Carbohydrate Research, 32 (1974) 115–126

SYNTHESES OF 2-ACETAMIDO-2-DEOXY-D-GLUCONO-1,4-LACTONE AND SOME ISOPROPYLIDENE ACETALS OF 2-ACETAMIDO-2-DEOXY-D-GLUCONIC ACID DERIVATIVES

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(Received July 12th, 1973; accepted August 20th, 1973)

ABSTRACT

Brief treatment of 2-acetamido-2-deoxy-D-gluconic acid (**3**) with boiling acetic acid affords, after purification, 2-acetamido-2-deoxy-D-glucono-1,4-lactone (**1**). The same lactone may also be prepared through hydrolytic cleavage of the isopropylidene group in 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucono-1,4-lactone (**4**). When the mixture of compounds obtained by the oxidation of 2-acetamido-2-deoxy-D-glucose was treated with an isopropylidenating agent, the crystalline lactone **4** was among the products isolated. In addition, in the course of that reaction, isopropylidene acetals of 2-acetamido-2-deoxy-D-gluconic acid esters, **6**–**8** and **9**, were formed.

When **4** was treated with *p*-toluenesulfonyl chloride in pyridine, β-elimination occurred to yield 2-acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-D-*erythro*-hex-2-enono-1,4-lactone (**10**). With methanol, **4** gave methyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-gluconate (**7**), which spontaneously reverted to **4**. The susceptibility of lactone derivatives to the action of alcohols is briefly discussed.

INTRODUCTION

In previous studies on the 2-acetamido-2-deoxyaldonolactones[1,2], 2-acetamido-2-deoxy-D-glucono-1,4-lactone (**1**) was detected as one of the components present in an equilibrated, aqueous solution which also contained 2-acetamido-2-deoxy-D-glucono-1,5-lactone (**2**) and 2-acetamido-2-deoxy-D-gluconic acid (**3**). The 1,4-lactone **1** has not, however, been isolated in pure form and, inasmuch as this compound may serve as an inhibitor[3] of 2-acetamido-2-deoxy-β-D-glucosidase ("β-*N*-acetylglucosaminidase"), we have undertaken a study of its preparation. Two different synthetic routes will be reported here.

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An initial approach was based on the possibility that appropriate derivatization of the equilibrium mixture containing **1**, **2**, and **3** might produce a readily isolable derivative of the 1,4-lactone, which could then be deblocked to yield **1**. Although this method was successful, a second one, based on the deliberate lactonization of 2-acetamido-2-deoxy-D-gluconic acid (**3**) in acidic medium, proved to be superior, and, because of its simplicity, will be discussed first.

RESULTS AND DISCUSSION

Brief treatment of **3** with boiling acetic acid afforded a mixture of lactones **1** and **2**, with the 1,4-lactone **1** preponderating. Separation of this mixture on a column of silica gel, eluted with acetone, gave chromatographically homogeneous 2-acetamido-2-deoxy-D-glucono-1,4-lactone (**1**), in 55% yield, in the form of an unstable foam; from acetone solution under carefully controlled conditions, it was obtained in crystalline form. The compound is dextrorotatory, conforming to the lactone rule[4]; its infrared spectrum showed the carbonyl absorption at 1770 cm^{-1} and was consistent with the 1,4-lactone structure[5].

It should be noted, in passing, that the use of a methanol-containing solvent mixture for the elution of the chromatographic column resulted in the isolation of methyl 2-acetamido-2-deoxy-D-gluconate[1,6].

We will now describe the synthesis of **1** *via* 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucono-1,4-lactone (**4**). As the source for the preparation of this isopropylidene derivative, the mixture of products obtained on oxidation of 2-acetamido-2-deoxy-D-glucose with unbuffered bromine solution[1] was used. Two isopropylidenating agents were employed: (*a*) acetone–copper(II) sulfate, and (*b*) 2,2-dimethoxypropane–*N*,*N*-dimethylformamide–*p*-toluenesulfonic acid. Separation of the reaction product from either reagent on a column of silica gel led to the isolation of a crystalline 2-acetamido-2-deoxy-*O*-isopropylidene-D-gluconolactone. As the mixture used for isopropylidenation contained two lactones, **1** and **2**, at least two isomeric isopropylidene derivatives were to be expected*. Therefore, special attention was given to the proof of homogeneity and ring-size of the product isolated, as well as to the unequivocal synthesis of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucono-1,5-lactone (**5**). These problems will be discussed later in this paper.

The byproducts in the isopropylidenating reactions, found in small proportions and isolated after repeated chromatography on silica gel, are: a methyl 2-acetamido-2-deoxy-di-*O*-isopropylidene-D-gluconate (**6**), methyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-gluconate (**7**), and methyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-gluconate (**8**). In addition, the presence of the free acid **3** was proved by the preparation of its dicyclohexylammonium salt[1].

The formation of methyl esters **6**, **7**, and **8** in these reactions is certainly dependent on the presence of methyl 2-acetamido-2-deoxy-D-gluconate in the mixture at the

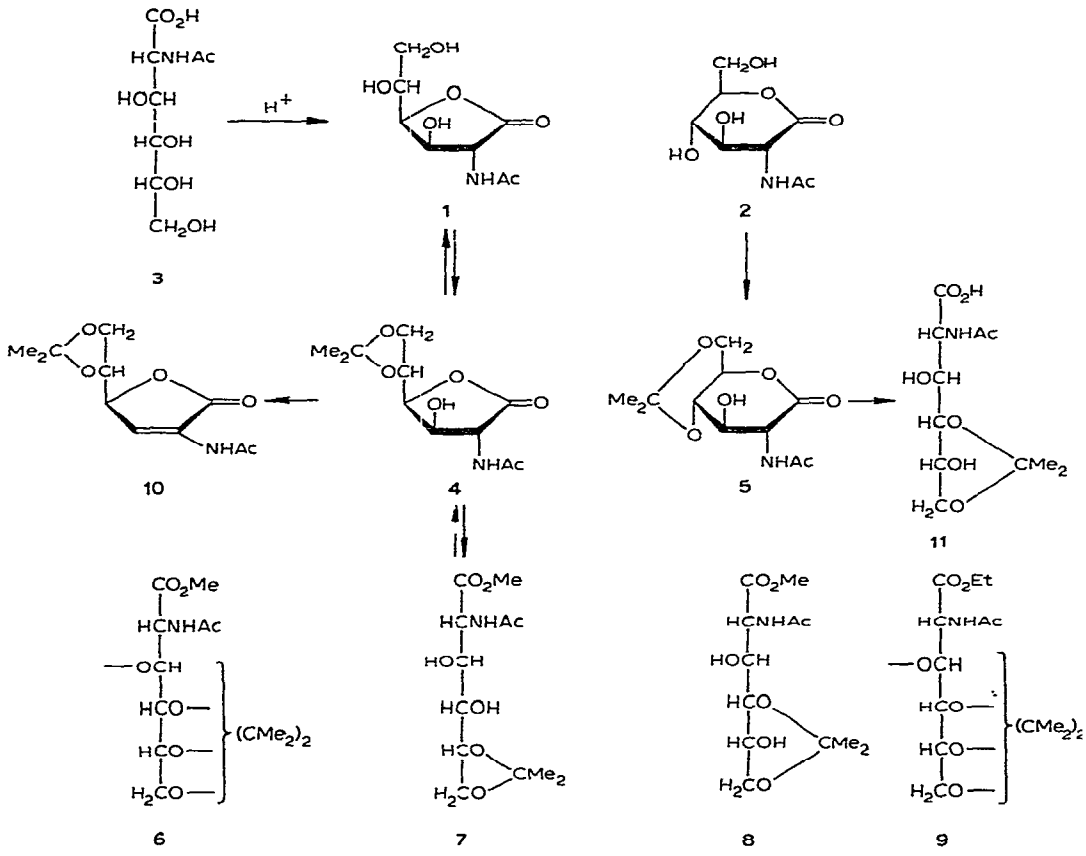
*Reagent *b*, especially, has been shown to favor the formation of 4,6-isopropylidene acetals[7].

outset. The usual processing of the reaction products from the oxidation of 2-acetamido-2-deoxy-D-glucose included overnight treatment with methanol[1]. When this step was omitted and the crude material was subjected to the action of isopropylidenating reagents, there was no evidence for the presence of such compounds as **6**, **7**, and **8**. In another experiment, the mixture of lactones **1** and **2** was obtained by the oxidation of 2-acetamido-2-deoxy-D-glucose with bromine–cadmium carbonate, as recently described[2]. In this experiment, crystalline **3** was separated after treatment with ethanol. The residue from the ethanolic mother-liquor, containing both lactones, was now allowed to react with reagent *b*. Here again, a crystalline 2-acetamido-2-deoxy-*O*-isopropylidene-D-gluconolactone was obtained, together with an ethyl 2-acetamido-2-deoxy-di-*O*-isopropylidene-D-gluconate (**9**). The fact that esters which are derived from the alcohol used in the treatment of the mixture are formed indicates the extreme susceptibility of at least some of the components of the mixture to alcoholysis. Although complete opening of the lactone ring requires quite a long period of time[1], partial alcoholysis seems to occur immediately.

Attention was now turned to the determination of the ring size of the 2-acetamido-2-deoxy-*O*-isopropylidene-D-gluconolactone. The compound appeared to be pure by all criteria available: its infrared spectrum showed carbonyl absorption at 1770 cm^{-1}, indicating the 1,4-lactone structure[5]; the n.m.r. spectrum was also in harmony with that structure. Through β-elimination, it was converted into the (known) 2-acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-D-*erythro*-hex-2-enono-1,4-lactone (**10**). On the basis of this evidence, we ascribed to the crystalline 2-acetamido-2-deoxy-*O*-isopropylidene-D-gluconolactone the five-membered ring-structure depicted in **4**. The compound is dextrorotatory ($[\alpha]_D$ +140.8°, in acetone) as expected from the lactone rule; however, in solution in methanol, the rotation changed quickly. With methanol during 12 days at room temperature, **4** was quantitatively converted into the crystalline methyl ester **7**. On prolonged storage, the ester partially reverted to **4**; a similar phenomenon had been observed with methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-gluconate[6]. Alcoholysis of lactones is known[8] to occur in the presence of alcoholic hydrogen chloride, 1,5-lactones being, in general, more reactive than 1,4-lactones. Some *O*-substituted (benzyl, benzoyl) derivatives both of 1,5-lactones[6,9] and 1,4-lactones[9,10] appear to be particularly labile, and may be esterified through the action of an alcohol alone. The alcoholysis of **4** into **7** is, thus, an additional example of such behavior.

It was of particular interest to prepare the isomeric 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucono-1,5-lactone (**5**) for comparison purposes. In the first attempt, 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucopyranose[7] was oxidized with bromine–cadmium carbonate. The lactone **5** was certainly an intermediate product that, in the course of the processing, was transformed into the final products, namely, 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-gluconic acid (**11**) and 2-acetamido-2-deoxy-D-gluconic acid (**3**). Next, the preparation of **5** starting from the 1,5-lactone[6] **2** was examined; treatment of **2** with reagent *b*, followed by separation on silica gel, led to the isolation of crystalline **5**. Although the chromatographic behavior of

the two isomeric derivatives **4** and **5** is almost identical, they are readily distinguished through their infrared spectra.



In order to obtain 2-acetamido-2-deoxy-D-glucono-1,4-lactone (**1**), the removal of the isopropylidene group was achieved with samples of **4** of high purity by using a dry, ion-exchange resin in the H^+ form[11], under very mild conditions, in a non-aqueous solvent. All of these precautions are necessary, because the free 1,4-lactone **1** could not readily be purified, as it changes quickly in the presence of water. 2-Acetamido-2-deoxy-D-glucono-1,4-lactone (**1**), thus prepared, was obtained in quantitative yield in the form of a syrup; nucleation with crystalline **1** prepared by lactonization of **3** (as described earlier in this paper) caused a very slow tendency to crystallize. However, the samples of **1** from the two sources were chromatographically indistinguishable. The comparison of optical rotations of the samples of **1** of various origins is not a reliable test, as the rotation changes rapidly (see the Experimental part for details). The direction of this change indicated the formation of 2-acetamido-2-deoxy-D-glucono-1,5-lactone (**2**) ($[\alpha]_D$ +137.7°)[6]. This observation was in agreement with the findings of the t.l.c. monitoring of the rotation solution.

Hydrolysis of the isopropylidene group from **4** can be also achieved through the action of 95% acetic acid.

In support of the identity of the samples of **1** prepared by the two different routes, we found that crystalline **1** obtained through lactonization of **3** was converted into 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucono-1,4-lactone (**4**). On the other hand, the syrupy **1** was characterized as a crystalline di-*N*-methylamide, which was shown to be identical with 2-acetamido-2-deoxy-di-*N*-methyl-D-mannonamide prepared earlier in our laboratory[6]; the epimerization occurring in the presence of an amine was discussed in that report[6].

In conclusion, it should be emphasized that the study of interconvertibility, and the formation of equilibrated mixtures, of the 2-acetamido-2-deoxy-D-glucono-lactones **1** and **2** in aqueous solution are of interest, especially from the viewpoint of their utilization as potent inhibitors of 2-acetamido-2-deoxy-β-D-glucosidase[3]. Preliminary studies on the inhibitory properties of some lactones, which will be published elsewhere[12], showed that all samples of **1** prepared in the course of this work, regardless of the method of preparation, exhibit the same inhibitory effect towards the enzyme. The results are given in Table I.

TABLE I

THE INHIBITORY EFFECT OF 2-ACETAMIDO-2-DEOXY-D-GLUCONO-1,4-LACTONE (**1**) OF VARIOUS ORIGINS ON THE 2-ACETAMIDO-2-DEOXY-β-D-GLUCOSIDASE FROM BULL EPIDIDYMIS[12]

Sample of **1**	50% *inhibition*[a] (μM)	K_i (μM)
Crystalline, from **3**, through lactonization	3.6±0.1	4.4
Amorphous, from **4**, by hydrolysis with Dowex 50 (H^+) resin	3.4±0.1	4.3
Amorphous, from **4**, by hydrolysis with AcOH	3.5±0.1	4.4

[a]Assay: the substrate (*p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside; 2.5 mM) was added to McIlvaine buffer of pH 4.8 (1.5 ml) containing 50 m E. U. of 2-acetamido-2-deoxy-β-D-glucosidase and various concentrations of inhibitor; then the volume was made to 2.0 ml with water, and the solution was incubated for 10 min at 38°. After incubation, the *p*-nitrophenol liberated was measured spectrophotometrically at 430 nm.

EXPERIMENTAL

General methods. — Column chromatography was performed on silica gel (E. Merck) of 0.05–0.20-mm particle size with the following solvent systems: *A*, 13:4 dichloromethane–methanol; *B*, 80:5:15:1 acetonitrile–acetone–water–acetic acid[13]; *C*, 4:1 ether–acetone; *D*, ether; *E*, 2:1:1 acetone–chloroform–ether; *F*, 6:1:1 ether–acetone–hexane; and *G*, 16:1:3 acetonitrile–acetone–water, all ratios being v/v. T.l.c. was conducted on plates (5 × 10 cm) of silica gel (E. Merck) in the solvent system

specified; the components were detected by spraying with 10% sulfuric acid and heating, or only by heating.

Specific rotations were measured at 20–24°. I.r. spectra were recorded on Perkin–Elmer 137 infracord and Model 257 spectrometers. The n.m.r. spectra were recorded at 60 MHz with a Varian A-60-*A* spectrometer, in the solvents specified, with tetramethylsilane as the internal standard.

*2-Acetamido-2-deoxy-*D*-glucono-1,4-lactone* (**1**). — (*A*) *From 2-acetamido-2-deoxy-*D*-gluconic acid* (**3**). To boiling acetic acid (40 ml) was added **3** (ref. 2; 1.0 g), and the solution was boiled for 3.5 min. The hot solution was immediately evaporated *in vacuo* to a light syrup which was dissolved in absolute ether (20 ml). The solution was evaporated *in vacuo*, the residue was dissolved in 1:1 2-methoxyethanol–acetone (10 ml), and the solution was applied to a column of silica gel (110 g) prepacked in acetone. The column was eluted with acetone, 12-ml portions of eluate being collected. Fractions were examined by t.l.c. in solvent *A*.

Fractions 35–58 contained homogeneous material; on evaporation, they afforded an amorphous, hygroscopic residue (550 mg) which, after being dried *in vacuo* at 25°/0.1 torr, became a foam; $[\alpha]_D$ +92.4° (*c* 0.5, water). Compound **1** crystallized slowly after a solution in acetone had been refrigerated for a few days; it can be also crystallized from 2-propanol–acetone or 2-methoxyethanol–ether; m.p. 104–107°, $[\alpha]_D$ +112.7° (*c* 1.0, water, 9 min after dissolution) → +107.1° (5 h) → +100.9° (2 days); ν_{max}^{Nujol} 3500–3300 (OH and NH), 1770 (C=O), and 1650 and 1540 cm^{-1} (Amide I and Amide II).

Anal. Calc. for $C_8H_{13}NO_6$: C, 43.83; H, 5.98; N, 6.39. Found: C, 43.78; H, 6.18; N, 6.20.

Fractions 59–70 contained a mixture of **1** and **2** (80 mg).

A sample of **1** (17 mg) was treated with reagent *b*, yielding 12 mg of product. After two recrystallizations from acetone–ether, the compound melted at 159–164°. Its chromatographic behavior and infrared spectrum were indistinguishable from those of a sample of **4** described later in this paper.

(*B*) *From 2-acetamido-2-deoxy-5,6-O-isopropylidene-*D*-glucono-1,4-lactone* (**4**). To a solution of **4** (twice-recrystallized sample, 150 mg) in 2-methoxyethanol (6 ml) was added dry Dowex-50 X-8 (H^+) ion-exchange resin (1.5 g), and the mixture was kept, with occasional stirring, for 20 h at room temperature. The progress of the reaction was monitored by t.l.c. with solvent *B*; a faint spot of **1** was visible after 30 min. The process is slow, but, at the termination of the reaction, **4** was not detectable by t.l.c. The suspension was filtered, the resin was washed several times with 2-methoxyethanol, and the filtrate and washings were combined, and evaporated under high vacuum at 30° (bath) to dryness, to give a colorless syrup in quantitative yield; $[\alpha]_D$ +73.2° (*c* 1.14, water, 5 min after dissolution) → +88.5° (30 min) → +100.3° (2 h) → +92.6° (3 h) → +74.5° (17 h, final); ν_{max}^{neat} 3400–3300 (OH and NH), 1770 (C=O), and 1650 and 1540 cm^{-1} (Amide I and Amide II); n.m.r. data (in Me_2SO-d_6) included signals at τ 1.55 (*J* 7.5 Hz, NH), 5.2–6.7 (unresolved multiplet), and 8.17 (NAc).

Anal. Calc. for $C_8H_{13}NO_6$: C, 43.83; H, 5.98; N, 6.39. Found: C, 44.11; H, 6.28; N, 6.23.

A solution of a sample of the material in 2-methoxyethanol was homogeneous on t.l.c. in solvent *B*, whereas t.l.c. of a solution of a sample in water showed spots of the slightly slower-moving 2-acetamido-2-deoxy-D-glucono-1,5-lactone (**2**) and 2-acetamido-2-deoxy-D-gluconic acid (**3**).

When the hydrolysis was conducted at the temperature of a steam bath, it required 30 min; the product was chromatographically homogeneous, but colored.

A sample of **1** afforded a crystalline product with anhydrous dimethylamine. Its chromatographic behavior, m.m.p., and i.r. spectrum identified it as 2-acetamido-2-deoxy-di-*N*-methyl-D-mannonamide[6].

(*C*) *From compound* **4**. A solution of **4** (60 mg) in 95% aqueous acetic acid (3 ml) was heated for 20 min on a steam bath, and then cooled to room temperature, and evaporated by means of a stream of air to a residue which was crystallized from 2-propanol. The crude product (20 mg) had an i.r. spectrum similar to that of crystalline **1**, obtained as already described.

Treatment of the mixture from the oxidation of 2-acetamido-2-deoxy-D-glucose with isopropylidenating reagents. — (*A*) *With acetone–copper(II) sulfate.* The crude, solvent-free mixture[1] (foam, 3.6 g) was shaken with anhydrous acetone overnight. To the suspension was added anhydrous copper(II) sulfate (12 g), and shaking was continued for 8–12 days, with the addition of a second portion (~10 g) of copper(II) sulfate after the fifth day; the progress of the reaction was monitored by t.l.c. in solvent *C*. The solid was removed by filtration, and thoroughly washed with warm acetone. The filtrate and washings were combined, and evaporated *in vacuo*, to give a foamy residue (3.4 g) which was chromatographed on a column of silica gel (60 g) with solvent *C*, 10-ml portions of eluate being collected.

Fractions 10–17 contained a yellowish oil (136 mg) which was rechromatographed on a column of silica gel with solvent *D*. Its chromatographic behavior in several solvent systems, its n.m.r. spectrum, and its optical rotation in chloroform identified it as methyl 2-acetamido-2-deoxy-di-*O*-isopropylidene-D-gluconate (**6**), described more fully in the next section.

Fractions 28–45 were pooled and evaporated, to yield a crystalline product (880 mg) which was recrystallized twice from absolute acetone, affording 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucono-1,4-lactone (**4**): m.p.* 163–164°, $[\alpha]_D$ +140.8° (*c* 0.49, acetone); $[\alpha]_D$ +141.8° (*c* 0.50, methanol; 3 min after dissolution). The optical rotation was observed during 3 days, and was found to change from an initial value of +0.705° to a final rotation of +0.02°. Compound **4** had ν_{max}^{KBr} 3500 (OH), 3400 (NH), 1770 (C=O), 1660 and 1540 (Amide I and Amide II), 1210, 1110, 970, and 870 cm^{-1}; n.m.r. data (in Me_2SO-d_6): τ 1.41 (doublet, *J* 7.8 Hz, removed by D_2O exchange, NH), 4.14 (doublet, *J* 5.0 Hz, removed by D_2O exchange, OH), 5.1–6.3 (multiplet, unresolved, 6 H), 8.13 (NAc), and 8.66 and 8.72 (CMe_2).

*The m.m.p. with 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-mannono-1,4-lactone[1] was depressed.

Anal. Calc. for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.71; H, 6.84; N, 5.26.

Fractions 46–70 consisted of a mixture (465 mg), which was rechromatographed, with solvent *E* for elution. An additional crop (135 mg) of **4** was obtained; total yield: 1.015 g. Further fractions contained a mixture of **7** and **8**, and were rechromatographed with the same solvent mixture to yield a few fractions of a homogeneous product in the form of a syrup: $[\alpha]_D$ −4.7° (*c* 1.02, acetone). The n.m.r. spectrum in acetone-d_6 showed signals at τ 2.62 (broad doublet, NH), 5.06 (quartet, H-2), 6.30 (OCH_3), 7.98 (NAc), and 8.69 (CMe_2), identifying it as methyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-gluconate (**8**).

Anal. Calc. for $C_{12}H_{21}NO_7$: C, 49.48; H, 7.27; N, 4.81. Found: C, 49.39; H, 7.63; N, 4.51.

Further elution of the "main" column with methanol afforded a syrup (1.01 g) that showed a strong carbonyl absorption in the infrared spectrum, and that gave, on a t.l.c. plate, a yellow spot when sprayed with Bromocresol Green. The syrup was colored and, therefore, was dissolved in water (15 ml); the solution was stirred with charcoal without heating, the suspension filtered, and the filtrate evaporated *in vacuo*. The residue was treated with ethanol (8 ml), the undissolved part was filtered off, and to the filtrate was added dicyclohexylamine (2 drops). The mixture was kept in a refrigerator overnight, and the crystals were then removed by filtration; wt. 200 mg. Its infrared spectrum, elemental analysis, m.p., and m.m.p. with an authentic sample[1] identified it as dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate.

(*B*) *With 2,2-dimethoxypropane.* To a solution of the solvent-free foam from the oxidation of 2-acetamido-2-deoxy-D-glucose[1] (3.5 g) in anhydrous *N,N*-dimethylformamide (40 ml) were added 2,2-dimethoxypropane (2 ml) and *p*-toluenesulfonic acid monohydrate (70 mg). The mixture was stirred for 3 h at room temperature (the progress of the reaction being monitored by t.l.c. with solvent *C*) and then stirred for 15 min with Amberlite IR-45 (OH^-) ion-exchange resin (~4.5 g), the suspension filtered, and the filtrate evaporated under high vacuum. The residue was chromatographed on a column of silica gel (60 g) with solvent *C*; 6-ml fractions were collected.

Fractions 11–15 contained methyl 2-acetamido-2-deoxy-di-*O*-isopropylidene-D-gluconate (**6**) (202 mg); it was rechromatographed twice on silica gel with solvent *F*, and it was chromatographed, prior to analysis, with ether: colorless syrup, $[\alpha]_D$ −12.9° (*c* 0.96, chloroform); ν_{max}^{neat} 3300 (NH), 1740 (C=O), and 1650 and 1520 cm^{-1} (Amide I and Amide II); n.m.r. data (in acetone-d_6): τ 2.79 (broad doublet, *J* 9.6 Hz, removed by D_2O exchange, NH), 5.07 (pair of doublets, $J_{2,3}$ 2.0, $J_{2,NH}$ 9.6 Hz, collapsed to a doublet by D_2O exchange, H-2), 5.52 (pair of doublets, $J_{2,3}$ 2.0, $J_{3,4}$ 7.5 Hz, H-3), 5.7–6.2 (unresolved, 4 H), 6.30 (OCH_3), 8.00 (NAc), and 8.64 and 8.70 (12 H, CMe_2).

Anal. Calc. for $C_{15}H_{25}NO_7$: C, 54.37; H, 7.60; N, 4.23. Found: C, 54.15; H, 7.52; N, 4.03.

After evaporation, fractions 21–26 afforded crystalline **4** (366 mg), and frac-

tions 27–58 gave a mixture (2.2 g) from which, by repeated trituration with acetone, **4** (530 mg) was separated. The acetone mother-liquor was rechromatographed on silica gel with solvent *E*, to give an additional crop of **4** (382 mg); total yield of **4**: 1.280 g. Each portion of **4** isolated was characterized by its chromatographic mobility and its i.r. spectrum.

From the column eluted with solvent *E*, further fractions contained a mixture (360 mg), and, finally, a homogeneous oil (260 mg). Prior to analysis, the latter was rechromatographed with the same solvent mixture; the syrup crystallized on being kept at room temperature. Its analysis corresponded to that calculated for $C_{12}H_{21}NO_7$; its chromatographic behavior and i.r. and n.m.r. spectra were indistinguishable from those of methyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-gluconate (**7**), described later in this paper; a m.m.p. was undepressed.

*Treatment of the mixture of 2-acetamido-2-deoxy-*D*-gluconolactones with 2,2-dimethoxypropane.* — 2-Acetamido-2-deoxy-D-glucose was oxidized with bromine–cadmium carbonate as described by Fletcher *et al.*[2]. The crude product was treated with absolute ethanol, and crystalline 2-acetamido-2-deoxy-D-gluconic acid (**3**) was filtered off. The mother liquor, which contained the lactones **1** and **2**, was evaporated *in vacuo* to a foamy residue (1.4 g), which was dissolved in anhydrous *N*,*N*-dimethylformamide (20 ml); 2,2-dimethoxypropane (4 ml) and *p*-toluenesulfonic acid monohydrate (40 mg) were added to the solution. The mixture was stirred overnight at room temperature and then for 15 min with Amberlite IR-45 (OH^-) ion-exchange resin (~3 g) to remove the acid present, the suspension filtered, and the filtrate evaporated *in vacuo*. The crude product was chromatographed on a column of silica gel (35 g) with solvent *C*; 5-ml fractions were collected.

Fractions 8–12 contained an oil (90 mg), which was rechromatographed with solvent *D* to give pure ethyl 2-acetamido-2-deoxy-di-*O*-isopropylidene-D-gluconate (**9**) in the form of a colorless syrup, $[\alpha]_D$ −5.1° (*c* 0.66, chloroform); n.m.r. data (in acetone-d_6): τ 2.85 (broad doublet, *J* 9.6 Hz, NH), 5.10 (pair of doublets, $J_{2,3}$ 2.1, $J_{2,NH}$ 9.6 Hz, H-2), 5.51 (pair of doublets, $J_{2,3}$ 2.1, $J_{3,4}$ 7.5 Hz, H-3), 5.83 (quartet, *J* 7.0 Hz, CH_2CH_3), 8.02 (NAc), 8.66 and 8.71 (CMe_2, 12 H), and 8.78 (triplet, *J* 7.0 Hz, CH_2CH_3).

Anal. Calc. for $C_{16}H_{27}NO_7$: C, 55.64; H, 7.88; N, 4.05. Found: C, 55.54; H, 8.07; N, 3.94.

Fractions 25–50 contained a partly crystalline material (800 mg) which was rechromatographed on silica gel with solvent *E*, to give **4** (367 mg). Its chromatographic behavior and i.r. spectrum were indistinguishable from those of a sample described earlier in this paper. Further fractions (195 mg) contained **4** contaminated with a slightly slower-moving compound. The n.m.r. spectrum of the mixture showed signals indicating that the second component was an ethyl 2-acetamido-2-deoxy-mono-*O*-isopropylidene-D-gluconate.

*Methyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-*D*-gluconate* (**7**) *from* **4**. — A solution of **4** (70 mg) in methanol (10 ml) was kept for 12 days at room temperature, the reaction being monitored by t.l.c. in solvent *C*. At the completion of the reaction,

the presence of **4** was not detectable. The solvent was removed *in vacuo*, and the residue was repeatedly treated with ether, to afford a colorless syrup in quantitative yield. On being kept overnight at room temperature, the compound crystallized: m.p. 83–85°, $[\alpha]_D$ +5.8° (*c* 0.98, acetone); ν_{max}^{KBr} 3360 (OH), 3200 (NH), 1715 (C=O), and 1650 and 1540 cm^{-1} (Amides I and II); n.m.r. data (in acetone-d_6): τ 2.72 (broad doublet, NH), 5.30 (quartet, H-2), 6.34 (OCH_3), 8.04 (NAc), and 8.70 and 8.74 (CMe_2).

Anal. Calc. for $C_{12}H_{21}NO_7$: C, 49.48; H, 7.27; N, 4.81. Found: C, 49.58; H, 7.32; N, 5.06.

On being kept for 2 weeks at room temperature, the sample was found to be heterogeneous; t.l.c. in solvent *C* revealed the presence of **4**.

*2-Acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-*D-erythro-*hex-2-enono-1,4-lactone* (**10**) *from* **4**. — To a solution of **4** (200 mg) in dry pyridine (5 ml) was added *p*-toluenesulfonyl chloride (0.6 g); the mixture was kept at room temperature, and the progress of the reaction was monitored by t.l.c. in solvent *F*. After 24 h, only a trace of the starting compound **4** could be detected, and the presence of a faster-moving product was evident. The mixture was diluted with water, poured onto crushed ice, and extracted with chloroform; the extracts were combined and successively washed with 2M hydrochloric acid, saturated sodium hydrogen carbonate solution, and water, dried (sodium sulfate), and evaporated *in vacuo*. The residue was chromatographed on a column of silica gel (30 g) with solvent *F*. Fractions containing homogeneous material gave, on evaporation, compound **10**. It was dissolved in ethanol, and the solution was treated with charcoal without heating, the suspension filtered, and the filtrate evaporated, to give colorless crystals (97 mg, 52%). The chromatographic behavior and i.r. spectrum of the product were indistinguishable from those of an authentic sample[1].

*2-Acetamido-2-deoxy-4,6-*O*-isopropylidene-*D*-gluconic acid* (**11**). — To a solution of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucopyranose[7] (1.0 g) in water (90 ml) were added cadmium carbonate (2.4 g) and bromine (0.3 ml), and the mixture was stirred at room temperature for 4 h, the progress of the reaction being monitored by t.l.c. with solvent *B*. The first product formed, presumably compound **5**, appeared as a spot moving slightly faster than the starting compound. The second component to appear was a slow-moving spot of compound **11**. As the reaction progressed further, the intensity of the zone of **11** increased, while that of **5** decreased; at the end of the reaction, the spot for the starting compound was undetectable.

The mixture was processed as described recently[2], and purification was checked by t.l.c. after each stage: (a) after treatment with silver carbonate, the spot for **5** was no longer detectable, and the only spot present was that of **11**; (b) after treatment with hydrogen sulfide, the presence of a new spot, corresponding to compound **3** was evident.

The aqueous solution of the product was evaporated *in vacuo* to give a glassy residue (600 mg) that showed, on a t.l.c. plate, a yellow spot when sprayed with

Bromocresol Green. It was chromatographed on a column of silica gel (30 g) with solvent *G*, 6-ml portions of eluate being collected.

Fractions 8–28 contained 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-gluconic acid (**11**). Fractions 12–18 were pooled, evaporated, and dried (for analysis) to give a friable glass (400 mg, 38%), m.p. ~72° (dec.), $[\alpha]_D$ −35.1° (*c* 0.97, water; 5 min after dissolution) → −31.8° (19 h) → −20.1° (4 days); n.m.r. data (in Me_2SO-d_6): τ 8.15 (NAc), and 8.68 and 8.78 (CMe_2).

Anal. Calc. for $C_{11}H_{19}NO_7$: C, 47.65; H, 6.91; N, 5.05. Found: C, 47.88; H, 7.28; N, 5.27.

*2-Acetamido-2-deoxy-4,6-*O*-isopropylidene-*D*-glucono-1,5-lactone* (**5**). — To a solution of **2** (ref. 3; 130 mg) in *N*,*N*-dimethylformamide (7 ml) were added 2,2-dimethoxypropane (1 ml) and *p*-toluenesulfonic acid (5 mg), and the mixture was kept overnight at room temperature, the progress of the reaction being monitored by t.l.c. in solvent *B*. At the end of the reaction, three components were detectable: **5**, **2**, and one showing the same mobility as **11**. The mixture was processed as already described, and the crude product was chromatographed on a column of silica gel (15 g) with solvent *E*, 2-ml fractions being collected .

Fractions 16–30 were evaporated to give compound **5** as a syrup that crystallized on trituration with acetone: yield 54 mg (35%), m.p. 148–150°, $[\alpha]_D$ +130.2° (*c* 0.72, acetone); ν_{max}^{KBr} 3400 (OH), 3290 (NH), 1740 (C=O), 1640 and 1540 (Amides I and II), 1380, 1235, 1200, 1080, 955, 870, and 850 cm^{-1}; n.m.r. data (in Me_2SO-d_6): τ 1.22 (doublet, NH), 4.44 (doublet, OH), 8.17 (NAc), and 8.53 and 8.68 (CMe_2).

Anal. Calc. for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.92; H, 6.46; N, 5.53.

ACKNOWLEDGMENTS

This work was supported, in part, by a P. L. 480 grant from the National Institutes of Health, Bethesda, Md., Agreement No. 02-010-1. The authors are indebted to Mr. B. Danilov, B. Sc., and Mrs. S. Babić for their valuable assistance, to Dr. O. Hadžija for microanalyses, and to Mrs. L. Tomić, B. Sc., for recording the n.m.r. spectra.

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