Summary

It has been already established that the three hydroxyl groups in kitigenin, a tetrahydroxy sapogenin, are located at C_3, C_4 and C_5, and that the C_3-hydroxyl group is β and the C_4- and C_5-hydroxyl groups are in *cis* relation. It is now, clarified that the one remaining hydroxyl group is situated at C_1, from the result of the conversion of kitigenin to 25D,5α-spirostan-1-one synthesized independently from tigogenone. And also, from the results of the C_3,C_4-acetonide formation and C_1,C_5-sulfite formation, it was clarified that the four hydroxyl groups are all β. Consequently, it was determined that kitigenin is 25D,5β-spirostane-1β,3β,4β,5-tetrol.

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112. Takeo Naito, Miyoshi Hirata, Tomoyoshi Kawakami, and Mitsuji Sano :
Studies on Nucleosides and Nucleotides. I. Synthesis of Glycosylthymines from Glycosylureas.

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The general method for synthesis of pyrimidine nucleosides includes those of Hilbert-Johnson[1] and of Fox,[2] and a new different route of synthesis was recently devised by Shaw.[3] A new synthetic process for pyrimidine nucleoside was attempted, starting with glycosylurea, by pyrimidine ring cyclization.

Goodman[4] reported the synthesis of 1-(tetra-O-acetyl-D-glucosyl)-6-aminouracil from tetra-O-acetyl-D-glucosylurea and cyanoacetic acid for the same purpose but he failed to give any details of this reaction. As a preliminary experiment for the present series of work, examinations were made on the mode of condensation of N-methylurea (I) and N-methylthiourea (II) with methyl 3-methoxy-2-methylacrylate (A), 3-methoxy-2-methylacryloyl chloride (B), and ethyl 2-formylpropionate (C) to form N-methylthymine and N-methylthiothymine.

Syntheses of N-methylthymine and N-methylthiothymine from methacrylic acid derivatives have been reported by Shaw[5] and Smith.[6] In the present series of work, condensation of (I) and (II) with (A) and (C) was attempted in the presence of sodium alkoxide and it was found that the reaction of (I) and (A) did not proceed but that of (II) and (A) afforded N-methyl-2-thiothymine (III), m.p. 230~232°, which, on treatment with monochloroacetic acid, was derived to N-methylthymine (IV), m.p. 282~284°. While the reaction of (I) and (C) afforded (IV), though in a minute amount, condensation of (II) and (C) afforded N-methylthiothymine (V) of m.p. 207~209°, different from (III). Treatment of (V) with

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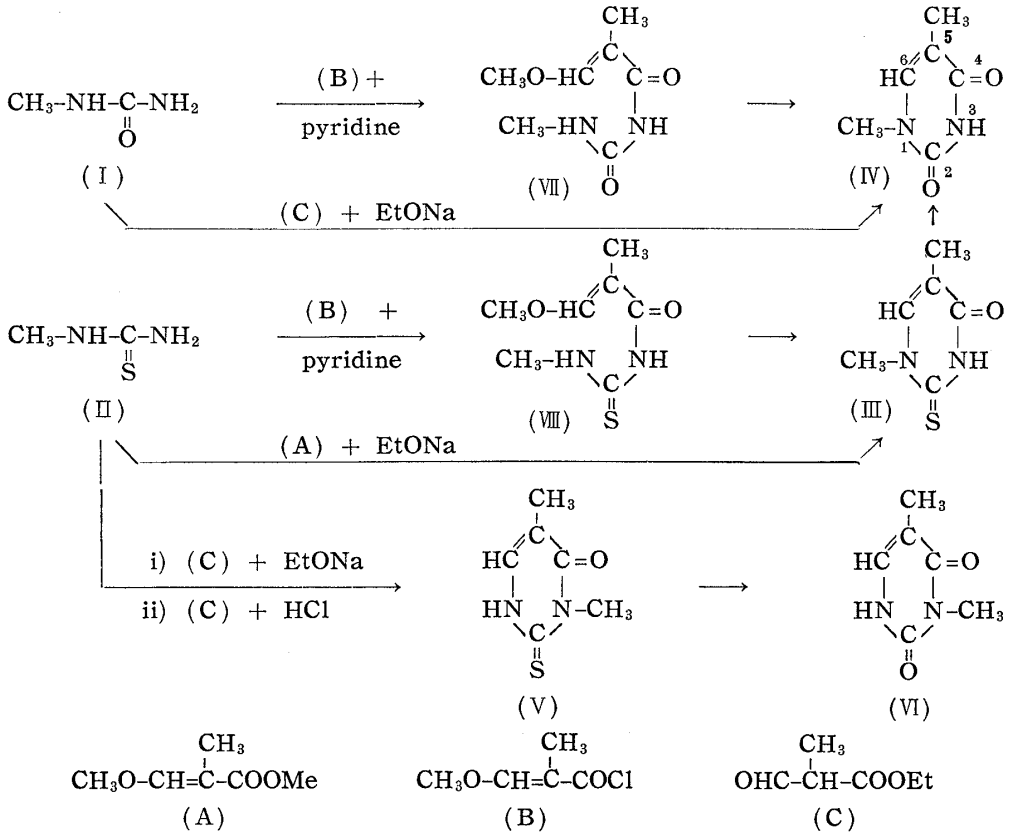
1) G.E. Hilbert, T.B. Johnson : J. Am. Chem. Soc., **52**, 4489 (1930); **58**, 60 (1936).
2) J.J. Fox : *Ibid.*, **78**, 2117 (1956).
3) G. Shaw : J. Chem. Soc., **1958**, 2295.
4) I. Goodman : Federation Proc., **15**, 264 (1956).
5) G. Shaw : J. Chem. Soc., **1958**, 157.
6) R.C. Smith : J. Org. Chem., **24**, 249 (1959).

monochloroacetic acid produced N-methylthymine (VI) of m.p. 202~205°, different from (IV). (V) was also obtained by condensation of (II) and (C) in the presence of a trace of hydrochloric acid. Condensation of (I) and (II) with (B) in pyridine-chloroform afforded (VII) and (VIII), respectively identical with N-(α-methyl-β-methoxyacryloyl)-N'-methylurea and N'-methylthiourea obtained by Shaw. Cyclization of (VII) and (VIII) with methylamine or sodium hydroxide produced (IV) and (III).

The compounds (III) and (V), and (IV) and (VI) are isomers of N-methylthiothymine and N-methylthymine. Johnson[7] stated that the substance (IV) of m.p. 280~282° is 1-methylthymine and that (VI) of m.p. 202~205°, 3-methylthymine. 1-Methylthymine was synthesized according to the method of Shaw[5] from N-ethoxycarbonyl-2-methyl-3-methoxyacrylamide and methylamine, and it was found to be identical with (IV). Accordingly, (VI) would be 3-methylthymine, and (III) and (V) would be 1-methyl-2-thiothymine and 3-methyl-2-thiothymine, respectively.



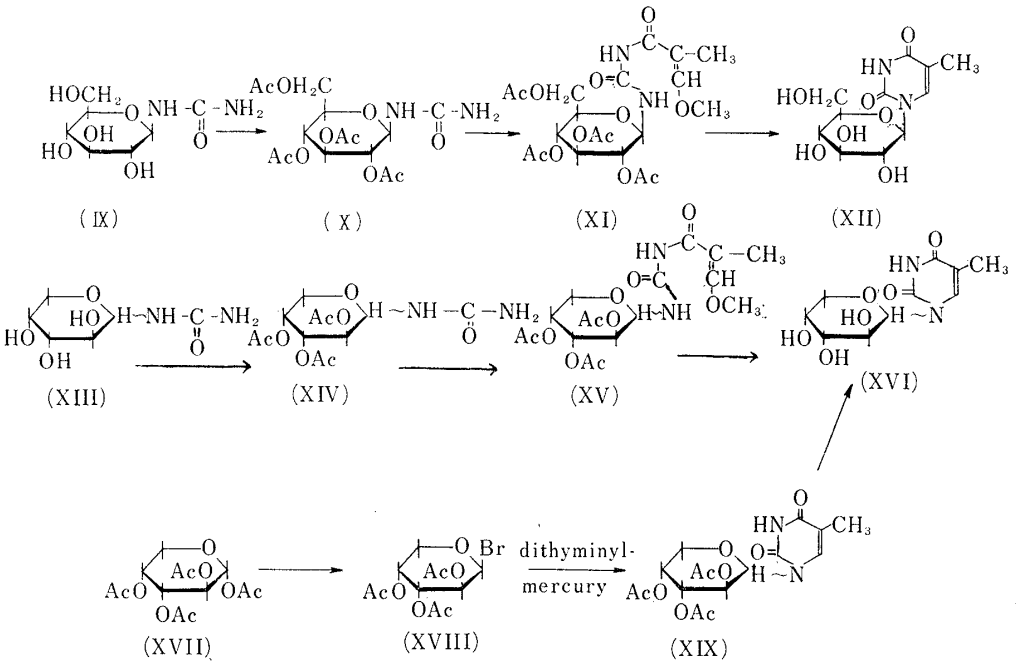
It was anticipated from the foregoing preliminary experiments that application of (B) to glycosylurea and of (A) and (B) to glycosylthiourea would produce 1-glycosylthymine and 1-glycosyl-2-thiothymine. These experiments also suggest the possibility that 3-glycosylthymine would be formed by application of (C) to glycosylthiourea and that synthesis of glycosyluracil would be possible through the use of 3-alkoxyacrylic acid derivative.

Based on these assumptions, reaction of D-glucosylurea and D-arabinosylurea with 2-methyl-3-methoxyacryloyl chloride was examined for the synthesis of corresponding pyrimidine nucleoside. D-Glucosylurea, the starting material for D-glucosylthymine, had been synthesized from D-glucose and urea, in the presence of mineral acid, by Helferich[8] and Hynd,[9] but the reaction required a long period of time. In the present series of

7) T.B. Johnson : J. Biol. Chem., **5**, 49 (1908).
8) B. Helferich : Ber., **56**, 69 (1926).
9) A. Hynd : Biochem. J., **20**, 195, 205 (1926).

work, the same reaction was carried out with the ion exchanger, Amberlite IR-120, as the catalyst, and the reaction temperature was raised, by which the reaction time was shortened and the objective was obtained in 52.6% yield. Benn[10] had proved the structure of this ureide as D-glucopyranosylurea but configuration at C-1 position is unknown.

This ureide was acetylated with acetic anhydride and pyridine to form tetra-O-acetyl-D-glucopyranosylurea (X), which was reacted with 2-methyl-3-methoxyacryloyl chloride in pyridine-chloroform by standing at room temperature for two days. 1-(Tetra-O-acetyl-glucopyranosyl)-3-(2-methyl-3-methoxyacryloyl)urea (XI), m.p. 147~148°, so obtained was warmed with dilute ammonia water and pyrimidine cyclization and deacetylation were effected at the same time to produce D-glucopyranosylthymine (XII), m.p. 266°. This nucleoside was identical in its melting point and paper partition chromatographic behavior with 1-β-D-glucopyranosylthymine (XII) synthesized by the method of Fox,[2] and its structure was thereby established. This has also proved that the starting ureide is 1-β-D-glucopyranosylurea (IX).



D-Arabinosylurea[11] (XIII), m.p. 192°, the starting material for synthesis of D-arabinosylthymine, was prepared by the method of preparation of L-arabinosylurea.[8] This ureide was acetylated with acetic anhydride and pyridine to tri-O-acetyl-D-arabinosylurea (XIV) and reacted with 2-methyl-3-methoxyacryloyl chloride in pyridine-chloroform to form 1-(tri-O-acetylarabinosyl)-3-(2-methyl-3-methoxyacryloyl)urea (XV), which was warmed with dilute ammonia water, finally producing D-arabinosylthymine (XVI), m.p. 243~244°. As for this nucleoside, Visser[12] obtained 1-D-arabinosylthymine, m.p. 250°, by the Hilbert-Johnson method and Fox[2] obtained 1-L-arabinosylthymine, m.p. 248~250°, by condensation of dithyminylmercury and acetobromo-L-arabinose. In the present work, 1-D-arabinosylthymine (XVI), m.p. 245~246°, was synthesized from β-acetobromo-D-arabinose (XVIII) and dithyminylmercury, and was found to be identical with the nucleoside described above, by mixed fusion. However, configuration at C-1 position and whether the sugar portion was in pyranose or furanose type remained uncertain. In order to elucidate these points,

10) M. H. Benn : Chem. & Ind. (London), **1959**, 997.
11) "Advances in Carbohydrate Chemistry," **13**, 235 (1958). Academic Press Inc., New York. I. Goodman (unpublished data) reported m.p. 185~187°.
12) D. W. Visser : J. Am. Chem. Soc., **70**, 1926 (1948).

examinations were made on the infrared spectrum of tetra-O-acetyl-D-arabinose, the starting material for β-acetobromo-D-arabinose used for the synthesis of this nucleoside. The infrared spectrum of this tetra-O-acetyl-D-arabinose was entirely identical with that of tetra-O-acetyl-D-arabopyranose (XVII) prepared by Barker[13] and the ring structure of the sugar portion was found to be the pyranose type. Judging from the reports of Baker[14] and Lemieux,[15] configuration at C-1 would be α-form and the structure of the compound synthesized would likely be 1-α-D-arabopyranosylthymine. It was thereby assumed that the ureide would be 1-α-D-arabopyranosylurea.

The foregoing experiments have proved that the new method for synthesis of pyrimidine nucleoside progresses through the same route of reactions as in the preliminary experiment for preparation of 1-methylthymine from N-methylurea, and pyrimidine cyclization from D-glucosylurea and D-arabinosylurea had successfully been concluded. It has become possible, through this method, to synthesize natural and non-nutural pyrimidine nucleosides.

Experimental

1-Methyl-3-(2-methyl-3-methoxyacryloyl)urea (VII)——To a suspension of (I) (0.6 g.) in dehyd. $CHCl_3$ (20 cc.), 2-methyl-3-methoxyacryloyl chloride (1.0 g.) was added, followed by pyridine (0.6 g.). The reaction mixture was refluxed for 2.5 hr., the clear solution thus obtained was concentrated *in vacuo*, and the crystalline residue was purified from EtOH to colorless needles, m.p. 129~131°; UV: $\lambda_{max}^{H_2O}$ 258.5 mμ (log ε 4.152). Yield, 0.9 g.

1-Methyl-3-(2-methyl-3-methoxyacryloyl) thiourea (VIII)——To a suspension of (II) (0.9 g.) in $CHCl_3$ (15 cc.), 2-methyl-3-methoxyacryloyl chloride (1.3 g.) was added followed by pyridine (0.9 g.). The reaction mixture was refluxed for 2 hr., the clear yellowish solution thus obtained was cooled, washed successively with 2.5% $NaHSO_4$, 5% $NaHCO_3$, and H_2O, dried over anhyd. Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in EtOH from which the precipitate of (VIII) separated. Recrystallized from EtOH, this separated to colorless plates, m.p. 141~143.5°; UV : λ_{max}^{EtOH} 270 mμ (log ε 4.10). Yield, 0.5 g. *Anal.* Calcd. for $C_7H_{12}O_2N_2S$: C, 44.68; H, 6.43; N, 14.88. Found : C, 44.57; H, 6.34; N, 14.57.

1-Methylthymine (IV)——(a) A mixture of 25% $MeNH_2$ (2.5 cc.) and (VII) (0.12 g.) was heated at 85° for 20 min. to a clear solution. After cool, the solution was acidified with dil. HCl and kept overnight, giving a precipitate of crude (IV), which was purified from hot water to colorless needles, m.p. 284°. UV : $\lambda_{max}^{H_2O}$ 274 mμ (log ε 3.95).

(b) A mixture of (III) (200 mg.) and 10% $CH_2ClCOOH$ (40 cc.) was refluxed for 5 hr. and evaporated *in vacuo*. The crystalline residue was recrystallized from hot water to colorless needles, m.p. 280~282°. This compound showed no depression of melting point on admixture with the specimen, synthesized by Shaw's method[5] and by (a).

1-Methyl-2-thiothymine (III)——(a) Suspension of (VIII) (0.1 g.) in 3.3% NH_4OH (6 cc.) was stirred on a water bath for 1 hr. The clear solution thus obtained was evaporated *in vacuo* and the residue was recrystallized from EtOH to colorless needles, m.p. 230~232; UV : $\lambda_{max}^{H_2O}$ 269 mμ (log ε 4.18). Yield, 50 mg.

(b) To a solution of Na (0.46 g.) in EtOH (20 cc.), (II) (1.8 g.) was added, followed by methyl α-methyl-β-methoxyacrylate (2.6 g.). The reaction mixture was refluxed for 5 hr. and allowed to stand overnight. The gelatinous product thus obtained was dissolved in H_2O (20 cc.) and evaporated to dryness *in vacuo*. The residue was dissolved in H_2O and acidified with dil. AcOH, separating colorless needles, m.p. 230~232°. Yield, 0.5 g. On admixture with the specimen from (a), this compound showed no depression of the melting point.

3-Methyl-2-thiothymine (V)——(a) To a solution of Na (0.46 g.) in EtOH (20 cc.), (II) (1.8 g.) was added, followed by ethyl α-formylpropionate (2.6 g.). The reaction mixture was refluxed for 4 hr., cooled, and filtered to obtain the crude (V). The solution of crude (V) in H_2O was acidified with dil. AcOH from which crystalline solid separated. When purified from MeOH, this formed pillars, m.p.

13) S. A. Barker : J. Chem. Soc., **1954**, 3468.
14) B. R. Baker : "Stereochemistry of Nucleoside Syntheses," Ciba Foundation Symposium, Chem. and Biol. Purines, 1957, 120. J. & A. Churchill Ltd., London.
15) R. U. Lemieux : "Advances in Carbohydrate Chemistry," **9**, 1 (1954). Academic Press Inc., New York.

207～208°(530 mg.). The alcoholic filtrate of reaction mixture was evaporated to dryness *in vacuo* and purified by the same procedure as described above. The impure material thus obtained was dissolved in Me_2CO and purified by a column of alumina to obtain the same compound as above, m.p. 207～208°(0.8 g.). UV : $\lambda_{max}^{H_2O}$ 279 mμ(log ε 4.20).

(b) A mixture of finely powdered (II)(1.8 g.), methyl 2-formylpropionate (2.6 g.), EtOH(0.5 cc.), and conc. HCl(1 drop) in glass dish was allowed to stand in a vacuum desiccator over H_2SO_4 with continuous evacuation for 10 days. The solidified mixture was dissolved with stirring in a hot (95°), solution (20 cc.) of NaOH (2 g.), cooled to about 50°, and acidified carefully with conc. HCl. After cool, the deposited precipitate was collected, washed with cold water and dried (0.95 g.). This was recrystallized from MeOH to colorless pillars, m.p. 206～207°, showing no depression on admixture with (V) synthesized by (a).

3-Methylthymine (VI)——A mixture of (V)(200 mg.) and 10% $CH_2ClCOOH$(40 cc.) was refluxed for 5 hr. and evaporated *in vacuo*. The crystalline residue was purified from hot water to colorless prisms, m.p. 204～206°. Yield, 50 mg. UV : $\lambda_{max}^{H_2O}$ 263 mμ(log ε 3.85).

1-β-D-Glucopyranosylurea (IX)——A mixture of anhyd. D-glucose(20 g.), urea (10 g.), water (20 cc.), and Amberlite IR-120 (H-form)(20 cc.) was shaken strongly at 75～80° for 4 days. The resin was filtered off and washed with H_2O. The washings were added to the filtrate and to the combined solution EtOH(200 cc.) was added. After standing in an ice box overnight, crystallized (IX), m.p. 205°, separated. Yield, 13 g.(52.6% from D-glucose). The product was recrystallized from dil. EtOH to colorless pillars, m.p. 207°; $[\alpha]_D^{27}$ −18.9°(c=1.603, H_2O). On admixture with the specimen synthesized by Helferich's method,[8] this product showed no depression.

2,3,4,5-Tetra-O-acetyl-β-D-glucopyranosylurea (X)——This compound was synthesized by Helferich's method.[8] Colorless crystalline powder, m.p. 95°.

1-(2, 3, 4, 6-Tetra-O-acetyl-β-D-glucopyranosyl)-3-(2-methyl-3-methoxyacryloyl)urea (XI)——To a solution of (X)(9.0 g.) in $CHCl_3$(20 cc.), 2-methyl-3-methoxyacryloyl chloride (3.0 g.) was added, followed by pyridine (2.0 g.). The reaction mixture was kept at room temperature for 2 days and concentrated *in vacuo*. The syrup which remained was recrystallized from EtOH to colorless needles, m.p. 145°; $[\alpha]_D^{25}$ −27.0°(c=0.844, pyridine); UV : $\lambda_{max}^{H_2O}$ 262.2 mμ(log ε 4.199). Yield, 7.35 g.(65.0%). *Anal.* Calcd. for $C_{20}H_{30}O_{12}N_2$: C, 48.97; H, 6.16; N, 5.72. Found : C, 49.18; H, 5.75; N. 5.64.

1-β-D-Glucopyranosylthymine (XII)——To 3.3% NH_4OH(30 cc.)(XI)(1.0 g.) was added. The mixture was heated at 80° for 2 hr. with occasional shaking by hand. The clear solution was concentrated *in vacuo* to a syrup, to which EtOH(50 cc.) was added and again evaporated *in vacuo*. This procedure was repeated three times and crude crystalline product was obtained. This was recrystallized from EtOH to colorless prisms, m.p. 266～267°; $[\alpha]_D^{27}$ +8.20° (c=1.987, H_2O); UV: $\lambda_{max}^{H_2O}$ 264.5 mμ (log ε 3.99). This compound showed no depression of melting point on admixture with the authentic specimen, synthesized by the mercury process.

1-D-Arabinopyranosylurea (XIII)——A solution of D-arabinose (20 g.) and urea (16 g.) in 0.7% HCl (20 cc.) was kept at 50° for 7 days. To the solution, EtOH (200 cc.) was added, kept overnight, and precipitate of (XIII) monohydrate separated. Yield, 14 g.(46.5% from D-arabinose). By purification through recrystallization from dil. EtOH colorless pillars, m.p. 193°, were obtained. $[\alpha]_D^{20}$ −52.1° (c=3.28, H_2O). *Anal.* Calcd. for $C_6H_{12}O_5N_2 \cdot H_2O$: C, 34.27; H, 6.71; N, 13.34. Found : C, 34.26; H, 6.56; N, 13.25.

2,3,4-Tri-O-acetyl-D-arabinopyranosylurea (XIV)——A mixture of (XIII)(10 g.), pyridine (100 cc.), and Ac_2O(50 cc.) was shaken at room temperature for 8 hr. to a clear solution and kept overnight. To the solution, H_2O(13 cc.) was added with stirring and ice-cooling, and the solution was concentrated *in vacuo* to a viscous syrup. Small quantity of H_2O was added to the syrup and oily acetate crystallized while scratching. This was filtered and recrystallized from EtOH to colorless pillars, m.p. 212～213°; $[\alpha]_D^{20}$ −44.0°(c=2.594, H_2O). Yield, 12.2 g.(80.4%). *Anal.* Calcd. for $C_{12}H_{18}O_8N_2$: C, 45.35; H, 5.70; N, 9.03. Found : C, 45.25; H, 5.70; N, 8.80.

1-(2, 3, 4-Tri-O-acetyl-D-arabinopyranosyl)-3-(2-methyl-3-methoxyacryloyl) urea (XV)——To a suspension of (XIV)(3.6 g.) in $CHCl_3$(20 cc.), 2-methyl-3-methoxyacryloyl chloride (1.5 g.) was added, followed by pyridine (0.9 g.). The reaction mixture was refluxed for 5 hr. and concentrated *in vacuo*. Crude crystals thus obtained were purified through recrystallization from EtOH to colorless pillars, m.p. 149°; $[\alpha]_D^{22}$ −15.7°(c=3.123, pyridine); UV : $\lambda_{max}^{H_2O}$ 262.0 mμ(log ε 4.17). Yield, 3.24 g.(68.8%). *Anal.* Calcd. for $C_{17}H_{24}O_{10}N_2$: C, 49.04; H, 5.81; N, 6.73. Found : C, 49.07; H, 6.00; N, 6.73.

1-D-Arabinopyranosylthymine (XVI)——To 3.3% NH_4OH(30 cc.), (XV)(1.0 g.) was added. The mixture was heated at 80° for 5 hr. to a clear solution. The solution was concentrated *in vacuo* to a syrup, to which EtOH(50 cc.) was added and again concentrated. This procedure was repeated three times and kept overnight. The crude crystals thus obtained were recrystallized from EtOH to colorless needles, m.p. 243°. Yield, 0.36 g.(58%). $[\alpha]_D^{20}$ −62.9°(c=1.51, H_2O); UV : $\lambda_{max}^{H_2O}$ 264.5 mμ(log ε 3.968).

(b) The solution of (XIX)(1.0 g.) in MeOH(50 cc.) was saturated with dry HCl and allowed to stand

at room temperature for 3 days. The solution was evaporated *in vacuo* to dryness, the residue was recrystallized from EtOH to colorless needles, m.p. 245~246°. Yield, 0.6 g. (84%). On admixture with the specimen synthesized by (a) this compound showed no depression. *Anal.* Calcd. for $C_{10}H_{14}O_6N_2$: C, 46.51; H, 5.46; N, 10.85. Found : C, 46.87; H, 5.48; N, 10.92.

1,2,3,4-Tetra-O-acetyl-α-D-arabinopyranose (XVII)——To a mixture of Ac_2O (125 g.) and anhyd. AcONa (15 g.), D-arabinose (25 g.) was added. After stirring for 4 hr. on a water bath, the reaction mixture was poured on cracked ice with vigourous agitation and oily substance separated which solidified to crystalline powder. After keeping overnight in an ice box, the precipitate was filtered and recrystallized from EtOH to colorless pillars, m.p. 95~97°. Yield, 25 g. (47.1%). *Anal.* Calcd. for $C_{13}H_8O_9$: C, 49.08; H, 5.70. Found : C, 49.05; H, 5.68.

2,3,4-Tri-O-acetyl-β-D-arabopyranosyl Bromide (XVIII)—— (XVII) (5 g.) was added to 70 g. of AcOH previously saturated with HBr(40%) and allowed to stand at room temperature with occasional shaking by hand for 2.5 hr. $CHCl_3$ (60 cc.) was then added and poured into ice-water with stirring. $CHCl_3$ layer was collected, washed successively with H_2O, 5% $NaHCO_3$, and H_2O, dried over anhyd. $CaCl_2$, and evaporated *in vacuo*. The crystalline residue was recrystallized from Et_2O to colorless pillars, m.p. 135~140°. This compound decomposed gradually on standing. Yield, 3.9 g. (73%).

1-(2,3,4-Tri-O-acetyl-D-arabopyranosyl)thymine (XIX)——Powdered dithyminylmercury (3 g.) was added to dehyd. xylene (100 cc.). The vigourously stirred suspension was dried by azetoroic destillation of xylene (ca. 15 cc.). (XVIII) (4.6 g.) was added and the stirred mixture was refluxed for 2 hr. The hot turbid solution was filtered and petr. ether was added. The precipitate was collected, dissolved in $CHCl_3$, washed successively with 30% KI and H_2O, and dried over anhyd. Na_2SO_4. After evaporating *in vacuo*, the crystalline residu was erecrystallized from MeOH to colorless needles, m.p. 135~140°. Yield, 1.7 g. (33%). *Anal.* Calcd. for $C_{16}H_{20}O_9N_2$: N, 7.28. Found : N, 7.04.

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Summary

As a new method for synthesis of pyrimidine nucleoside, synthesis of glycosylthymine from glycosylurea was carried out. As a model experiment, pyrimidine cyclization was examined with N-methylurea and N-methylthiourea with methyl 2-methyl-3-methoxyacrylate, 2-methyl-3-methoxyacryloyl chloride, and ethyl β-formylpropionate. Starting from 1-D-glucosylurea and 1-D-arabinosylurea, protecting the hydroxyls in the sugar portion by acetylation, condensation with 2-methyl-3-methoxyacryloyl chloride afforded the intermediates, (XI) and (XV), and their cyclization successfully produced 1-β-D-glucopyranosylthymine and 1-D-arabinosylthymine.

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