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3-Oxoglucuronic Acid. III.[1] Synthesis of 3-Amino-3-deoxy-D-allouronic Acid

Akio Tsuji, Toshio Kinoshita, and Masako Maeda

School of Pharmaceutical Sciences, Showa University[2]

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3-Amino-3-deoxy-D-allouronic acid hydrochloride (VII) was synthesized. Barium 3-oxo-1,2-O-isopropylidene-D-glucofuranuronate (IV) was converted into the corresponding phenylhydrazone (V) which was hydrogenated to give the 3-amino compound (VI). Acidic hydrolysis of VI gave VII crystalline.

The oxime (IX) of methyl 5-O-acetyl-3-oxo-1,2-O-isopropylidene-D-glucofuranuronate (VIII) was found to undergo stereospecific reduction with lithium aluminum hydride to give 3-amino-3-deoxy-D-allose derivative (IIIb[14]) predominantly.

Displacement at C-3 of methyl 5-O-acetyl-3-O-methanesulfonyl-1,2-O-isopropylidene-D-glucofuranuronate (Ib) with sodium azide and subsequent catalytic hydrogenation gave methyl 5-O-acetyl-3-amino-3-deoxy-1,2-O-isopropylidene-D-allofuranuronate (IIb) in low yield.

The previous papers[1,3] described the investigation on 3-oxo-D-glucuronic acid. In the course of the work, 3-unsubstituted (*e.g.* Ia) and 3-oxo (*e.g.* IV, VIII) derivatives of D-glucuronic acid have been prepared. These substances were expected to be useful intermediates for synthesis of aminated uronic acids. Aminodeoxyuronic acids have often been supposed[4] to exist in natural substances and several of them have been isolated. Webster and coworkers[5,6] have demonstrated the presence of aminohexuronic acid in Vi-antigen of *E. Coli* and this acid was afterwards proven[7] to be 2-amino-2-deoxy-D-galacturonic acid.[8] Neuraminic acid,[9] an important component of glycoproteins, has been shown to have a polyhydroxyamino acid structure. Gougerotin,[10] a broad spectrum antibiotic of recent discovery, is claimed to be a nucleoside containing 4-amino-4-deoxy-D-galacturonic acid moiety.[11] Since aminodeoxyuronic acids have been found in substances possessing remarkable biological activity, it would seem to be of interest to prepare these sugars and investigate their chemical behavior. The present report is concerned with the synthesis of 3-amino-3-deoxy-D-allouronic acid. This amino sugar is of particular interest in that it is a 5-carboxy-derivative of 3-amino-3-deoxy-D-ribose which is a constituent of puromycin, one of the most powerful antitumor antibiotics.

Treatment of methyl 5-O-acetyl-1,2-O-isopropylidene-D-glucofuranuronate[3] (Ia) with methanesulfonyl chloride in pyridine gave the corresponding 3-O-methanesulfonyl derivative (Ib) in excellent yield. By heating Ib with sodium azide in dimethylformamide, there was obtained a glassy substance (IIa) having the characteristic azido group absorption at 2160 cm^{-1}.

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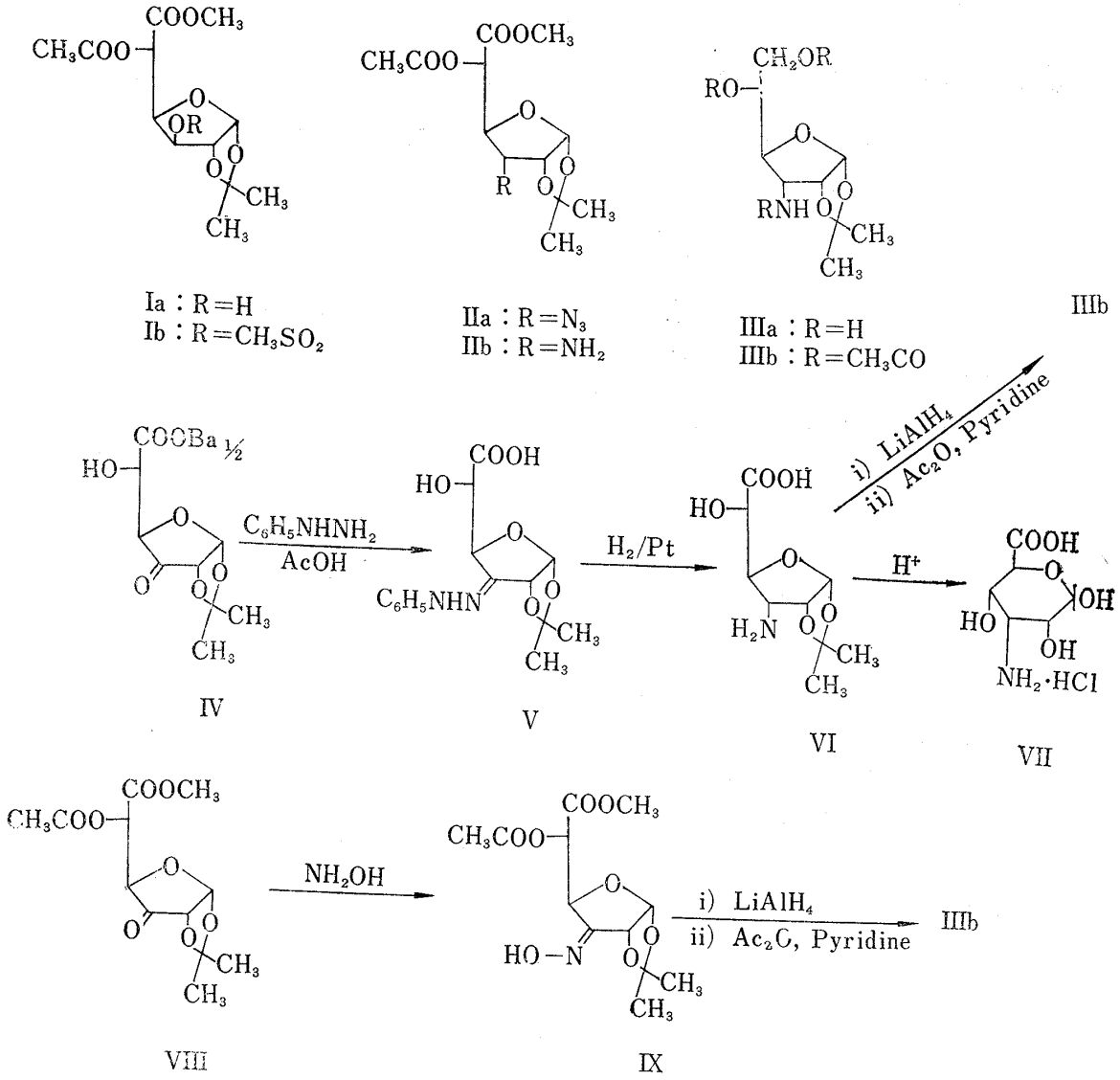

Catalytic hydrogenation of this product using Adams' catalyst afforded a material (IIb) showing amino absorption at 1640 cm^{-1} and no absorption of azido group.

IIb was converted into the known 3-acetamido-3-deoxy-5,6-di-O-acetyl-1,2-O-isopropylidene-D-allofuranose (IIIb) by means of lithium aluminum hydride reduction followed by acetylation. IIb was accordingly assigned as methyl 5-O-acetyl-3-amino-3-deoxy-1,2-O-isopropylidene-D-allofuranuronate. However, IIb did not crystallize and gave IIIb in poor yield presumably due to the impurity. Attempted hydrolysis of IIb could not afford the crystalline free sugar (VII). In order to obtain an intermediate more suitable for preparation of the free aminodeoxyuronic acid, amination of the 3-keto derivatives was investigated.

Treatment of barium 3-oxo-1,2-O-isopropylidene-D-glucofuranuronate[3] (IV) with phenylhydrazine in 50% aqueous acetic acid gave 3-oxo-1,2-O-isopropylidene-D-glucofuranuronic acid phenylhydrazone (V). Catalytic hydrogenation employing Adams' catalyst transformed V into 3-amino-3-deoxy-1,2-O-isopropylidene-D-allofuranuronic acid (VI). This material gave a positive ninhydrin test and absorption in amino region (1615 cm^{-1}). The structure of VI was supported by reduction with lithium aluminum hydride and subsequent acetylation to give IIIb in 72% yield.

The reactivity of C-3 substituent of 1,2-O-isopropylideneglucofuranose derivatives are often much influenced by its configuration. Wolfrom and co-workers[12] showed that the sulfonyloxy group of 3-O-*p*-toluenesulfonyl-1,2;5,6-di-O-isopropylidene-D-glucofuranose is hardly displaced by azide ion whereas that of 3-O-*p*-toluenesulfonyl-1,2;5,6-di-O-isopropylidene-D-allofuranose is readily replaced[13] to give the corresponding 3-azido-D-glucofuranose derivative. The previous paper discussed[3] on the lithium aluminum hydride reduction of methyl 5-O-acetyl-3-oxo-1,2-O-isopropylidene-D-glucofuranuronate to give exclusively 1,2-O-isopropylidene-D-allofuranose. This fact implied that the steric hindrance caused by the isopropylidene group strongly inhibits the attack of the reagents from the α-side of D-glucofuranuronic acid derivatives as well as D-glucose derivatives.

In the present experiment, the 3-azido derivative (IIa) of 1,2-O-isopropylidene-D-allofuranuronic acid was obtained although the reaction condition was drastic and the product did not crystallize. Catalytic hydrogenation of V seems to have proceeded stereospecifically because the yield was considerably high (41.9%) but it was difficult to prove because the acetamidoglucose derivative corresponding to IIIb was not available for comparison. For further investigation on the behavior of C=N bonding located at C-3 of D-glucuronic acid 1,2-O-isopropylidene acetal, the oxime (IX) of methyl 5-O-acetyl-3-oxo-1,2-O-isopropylidene-D-glucofuranuronate (VIII) was prepared and reduced with lithium aluminum hydride. The product was isolated in the crystalline state through acetylation. After recrystallization, the acetamidoallose derivative IIIb was obtained in the yield of 64.5% showing that the reduction proceeded along a stereospecific course. In addition, this reduction provided a way to prepare the 3-amino-3-deoxy-D-allose derivatives IIIa and IIIb under mild conditions. These compounds have been prepared[14] from 3-O-methanesulfonyl-1,2-O-isopropylidene-D-glucofuranose but the latter method requires treatment with anhydrous hydrazine, extremely unpleasant reagent, at high temperature. The oxime (IX) was considerably unstable and the attempted catalytic hydrogenation to give amino-deoxyuronic acid derivative was unsuccessful.

Hydrolysis of the amino derivative VI with dilute hydrochloric acid gave 3-amino-3-deoxy-D-allouronic acid hydrochloride (VII) as fine needles. This substance is soluble in water and alcohol, sparingly soluble in acetone and insoluble in ether. It gives a positive ninhydrin test, shows purple-red color in Elson-Morgan test, reduces ammoniacal silver nitrate solution, decolorizes neutral potassium permanganate solution and reacts with periodate-benzidine solution. In the infrared studies, as a KBr tablet, VII had absorption of C=O stretching vibration at 1735 cm^{-1} and that of N-H deformation at 1640 cm^{-1}. 2-Amino-2-deoxy-D-galacturonic acid isolated from Vi antigen is reported[7] to have carbonyl absorption at 1740 cm^{-1} and amino absorption at 1600 cm^{-1}. Location of the amino group seems to affect the position of the absorption band.

On paperchromatogram VII moved as a single, well defined spot (*Rf* 0.75 sprayed by ninhydrin reagent) in a neutral solvent (buthanol-ethanol-water 3:2:2) whereas 2-amino-2-deoxy-D-galacturonic acid[5] was reported to move only slightly with streaking in neutral and basic solvents.

Experimental

All evaporations were carried out under reduced pressure.

Methyl 5-O-Acetyl-3-O-methanesulfonyl-1,2-O-isopropylidene-D-glucofuranuronate (Ib)——To a solution of methyl 5-O-acetyl-1,2-O-isopropylidene-D-glucofuranuronate[3] (Ia, 10.0 g) in anhydrous pyridine (50 ml) on an ice bath was added a solution of methanesulfonyl chloride (7.0 g) in anhydrous pyridine (50 ml) dropwise

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over the period of 40 min and the resulting mixture was allowed to stand overnight at room temperature. To the reaction mixture was added ice water (200 ml) portionwise under stirring and deposited crystals were collected by filtration. After recrystallization from MeOH, the product showed mp 135—136°. Yield 9.44 g (61.7%). IR ν_{max}^{KBr} cm^{-1}: 1320 (ν_{S-O}). $[\alpha]_D^{25}$ −15.8° (c=5, $CHCl_3$) (*Anal.* Calcd. for $C_{19}H_{25}O_{10}S$: C, 42.12; H, 5.43. Found: C, 42.47; H, 5.55.).

Methyl 5-O-Acetyl-3-azido-3-deoxy-1,2-O-isopropylidene-D-allofuranuronate (IIa)——A mixture of Ib (3.0 g), NaN_3 (3.0 g) and dimethylformamide (20 ml) was heated for 18 hr under reflux. The mixture was then filtered and the filtrate was evaporated. The obtained syrupy residue was extracted with chloroform, the chloroform extract was dried with Na_2SO_4 and evaporated to give a yellowish glass (1.9 g) IR ν_{max}^{Cap} cm^{-1}: 2160 (ν_{N_3}), 1748 ($\nu_{C=O}$ of methyl ester), 1730 ($\nu_{C=O}$ of acetyl).

The crude azido derivative was submitted to the following procedure without further purification.

Catalytic Hydrogenation of IIa——A sample of IIa (900 mg) was dissolved in absolute MeOH and hydrogenated at room temperature under 1 atm. using 10% palladium charcoal (500 mg) as a catalyst.

After 48 hr of hydrogenation, the catalyst was removed and the obtained yellowish solution was evaporated to give a thick syrup (IIb, 701 mg). IR ν_{max}^{Cap} cm^{-1}: 1740 ($\nu_{C=O}$ of methyl ester), 1730, ($\nu_{C=O}$ of acetyl), 1620 (δ_{N-H}) of amino group, absorption of azido group disappeared.

Reduction of IIb with Lithium Aluminum Hydride——To a solution of IIb in dry dioxane (5 ml) was added a solution of $LiAlH_4$ (400 mg) portionwise and the resulting mixture was stirred at room temperature for 1 hr and then at 50° for 2 hr.

The reducing agent was finally destroyed by addition of water diluted with dioxane (1:5) and the resulting mixture was evaporated to dryness. The residue was acetylated with acetic anhydride and pyridine in a usual manner to give 20.05 mg (17.2%) of 5,6-di-O-acetyl-3-amino-3-deoxy-1,2-O-isopropylidene-D-allofuranose (IIIb)[14] crystalline. The product obtained did not show depression of melting point (162—164°) when admixed with the authentic specimen and gave the identical infrared spectrum with the authentic.

3-Oxo-1,2-O-isopropylidene-D-glucofuranuronic Acid Phenylhydrazone (V)——To a chilled solution of barium 3-oxo-1,2-O-isopropylidene-D-glucofuranuronate (IV, 1.0 g) in 50% AcOH (10 ml) was added dropwise a solution of phenylhydrazine (0.4 g) in AcOH (10 ml) and the resulting clear solution was allowed to stand overnight at 5°.

Deposited crystals were collected and recrystallized from EtOH, mp 186—187°. Yield 998 mg (92.8%). IR ν_{max}^{KBr} cm^{-1}: 1740 (ν_{C-O} of carboxyl), 1638 ($\nu_{C=N}$); carbonyl absorption of 3-oxo function of IV (1770 cm^{-1}) disappeared. $[\alpha]_D^{22}$ =+38.7° (c=0.5, MeOH) (*Anal.* Calcd. for $C_{15}H_{18}O_6N_2$: C, 55.89; H, 5.63; N, 8.69. Found: C, 56.15; H, 5.98; N, 8.60).

3-Amino-3-deoxy-1,2-O-isopropylidene-D-allofuranuronic Acid (VI)——A solution of V (250 mg) in EtOH (50 ml) was shaken with H_2 under 1 atm at room temperature for 40 hr in the presence of Adams' catalyst. The reaction mixture was then filtered and the filtrate was evaporated under reduced pressure on a waterbath below 30°. Obtained colorless syrup was dried over P_2O_5 overnight. The hard glassy material (184 mg) showed a single spot on thin-layer chromatogram (*Rf* 0.87) using Kieselgel G (Merck) plate and BuOH–EtOH–H_2O 3:2:2 as a solvent. This substance was dissolved in minimum amount of EtOH and precipitated with ether to give fine crystals which were washed several times with *n*-hexane. The product (VI) decomposed at 155°, yield 75.8 mg (41.9%), showed purple color by ninhydrin test and yellow by Elson-Norgan reaction. IR ν_{max}^{KBr} cm^{-1}: 1740 ($\nu_{C=O}$), 1615 (δ_{N-H}).

For further identification, this product was converted into the known 5,6-di-O-acetyl-3-acetamido-3-deoxy-1,2-O-isopropylidene-D-allofuranose (IIIb)[14] in the following manner. To a suspension of $LiAlH_4$ (40 mg) in dioxane (11 ml) was added crystals of VI (20.6 mg) under ice cooling and stirring. The resulting mixture was stirred at room temperature for 1 hr and at 50° for additional 2 hr. To the reaction mixture was added water (2 ml) diluted with dioxane (5 ml) and the resulting mixture was evaporated below 25°. Remaining moisture was removed from the residue by evaporation with absolute ethanol. The obtained white solid was dried over P_2O_5 overnight and acetylated with Ac_2O–pyridine in a usual manner to give IIIb. After recrystallization from isopropylether, the product showed no depression of melting point (162—164°) when admixed with the authentic sample and gave the identical infrared absorption spectrum with the authentic. Yield, 21.8 mg (71.7%).

Methyl 5-O-Acetyl-3-oxo-1,2-O-isopropylidene-D-glucofuranuronate Oxime (IX)——To a solution of VIII (3.5 g) in 95% EtOH (25 ml) was added a solution of hydroxyamine hydrochloride (2.4 g) in water (10 ml) and N NaOH was added dropwise to keep the pH of the solution in the range of 4.0 to 6.8 over a period of 2 hr, the resulting mixture was then heated at 60° for 6 hr and evaporated. After removal of moisture by evaporations with absolute EtOH, 2.4 g (65.2%) of the solid product was obtained. IR ν_{max}^{KBr} cm^{-1}: 1640 ($\nu_{C=N}$), 1740 ($\nu_{C=O}$ of methyl ester), 3390 (ν_{O-H} of oxime), the peak atb 1873 ased on carbonyl streching absorption of 3-keto group of VIII disappeared.

Reduction of IX with Lithium Aluminum Hydride——To a chilled solution (5°) of IX (520 mg) in dioxane (2 ml) was added a solution of $LiAlH_4$ (500 mg) in dioxane (5ml) and the resulting mixture was heated under reflux for 18 hr. After chilling the mixture, excess hydride was destroyed by addition of AcOEt and then EtOH–water (4:1). Gray precipitates formed were removed by centrifugation and the supernatant was evaporated. The obtained syrupy residue was acetylated with Ac_2O and pyridine in a usual manner.

Pyridine and Ac_2O was removed by evaporation with absolute EtOH to give a thick syrup (540 mg) which gave crystals on addition of isopropylether and scratching. After recrystallization from isopropylether, there was obtained 383 mg (64.5%) of IIIb which showed no depression of melting point when admixed with the authentic sample and gave the identical infrared spectrum with the authentic.

3-Amino-3-deoxy-D-allouronic Acid Hydrochloride (VII)——To a solution of VI (200 mg) in MeOH (0.3 ml) was added 2 N HCl (0.6 ml) and the mixture was stirred for 2 hr at 85°. The brown substance formed were removed by extraction with ether (10 ml × 3). The aqueous layer was then decolorized with charcoal and evaporated to a slightly yellow syrup. On scratching with absolute EtOH, this syrup gave needles which was recrystallized from absolute EtOH, mp 200°. Yield 25 mg (13.6%). IR cm^{-1} 1735 ($\nu_{C=O}$ of carboxyl); 1640 (δ_{N-H}); no absorption of isopropylidene group was observed. (*Anal.* Calcd. for $C_6H_{12}O_6NCl \cdot 1.5H_2O$: C, 28.08; H, 5.90; N, 5.46. Found: C, 28.47; H, 6.50; N, 5.10). The water of crystallization was distilled and was detected by cobaltous chloride. 2-Aminohexuronic acid from Vi-antigen was also reported[5] to contain either 1 or 2 mole water of crystallization. This aminodeoxyuronic acid (VII)showed positive ninhydrin test and Elson-Morgan test (purple-violet). VII reduces Fehling solution and ammoniacal silver nitrate solution at room temperature and reacts with neutral potassium permanganate solution and periodate-benzidine reagent.

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