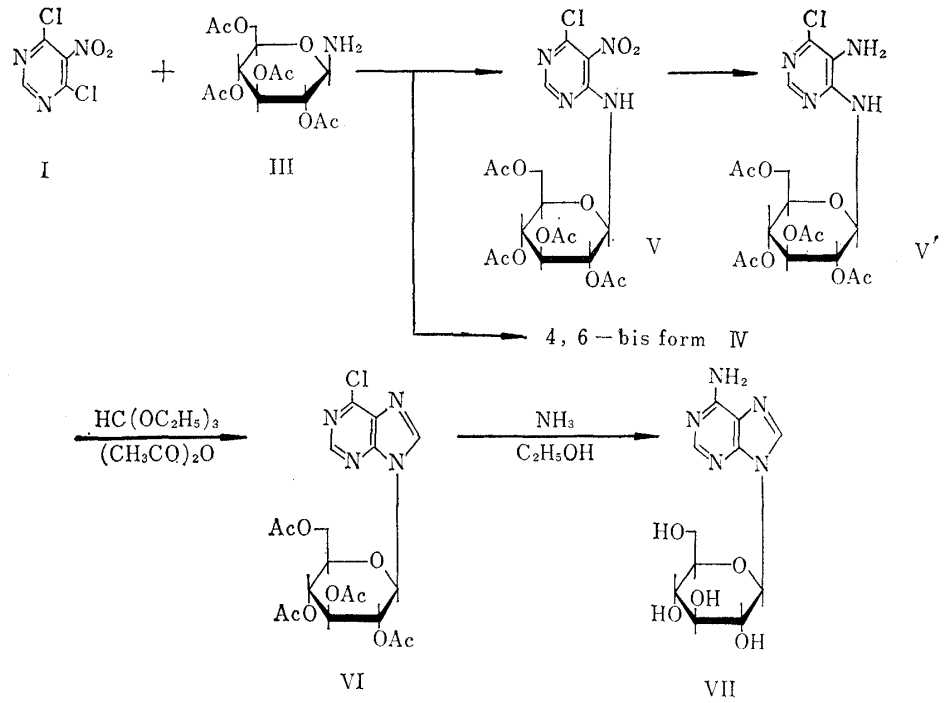


Chart 1.

a yellowish glass having λ_{max} 246 and 284 mμ was obtained. This spectrum was closely resembled with λ_{max} 260 and 288 mμ of 4,5-diamino-6-chloropyrimidine.[8]

Compound (V), thus obtained, was cyclized to chloropurine (VI) by the reflux with acetic anhydride-ethyl orthoformate mixture. In order to avoid the hydrolysis of 6-chlorine atom by the recrystallization procedure, VI was heated with ethanol saturated with ammonia to afford 9-β-D-glucopyrancsyladenine (VII), m.p. 225~228°. Direct comparison with the authentic sample[1] and melting point of picrate derived from it established the structure of VII. This fact also indicated the validity of 1,2-*trans* rule[9] in the case of purine ring closure of this type.

In order to extend this reaction to the synthesis of 8-keto or thioketopurine nucleoside, 4-amino-5-nitro-6-chloropyrimidine (VIII)[10] was taken as the starting material. The compound of the type (V') derived from 4,6-dichloro-5-nitropyrimidine would give an undesirable thiazole derivative (C), when carbon disulfide and pyridine[11] was utilized as cyclizing agent.

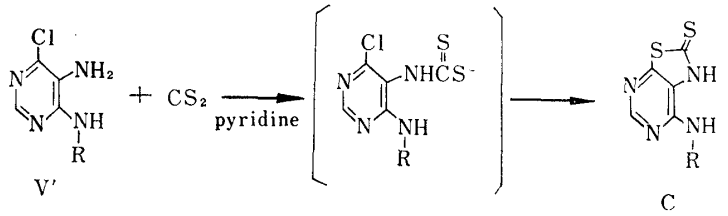


Chart 2.

Compound (VII) was reacted with tetra-acetylglucosylamine at 60~70° for 8 hours and 4-amino-5-nitro-6-(2,3,4,6-tetra-O-acetyl-D-glucopyranosylamino)pyrimidine (IX) was obtained as a crystalline compound, m.p. 161~161.5°, in a yield of 45.8%. Nitro group of compound (IX) was hydrogenated with Raney nickel at 50 atomospheric pressure.

9) B.R. Baker: "Chemistry and Biology of Purines" CIBA Foundation Symposium, p. 120 (1957).
10) W.L. Boon, W.G.M. Jones, G.R. Ramage: J. Chem. Soc., **1951**, 61.
11) R.W. Balsiger, A. Fikes, T.P. Johnson, J.A. Montgomery: J. Org. Chem., **26**, 3386 (1961).

Ultraviolet absorption maximum at 328 mμ of IX was diminished and a new maximum at 292 mμ appeared after the reaction was over. Rf value of paper chromatogram also altered from 0.88 to 0.13. Although it is reasonably concluded that this compound is identical with that of Holland, *et al.*,[12] the direct comparison of both samples were not performed, because the latter was described as having no definite melting point.

Triamino derivative (X), thus obtained, was refluxed with carbon disulfide in pyridine and a yellow crystalline substance, m.p. 265°, was obtained. The structure of this compound was characterized as 8-mercapto-9-(2,3,4,6-tetra-O-acetyl-D-glucopyranosyl)-adenine (XI) from the optical behavior and elementary analysis. Further transformation, desulfurization with Raney nickel and de-acetylation with methanol-ammonia of XI gave 9-β-D-glucopyranosyladenine. From the mother liquor of XI a glassy residue, the structure of which was assumed to be 8-mercapto-6-(2,3,4,6-tetra-O-acetyl-D-glucopyranosyl)-adenine (XII), was obtained. XII was transformed to a compound presumably represented the structure of N^6-glucosyladenine. Whereas these two compounds had the same Rf value (in solvent A) in acetylated form, bared nucleosides had Rf 0.52 and 0.62, respectively. The ratio of cyclization of X to N^9-glucosyladenine *vs.* N^6-glucosyladenine was

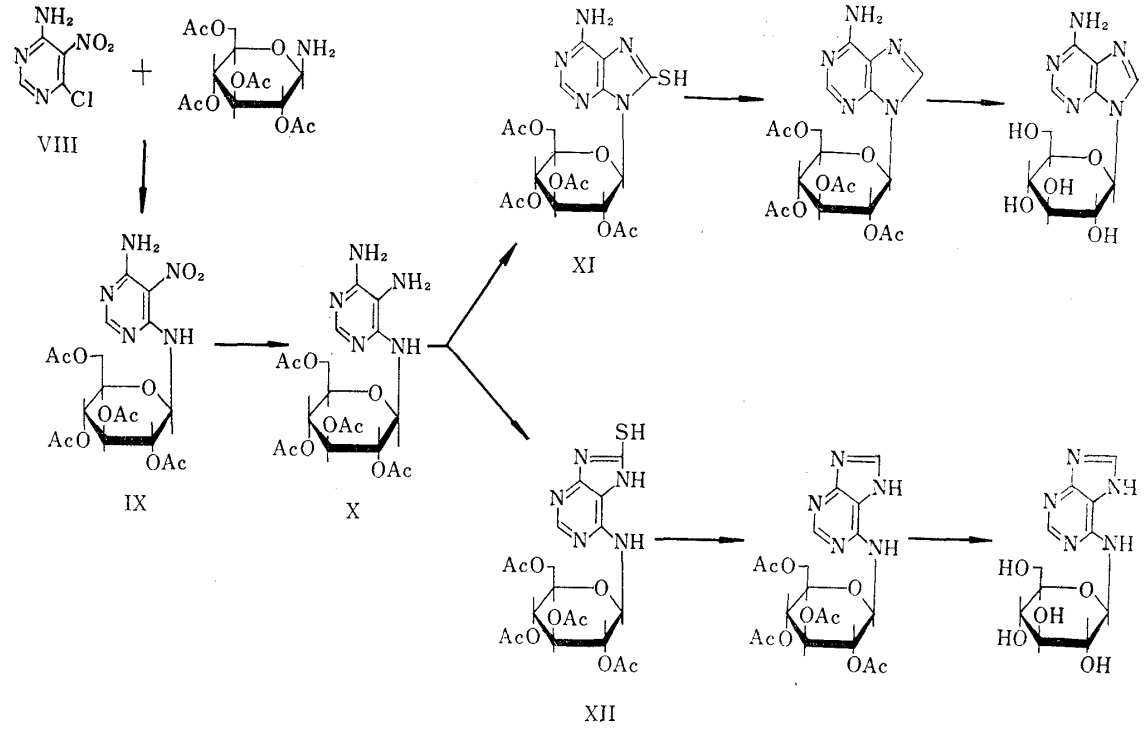


Chart 3.

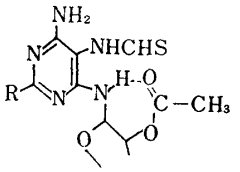


Chart 4.

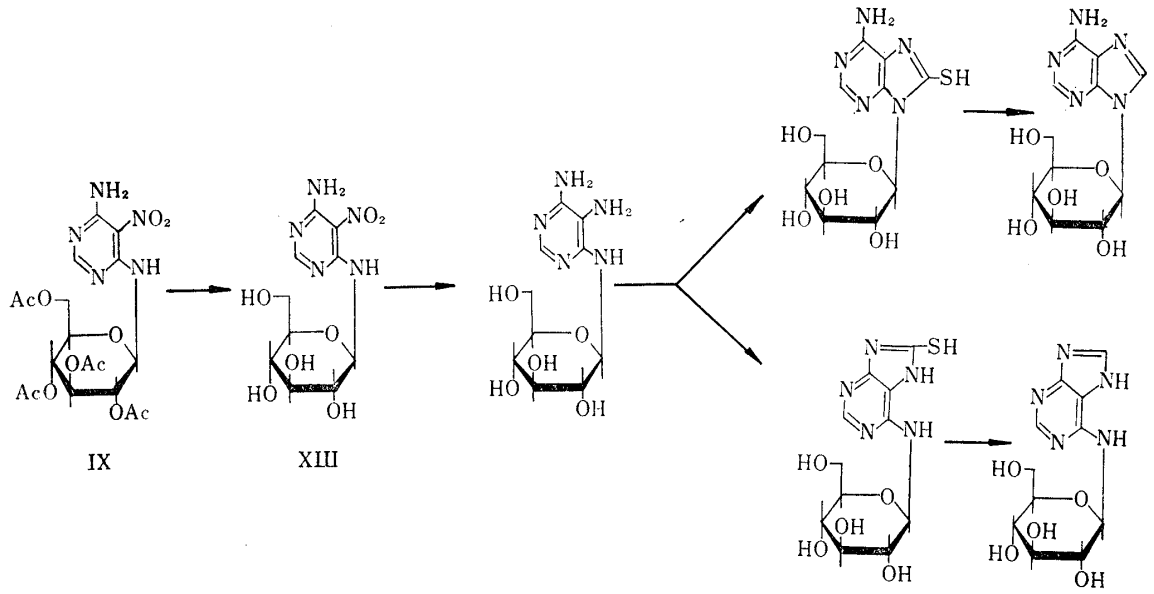
2:9. This fact was consistent with results obtained in the experiments of Kenner, *et al.*[13] using dithioformic acid as cyclizing agent. The reason of the surpassing formation of XII may be attributed to hydrogen-bond formation between 2′-O-acetyl group and 6-NH group of pyrimidine as shown in Chart 4.

We attempted, therefore, the cyclization after deacetylation of compound (IX). Treatment of IX with ammonia-methanol easily gave 4-amino-5-nitro-6-D-glucosylaminopyrimidine (XIII) in a yield of 76.5%. Reduction of nitro group and reflux with carbon

12) A. Holland, B. Lythgoe, A.R. Todd: J. Chem. Soc., **1948**, 965.
13) G.W. Kenner, A.R. Todd: J. Chem. Soc., **1946**, 852.

disulfide in pyridine gave a mixture, having Rf 0.52 and 0.62 on paper chromatogram. The former showes λ_{max} 257 mμ in neutral media and the latter showed λ_{max} 262 and 269 mμ. The ratio of both compounds involved in the mixture estimated photometrically from the eluted spots was 9:4. These facts indicated that by the cyclization with carbon disulfide in pyridine of XII a larger amount of N^9-glucosyladenine was formed.

From the results described above it was concluded that the new method will be of practical value in order to obtain the nucleoside analogs, especially modified in the sugar moieties.



IX XIII

Chart 5.

Experimental

Paper Chromatography——Solvent A, H_2O adjusted to pH 10 with NH_3; solvent B, BuOH-H_2O (86:14); solvent C, BuOH-AcOH-H_2O(5:2:3). All chromatography was carried out by ascending technique and by using Toyo Filter Raper, No. 51 and 51A.

Reaction of 2, 6-Dichloro-5-aminopyrimidine with 2, 3, 4, 6-Tetra-O-acetylglucosylamine——2, 6-Dichloro-5-aminopyrimidine (1.54 g., 10 m mole), 2, 3, 4, 6-tetra-O-acetyl-D-glucosylamine[14] (3.47 g., 10 m mole) and triethylamine (1.05 g., 10 m mole) was dissolved in 35 ml. of dioxane and refluxed for 25 hr. Solvent was removed under reduced pressure and the residue was recrystallized from EtOH. Fine needles, m.p. 200~201°, were obtained (yield was 1.5 g.). *Anal.* Calcd. for $C_{28}H_{39}O_{18}N$(octaacetyl-diglucosylamine): C, 49.96; H, 5.81; N, 2.09. Found: C, 49.78; H, 5.78; N, 2.25.

Evaporation of the mother liquor of the above recrystallization gave 1.5 g. of glass, from which 2,6-dichloro-5-aminopyrimidine (0.82 g.) was recovered by hexane extraction.

4-Chloro-5-nitro-6-(2,3,4,6-tetra-O-acetyl-D-glucopyranosylamino)pyrimidine (A) and 4,6-bis(2,3,4,6-Tetra-O-acetyl-D-glucopyranosylamino)-5-nitropyrimidine (B)——Into a solution of 2,8-dichloro-5-nitropyrimidine (1.94 g., 10 m mole) and triethylamine (1.01 g., 10 m mole) in 40 ml. of dioxane, 2,3,4,6-tetra-O-acetyl-D-glucosylamine (3.47 g., 10 m mole) dissolved in 10 ml. of dioxane was added dropwise at 40~50° under vigorous stirring. White precipitate appeared on the wall of the reaction flask. The whole was poured onto cracked ice (ca. 300 g.) and extracted with $CHCl_3$(100 ml.×3). $CHCl_3$-layer was dried over $MgSO_4$ and evaporated *in vacuo*. Residual thick syrup was extracted with Et_2O(10 ml.×5) and the residue was recrystallized from EtOH-Me_2CO-petr. Et_2O mixture. A pale yellow crystalline material (B), m.p. 170°(decomp.), was obtained (yield was 1.5 g., 18.8%). *Anal.* Calcd. for $C_{32}H_{21}O_{20}N_5$: C, 47.09; H, 5.12; N, 8.58. Found: C, 47.01; H, 4.86; N, 9.02. UV λ_{max}^{EtOH} mμ: 234, 320.
Paper chromatography: Rf 0.90 (solvent B). Evaporation of the above Et_2O-extract gave glassy residue, which was recrystallized from benzene (A), m.p. 150~155°(yield 2.3 g., 45.5%). *Anal.* Calcd. for

14) A. Bertho: Chem. Ber., **63**, 836 (1930).

$C_{18}H_{21}O_{11}N_4Cl$: C, 42.82; H, 4.19; N, 11.11. Found: C, 42.40; H, 4.58; N, 11.02. UV λ_{max}^{EtOH} mμ: 230, 322.

Mixed melting point test with the sample described above showed a depression. The compound (B) showed UV: $\lambda_{max}^{H_2O}$ 319 mμ when treated with NH_3.

4-Chloro-5-amino-6-(2,3,4,6-tetra-O-acetyl-D-glucopyranosylamino)pyrimidine——Above 4-chloro-5-nitro-6-(2,3,4,6-tetra-O-acetylglucosylamino)pyrimidine (100 mg.) was heated at 90~95° with Zn dust (500 mg.) in H_2O (5 ml.) for 15 min. The suspension of nitro compound dissolved into solution at the end of the reaction. After removal of Zn, pale yellow filtrate was evaporated to a half of its volume. Solution was extracted with $CHCl_3$ (10 ml. × 3) and $CHCl_3$-layer was dried over $MgSO_4$. Evaporation of $CHCl_3$ under reduced pressure gave a yellow glass (95 mg.), UV $\lambda_{max}^{H_2}$: 246, 284 mμ. In order to avoid decomposition, this material was used for further reaction without purification.

Reduction of 2,4-bis(2,3,4,6-Tetra-O-acetyl-D-glucopyranosylamino)-5-nitropyrimidine——4-Chloro-5-amino-6-(2,3,4,6-tetra-O-acetylglucosylamino)pyrimidine (100 mg.), obtained above, was reduced with Zn dust (500 mg.) at 90~95° for 15 min. UV $\lambda_{max}^{H_2O}$ changed from 234 and 320 mμ to 278 mμ. This indicated the occurrence of triamino compound.

6-Chloro-9-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)purine——Above 4-chloro-5-amino-6-(2,3,4,6-tetra-O-acetylglucosylamino)pyrimidine (1.0 g.) was dissolved in Ac_2O (10 ml.) and $HC(OEt)_3$ (10 ml.). Reaction mixture was refluxed for 30 min. so as to evaporate EtOAc. The bath temperature was raised to 130° and reflux was maintained for 2.5 hr. Reaction mixture was taken into dryness under reduced pressure at 30°. Residual brown syrup showed $\lambda_{max}^{H_2O}$ 258 mμ. This was used in the next reaction without purification.

4-Amino-5-nitro-6-(2,3,4,6-tetra-O-acetyl-D-glucopyranosylamino)pyrimidine——Into a solution of 4-amino-5-nitro-6-chloropyrimidine (2.73 g., 15.6 m mole) in 30 ml. of dry dioxane, a mixture of triethylamine (1.58 g., 15.6 m mole) and 2,3,4,6-tetra-O-acetyl-D-glucosylamine (5.43 g., 15.6 m mole) in 25 ml. of dry dioxane was added dropwise under vigorous stirring. Reaction was continued for 8 hr. at 60~70°. Triethylamine hydrochloride gradually appeared as white precipitate, which was filtered off. Filtrate was evaporated under reduced pressure and residual brown glass was taken up in 30 ml. of $CHCl_3$. $CHCl_3$ was removed by vacuum distillation and the residue was recrystallized from EtOH, m.p. 161~161.5° (yield 3.5 g., 45.5%). *Anal.* Calcd. for $C_{18}H_{22}O_{11}N_5$: C, 44.63; H, 4.78; N, 14.43. Found: C, 44.66; H, 4.62; N, 14.54.

Paper chromatography: 0.88 (solvent C).

4,5-Diamino-6-(2,3,4,6-tetra-O-acetyl-D-glucopyranosylamino)pyrimidine——Amino-nitro-acetylglucosylaminopyrimidine (500 mg.), obtained as described above, was dissolved in 20 ml. of dry pyridine and hydrogenated in the presence of 1.5 ml. (wet) of Raney Ni under 50 atm. of H_2 for 18 hr. At this stage λ_{max} of 328 mμ was diminished and λ_{max}^{pH1} 292 mμ appeared. Paper chromatography: Rf 0.13 (solvent C). This was applied to the next reaction without purification.

Carbon Disulfide Ring-closure of 4,5-Diamino-6-(2,3,4,6-tetra-O-acetyl-D-glucopyranosylamino)-pyrimidine——Raney Ni was removed from the above pyridine solution and 10 ml. of CS_2 was added. After reflux for 5 hr., paper chromatography (solvent B) of an aliquot showed a spot of Rf 0.82. Solvent was removed under reduced pressure and residue was recrystallized from EtOH. Yellow needles, m.p. 265° (decomp.) was obtained (yield 100 mg., 5.7%). *Anal.* Calcd. for $C_{19}H_{23}O_9N_5S$ (as 8-mercapto-glucosyladenine): C, 45.86; H, 4.66; N, 14.14. Found: C, 46.03; H, 4.67; N, 14.01.

Evaporation of the mother liquor of above recrystallization gave a glass (yield 450 mg., 38.8%). This material corresponded to N^6-glucosyladenine derivative.

9-β-D-Glucopyranosyladenine——i) 6-Chloro-9-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)purine (1.0 g.) was dissolved in 30 ml. of abs. EtOH saturated previously with NH_3 at 0°. Reaction was carried out in a fused tube at 120° for 15 hr. EtOH was evaporated *in vacuo* and residue was shaken with a mixture of H_2O (20 ml.) and $CHCl_3$ (10 ml. × 3). H_2O-layer was evaporated *in vacuo* and residue was triturated with EtOH. Cream colored powder, m.p. 170~175°, was obtained (yield 520 mg.). This was recrystallized from EtOH-H_2O, m.p. 220~222° (yield 210 mg.). Mixed melting point test with an authentic sample synthesized according to Davoll[1] showed m.p. 223~225°. Mother liquor of this crystallization was mixed with 300 mg. of picric acid and resulting yellow precipitate was recrystallized from EtOH, m.p. 245~247° (150 mg.). Mixed melting point test with an authentic adenineglucoside picrate showed m.p. 247~248°.

ii) 8-Mercapto-9-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)adenine (20 mg.) was refluxed with 10 ml. of EtOH with ca. 1 ml. (wet) of Raney Ni. Catalyst was removed by filtration and EtOH was evaporated off. Residual colorless glass was kept for standing overnight in 10 ml. of EtOH saturated previously with NH_3 at 0°. Residue obtained after removal of EtOH under reduced pressure gave a spot of Rf 0.52 on paper chromatogram (solvent A). This was identical with that of authentic 9-β-D-glucopyranosyladenine revealed by co-chromatography. UV $\lambda_{max}^{H_2O}$ of the extract from the spot of Rf 0.52 was 257 mμ.

N^6-Glucosyladenine——Desulfurization and deacetylation of N^6-(2,3,4,6-tetra-O-acetylglucosyl)adenine, obtained above, by the analogous procedure described in the section of 9-glucosyladenine gave a glass. Paper chromatography (solvent A) of this residue showed a single spot of Rf 0.62. UV absorption spectra of the H_2O-extract of this spot was UV : $\lambda_{max}^{0.1N\,HCl}$ 262, 269 mμ (shoulder); $\lambda_{max}^{H_2O}$ 262, 269 mμ (shoulder); $\lambda_{max}^{0.1N\,NaOH}$ 271, 279 mμ (shoulder). Treatment with HNO_2 in AcOH gave no change in spectra. From these optical behaviors, this compound seems to be corresponding to N^6-glucosyladenine.

4-Amino-5-nitro-6-D-glucosylaminopyrimidine——4-Amino-5-nitro-6-(2, 3, 4, 6-tetra-O-acetyl-D-glucopyranosylamino)pyrimidine (600 mg.) was deacetylated with 50 ml. of anhyd. MeOH saturated previously with NH_3 overnight at room temperature. Residual syrup obtained after removal of EtOH was recrystallized from EtOH, m.p. 220°(decomp., colorization began at 200°), yield 76.5%. *Anal.* Calcd. for $C_{10}H_{15}O_7N_5$: C, 37.95; H, 4.76; N, 22.07. Found : C, 37.36; H, 4.94; N, 22.06.

Carbon Disulfide Ring-closure of 4-Amino-6-D-glucosylaminopyrimidine after Reduction of Nitro Group——Deacetylated pyrimidine (300 mg.), obtained as above, was refluxed for 1 hr. in 60 ml. of anhyd. pyridine containing Raney Ni (2 ml., wet). After the filtration of Ni and the addition of CS_2 (8 ml.), reaction mixture was refluxed for 9 hr. Solvent was removed under reduced pressure and a yellow glass was obtained. Paper chromatography of the residue revealed two spots having Rf 0.52 and 0.62 (solvent A). The ratio of the occurrence of both substances estimated photometrically (as having the same ε) was 9:4. The former was identical with the Rf of co-chromatographed N^9-glucosyladenine[1] and having UV : $\lambda_{max}^{0.1N\,HCl}$ 255, $\lambda_{max}^{H_2O}$ 257, $\lambda_{max}^{0.1N\,NaOH}$ 257 mμ. The spot having Rf 0.62 showed UV : $\lambda_{max}^{0.1N\,HCl}$ 262, 269 mμ (shoulder); $\lambda_{max}^{H_2O}$ 262, 269 mμ (shoulder); $\lambda_{max}^{0.1N\,NaOH}$ 270, 279 mμ (shoulder). These data showed the optical behavior corresponding to N^6-glucosyladenine.

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

Summary

The reaction between 4,6-dichloro-5-nitropyrimidine or 4-amino-5-nitro-6-chloropyrimidine and 2,3,4,6-tetra-O-acetyl-D-glucopyranosylamine was investigated. In the former case, in addition to 4-chloro-5-nitro-6-(2,3,4,6-tetra-O-acetylglucosylamino)pyrimidine, 4,6-bis-glucosylamino derivative was obtained. Ring-closure of 4-chloro-5-amino-6-(2,3,4,6 tetra-O-acetylglucosylamino)pyrimidine was achieved by acetic anhyd. ethyl orthoformate and further two step reaction afforded 9-β-D-glucopyranosyladenine. By the carbon disulfide ring-closure of 4,5-diamino-6-(2,3,4,6-tetra-O-acetylglucosylamino) pyrimidine, 9-β-D-glucosyladenine and N^6-glucosyladenine was obtained in the ratio of 2:9. After deacetylation of triaminopyrimidine derivative, however, the reaction occurred in the reversed ratio of 9:4.

(Received March 28, 1963)