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Morio Ikehara and Hitoshi Uno: Studies of Nucleosides and Nucleotides. XXVI.[*1] Further Studies on the Chlorination of Inosine Derivatives with Dimethylformamide-Thionyl Chloride Complex.

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As described in the earlier paper[1] of this series, it seemed to be diffiicult to isolate the chlorinated inosine derivatives in a pure state. After several attempts to improve the work-up procedure, we have now isolated 2′,3′-O-isopropylidene-5′-O-benzoyl derivative of 6-chloro-9-β-D-ribofuranosylpurine (I) in a crystalline form and starting from this compound several N^6-substituted purine nucleosides were synthesized. 2′,3′-O-Isopropylidene-5′-O-benzoylinosine was refluxed with dimethylformamide (DMF)-thionyl chloride complex as described earlier.[1] The reaction mixture was poured into ice-water followed by the extraction with chloroform and thorough washing with sodium bicarbonate solution. Residue obtained after evaporation of chloroform was recrystallized from ethanol as slightly yellow rods, m.p. 122~124°, in the yield around 87%. Elemental analysis and spectrophotometrical data suggested the structure to be correct. Earlier

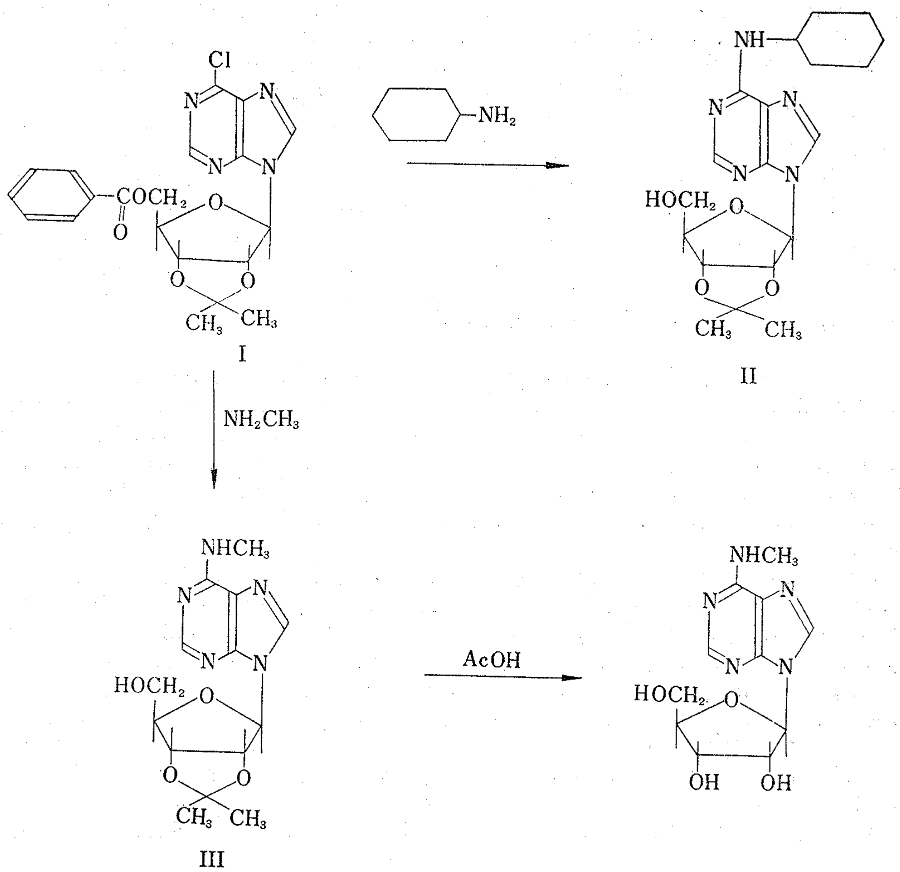

Chart 1.

[*1] Part XXV. M. Ikehara, H. Tada, K. Muneyama: This Bulletin, in Press.

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1) M. Ikehara, H. Uno: This Bulletin, **12**, 267 (1964).

difficulty of crystallization may be due to the partial reaction of 6-chloro atom with ethanol, which had been used in order to remove the traces of thionyl chloride. When the reaction described above was carried out at room temperature for two days, the starting material was recovered.

The reaction of this 6-chloro-9-(2′,3′-O-isopropylidene-5′-O-benzoyl-β-D-ribofuranosyl)purine with cyclohexylamine or monomethylamine was studied. When compound (I) was refluxed with excess cyclohexylamine in ethanol for 15 hours, a dark brown glass (II) was obtained. Recrystallization as the picrate gave an analytically pure sample m.p. 182~184°. Ultraviolet absorption property indicated N^6-monosubstituted purine.*3

The reaction of I with monomethylamine in the analogous procedure as described above gave 2′,3′-O-isopropylidene derivative of 6-methylamino-9-β-D-ribofuranosylpurine (III) as a glass. The structure of III was elucidated by the deprotection of isopropylidene group with acetic acid to afford 6-methylamino-9-β-D-ribofuranosylpurine.[2]

Thus, chlorination with DMF-thionylchloride complex of inosine derivatives has been proved useful to obtain 6-chloropurine nucleoside as well as N^6-substituted derivatives.

Experimental

Paper Chromatography——Solvent A, water adjusted to pH 10.0 with ammonia (measured by pH test paper); solvent B, butanol-water, 86:14. All the paper chromatographies were performed by ascending technique.

6-Chloro-9-(2′,3′-O-isopropylidene-5′-O-benzoyl-β-D-ribofuranosyl)purine——Freshly distilled thionyl chloride (1.8 g.) was dissolved in 30 ml. of dry chloroform, followed by the addition of 0.5 ml. of DMF (freshly distilled from anhydrous cupric sulfate). After the reaction mixture was set aside for 10 min. at room temperature, 2′,3′-O-isopropylidene-5′-O-benzoylinosine[1] (2.06 g., 5.0 mmoles) was added. During the reflux of this solution for 3 hr. an acidic gas evolved (presumably hydrochloric acid). After cooling, chloroform was evaporated *in vacuo* and the residue was poured into ice-water (150 ml.) under stirring. Precipitated crude substance (quantitative yield)*4 was extracted with chloroform and the chloroform layer was washed thoroughly with 5% sodium bicarbonate solution and water until it was neutral. Drying over sodium sulfate and evaporation of chloroform *in vacuo* afforded a solid.*5 Dried material weighed 1.88 g. (yield 87.4%). Recrystallization from ethanol gave slightly yellow tiny rods, m.p. 122~124°. UV $\lambda_{max}^{0.1N\,HCl}$ mμ: 234, 264; $\lambda_{max}^{H_2O}$ mμ: 237, 265~266; $\lambda_{max}^{0.1N\,NaOH}$ mμ: 237, 265~266. Paper chromatography: Rf 0.83 (solvent B). *Anal.* Calcd. for $C_{20}H_{19}O_5N_4Cl$: C, 55.72; H, 4.68; N, 13.00. Found: C, 55.48; H, 4.53; N, 13.11.

Reaction of 2′,3′-O-Isopropylidene-5′-O-benzoylinosine with DMF-Thionyl Chloride at Room Temperature——The reaction mixture of the same composition as described above was allowed for standing at room temperature under exclusion of moisture for 2 days. During the reaction a kind of gelatinization occurred. The mixture was poured into ice-water and treated as described above. 2′,3′-O-Isopropylidene-5′-O-benzoylinosine, m.p. 195~197° (from ethanol) was obtained (yield 540 mg.). Paper chromatography: Rf 0.80 (solvent B). *Anal.* Calcd. for $C_{20}H_{19}O_6N_4$: C, 58.49; H, 4.89; N, 13.55. Found: C, 57.86; H, 5.28; N, 13.87.

This sample was identical with an authentic specimen[2] by mixed melting point.

6-Cyclohexylamino-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine——6-Chloro-9-(2′,3′-O-isopropylidene-5′-O-benzoyl-β-D-ribofuranosyl)purine (2.25 g., m.p. 110~120°) was dissolved in 30 ml. of ethanol, followed by the addition of 3 g. of cyclohexylamine. After reflux for 6.5 hr., an aliquot from the reaction mixture was examined by paper chromatography (solvent A). Spot having Rf's 0.64 and 0.76 were observed. Cyclohexylamine (1 g.) and ethanol (10 ml.) were further added and the mixture was refluxed for additional 9 hr. Paper chromatographical examination showed a single spot, Rf 0.76 and no starting material was detected. Ethanol was evaporated *in vacuo* (a precipitate appeared) and applied to the steam distillation. From the distillate (ca. 200 ml.), water layer was removed by decantation and a brown glassy substance was taken up in ethanol. Charcoal decolorization and the addition of water gave a brown

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*3 When the reaction was carried out in the absence of the solvent (ethanol), a crystalline substance, m.p. 115~122°, was obtained.

*4 This material could be used as such for the next reaction.

*5 Ethanol trituration was helpful to induce crystallization.

[2] M. Ikehara, E. Ohtsuka, F. Ishikawa: This Bulletin, **9**, 173 (1961).

precipitate, which was dissolved in a large amount of water and set aside. A gray-white crystalline sub stance was obtained (194.9 mg.). UV : $\lambda_{max}^{0.1N\,NaOH}$ 270 mμ; $\lambda_{max}^{H_2O}$ 270 mμ; $\lambda_{max}^{0.1N\,HCl}$ 268 mμ. IR : No band at ν_{max}^{Nujol} 1740 cm^{-1} (benzoyl). A part of the above glass was dissolved in ethanol and a solution of picric acid in ethanol was added. Addition of a small amount of water gave a precipitate, which was recrystallized twice from aqeous ethanol. Greenish yellow crystal, m.p. 182~184°, was obtained. *Anal.* Calcd. for $(C_{19}H_{27}O_4N_5)_2 \cdot C_6H_3O_7N_3 \cdot H_2O$: C, 51.96; H, 5.40. Found : C, 51.51; H, 5.76.

6-Methylamino-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine——6-Chloro-9-(2′, 3′-O-isopropylidene-5′-O-benzoyl-β-D-ribofuranosyl)purine (2.43 g.) was dissolved in 40 ml. of ethanol. The solution was saturated with dry methylamine at 0° and heated at 100° for 3 hr. in a sealed tube. After cooling methylamine was bubbled again through the reaction mixture at 0° and heated further for 3.5 hr. The reaction mixture gave a single spot having Rf 0.74 (solvent A) and UV absorption properties of the spot excized from the paper chromatogram was $\lambda_{max}^{0.1N\,HCl}$ 263 mμ; $\lambda_{max}^{0.1N\,NaOH}$ 268 mμ. A small amount of insolubles were filtered off and the filtrate was concentrated *in vacuo*. Codistillation several times with methanol gave a colorless glass which could be utilized as such for further reaction.

6-Methylamino-9-β-D-ribofuranosylpurine——A part of the above glass was dissolved in 20% acetic acid and heated at 100° for 1 hr. Acetic acid was removed by vacuum distillation and codistillation with methanol was repeated until it gave a white solid. Recrystallization of this solid from ethanol gave m.p. 130~132°, which was same as reported earlier.[2] UV : $\lambda_{max}^{0.1N\,HCl}$ 263 mμ; $\lambda_{max}^{H_2O}$ 268 mμ. Paper chromatography : Rf 0.67 (solvent A), Rf 0.53 (solvent B); co-chromatographed isopropylidene derivative Rf 0.84 (solvent B). The former 2 spots were detected by metaperiodate spray.[3]

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[3] M. Viscontini, D. Hoch, P. Karrer : Helv. Chim. Acta, 38, 642 (1955).