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Studies on Amino Sugars. I. Synthesis of 5-Amino-5-deoxyuronic Acid and 5-Amino-5-deoxysaccharic Acid Derivatives from D-Glucuronolactone[1]

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Synthesis of 5-amino-5-deoxyuronic acid and 5-amino-5-deoxysaccharic acid derivatives from D-glucuronolactone is described. 1,2-O-Isopropylidene-D-glucofuranurono-6,3-lactone (I) was oxidized to 5-ulose (II), sodium salt of which was derived to the oxime (IVb). Catalytic hydrogenation of IVb using Adams' catalyst gave crystalline 5-aminated 1,2-O-isopropylidene-hexuronic acid (V) consisting of D-gluco and L-ido isomers. N-Acetylation of V gave each isomer separated as crystals of 6,3-lactone. Acid hydrolysis of the isopropylidene group in 5-acylamino-5-deoxy-1,2-O-isopropylidene-hexofuranurono-6,3-lactones (VI and VII) gave 5-acylamino-5-deoxy-D-glucofuranurono-6,3-lactone (VIII) and 5-acylamino-5-deoxy-L-idofuranurono-6,3-lactone (IX), respectively. Catalytic oxidation of IXa and VIIIa using Adams' catalyst gave 5-acetamido-5-deoxysaccharo-6,3-lactones (XI and XII), and gave 5-acetamido-4,5-dideoxy-L-*threo*-hex-4-enaro-6,3-lactone (XIVa) in the presence of sodium hydrogen carbonate. Some derivatives of XIVa were prepared.

In recent years, many organic chemists and biologists have been interested in 5-amino-5-deoxyhexoses. 5-Amino-5-deoxy-D-glucose (Nojirimycin)[3] and 5-amino-5-deoxy-D-allofuranosyluronic acid (a sugar moiety of Polyoxin analogs)[4] are known as natural antibiotics. Inoue and his co-workers[5] found that synthetic 5-amino-5-deoxy-D-glucono-1,5-lactam and 5-amino-5-deoxy-D-glucaro-1,5-lactam powerfully inhibited β-glucosidase and β-glucuronidase activity, respectively. We took an interest in the remarkable biological activity and chemical behavior of these aminodeoxyuronic and -saccharic acid derivatives and attempted the synthesis of their analogs. The present report is concerned with the synthesis of 5-amino-5-deoxy-1,2-O-isopropylidene-hexuronic acid and 5-amino-5-deoxysaccharic acid derivatives from D-glucuronolactone.

Kinoshita and his co-workers[6] prepared 3-amino-3-deoxy-D-alluronic acid *via* methyl 5-O-acetyl-3-oxo-1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone from 1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone[7] (I). We tried to prepare these 5-amino-5-deoxyuronic and -saccharic acid derivatives *via* 1,2-O-isopropylidene-D-xylo-hexofuranurono-6,3-lactone-5-ulose (II) (namely 5-oxo-1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone) from I. II has been prepared from I by oxidation of the hydroxyl group at C-5 using manganese dioxide,[8] chromium trioxide in acetic acid,[9] or dimethyl sulfoxide-phosphorus pentoxide.[10] Catalytic

1) A part of this work was presented at the 92nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April, 1972.
2) Location: *Takada 3-41-8, Toshima-ku, Tokyo, 171, Japan.*
3) S. Inoue, T. Tsuruoka, T. Ito, and T. Niida, *Tetrahedron*, **23**, 2125 (1968); H. Saeki and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2477 (1968).
4) K. Isono and S. Suzuki, *Tetrahedron Letters*, **1968**, 203.
5) T. Niwa, T. Tsuruoka, S. Inoue, Y. Naito, T. Koeda, and T. Niida, *J. Biochem.* (Tokyo), **72**, 207 (1972).
6) A. Tsuji, T. Kinoshita, and M. Maeda, *Chem. Pharm. Bull.* (Tokyo), **16**, 539 (1968).
7) L.N. Owen, S. Peat, and W.J.G. Jones, *J. Chem. Soc.*, **1941**, 339.
8) H. Weidmann, *Monatsh. Chem.*, **96**, 766 (1965).
9) W. Mackie and A.S. Perlin, *Can. J. Chem.*, **43**, 2921 (1965).
10) K. Onodera, H. Hirano, and N. Kashimura, *Carbohydrate Res.*, **6**, 276 (1968).

oxidation of I[11] or 1,2-O-isopropylidene-D-glucofuranose[12] using Adams' catalyst also gave II. We prepared II in a relatively high yield (62.4%) by the oxidation of I using chromium trioxide in ethyl acetate, in which I was moderately oxidized and II was easily separated from the reaction mixture. No absorption band corresponding to the carbonyl group at C-5 could be observed in the infrared (IR) spectrum of II, but in its nuclear magnetic resonance (NMR) spectrum, two singlets corresponding to *gem*-hydrate form[8] ($C\langle^{OH}_{OH}$) of the carbonyl group was observed at δ 7.37 and 7.19 measured in Me_2SO-d_6. II was liable to react with hydrazine

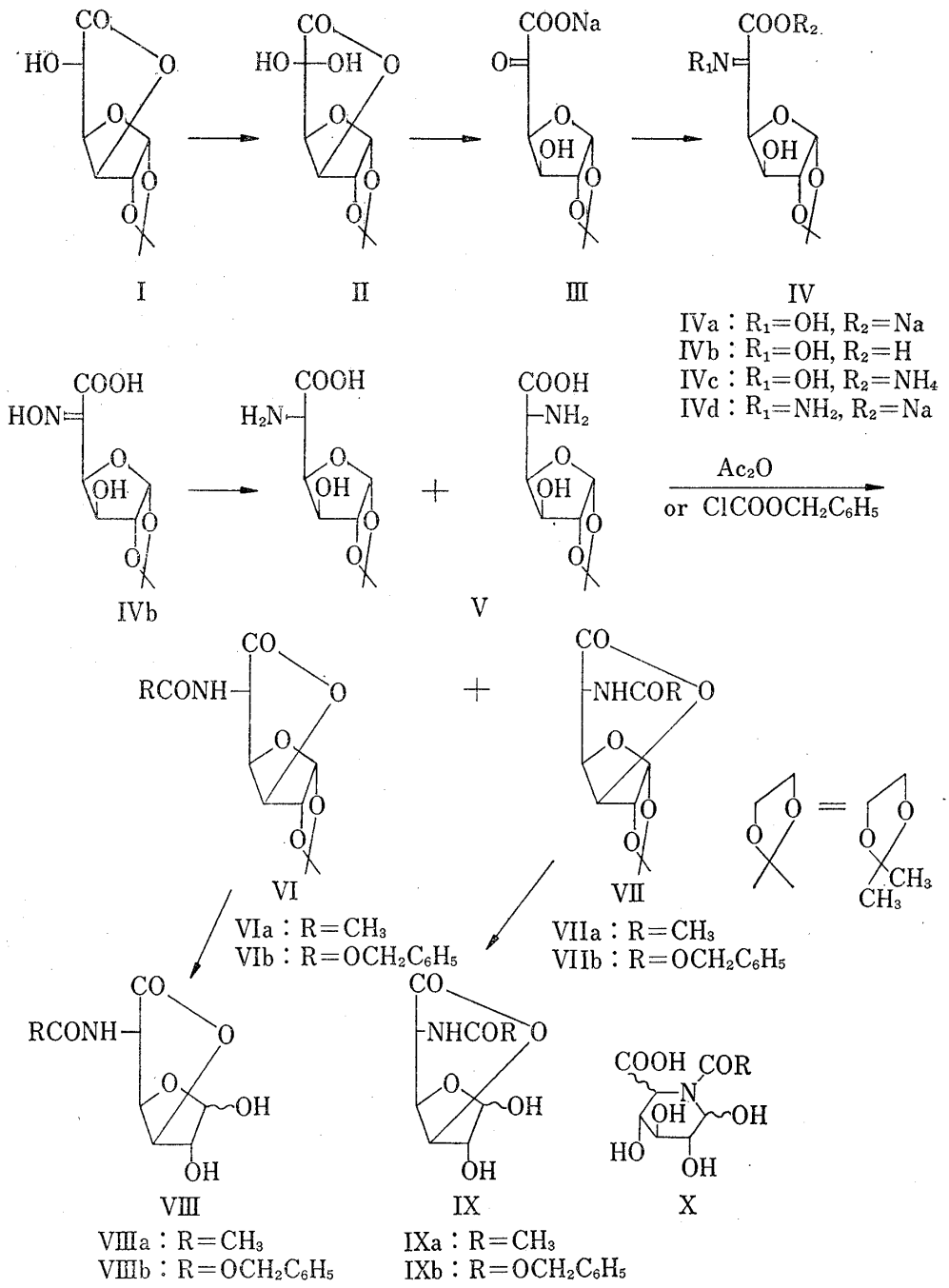


Chart 1

11) K. Heyns, E. Alpers, and J. Weyer, *Chem. Ber.*, **101**, 4209 (1968).
12) T. Olof, Ger. Patent 1812033 (1970) [*C.A.*, **72**, 121875 (1970)].

or phenylhydrazine to form 5-hydrazino derivatives,[13] and with amino compounds to form unstable products in some cases because of the two reactive carbonyl groups at C-5 and adjacent C-6. Actually, we failed to prepare the oxime and hydrazone of II as pure crystals. Treatment of II with 10% aqueous solution of sodium hydroxide gave sodium 5-oxo-1,2-O-isopropylidene-D-glucofuranuronate (III), which was obtained as a white powder after evaporation of the aqueous solution and showed IR absorption bands due to carbonyl stretching vibration at 1720 and 1630 cm^{-1} indicating a ketone form at C-5. Treatment of the aqueous solution of III with hydroxylamine or hydrazine hydrate gave a crystalline oxime (IVa) and hydrazone (IVd) of III, respectively. 5-Hydroxyimino-5-deoxy-1,2-O-isopropylidene-D-glucofuranuronic acid (IVb) was obtained by deionization of IVa with dry Amberlite IR-120 (H^+) in methanol, and subsequent treatment of IVb with dry ammonia gave its ammonium salt (IVc). Catalytic hydrogenation of IVa-d at an atmospheric pressure using Adams' catalyst afforded aminated substances, which could not be separated as pure crystals. On paper chromatogram in a solvent (*n*-butanol–acetic acid–water=4: 1: 1), there were many spots of unknown compounds positive to the ninhydrin reagent. Catalytic hydrogenation of IVb in methanol under high pressure using the same catalyst gave 5-amino-5-deoxy-1,2-O-isopropylidene-hexuronic acid (V) as fine needle crystals. V consisted of two isomers, which were 5-amino-5-deoxy-1,2-O-isopropylidene-D-glucofuranuronic acid and 5-amino-5-deoxy-1,2-O-isopropylidene-L-idofuranuronic acid. It was difficult to separate these isomers from each other by a usual method. Recrystallized pure V moved as a single spot on paper chromatogram [*Rf* 0.59 (*tert*-butanol–methyl ethyl ketone–formic acid–water=8: 6: 3: 3), *Rf* 0.48 (*n*-butanol–acetic acid–water=4: 1: 5), visualized with the ninhydrin reagent]. N-Acetylation of V with acetic anhydride in water gave 5-acetamido-5-deoxy-1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone (VIa) and 5-acetamido-5-deoxy-1,2-O-isopropylidene-L-idofuranurono-6,3-lactone (VIIa), both separated as crystals. A crystalline precipitate of VIIa separated out from the reaction mixture and VIa was obtained from the mother liquor by column chromatography on silica gel by eluting the column with benzene containing methanol. The ratio of the yield of VIIa to VIa was about 3: 1. Treatment of V with benzyloxycarbonyl chloride and sodium hydroxide gave the corresponding N-benzyloxycarbonyl isomers, 5-benzyloxycarbonylamino-5-deoxy-1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone (VIb) and 5-benzyloxycarbonylamino-5-deoxy-1,2-O-isopropylidene-L-idofuranurono-6,3-lactone (VIIb). The structure of each separated isomer was characterized by NMR, optical rotatory dispersion (ORD), and circular dichroism (CD) spectra. In ORD spectra, a positive Cotton effect was observed for VIa and VIb (D-gluco isomer) having a peak at 231 nm and 233 nm, respectively, and this effect was not observed in VIIa and VIIb (L-ido isomer). In CD spectra, each of D-gluco isomers had a plus maximum peak at 221 nm (VIa) and 224 nm (VIb), and each of L-ido isomers had a minus peak at 229 nm (VIIa) and 235 nm (VIIb). These results were consistent with a sign of the Cotton effect of sugar lactones reported by Okuda and his co-workers.[14] Acid hydrolysis of 5-acylamino-5-deoxy-1,2-O-isopropylidene-hexofuranurono-6,3-lactones (VIa,b and VIIa,b) with Amberlite IR-120 (H^+) gave the corresponding 5-acylamino-5-deoxy-hexofuranurono-6,3-lactones (VIIIa,b and IXa,b). On paper and thin-layer chromatograms, VIIIa,b and IXa,b were detected as a pale yellow spot by *p*-anisidine reagent. The lactone in VIIIa,b and IXa,b was difficult to be hydrolyzed with Amberlite IR-120 (H^+) to carboxylic acid. Therefore transformation of a furanose into pyranose structure [namely N-acylpiperidinose form[15] (X)] was not observed in this condition.

Catalytic oxidation of VIIIa and IXa using Adams' catalyst gave two kinds of 5-acetamido-hexosaccharo-6,3-lactone from each other under different conditions. VIIIa and IXa were

13) H. Paulsen and H. Kuhn, *Carbohydrate Res.*, **13**, 289 (1970).
14) T. Okuda, S. Harigaya, and A. Kiyomoto, *Chem. Pharm. Bull.* (Tokyo), **12**, 504 (1964).
15) H. Paulsen and K. Todt, *Adv. Carbohydrate Chem.*, **23**, 105 (1968); H. Paulsen, *Angew. Chem.*, **78**, 495 (1966).

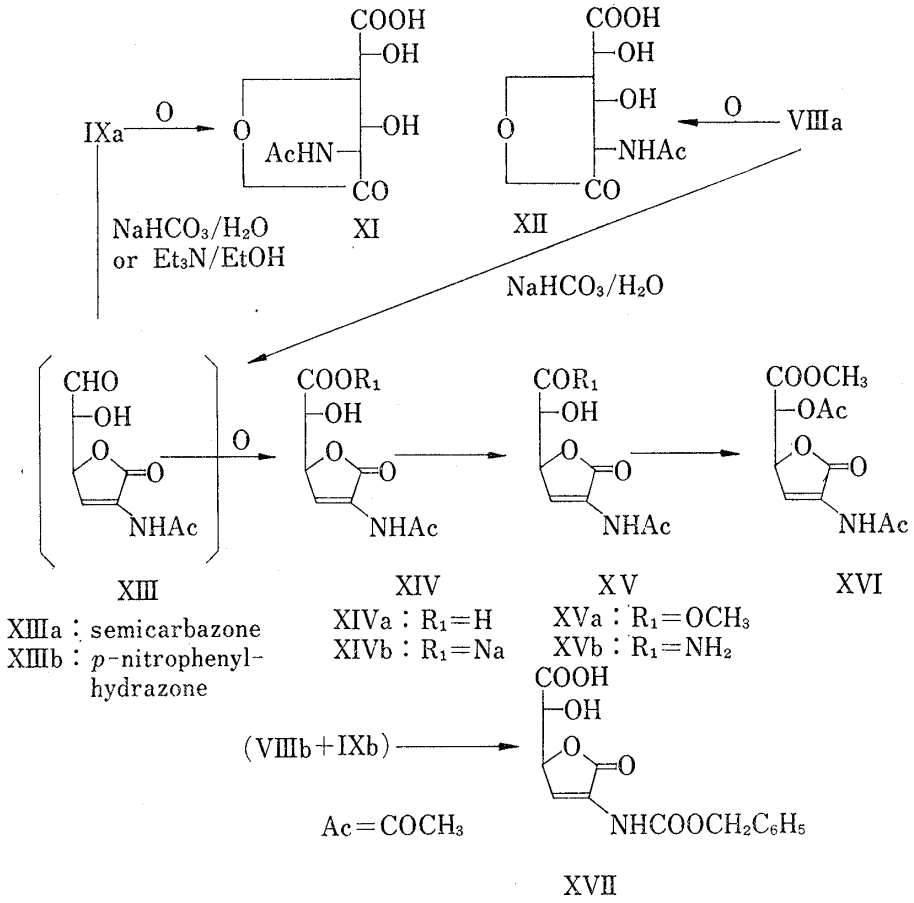


Chart 2. Catalytic Oxidation of 5-Acylamino-5-deoxyuronic Acids (VIII and IX)

respectively oxidized to the corresponding 5-acetamido-5-deoxy-D-glucosaccharo-6,3-lactone (XII) as a syrup and 5-acetamido-5-deoxy-L-idosaccharo-6,3-lactone (XI) as crystals by the condition that the reaction solution changed gradually from neutral in the first time to acid state by saccharic acid formed. In the presence of sodium hydrogen carbonate, both VIIIa and IXa were converted to 5-acetamido-4,5-dideoxy-L-*threo*-hex-4-enaro-6,3-lactone (XIVa) because of β-elimination at C-4 and loss of hydrogen located at C-5. XIVa is a saccharolactone having α,β-unsaturated lactone ring in the structure and this was supported

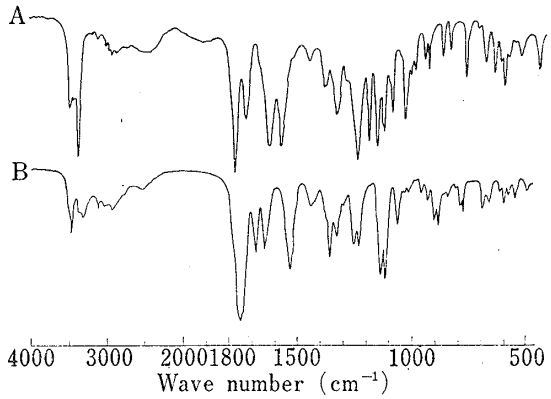


Fig. 1

A: IR spectrum of XI (KBr tablet)
B: IR spectrum of XIVa (KBr tablet)

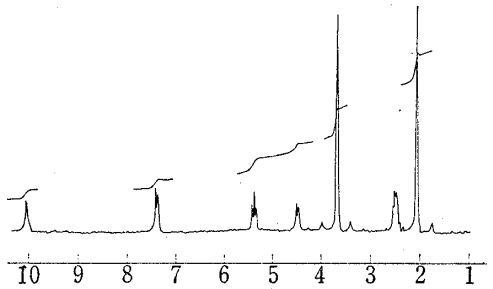
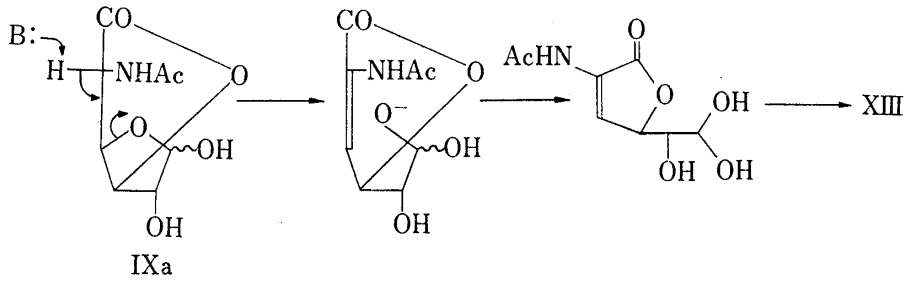


Fig. 2. NMR Spectrum of XVa (Me_2SO-d_6)

by the characteristic ultraviolet (UV) absorption at 247 nm corresponding to the double bond conjugated with carbonyl group at C-6.

Treatment of XIVa with diazomethane gave a methyl ester (XVa) and the presence of α,β-unsaturated lactone ring in the structure was supported by its NMR spectrum exhibiting no signal corresponding to H-5 and with the signal of an amide proton changed to a singlet shifting to the lower field on account of the double bond. Treatment of XVa with ammonia in methanol gave an amide derivative (XVb). Acetylation of XVa with acetic anhydride and pyridine gave 2-O-acetyl-5-acetamido-4,5-dideoxy-L-*threo*-hex-4-enaro-6,3-lactone methyl ester (XVI).

In the course of the oxidation of VIIIa and IXa in the presence of sodium hydrogen carbonate, there should have been an intermediate (XIII) having an unsaturated lactone ring and an aldehyde group. It was assumed that a nucleophilic hydroxyl ion attacked the reactive proton located at C-5 in VIIIa and IXa and then β-elimination occurred at C-4, that is, the furanose ring in VIIIa and IXa should be cleaved to give an aldehyde group or its hydrate form in XIII, which was subsequently oxidized to carboxyl group.



Ac=$COCH_3$ B=base

Chart 3

In order to estimate XIII, the following examinations were carried out. A solution of IXa in 0.01 M solution of sodium hydrogen carbonate showed an increasing absorption intensity at 244 nm in its UV spectrum. The solution of IXa dissolved in deuterium oxide and warmed at 60° showed new signals in its NMR spectrum different from that of IXa and by comparison of the spectrum before and after warming, the shifts of clearly different signals from IXa were at δ 7.51 (doublet), 3.70—3.87 (quartet), and 2.20 (singlet). It was thought that IXa was converted to XIII which showed the signals due to H-4, H-2, and methyl proton of N-acetyl group. On thin-layer chromatogram using silica gel, XIII moved as a single spot *Rf* 0.66, sprayed with ammonium metavanadate-sulfuric acid reagent) developed with *n*-butanol–acetic acid–water (4:1:5). Treatment of IXa with triethylamine in ethanol also gave XIII and that with *p*-nitrophenylhydrazine or semicarbazide gave the corresponding *p*-nitrophenylhydrazone (XIIIb) and semicarbazone (XIIIa). IR spectra of XIIIa and XIIIb showed absorptions of the carbonyl stretching vibration of the unsaturated lactone at 1761 and 1767 cm^{-1}, respectively. Catalytic oxidation of a mixture of VIIIb and IXb over Adams' catalyst in the presence of sodium hydrogen carbonate gave 5-benzyloxycarbonylamino-4,5-dideoxy-L-*threo*-hex-4-enaro-6,3-lactone (XVII).

Experimental[16]

1,2-O-Isopropylidene-D-xylo-hexofuranurono-6,3-lactone-5-ulose Hydrate (II)——CrO_3 (20 g) was gradually added in small portions to the solution of I (20 g) in AcOEt (200 ml) under stirring and cooling with H_2O for 2 hr. The solution was stirred at room temperature and allowed to stand overnight. The dark

16) All melting points are uncorrected. All evaporations were carried out under a reduced pressure, keeping the bath temperature below 40°. The NMR spectrum was measured at 60 MHz, using tetramethylsilane or 3-(trimethylsilyl)-propanesulfonic acid sodium salt as an internal standard.

reaction mixture was filtered and the black cake was extracted with hot acetone (100 ml). The mixture of filtrate and extract was decolorized with charcoal at 50°. The clear filtrate was evaporated and the crystals that separated were recrystallized from AcOEt to II (13.4 g, 62.4%) as colorless needles, mp 157—158°. *Anal.* Calcd. for $C_9H_{12}O_7$: C, 46.56; H, 5.21. Found: C, 46.23; H, 5.18.

Sodium 5-Oxo-1,2-O-isopropylidene-D-glucofuranuronate (III)——To a solution of II (2.3 g) dissolved in H_2O (60 ml), 10% NaOH (4 ml) was added dropwise, maintaining the pH between 8 and 9 at room temperature. The resultant solution (pH 8) was evaporated to dryness to give III (2.6 g) as a white powder. IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O), 1630 (COONa); lactone absorption was not observed.

Sodium 5-Oxo-1,2-O-isopropylidene-D-glucofuranuronate Oxime (IVa)——The solution of III was prepared from II (10.0 g) and 10% NaOH solution (16 ml) under the same condition as above. To the resultant solution, $NaHCO_3$ (5.4 g) was added and $NH_2OH \cdot HCl$ (4.5 g) was added gradually. The solution was warmed at 45° for 3 hr after CO_2 evolution ceased and allowed to stand overnight at room temperature. Evaporation of the solution gave the crude IVa, which contained NaCl. It was washed with MeOH and recrystallized from H_2O–MeOH to IVa (7.7 g, 66.4%) as colorless leaflets, mp 246° (decomp.). *Anal.* Calcd. for $C_9H_{12}O_7NNa$: C, 40.16; H, 4.49; N, 5.20. Found: C, 40.44; H, 4.92; N, 5.23.

5-Hydroxyimino-5-deoxy-1,2-O-isopropylidene-D-glucofuranuronic Acid (IVb)——IVa (3.0 g) was suspended in MeOH (50 ml) and stirred with dry Amberlite IR-120 (H^+) (6.0 g) at room temperature until IVa dissapeared. The resin was filtered off and the clear filtrate was concentrated to a white solid (2.4 g). Recrystallization from ether gave IVb as colorless needles, mp 152—153° (decomp.). *Anal.* Calcd. for $C_9H_{13}O_7N$: C, 43.73; H, 5.30; N, 5.67. Found: C, 43.91; H, 5.21; N, 5.75.

Ammonium 5-Hydroxyimino-5-deoxy-1,2-O-isopropylidene-D-glucofuranuronate (IVc)——MeOH solution of IVb prepared from IVa (10.0 g) and Amberlite IR-120 (H^+) (20 g), as above, was cooled in an ice bath. Dry NH_3 was introduced into the solution and the resulting weak alkaline solution was evaporated to dryness. Recrystallization from EtOH–MeOH (1: 1) mixture gave IVc (8.6 g, 87.6%) as colorless needles, mp 185° (decomp.). *Anal.* Calcd. for $C_9H_{16}O_7N_2$: C, 40.91; H, 6.10; N, 10.60. Found: C, 40.94; H, 6.06; N, 10.55.

Sodium 5-Oxo-1,2-O-isopropylidene-D-glucofuranuronate Hydrazone (IVd)——$H_2NNH_2 \cdot H_2O$ (83%, 2.5 g) was added to the solution of III prepared from II (2.5 g) and 10% NaOH solution (4 ml). The mixture was heated at 90° for 2 hr, allowed to stand overnight at room temperature, and evaporated to dryness. The resultant white solid was recrystallized from aqueous EtOH to give IVd (1.4 g, 48.5%) as colorless needles, mp 270° (decomp.). *Anal.* Calcd. for $C_9H_{13}O_6N_2Na$: C, 40.31; H, 4.89; N, 10.44. Found: C, 40.40; H, 5.12; N, 10.49.

5-Amino-5-deoxy-1,2-O-isopropylidene-D-glucofuranuronic Acid and 5-Amino-5-deoxy-1,2-O-isopropylidene-L-idofuranuronic Acid (V)——The solution of IVb in MeOH (100 ml), prepared from IVa as above, was hydrogenated catalytically over PtO_2 (0.3 g) under the pressure in a 300 ml-autoclave at 40—60° for 4 hr. The initial pressure of H_2 was 75 kg/cm^2 at 25°. The reaction mixture was decolorized with charcoal and evaporated to a white solid. Recrystallization from aqueous EtOH gave V (1.8 g, 41.5%) as colorless needles, mp 195° (decomp.). *Anal.* Calcd. for $C_9H_{15}O_6N$: C, 46.35; H, 6.48; N, 6.01. Found: C, 46.47; H, 6.57; N, 5.99.

5-Acetamido-5-deoxy-1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone (VIa) and 5-Acetamido-5-deoxy-1,2-O-isopropylidene-L-idofuranurono-6,3-lactone (VIIa)——To the solution of V (8.5 g) in H_2O (20 ml), 10% NaOH solution (17 ml) was added and followed by dropwise addition of Ac_2O (11 g) through a dropping funnel during 40 min under ice cooling and the mixture was stirred for 2 hr at room temperature. VIIa was separated from the reaction mixture by filtration, the mother liquor was passed through a column of Amberlite IR-120 (H^+) (50 ml), and the eluate was evaporated to a syrup. VIIa was also separated from EtOH solution of the syrup. Recrystallization of VIIa obtained from the above treatment from EtOH gave 3.0 g of colorless needles, mp 184—186° (decomp.), $[\alpha]_D^{22}$ +108.8° (c=0.5, dioxane). *Anal.* Calcd. for $C_{11}H_{15}O_{16}N$: C, 51.36; H, 5.88; N, 5.44. Found: C, 51.26; H, 5.81; N, 5.49. NMR (10% solution in Me_2SO-d_6) δ: 8.91 (1H, doublet, NH, J=7.8 cps), 5.2—4.8 (3H, multiplet, H-2 H-3 H-4), 3.90 (1H, doublet, H-5), 1.84 (3H, singlet, CH_3 of N-acetyl group).

The filtrate left after separation of VIIa was chromatographed over a column of silica gel and the column was eluted with 4% MeOH–benzene. VIa (1.05 g) and diacetyl derivative (0.3 g) were separated. VIa was recrystallized from EtOH, mp 166—167.5° (decomp.), $[\alpha]_D^{22}$ +68.1° (c=0.4, dioxane). *Anal.* Calcd. for $C_{11}H_{15}O_6N$: C, 51.36; H, 5.88; N, 5.44. Found: C, 51.26; H, 5.81; N, 5.49. NMR (10% solution in Me_2SO-d_6) δ: 8.42 (1H, doublet, NH, J=7.2 cps), 5.22 (1H, quartet, H-5, J=4.5 cps), 5.20—4.75 (3H, multiplet, H-2 H-3 H-4), 1.92 (3H, singlet, CH_3 of N-acetyl group).

We estimated the diacetyl derivative to be 3-O-acetyl-5-acetamido-5-deoxy-1,2-O-isopropylidene-D-gluco (or L-ido)furanuronic acid from NMR spectrum and elemental analysis (*Anal.* Calcd. for $C_{13}H_{19}O_8N$: C, 49.21; H, 6.04; N, 4.41. Found: C, 48.92; H, 6.00; N, 4.43). VIa: ORD (c=0.4, dioxane) $[\alpha]^{27}$ (nm): +2689° (210), +2169° (218) (trough), +2594° (231) (peak), +755° (300), +340° (400). CD (c=0.4, dioxane) $[\theta]^{25}$ (nm): +45 (221) (positive maximum). VIIa: ORD (c=0.4, dioxane) $[\alpha]^{27}$ (nm): +1352° (250), +812° (300), +367° (400), +217° (500). CD (c=0.4, dioxane) $[\theta]^{25}$ (nm): −28 (229) (negative maximum).

5-Benzyloxycarbonylamino-5-deoxy-1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone (VIb) and 5-Benzyloxycarbonylamino-5-deoxy-1,2-O-isopropylidene-L-idofuranurono-6,3-lactone (VIIb)——To the solution of V (20 g) in H_2O (200 ml), 10% NaOH solution (50 ml) was added, then benzyloxycarbonyl chloride (29.4 g) in dioxane (80 ml) was added dropwise through a dropping funnel, with vigorous stirring at room temperature, maintaining the alkaline condition by the dropwise addition of 10% NaOH solution. The reaction mixture was stirred for 4 hr after completion of addition and extracted with AcOEt. The extract was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave a syrup. VIIb was separated from EtOH solution of the syrup and recrystallized from EtOH to colorless needles (6.0 g), mp 150—153°, $[\alpha]_D^{22}$ +141° (c=1, dioxane). *Anal.* Calcd. for $C_{17}H_{19}O_7N$: C, 58.45; H, 5.48; N, 4.01. Found: C, 58.05; H, 5.36; N, 4.11.

The aqueous layer left after extraction with AcOEt was acidified with 10% HCl solution (70 ml) and the oily substance that separated was extracted with AcOEt. The extract was evaporated to a syrup by the same treatment as above. The crystals, a mixture of VIIb and VIb, were separated from EtOH solution of the syrup. Recrystallization from EtOH gave (3.2 g) of the mixture of VIIb and VIb as colorless needles, mp 156—158°. *Anal.* Calcd. for $C_{17}H_{19}O_7N$: C, 58.45; H, 5.48; N, 4.01. Found: C, 58.25; H, 5.29; N, 4.20. The pure VIb was separated by the same treatment as N-acetylation, as colorless needles, mp 131—132°, $[\alpha]_D^{22}$ +128.8° (c=0.4, dioxane). *Anal.* Found: C, 58.30; H, 5.36; N, 4.12. VIIb: ORD (c=0.4, dioxane) $[\alpha]^{22}$ (nm): +1505° (230), +671° (246) (trough), +718° (260 (peak), +509° (300), +241° (400). CD (c=0.4, dioxane) $[\theta]^{25}$ (nm): −58 (negative maximum). VIb: ORD (c=0.4, dioxane) $[\alpha]^{22}$ (nm): +108.2° (220), +1126° (233) (peak), +325° (300), +160° (400). CD (c=0.4, dioxane) $[\theta]^{25}$ (nm): +34 (224) (positive maximum).

5-Acetamido-5-deoxy-L-idofuranurono-6,3-lactone (IXa) and 5-Acetamido-5-deoxy-D-glucofuranurono-6,3-lactone (VIIIa)——VIIa (0.3 g) suspended in H_2O (30 ml) was stirred with Amberlite IR-120 (H^+) for 3 hr at 60—70°. The resin was removed by filtration and the filtrate was evaporated to a syrup. IXa was separated from EtOH solution of the syrup and recrystallized from EtOH to (0.2 g, 79.1%) of colorless needles, mp 173—174° (decomp.), $[\alpha]_D^{22}$ +152.5° (c=0.5, H_2O). IR ν_{max}^{KBr} cm^{-1}: 1762 (lactone), 1634 (amide-I), 1548 (amide-II). *Anal.* Calcd. for $C_8H_{11}O_6N$: C, 44.24; H, 5.11; N, 6.45. Found: C, 44.11; H, 5.08; N, 6.43.

The same method was used for VIa (1.3 g) to give VIIIa (1.1 g) as a syrup. IR ν_{max}^{KBr} cm^{-1}: 1775 (lactone), 1645 (amide-I), 1525 (amide-II) (measured as a dried sirup).

5-Benzyloxycarbonylamino-5-deoxy-L-idofuranurono-6,3-lactone (IXb) and 5-Benzyloxycarbonylamino-5-deoxy-D-glucofuranurono-6,3-lactone (VIIIb)——The solution of VIIb (4.0 g) in 50% aqueous dioxane was stirred vigorously with Amberlite IR-120 (H^+) (10 ml) for 16 hr at 75—80°. The resin was removed by filtration and the filtrate was decolorized with charcoal. The clear solution was evaporated to dryness and the resulting white solid was recrystallized from hot H_2O to IXb as colorless leaflets (2.8 g, 79.1%), mp 147—151°, $[\alpha]_D^{22}$ +87.7° (c=0.5, dioxane). *Anal.* Calcd. for $C_{14}H_{15}O_7N$: C, 54.37; H, 4.49; N, 4.53. Found: C, 54.13; H, 4.78; N, 4.71.

The same method as above was used for VIb containing a little VIIb. VIIIb (2.2 g, 49.7%), which contained a small quantity of IXb, was obtained from VIb (5.0 g) (contained a small quantity of VIIb), mp 172—173°. *Anal.* Found: C, 54.13; H, 4.70; N, 4.58.

5-Acetamido-5-deoxy-L-idosaccharo-6,3-lactone (XI) and 5-Acetamido-5-deoxy-D-glucosaccharo-6,3-lactone (XII)——IXa (0.5 g) was dissolved in H_2O (30 ml) and oxidized catalytically at 60—70° for 6 hr by bubbling of O_2 stream in the presence of Pt, which was prepared by reducing PtO_2 (0.4 g) in H_2 at atmospheric pressure for 30 min. The reaction mixture was decolorized with charcoal, filtered, and the filtrate was evaporated to dryness, which solidified. Recrystallization from EtOH–H_2O gave XI (235 mg, 43.8%) as colorless needles, mp 210—215° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 1767 (lactone), 1712 (COOH), 1613 (amide-I), 1562 (amide-II). *Anal.* Calcd. for $C_8H_{11}O_7N$: C, 41.21; H, 4.76; N, 6.01. Found: C, 40.90; H, 4.59; N, 5.89.

Similarly, XII (1.1 g) was prepared as an acidic syrup by oxidation of VIIIa (1.2 g) but it was not separated as crystals.

5-Acetamido-4,5-dideoxy-L-*threo*-hex-4-enaro-6,3-lactone (XIVa)——IXa (0.5 g) dissolved in H_2O (30 ml) was oxidised catalytically at 60—70° by bubbling of O_2 stream in the presence of Pt catalyst prepared from PtO_2 (0.4 g). The solution was maintained at pH 7—8 by dropwise addition of 5% $NaHCO_3$ solution during oxidation. After 5 hr, the reaction mixture was filtered with charcoal and the filtrate was passed through a column of Amberlite IR-120 (H^+) (20 ml). The effluent was evaporated to dryness and the separated crystals were recrystallized from hot H_2O to XIVa (0.20 g, 40.4%) as colorless granules, mp 250° (decomp.), $[\alpha]_D^{22}$ +152.5° (c=0.5, H_2O). IR ν_{max}^{KBr} cm^{-1}: 1748 (lactone), 1648 (COOH), 1643 (amide-I), 1515 (amide-II). UV $\lambda_{max}^{H_2O}$ $m\mu(\varepsilon)$: 247 (9100). *Anal.* Calcd. for $C_8H_9O_6N$: C, 44.66; H, 4.22; N, 6.51. Found: C, 44.55; H, 4.25; N, 6.37.

Similarly XIVa (245 mg, 43.8%) was prepared from VIIIa (0.6 g) under the same condition.

Sodium Salt (XIVb) of XIVa——XIVa (112 mg) dissolved in H_2O (5 ml) was neutralized with $NaHCO_3$ (44 mg) and the weak alkaline solution was evaporated to dryness. The resulting crystals were recrystallized from EtOH–H_2O to XIVb (87 mg, 61.2%) as colorless leaflets, mp 171° (decomp.). IR ν_{max}^{KBr} cm^{-1}:

1770 (lactone), 1635 (amide-I), 1595 (COONa), 1538 (amide-II). *Anal.* Calcd. for $C_8H_8O_6NNa \cdot 2H_2O$: C, 35.17; H, 4.43; N, 5.13. Found: C, 35.07; H, 4.04; N, 5.13.

5-Acetamido-4,5-dideoxy-L-*threo*-hex-4-enaro-6,3-lactone Methyl Ester (XVa)——XIVa (0.1 g) was suspended in MeOH (10 ml), and a solution of CH_2N_2 in ether was added dropwise until the MeOH solution changed to a pale yellow color. The reaction mixture was evaporated to dryness and MeOH (10 ml) was added to this residue. This procedure was repeated three times until no more CH_2N_2 was consumed. The residual solid was recrystallized from EtOH to XVa (0.10 g, 93.9%) as colorless needles, mp 150—153°. NMR (10% solution in Me_2SO-d_6) δ: 10.1 (1H, singlet, NH), 7.36 (1H, doublet, H-4, J=2.4 cps), 5.37 (1H, triplet, H-3), 4.48 (1H, doublet, H-2, J=3.0 cps), 3.68 (3H, singlet, CH_3 of ester), 2.05 (3H, singlet, CH_3 of N-acetyl group). *Anal.* Calcd. for $C_9H_{11}O_6N$: C, 47.17; H, 4.84; N, 6.11. Found: C, 46.97; H, 4.77; N, 6.29.

2-O-Acetyl-5-acetamido-4,5-dideoxy-L-*threo*-hex-4-enaro-6,3-lactone Methyl Ester (XVI)——XVa (0.1 g) was acetylated with Ac_2O (2 ml) and pyridine (1 ml). After standing overnight, the reaction mixture was evaporated to a syrup and it was extract with $CHCl_3$. The extract was washed with 5% $NaHCO_3$ solution, dried over anhyd. Na_2SO_4, and evaporated to a syrup. XVI was separated from EtOH solution of the syrup and recrystallized from EtOH–petr. ether to colorless leaflets (110 mg, 93.1%), mp 107—109°. *Anal.* Calcd. for $C_{11}H_{13}O_7N$: C, 48.71; H, 4.83; N, 5.16. Found: C, 48.46; H, 4.75; N, 5.09.

Amide (XIVb) from XVa——XVa (51 mg) was suspended in EtOH (2 ml) and dry NH_3 was introduced at 0—5° until the solution became clear. After 3 hr, the solution was evaporated to a syrup. The crystals that separated from EtOH solution of the syrup were recrystallized from EtOH to XIVb (24 mg, 50.0%) as colorless needles, mp 209—211° (decomp.). *Anal.* Calcd. for $C_8H_{10}O_5N_2$: C, 44.86; H, 4.71; N, 13.07. Found: C, 44.58; H, 4.71; N, 12.74.

5-Benzyloxycarbonylamino-4,5-dideoxy-L-*threo*-hex-4-enaro-6,3-lactone (XVII)——Catalytic oxidation of VIIIb+IXb (a mixture of about 1 : 1) (0.6 g) over PtO_2 (0.4 g) gave XVII (220 mg, 36.9%) under the same condition and treatment as for XIVa. Recrystallization from EtOH–H_2O gave needle crystals, mp 197—200° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 1788, 1745 (lactone), 1719 (COOH), 1664 (amide-I), 1547 (amide-II). UV λ_{max}^{MeOH} nm(ε): 207 (9400), 242 (9700). *Anal.* Calcd. for $C_{14}H_{13}O_7N$: C, 54.73; H, 4.26; N, 4.56. Found: C, 54.44; H, 4.08; N, 4.71.

Semicarbazone (XIIIa) and *p*-Nitrophenylhydrazone (XIIIb) of the Intermediate (XIII)——To the solution of IXa (0.5 g) in EtOH (10 ml), 3 drops of NEt_3 was added at room temperature. The solution was evaporated to a syrup after standing overnight. To the solution of the syrup in EtOH, $H_2NCONHNH_2 \cdot HCl$ and AcONa or *p*-nitrophenylhydrazine was added. The mixture was refluxed for 10 min and allowed to stand overnight. XIIIa or XIIIb was separated from the reaction mixtures directly. XIIIa: Yellow powder from H_2O, mp 213—216° (decomp.). *Anal.* Calcd. for $C_9H_{12}O_5N_4$: C, 42.19; H, 4.72; N, 21.87. Found: C, 41.92; H, 4.64; N, 21.51. XIIIb: Brown needles from aqueous EtOH, mp 210—214° (decomp.). *Anal.* Calcd. for $C_{14}H_{14}O_6N_4$: C, 50.30; H, 4.22; N, 16.76. Found: C, 50.24; H, 4.02; N, 16.69.

NMR Spectrum of the Intermediate XIII——IXa (50 mg) was dissolved in D_2O (0.5 ml) and $NaHCO_3$ (5 mg) was added. The solution was warmed at 60° for 15 min, and the color of the solution changed gradually to yellow. The reaction mixture was measured. δ: 7.51 (doublet, H-4 of XIII), 4.01—5.60 (multiplet, the shape of signals were different from these of IXa), 3.70—3.87 (quartet, H-2 of XIII), 2.20 (singlet, CH_3 of N-acetyl group of XIII), 2.08 (singlet, CH_3 of N-acetyl group of IXa). The relative intensities of the singlets at δ 2.20 and 2.08 suggested that the ratio of XIII to IXa was about 10 : 7.

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