269° (decomp.); $[\alpha]_D^{22}$ +11° (c=1.00, H_2O); UV; $\lambda_{max}^{H_2O}$ 265 mμ (ε, 11890); $\lambda_{min}^{H_2O}$ 233.5 mμ (ε, 3060). *Anal.* Calcd. for $C_{11}H_{14}O_8N_2 \cdot C_2H_5OH \cdot 1/2H_2O$: C, 43.60; H, 5.77; N, 7.82. Found: C, 43.73; H, 5.91; N, 8.18.

b) XVIII was also obtained from XVI in a yield of 80% by the same procedure described for the synthesis of VI by alkaline hydrolysis of V.

Oxidation of 1-Deoxy-1-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)-β-D-glucopyranose (XIX) with a Stream of Oxygen——A solution of XIX (0.28 g.) in water (100 ml.) was oxidized with a stream of purified O_2 in the presence of a Pt-catalyst (prepared from Adam's platinum-oxide (0.15 g.) by hydrogenation in an usual procedure) at 60~65° for 50 hr., neutrality or mild alkalinity being maintained by addition of 0.5*N* $NaHCO_3$ until the reaction was completed. The catalyst was removed by filtration, and the filtrate was passed through a column of Amberlite IR-120 (H^+). The effluent and washings were combined and evaporated at 40° *in vacuo*, giving a crude gummy product which on twice recrystallization from water had m.p. 277~278° (decomp.). Yield, 0.12 g. (42%). $[\alpha]_D^{23}$ +11.3 (c=0.82, H_2O). No depression of melting point was observed on admixture with VI prepared by Hilbert-Johnson's method in Chart 2, IR spectra and $[\alpha]_D$ of both compounds were identical.

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Summary

D-Glucuronic acid nucleotides of uracil (VI, VIII), thymine (XVIII), cytosine (VII), 5-bromo- and 5-iodo-uracil (IX, X) were prepared by Hilbert-Johnson and Fox's procedures for the syntheses of pyrimidine nucleosides. VI was also obtained from 1-deoxy-1-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)-β-D-glucopyranose by O_2-oxidation in the presence of Pt-catalyst. The structure and properties of the nucleosides synthesized were briefly discussed.

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183. Torahiko Kishikawa*1 and Hidetaka Yuki*2 : Studies on Chemotherapeutic Agents. II.*3 A Synthesis of Purine Nucleosides of D-Glucuronic Acid.

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A new nucleoside-type antibiotic, gougerotin, which had been recently isolated from *Streptococcus gougerotii*[1] was found to show a broad spectrum anti-bacterial activity and to contain an amino-uronic acid as the sugar moiety in cytidine-like structure.[2]

Though it has been well known that some of synthetic nucleosides and antibiotic nucleosides exhibit biological activities against various bacteria, viruses and tumors, there has been little report on a study of nucleosides containing hexuronic acid or their derivatives[3] as a sugar component.

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3) I. Goodman : Federation Proc., **12**, 210 (1953).

The advent of gougerotin led the authors to a study of pyrimidine and purine nucleosides of hexuronic acids and amino hexuronic acids. In a previous paper,*1 they undertook the synthesis of pyrimidine nucleosides of D-glucuronic acid. This paper deals with a preparation of purine nucleosides of D-glucuronic acid.

Wolfrom and McWain[4] have lately described the synthesis of adenine D-glucuronic acid nucleoside by the method of Davoll and Lowy,[5] which involved coupling of α-acetobromoglucuronic acid with 6-acetamido-9-chloromercuripurine in refluxing toluene followed by treatment with methanolic ammonia.

The authors have tried to prepare the nucleoside derivatives of D-glucuronic acid by modifying the procedure for the synthesis of D-glycosyladenine established by Ikehara and Tada,[6] using 4-ethylthio-5-nitro-6-chloro-pyrimidine instead of 5-nitro-4,6-dichloropyrimidine as a starting material.

The chlorine atoms of 5-nitro-4,6-dichloropyrimidine were highly reactive for the substitution reaction with an alkyl- or aryl-amine and a 4,6-bis-alkylamino (or -arylamino) derivative was usually formed even if a stoichiometric proportion of the amine was used.[7~9] But at low reaction temperature, 5-nitro-4,6-dichloropyirmidine could be used for the mono-substitution reaction, sometimes, being accompanied with formation of some 4,6-bis form.[6,10~12] In order to avoid such a side reaction completely and to establish a general procedure for the synthesis of purine nucleosides, compound I, II or 4-ethylthio-5-nitro-6-chloropyrimidine was caused to react with triethylamine and methyl 1-amino-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (III)[13] (Chart 1), which was readily prepared from the corresponding azido derivative by hydrogenation in the presence of Raney nickel or platinum-catalyst. The chlorine atom of I or II was not so reactive that the coupling to be aimed did not take place and a crystalline substance (V) having no UV absorption, decomp., 237~238°, was obtained. Though the structure of this compound was assumed to be V by the elemental analytical data and an analogous reaction,[6,14] it was confirmed by the mixed melting point test and the comparison of infrared spectrum of V with that of the authentic sample[15] prepared by an umambigous synthetic method.

The condensation of 4-ethylthio-5-nitro-6-chloropyrimidine with III in dioxane solution at 50~55° was effected and methyl 1-deoxy-1-(4-ethylthio-5-nitro-6-pyrimidinylamino)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (VI) was obtained as pale yellow crystals, m.p. 168~170°, in a yield of 71.7%. In this case, only trace of compound V was formed.

Hydrogenation of the nitro group of VI in the presence of platinum-catalyst at low temperature was achieved.

The amino-derivative (VII) which was obtained as a yellowish glass after evaporation of the solvent, was used in further reaction without purification.

An attempt to cyclize VII to purine derivative with a mixture of acetic anhydride and ethyl orthoformate failed.

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10) R. K. Robins, H. H. Lin : J. Am. Chem. Soc., **79**, 490 (1957).
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12) *Idem* : *ibid.*, **1959**, 481.
13) M. Kuranari : Yakugaku Zasshi, **81**, 1189 (1961).
14) R. S. Tipon : J. Org. Chem., **26**, 2462 (1962).
15) J. Ide : Yakugaku Zasshi, **85**, 226 (1965).

When VII was allowed to react with carbon disulfide in pyridine under refluxing condition,[6,16,17] cyclisation was effected to afford methyl 1-deoxy-1-(6-ethylthio-8-mercapto-9-purinyl)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (VIII), m.p. 208~209°.

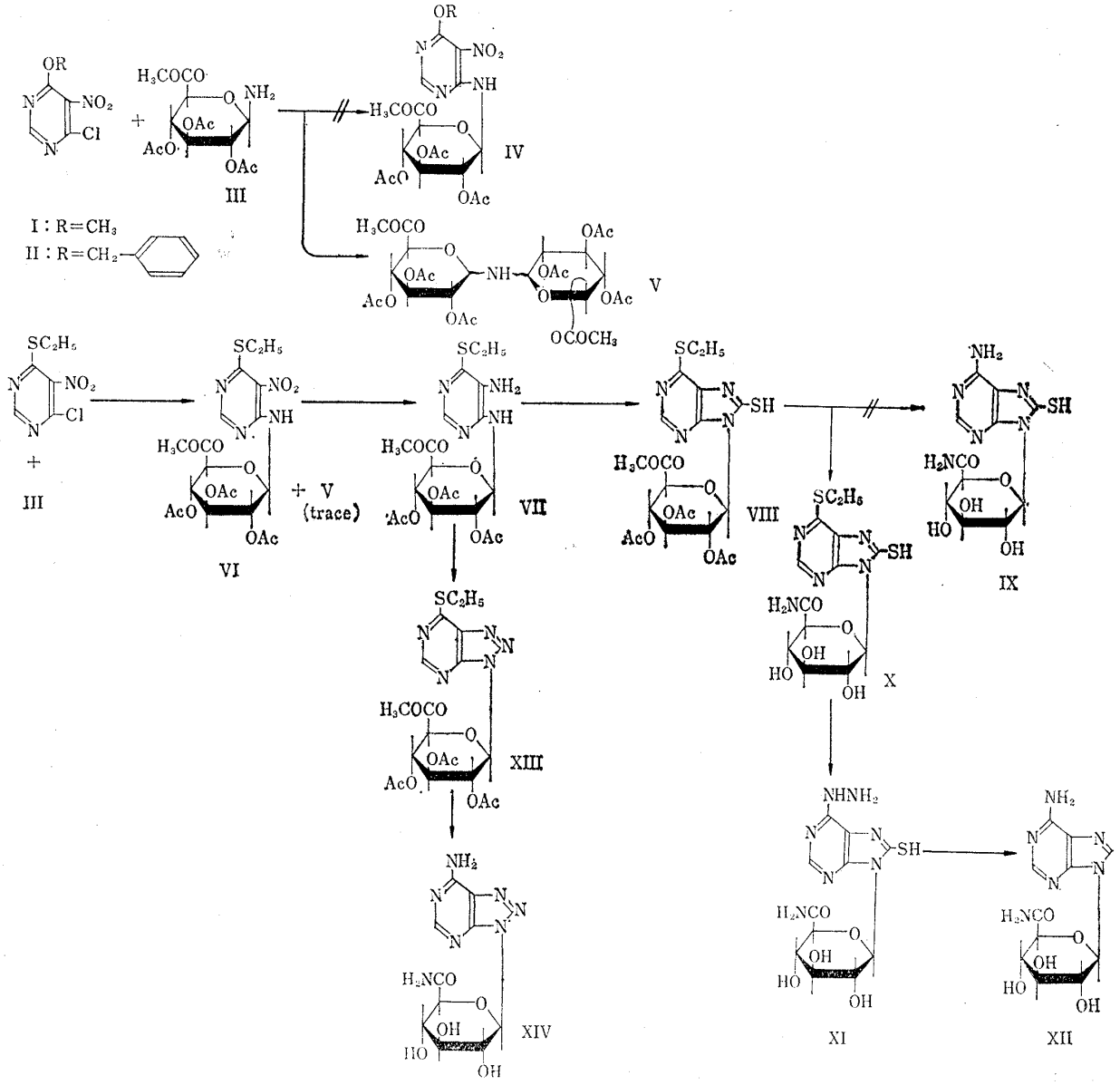

Chart 1.

Amination of 6-ethylthio group of VIII with ethanolic or methanolic ammonia could not be accomplished and, instead of the expected IX, compound X unchanged on purine nucleus but deacetylated and amidated in D-glucuronate moiety was obtained, showing wide ranged melting point of 221~230°.

However, 6-ethylthio group of compound X was easily reacted with hydrazine solution and resulted in formation of the corresponding 6-hydrazino derivative XI which was followed by treatment with Raney nickel without isolation. Desulfurization of 8-mercapto group and reduction of 6-hydrazino group in compound XI concurred to afford 1-deoxy-1-(6-amino-9-purinyl)-β-D-glucopyranuronamide (XII) in a crystalline form, m.p. 269~270° (decomp.), but the yield was low (17% from compound (X)). The confirmation

16) R.W. Balsiger, *et al.*: J. Org. Chem., **26**, 3386 (1961).
17) E. Bühler, W. Pfleiderer: Ang. Chem., **76**, 713 (1964).

of the structure of XII was made by comparison with the authentic specimen synthesized according to the method of Wolfrom and McWain.[4]

On the other hand, compnund VII was allowed to react in acetic acid with sodium nitrite to afford triazolo-derivative (XIII), m.p. 185~186°.

In contrast with difficulties that were encountered with amination of 6-ethylthio group of VIII, that of compound XIII was readily aminated with ethanolic ammonia in a sealed tube at 80° to give 1-deoxy-1-(7-amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-β-D-glucopyranuronamide (XIV) in a crystalline form, m.p. 254~255° (decomp.).

Thus, the reaction described above will be of practical use for the preparation of purine nucleosides and their triazolo derivatives.

Experimental*5

Methyl 1-Amino-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (III)[13]——Methyl 1-azido-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (10 g.) was dissolved in 200 ml. of dioxane and hydrogenated in the presence of Pt-catalyst (prepared from 1.0 g. of PtO_2 in an usual way) at room temperature for 6 hr. Nitrogen produced during the reaction was removed by flushing the apparatus with hydrogen gas at intervals of about 1 hr. After removal of the catalyst, the filtrate was evaporated to dryness to leave pale yellow solid. Recrystallization from EtOH afforded 8.4 g. of III (91.5%), m.p. 141~142°.

Reaction of I or II with methyl 1-amino-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (III)——To a solution of 4-benzyloxy-5-nitro-6-chloropyrimidine (2.65 g.) and triethylamine (1.01 g.) in dioxane (30 ml.) was added a solution of III (3.32 g.) in dioxane with stirring, and the mixture was heated at 50~55° for 8 hr. The reaction mixture was filtered and the filtrate was evaporated under a reduced pressure to give a residue, which was dissolved in EtOH. The insoluble material was filtered, collected and recrystallized from dioxane in colorless needles. Yield, 1.2 g. m.p. 237~238°, undepressed on admixture with hexa-O-acetyl-di-D-glucopyranuronosylamine dimethyl ester prepared from α-acetobromoglucuronic acid and III by König-Knor reaction.[15] IR spectra of both were identical; $[\alpha]_D^{25}$ +135° (c=1.08, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{35}O_{18}N$; C, 48.07; H, 5.43; N, 2.16. Found: C, 48.39, H, 5.35; N, 1.97.

Methyl 1-Deoxy-1-(ethylthio-5-nitro-6-pyrimidinylamino)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (VI)——4-Ethylthio-5-nitro-6-chloropyrimidine (2.19 g., 10 mmole.) and triethylamine (1.0 g., 10 mmole) were dissolved in 30 ml. of dioxane. To this solution was added dropwise at 50~55° with vigorous stirring III (3.33 g., 10 mmole) in 10 ml. of dioxane and the reaction was continued for 8 hr. After cooling the reaction mixture, the precipitated triethylamine hydrochloride was removed by filtration and the filtrate was evaporated *in vacuo* at 40° to give crystalline crops, which were dissolved in EtOH. Trace of insoluble material gave m.p. 237~238° (decomp.) on recrystallization from dioxane. The substance was identified as compound V by mixed melting point test and infrared spectrum. The filtrate of the EtOH solution was evaporated to give a residue which was recrystallized from EtOH. Pale yellow needles, m.p. 168~170°, were obtained in a reasonable yield (3.7 g. 71.7%). $[\alpha]_D^{22}$ +28.9° (c=1.02, $CHCl_3$); UV; λ_{max}^{MeOH} 255.5 mμ (ε, 27180), 338 mμ (ε, 8350); λ_{min}^{MeOH} 384 mμ (ε, 1430). *Anal.* Calcd. for $C_{19}H_{24}O_{11}N_4S$: C, 44.18; H, 4.68; N, 10.85. Found; C, 44.37; H, 4.89; N, 10.53.

Methyl 1-Deoxy-1-(4-ethylthio-5-amino-6-pyrimidinylamino)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (VII)——1.5 g. or VI and 0.3 g. of PtO_2 were suspended in 50 ml. of MeOH and hydrogenated at 5~10° under cooling with ice water for 3 hr. After filtration of the catalyst, the clear filtrate was evaporated to dryness *in vacuo* at 30° to leave 1.4 g. of a pale yellow glass, which was used in further reaction without purification.

Methyl 1-Deoxy-1-(6-ethylthio-8-mercapto-9-purinyl)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (VIII)——The 5-amino-derivative (VII), prepared from 1.5 g. of VI by hydrogenation as described above, was dissolved in a mixture of 20 ml. of CS_2 and 40 ml. of pyridine and heated on a steam bath under reflux for 7 hr. The reaction mixture was treated with activated carbon and filtered. The filtrate was evaporated to dryness *in vacuo*, the residual solid was recrystallized from EtOH-ligroin in an amorphous powder. m.p. 208~209°. Yield was 1.4 g. (93.5% from VI); $[\alpha]_D^{25}$ −37° (c=1.08, MeOH), UV: λ_{max}^{MeOH} 257.5 mμ (ε, 16490), 329 mμ (ε, 31180); λ_{min}^{EtOH} 290 mμ (ε, 6360). *Anal.* Cacld. for $C_{20}H_{24}O_9N_4S_2$: C, 45.43; H, 4.57; N, 10.65. Found: C, 44.96; H, 4.92, N, 10.52.

Methyl 1-Deoxy-1-(7-ethylthio-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (XIII)——To a solution of VII (1.5 g.) in AcOH (15 ml.) was added dropwise a solution of $NaNO_2$ (350 mg.) in H_2O (2 ml.) with vigorous stirring at room temperature. The reaction mixture was stirred for further one hour and poured into ice water. The precipitate formed was collected, washed with water and

*5 All melting points are uncorrected.

recrystallized from EtOH. Colorless needles, m.p. 185~186° was obtained in a yield of 80.5% (1.2 g.); $[\alpha]_D^{25}$ −35.5° (c=0.98, $CHCl_3$), UV : λ_{max}^{MeOH} 228 mμ (ε, 13000), 301 mμ (ε, 16800); λ_{min}^{MeOH} 247 mμ (ε, 2460). *Anal.* Calcd. for $C_{19}H_{23}O_9N_5S$: C, 45.87; H, 4.66; N, 14.08. Found : C, 45.53; H, 4.44; N, 14.27.

1-Deoxy-1-(7-amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-β-D-glucopyranuronamide (XIV)——A mixture of 0.2 g. of XIII and 8 ml. of anhyd. EtOH saturated with NH_3 at 0° was heated in a glass tube at 80° for 8 hr. On cooling the reaction mixture, crystals separated. Recrystallization from aq. EtOH gave 0.1 g. (76%) of fine needles. m.p. 254~255° (decomp.); $[\alpha]_D^{25}$ −5.4 (c=0.73, H_2O); UV : $\lambda_{max}^{H_2O}$ 280 mμ (ε, 11930); $\lambda_{min}^{H_2O}$ 235 mμ (ε, 3590). *Anal.* Calcd for $C_{10}H_{13}O_5N_7 \cdot H_2O$: C, 36.47; H, 4.59; N, 29.77. Found : C, 36.61; H, 4.23; N, 29.46.

1-Deoxy-1-(6-ethylthio-8-mercapto-9-purinyl)-glucopyranuronamide (X)——One gram of VIII was dissolved in 40 ml. of ethanolic NH_3 saturated at 0° and allowed to stand at room temperature overnight. EtOH and NH_3 were removed *in vacuo* and the residual yellowish powder was recrystallized from water. Pale yellow crystals were obtained in a yield of 75% (0.7 g.). m.p. 221~230° (decomp. with effervescence); $[\alpha]_D^{25}$ +60° (c=1.10, H_2O); UV : $\lambda_{max}^{H_2O}$ 257 mμ (ε, 15600), 330 mμ (ε, 27510), $\lambda_{min}^{H_2O}$ 284 mμ (ε, 6200). *Anal.* Calcd. for $C_{13}H_{17}N_5O_5S_2 \cdot 2H_2O$: C, 36.87; H, 5.00; N, 16.54. Found; C, 36.71; H, 4.84; N, 16.67.

1-Deoxy-1-(6-amino-9-purinyl)-β-D-glucopyranuronamide (XII)——A solution of 425 mg. of X in 10 ml. of 30% NH_2NH_2 was heated on a steam bath for 3 hr. The reaction mixture was evaporated *in vacuo* to dryness. Water addition and evaporation were repeated several times to remove the excess NH_2NH_2. Complete removal of water and NH_2NH_2 gave the residual solid which was dissolved in 25 ml. of water. To this mixture was added 2 ml. of W-7 Raney Ni and refluxed for 1 hr. After another addition of 1 ml. of the Raney Ni, refluxing was continued for further 30 min. The catalyst was filtered and the clear filtrate was concentrated to a small volume (ca. 2 ml.) and left standing in a refrigerator several days. Crystalline crops separated. m.p. 269~270° (decomp.). The yield was 55 mg. (17 % from Compound X). $[\alpha]_D^{28}$ +26.1° (c=1.03, H_2O), while Wolfrom and McWain[4] described m.p. 257~259° (decomp.) and $[\alpha]_D^{22}$ +20° (c=0.30, H_2O). UV : $\lambda_{max}^{H_2O}$ 259 mμ (ε, 15700); $\lambda_{min}^{H_2O}$ 226 mμ (ε, 2640). *Anal.* Calcd. for $C_{11}H_{14}O_5N_6$: C, 42.58; H, 4.55; N, 27.08. Found : C, 42.35; H, 4.72; N, 26.85.

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Summary

Condensation of 4-ethylthio-5-nitro-6-chloropyrimidine with methyl 1-amino-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate was effected to give methyl 1-deoxy-1-(4-ethylthio-5-nitro-6-pyrimidinylamino)-2,3,4-tri-O-acetyl-D-glucopyranuronate (VI). Hydrogenation of nitro group of VI in the presence of platinum-catalyst and subsequent ring closure of 4,5-diamino-derivative (VII) with carbon disulfide were achieved. 6-Ethylthio group of X was replaced to 6-hydrazino group followed by treatment with Raney nickel to afford 1-deoxy-1-(6-amino-9-purinyl)-β-D-glucopyranuronamide (XII). VII was reacted with sodium nitrite to give *v*-triazolo derivative (XIII), 6-ethylthio group of which was readily aminated to yield 1-deoxy-1-(7-amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-β-D-glucopyranuronamide (XIV).

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