Rf 0.33, the spot of uridine being extremely small. Further extension of the reaction failed to increase the amount of uridine.

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Summary

Synthesis of glycosylthiouracil from glycosylthiourea was carried out by the method for synthesis of glycosylthymine described in Part II of this series. 1-Methyl-3-(3-ethoxyacryloyl)thiourea, obtained from N-methylthiourea and ethyl 3,3-diethoxyacryloyl chloride, was submitted to pyrimidine cyclization and 1-methyl-2-thiouracil was found to be produced. In accordance with this model experiment, 1-(β -D-glucopyranosyl)-2-thiouracil was obtained from 1-(tetra-O-acetyl- β -D-glucopyranosyl)thiourea and ethyl 3,3-diethoxyacryloyl chloride, and 1-(β -D-ribofuranosyl)-2-thiouracil (2-thiouridine) from 1-(tri-O-benzoyl- β -D-ribofuranosyl)thiourea.

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49. Mitsuji Sano : Studies on Nucleosides and Nucleotides. IV.*¹ Synthesis of Isoglycosyluracils and Isoglycosyl-2-thiouracils from Glycosylureas and Glycosylthioureas.

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Naito and others reported in Part I of this series¹⁾ on a new method for synthesis of pyrimidine nucleoside and assumed that 3-glycosyl-2-thiothymine (pyrimidines with a glycosyl group in 3-N position will tentatively designated hereafter as isopyrimine nucleoside) would be obtained from N-glycosylthiourea by application of this method, since, in preliminary experiment, condensation of N-methylthiourea and ethyl 2-formylpropionate afforded 3-methyl-2-thiothymine.*³ Based on this assumption, reaction of glycosylthiourea and ethyl 2-formylpropionate or ethyl 3,3-diethoxypropionate was examined and isopyrimidine nucleoside was successfully obtained.

Isopyrimidine nucleosides are unknown substances and are extremely bitter compounds, possessing a glycosyl group bonded to the nitrogen in 3-position of the pyrimidine

*¹ Part III : This Bulletin, 9, 308 (1961).

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*³ Nomenclature of uracil and thymines in this paper followed that used in the Chemical Abstracts, i.e.

1) Part I : This Bulletin, 9, 703 (1961).

ring, whereas natural pyrimidine nucleoside has the glycosyl group bonded to the nitrogen in 1-position. Synthesis of such a nucleoside would open a new field for elucidation of its chemical and biochemical properties.

Before carrying out this synthesis, preliminary experiment was carried out on the reaction of N-methylthiourea and ethyl 3,3-diethoxypropionate (b), in accordance with that reported in Part I¹⁾, and the reaction proceeded as described for the reaction of 1-methylthiourea (I) and ethyl 2-formylpropionate (a). However, some difficulty was experienced in the separation of a by-product and the reaction product was purified by chromatography through activated charcoal, from which 3-methyl-2-thiouracil (II), m.p. 283°, was obtained. Treatment of (II) with monochloroacetic acid converted it to 3-methyluracil (III) of m.p. 174~175°, which agreed with the melting point recorded by Johnson and others²⁾.

Based on the foregoing preliminary experiment, synthesis of 3-glycosyl-2-thiothymine and 3-glycosyl-2-thiouracils were carried out.

For the synthesis of 3-(β-D-glucopyranosyl)-2-thiothymine (VIa), a mixture of 1-(tetra-O-acetyl-β-D-glucopyranosyl)thiourea³⁾ (IVa) and ethyl 2-formylpropionate (a) in a small quantity of hydrochloric acid and ethanol was allowed to stand in evacuated desiccator at room temperature. The solid herein obtained was assumed to be 1-(tetra-O-acetyl-β-D-glucopyranosyl)-3-(2-ethoxycarbonyl-propenyl)-2-thiourea (Va), since ethyl β-ureidocrotonate was obtained as an intermediate in the reaction of urea and ethyl acetoacetate for the preparation of 6-methyluracil.⁴⁾ Without isolation or purification, this solid was dissolved in hot sodium hydroxide solution and dealkalified with cation-exchange resin by which pyrimidine cyclization was effected at the same time. Ultra-violet spectrum of the reaction mixture showed appearance of new absorptions at 276 and 300 mμ, and paper chromatography of the reaction mixture, developed with methyl ethyl ketone indicated the presence of the starting material with a spot*³ at R_f 0.04 and a substance giving a spot*³ at R_f 0.19. It was thereby concluded that the pyrimidine cyclization had been effected.

*³ The spot shows dark purple absorption on irradiation of ultraviolet ray of 2536 Å.

2) J. B. Johnson: Am. Chem. J.: 42, 35(1920).

3) Part II: This Bulletin, 9, 709 (1961).

4) Org. Syntheses, Coll. Vol. 2, 422.

The reaction mixture was purified by column chromatography through Celite 535, using methyl ethyl ketone saturated with water, and 3-(β -D-glucopyranosyl)-2-thiothymine (VIa) was obtained as colorless prisms, m.p. 222° (decomp.). Treatment of (VIa) with monochloroacetic acid to form 3-(β -D-glucopyranosyl)thymine (VIc) did not materialize.

In a similar manner, synthesis of 3-glucosyl-2-thiouracils was carried out. Reaction of 1-(tetra-O-acetyl- β -D-glucopyranosyl)thiourea (IVa) and ethyl 3,3-diethoxypropionate (b), as in the case of (VIa), afforded a crude product (VIb) through an intermediate assumed to be (Vb). Purification of this crude product was not sufficient by partition chromatography using Celite 535 and methyl ethyl ketone, and further purification by chromatography through activated charcoal, according to the method of Stambaugh⁵⁾, finally afforded 3-(β -D-glucopyranosyl)-2-thiouracil (VIb) of m.p. 213~214° (decomp.). Treatment of (VIb) with monochloroacetic acid gave 3-(β -D-glucopyranosyl)uracil (VIc) of m.p. 243~244° (decomp.), though in a low yield. This substance was also obtained, without passing through the thiouracil compound, directly from 1-(tetra-O-acetyl- β -D-glucopyranosyl)urea¹⁾ (IVb) by the same reaction as above. The reaction proceeded in a same manner but isolation and purification of (VIc) required column chromatography through activated charcoal and column chromatography with cellulose powder, developing with the upper layer of butanol-acetic acid-water (4:1:5), affording 3-(β -D-glucopyranosyl)uracil (VIc) of m.p. 243~244° (decomp.), identical with the same compound obtained from (VIb).

3-(β -D-Ribofuranosyl)-2-thiouracil (2-isothiouridine) (IX) is a compound corresponding to 2-thiouridine reported in the preceding paper.*¹ Its synthesis was carried out in the same manner as in the case of (VIa) from 1-(tri-O-benzoyl- β -D-ribofuranosyl)thiourea³⁾ (VII) and (b), and (IX) was obtained through an intermediate assumed to be (VIII). Purification of (IX) was easily effected by distribution chromatography through Celite 535, using methyl ethyl ketone, as in the case of (VIa) and 3-(β -D-ribofuranosyl)-2-thiourea (IX), m.p. 187° (decomp.), was obtained. Attempted derivation of (IX) to 3-(β -D-ribofuranosyl)uracil (X) by treatment with monochloroacetic acid did not materialize, same as in the case of (VIa).

The presence of glycosyl group in 3-N of the pyrimidine ring in these isopyrimidine nucleosides obtained in these experiments seems certain from the preliminary experiments and from their ultraviolet absorption spectra, which will be reported in the following paper. In order to make further proof, the following experiment was carried out.

Levene and others⁶⁾ carried out methylation of uridine with dimethyl sulfate in the presence of alkali to obtain mono-N-methyluridine and its hydrolysis with a strong acid afforded 3-methyluracil, thereby proving that the sugar portion is bonded to 1-N of uracil. Bredereck and others⁷⁾ also obtained proof of thymidine and deoxycytidine structures by the same method. Levene and others⁶⁾ carried out methylation with diazomethane on uridine whose hydroxyls were protected. Miles⁸⁾ recently reported that 3-

5) R. L. Stambaugh : J. Chromatog., **3**, 22 (1960).

6) P. A. Levene, R. S. Tipson : J. Bion. Chem., **104**, 385 (1934).

7) H. Bredereck, G. Müller : Ber., **73**, 1058 (1940).

8) H. D. Miles : J. Am. Chem. Soc., **79**, 2565 (1957).

methylthymidine was obtained by direct methylation of thymidine and this method was adopted in the present case. Methylation of 3-(β -D-glucopyranosyl)uracil (VIc) with diazomethane in methanol and purification of the reaction mixture by chromatography afforded an amorphous methylated compound (XI). Its hydrolysis with sulfuric acid gave 1-methyluracil (XII) of m.p. 230~232°.

This fact proves that (VIc) is 3-(β -D-glucopyranosyl)uracil and that (VIb), which was derived to (VIc) by treatment with monochloroacetic acid, is 3-(β -D-glucopyranosyl)-2-thiouracil. Consequently, a series of compounds synthesized by these methods are isopyrimidine nucleosides possessing a glycosyl group in the nitrogen at 3-position.

Pyrimidine nucleosides in general are said to be inert to orcinol reaction and this is due to a much stronger glycosyl bonding against acid than purine nucleosides. The orcinol reaction of 2-isothiouridine obtained in the present series of experiments showed that it easily underwent coloration and this fact indicates that 2-isothiouridine is easily hydrolyzed by an acid to form the sugar and the base, showing a marked difference from 2-thiouridine. In order to confirm this point further, the hydrolysis rate of 2-isothiouridine, when heated with 10% hydrochloric acid at 100°, was compared with that of 2-thiouridine. As shown in Fig. 1, 2-isothiouridine was almost completely hydrolyzed in ca. 20 minutes, while only about 50% of 2-thiouridine was hydrolyzed in 3 hours. Such a tendency was noticed in other isopyrimidine nucleosides obtained in this experiment. Comparison of the rate of hydrolysis between 1-(β -D-glucopyranosyl)-2-thiouracil and 3-(β -D-glucopyranosyl)-2-thiouracil is illustrated in Fig. 2 and it will be seen that the result is the same; while the former is hydrolyzed to about 20% in 4 hours, the latter is almost completely hydrolyzed in ca. 2 hours. The fact that isopyrimidine nucleoside is much more labile toward acid than pyrimidine nucleoside is of interest as the specific characteristics of isopyrimidine nucleoside.

Fig. 1. Hydrolysis of 2-Thiouridines
(with 10% HCl at 100°)

Fig. 2. Hydrolysis of Glycosyl-2-thiouracils
(with 10% HCl at 100°)

The failure to derive (VIa) and (VIb) to (VIc) by treatment with monochloroacetic acid is considered to be due to the specific lability of (VIa) and (VIb) to acid, since uracil or thymine had been detected in these reaction mixtures, and hydrolysis of the sugar portion occurs at the time of conversion of sulfur atom to oxygen atom by monochloroacetic acid.

Synthesis of isopyrimidine nucleosides and their specific chemical properties increase biochemical interest in these compounds.

Experimental

3-Methyl-2-thiouracil—In a petri dish, 0.9 g. (0.01 mole) of well-powdered N-methylthiourea (I) was placed, 2.5 g. (0.013 mole) of ethyl 3,3-diethoxypropionate (b), 0.5 cc. of EtOH, and 1 drop of conc. HCl were added, and the whole was mixed thoroughly. This mixture was allowed to stand over H_2SO_4 in an evacuated desiccator for ca. 2 weeks with occasional stirring, by which the mixture turned into a yellow solid. The solid was warmed on a water bath with 1 g. of NaOH and 10 cc. of water to effect dissolution, the solution was warmed for 5 min., and cooled. Acidification with conc. HCl precipitated yellow crystalline powder which was collected. Yield of the dried product, 0.8 g. Recrystallization of this crude product showed that it was composed of two substances.

The crude product was therefore submitted to chromatography by the apparatus shown in Fig. 2 of Part II of this series.³⁾ A column of 2.7 cm. in diameter, with a mixture of 10 g. each of activated charcoal and cellulose powder placed in A, was used. A solution of the sample dissolved in 30 cc. of warm water and adjusted to pH 2.0 was passed through the column and the column was eluted with distilled water until the effluent was no longer acid. In the upper part of A and in B, 200 and 1000 cc. of water and were placed, 3000 cc. of 5:1:13 mixture of conc. NH_4OH -EtOH- H_2O in C, and the column was developed. The fractions showing ultraviolet absorption peaks at 258 and 311 $\text{m}\mu$ in NH_3 -alkalinity were collected and the solvent was evaporated in reduced pressure. Recrystallization of the residue from a large amount of MeOH afforded 0.3 g. (21%) of colorless plates, m.p. 283° . UV: $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH } 6.5)}$ 277 $\text{m}\mu$ (log ϵ 4.17). Anal. Calcd. for $\text{C}_5\text{H}_6\text{ON}_2\text{S}$: C, 42.25; H, 4.26; N, 19.71. Found: C, 42.46; H, 4.34; N, 19.54.

3-Methyluracil (III)—A mixture of 0.1 g. of (II), 2 g. of monochloroacetic acid, and 20 cc. of water was refluxed in an oil bath for 8 hr., the reaction mixture was concentrated in reduced pressure, and the syrupy residue was allowed to stand with addition of EtOH. The crystals that separated out were collected by filtration and recrystallized from MeOH to 50 mg. of colorless prisms, m.p. $174\sim 175^\circ$. Anal. Calcd. for $\text{C}_5\text{H}_6\text{O}_2\text{N}_2$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.38; H, 4.52; N, 21.85.

3-(β -D-Glucopyranosyl)-2-thiothymine (VIa)—Well-powdered 5 g. (0.0125 mole) of 1-(tetra-O-acetyl- β -D-glucopyranosyl)thiourea³⁾ (IVa) was placed in a petri dish, 2.4 g. (0.018 mole) of ethyl 3-formylpropionate (a), 4 cc. of EtOH, and 2 drops of conc. HCl were added, and the mixture was placed over H_2SO_4 in an evacuated desiccator. This was allowed to stand at room temperature with occasional stirring, by which the mixture became syrupy in ca. 3 days and formed a colloidal solid in ca. 2 weeks. This mixture was added to a hot solution of 2 g. of NaOH in 40 cc. of water and warmed with stirring for 30 min. After cool, some insoluble matter that remained was filtered off, 40 cc. of Amberlite IR-120 was added to effect dealkalization, and the acid solution so obtained was concentrated to ca. 20 cc. in reduced pressure. To this solution, 30 cc. of MeOH was added, glucosylthiourea was inoculated, and the mixture was allowed to stand in an ice chamber. The crystals that separated out were filtered off, the filtrate was evaporated to dryness, and 3.0 g. of a syrupy residue was obtained. This syrup showed ultraviolet absorption peaks at 276 and 300 $\text{m}\mu$, and its paper partition chromatography with MeCOEt showed two spots*³ at Rf 0.19 and 0.04.

A suspension of 90 g. of Celite 535 wetted with 90 cc. of water saturated with MeCOEt was filled in a chromatographic tube of 3.8 cm. in diameter by suspending in water-saturated MeCOEt. The sample syrup was mixed with 10 g. of Celite 535 and placed on top of the column. The column was developed with watersaturated MeCOEt and the effluent was collected in 25-cc. fractions. The substance showing a spot at Rf 0.19 eluted in the fraction Nos. 32~71, these fractions were combined, and evaporated in reduced pressure. The residue, when allowed to stand with addition of EtOH, crystallized and recrystallization from EtOH afforded 250 mg. (7%) of colorless prisms, m.p. 222° (decomp.). UV $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH } 6.5)}$ $\text{m}\mu$ (log ϵ): 276.5 (3.99), 301 (4.04). $[\alpha]_D^{20} + 34.8$ (c=0.49, H_2O). Anal. Calcd. for $\text{C}_{11}\text{H}_{16}\text{O}_6\text{N}_2\text{S}$: C, 43.42; H, 5.30; N, 9.21. Found: C, 43.40; H, 5.32; N, 9.08.

3-(β -D-Glucopyranosyl)-2-thiouracil (VIb)—In a petri dish, 5 g. (0.0125 mole) of well-powdered 1-(tetra-O-acetyl- β -D-glucopyranosyl)thiourea (IVa) was placed, 3.1 g. (0.016 mole) of ethyl 3,3-diethoxypropionate (b), 4 cc. of EtOH, and 4 drops of conc. HCl were added, and the mixture was placed in an evacuated desiccator. The desiccator was allowed to stand at room temperature for a week, the colloidal solid so formed was warmed for 20 min. with a hot solution of 3 g. of NaOH in 60 cc. of water, and the solution was allowed to cool. Some insoluble matter that remained was filtered off and the solution of dealkalified with 50 cc. of Amberlite IR-120. The solution was concentrated and residual syrup was submitted to paper partition chromatography to find a suitable solvent for separation of the crude product. Separation of the spot was insufficient with various solvents except with BuOH-AcOH- H_2O system.

The objective substance appeared as a spot at Rf 0.30 with BuOH-AcOH- H_2O (4:1:5) or at Rf 0.36 (5:2:3). The purification of this substance was effected, as with (VIa), by the use of 100 g. of Celite 535 and developed with MeCOEt. The effluent was collected in 23-cc. fractions and the objec-

tive was found to be eluted in fraction Nos. 45~110. These fractions were collected and purified by chromatography through activated charcoal by the method given for (II).

The column of 3 cm. in diameter, with 20 g. of each activated charcoal and cellulose powder placed in A, was used and the aqueous solution of the sample, adjusted to pH 2 with dil. HCl, was passed through the column. The column was washed thoroughly with distilled water until the effluent was no longer acid. The upper part of A and in B were placed 100 and 800 cc. of water, 1000 cc. of conc. NH_4OH -EtOH- H_2O (5:1:13) was placed in C, and the column was duly developed. The effluent was collected in 20-cc. fractions and the presence of the objective substance in the effluent was examined by paper partition chromatography. The substance was found to be eluted in the fraction Nos. 37~44, which were collected, evaporated to dryness in reduced pressure, and the residue was allowed to stand with addition of EtOH. The crystallized residue was recrystallized from 50% EtOH to 240 mg. (7%) of colorless prisms, m.p. $213\sim 214^\circ$ (decomp.). $[\alpha]_D^{20} + 28.0^\circ$ ($c=0.21$, H_2O). UV $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH } 6.5)} \text{ m}\mu$ (log ϵ): 270.5 (3.96), 300 (4.04). Anal. Calcd. for $\text{C}_{10}\text{H}_{14}\text{O}_6\text{N}_2\text{S}\cdot\text{C}_2\text{H}_5\text{OH}$: C, 42.85; H, 6.01; N, 8.33. Found: C, 43.02; H, 5.55; N, 9.81.

The crystals were well powdered and dried for 8 hr. at $120\sim 130^\circ$ in reduced pressure and the crystals lost ethanol but the m.p. remained unchanged. Anal. Calcd. for $\text{C}_{10}\text{H}_{14}\text{O}_6\text{N}_2\text{S}$: C, 41.38; H, 4.86; N, 9.65. Found: C, 41.28; H, 4.60; N, 8.91.

3-(β -D-Glucopyranosyl)uracil (VIc)—Well-powdered 5 g. (0.013 mole) of 1-(tetra-O-acetyl- β -D-glucopyranosyl)urea¹ (IVb) was placed in a petri dish, 3.2 g. (0.017 mole) of ethyl 3,3-diethoxypropionate (b), 4 cc. of EtOH, and 4 drops of conc. HCl were added, and the mixture was allowed to stand in an evacuated desiccator at room temperature. The mixture became syrupy in a few days and completely solidified in ca. 10 days. The solid mixture was warmed for ca. 10 min. with hot solution of 4 g. of NaOH in 80 cc. of water, the mixture was cooled, and filtered to remove some insoluble material. The filtrate was dealkalified with 70 cc. of Amberlite IR-120 and the yellow solution so obtained was evaporated to dryness in reduced pressure, leaving 3.5 g. of a residue. This crude product showed ultraviolet absorption at 263 $\text{m}\mu$ and indicated that pyrimidine cyclization had been effected. Paper partition chromatography of this residue with a solvent system of BuOH-AcOH- H_2O (4:1:5) indicated a spot of the main product at R_f 0.19 and in the same solvent system of 5:2:3, at R_f 0.22.

Purification of the product was effected by column chromatography through activated charcoal as used in the case of (VIb). A chromatographic column of 5 cm. in diameter, with 30 g. of each activated charcoal and cellulose powder placed in A, was used. The sample was passed through the column in a usual manner and the column was developed by 200 and 2000 cc. of water placed respectively in the upper part of A and in B, and 2000 cc. of conc. NH_4OH -EtOH- H_2O (5:1:13) placed in C. The effluent was collected in 25-cc. fractions and the objective substance was found to be eluted in the fraction Nos. 6~18. These fractions were combined, the solvent was evaporated, and the residue was allowed to stand with a small quantity of water. Some more crop of crystals were obtained from the mother liquor. All the crystals melted at $148\sim 152^\circ$ with decomposition and possessed a water of crystallization. Recrystallization from 90% EtOH afforded 400 mg. (11%) of colorless needles, m.p. $243\sim 244^\circ$ (decomp.). $[\alpha]_D^{20} + 7.52^\circ$ ($c=0.66$, H_2O). UV: $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH } 6.5)} 264 \text{ m}\mu$ (log ϵ 3.87). Anal. Calcd. for $\text{C}_{10}\text{H}_{14}\text{O}_7\text{N}_2$: C, 43.80; H, 5.15; N, 10.22. Found: C, 44.04; H, 4.98; N, 10.56.

Formation of 3-(β -D-Glucopyranosyl)uracil (VIc) from 3-(β -D-Glucopyranosyl)-2-thiouracil (VIb)—A mixture of 100 mg. of 3-(β -D-glucopyranosyl)-2-thiouracil (VIb), 2 g. of monochloroacetic acid, and 20 cc. of water was refluxed for 10 hr. Paper partition chromatography of the reaction mixture, using 4:1:5 mixture of BuOH-AcOH- H_2O , showed two spots*³ at R_f 0.16 and 0.43, and these spots agreed respectively with (VIc) and uracil.

For separation of (VIc), 2 g. of activated charcoal was added to the reaction mixture, thoroughly mixed, and filtered. The mixture on the filter was washed with water, stirred into 100 cc. of 10% NH_4OH , and filtered. The filtrate was evaporated to dryness in reduced pressure and residual syrup was allowed to stand after addition of a small amount of water. The crystals that precipitated out were collected by filtration, the filtrate was evaporated to dryness, and the residue was allowed to stand with EtOH. A small amount of crystals was obtained as colorless prisms, m.p. $243\sim 244^\circ$ (decomp.), showing no depression on admixture with 3-(β -D-glucopyranosyl)uracil synthesized earlier.

3-(β -D-Ribofuranosyl)-2-thiouracil (IX) (2-Isothiouridine)—In a petri dish, 5.2 g. (0.01 mole) of well-powdered 1-(tri-O-benzoyl- β -D-ribofuranosyl)thiourea (VII) was placed, 2.5 g. (0.013 mole) of ethyl 3,3-diethoxypropionate, 4 cc. of EtOH, and 5 drops of conc. HCl were added, and mixed thoroughly. The mixture was allowed to stand in an evacuated desiccator at room temperature, by which it turned into a syrup in a few days and formed a colloidal solid after 10 days. The solid was warmed on a water bath for 30 min. with a hot solution of 3 g. of NaOH, 50 cc. of water, and 40 cc. of EtOH, and the solution was cooled. The solution was dealkalified with 50 cc. of Amberlite IR-120, BzOH that precipitated and the ion exchanger were removed by filtration, and the filtrate was evaporated to dryness in reduced pressure to leave 2.1 g. of a crude product. Paper partition chromatography

of this crude product showed the presence of three spots at Rf 0.43, 0.25, and 0.14, when developed with watersaturated MeCOEt. The spot at Rf 0.14 is that of ribosylthiourea, that of 0.25 was assumed to be the main product, and the small spot at 0.43 to be that of a by-product. The paper chromatogram developed with BuOH-AcOH-H₂O (5:2:3) showed the presence of spots for two more by-products, but the both spots were very small.

The reaction product was purified by partition chromatography using Celite 535 (150 g.) and developed with MeCOEt, as in the case of (VIa), and the effluent was collected in 23-cc. fractions. The main product was found to be eluted in the fraction Nos. 34~52. These fractions were combined, the solvent was evaporated, and the syrupy residue thereby obtained was allowed to stand with a few drops of water and 1 cc. of EtOH. The crystals that separated out were collected, washed with EtOH, and 120 mg. (5%) of crude crystals were recrystallized from hydr. EtOH to colorless prisms, m.p. 187° (decomp.). UV $\lambda_{\text{max}}^{\text{H}_2\text{O}(\text{pH } 6.5)}$ m μ (log ϵ): 271.5 (3.92), 300.5 (4.01). *Anal.* Calcd. for C₉H₁₂O₆N₂S: C, 41.54; H, 4.65; N, 10.77. Found: C, 41.38; H, 4.76; N, 10.52.

Methylation of 3-(β -D-Glucopyranosyl)uracil and Hydrolysis of its Product—To a solution of 200 mg. of (VIc) dissolved in 2 cc. of water and added with 20 cc. of MeOH, Et₂O solution of CH₂N₂ was added with ice-cooling. The reaction proceeded immediately with evolution of N₂, excess CH₂N₂ was added, and the mixture was allowed to stand for 2 hr. with ice-cooling. The solvent was evaporated in reduced pressure and paper partition chromatography of the amorphous product showed two spots*³ at Rf 0.08 and 0.20 when developed with MeCOEt saturated with water, but at Rf 0.29 and 0.41 when developed with BuOH-AcOH-H₂O (4:1:5).

The amorphous product was purified by column chromatography through 40 g. of cellulose powder, as in the case of (VIc), and the effluent was collected in 25-cc. fractions. The main product eluted in the fraction Nos. 14~17, these fractions were combined, and the solvent was evaporated to dryness, leaving 110 mg. of an amorphous substance. This product failed to crystallize but was assumed to be 3-(β -D-glucopyranosyl)-1-methyluracil (XI).

A solution of 110 mg. of the amorphous methylated compound (XI) dissolved in 5 cc. of 10% H₂SO₄ was placed in a sealed tube and heated at 120~125° for 2.5 hr. 3% Ba(OH)₂ was added to the reaction mixture until no more precipitate formed and the precipitate was separated by centrifugation. The filtrate was evaporated to dryness, the residue was dissolved in a small amount of MeOH, and insoluble matter was filtered off. The filtrate was evaporated and the crystals that separated out were recrystallized from EtOH, affording 1-methyluracil of m.p. 229~230°, undepressed on admixture with an authentic specimen.

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Summary

From the formation of 3-methyl-2-thiothymine or 3-methyl-2-thiouracil from 1-methylthiourea and ethyl 2-formylpropionate or ethyl 3,3-diethoxypropionate, the same reaction was applied to 1-(poly-O-acyl- β -D-glycosyl) thiourea and -urea, and 3-(β -D-glucopyranosyl) uracil, 3-(β -D-glucopyranosyl)-2-thiouracil 3-(β -D-glucopyranosyl)-2-thiothymine, and 3-(β -D-ribofuranosyl)-2-thiouracil(2-isothiouridine) were obtained. These compounds have the glycosyl group bonded to the nitrogen in 3-position of the pyrimidine ring, as against that in the nitrogen in 1-position in pyrimidine nucleoside, and was tentatively designated as isopyrimidine nucleoside. The structure of these isopyrimidine nucleosides was established and it was revealed that these iso compounds have specific nature of being extremely labile to acids.

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