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THE PREPARATION AND PROPERTIES OF SOME 2-ACETAMIDO-2-DEOXYHEXONIC ACIDS*

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ABSTRACT

The oxidation of 2-acetamido-2-deoxy-D-mannose (Scheme I, **1**) with aqueous bromine in the presence of barium benzoate leads to the isolation of crystalline 2-acetamido-2-deoxy-D-mannonic acid (**2**). With aqueous dicyclohexylamine, **2** gives dicyclohexylammonium 2-acetamido-2-deoxy-D-mannonate (**4**), a crystalline salt readily distinguishable from its known D-*gluco* isomer (**5**). Both **2** and **4** are convertible into the known 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**3**), showing that the configurations assigned to these structures are correct. As the salt (**4**) is stable in saturated aqueous dicyclohexylamine solution, the conversion of **3** into **5** by aqueous dicyclohexylamine, reported earlier, probably involves an inversion prior to opening of the lactone ring. The behavior of **3** with aqueous dicyclohexylamine has been reinvestigated and found to produce **4** as well as **5**. With anhydrous dicyclohexylamine, **3** slowly undergoes a β-elimination to give **6**.

2-Acetamido-2-deoxy-D-gluconic acid (Scheme II, **8**) can be prepared through the bromine–barium benzoate oxidation of 2-acetamido-2-deoxy-D-glucose, (**7**) but the yield is higher when bromine–cadmium carbonate is used. The acid (**8**) may also be made from **5** through its silver salt. The conversion of the lactone **10** into **3** through the action of anhydrous dicyclohexylamine, reported earlier, has been confirmed; the available evidence appears to indicate that **3** and the as yet uncharacterized lactone **10** are readily interconvertible under alkaline conditions.

The preparation of a crystalline hydrate of 2-acetamido-2-deoxy-D-galactonic acid (**11**) from the corresponding dicyclohexylammonium salt is described. On dehydration *in vacuo*, **11** gives the known 2-acetamido-2-deoxy-D-galactono-1,4-lactone (**12**) in high yield.

INTRODUCTION

The oxidation of 2-acetamido-2-deoxy-D-glucose (**7**) with unbuffered aqueous bromine has been shown[1] to afford a mixture from which 2-amino-2-deoxy-D-gluconic acid can be removed by direct crystallization. Treatment of the material

*Dedicated to Dr. Louis Long, Jr., in honor of his 70th birthday.

remaining in the mother liquor with dicyclohexylamine under essentially anhydrous conditions gave the crystalline dicyclohexylammonium salt of 2-acetamido-2-deoxy-D-gluconic acid (**5**); the mother liquor then yielded 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**3**) in crystalline form. More recently, aqueous dicyclohexylamine has been found[2] to convert 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**3**) into dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate (**5**). The objective of the present research was to throw some light on the nature of these epimerizations.

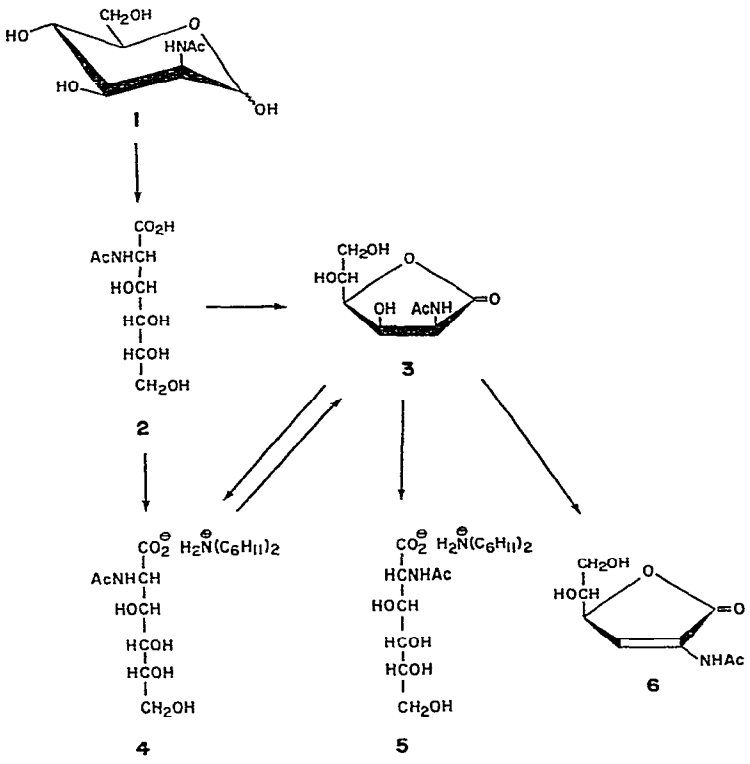
In view of the strongly basic nature of dicyclohexylamine[3] the observed inversions are not unexpected. However, the actual chemical species undergoing inversion remains in doubt. Does 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**3**) isomerize into its D-*gluco* analog (**10**) prior to ring opening or is dicyclohexylammonium 2-acetamido-2-deoxy-D-mannonate (**4**) the immediate product, epimerizing subsequently to the salt of D-*gluco* configuration? As the properties of dicyclohexylammonium 2-acetamido-2-deoxy-D-mannonate are obviously central to these questions, we undertook the preparation of an authentic sample of this salt and of some related compounds.

RESULTS AND DISCUSSION

The oxidation of aldoses with unbuffered bromine-water produces a highly acidic medium which would be expected to favor the formation of aldono-1,4-lactones, and it is not surprising that such an oxidation of 2-acetamido-2-deoxy-D-mannose (**1**) was found[1] to lead to 2-acetamido-2-deoxy-D-manno-1,4-lactone (**3**), isolated crystalline in 51% yield. The use of barium benzoate as a buffer, apparently introduced by Antoniani[4], permits the bromine oxidation of aldoses to be carried out under less acidic conditions. When 2-acetamido-2-deoxy-D-mannose (**1**) was oxidized in this manner, a crystalline product, markedly less soluble than **3**, was obtained in 55% yield and this proved to be a 2-acetamido-2-deoxyaldonic acid. Brief treatment of the acid with hot glacial acetic acid converted it in high yield into the lactone **3**; the acid is, therefore, assigned the D-*manno* configuration depicted by **2**.

In both the buffered and unbuffered oxidations, hydrogen bromide was ultimately removed through the addition of an excess of silver carbonate, the silver remaining in solution then being precipitated as silver sulfide. It is probable that the 1,4-lactone **3**, which is presumed to be the preponderant product when unbuffered bromine is used, is largely unaffected by the silver carbonate. The buffered oxidation mixture, on the other hand, should contain a substantial quantity of the as yet unknown 2-acetamido-2-deoxy-D-mannono-1,5-lactone as well as of the free acid **2** and these would be expected to give the silver salt of **2**; hence **2** rather than **3** is the main product isolated.

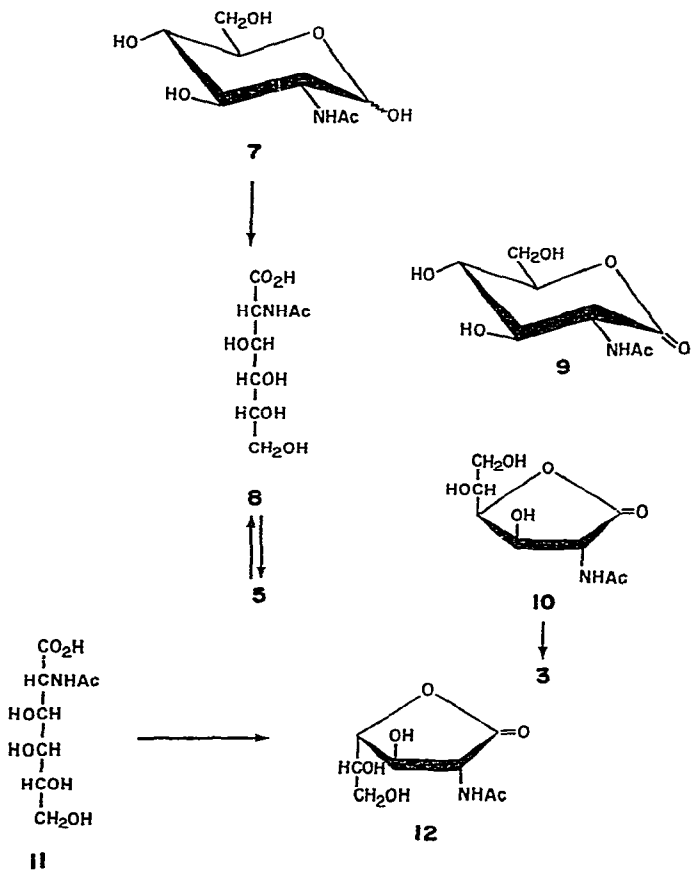
Cautious neutralization of 2-acetamido-2-deoxy-D-mannonic acid with dicyclohexylamine yielded a crystalline dicyclohexylammonium salt that clearly differed in melting point, specific rotation, and infrared spectrum from **5**; that no configura-



SCHEME I

tional change had taken place was shown by conversion of the salt into the lactone 3. With saturated aqueous dicyclohexylamine, the dicyclohexylammonium 2-acetamido-2-deoxy-D-mannonate (**4**) was not detectably altered. We may, therefore, conclude that **4** is not an intermediate in the conversion of **3** into **5**. As an alternative scheme, one may postulate that inversion takes place at the lactone stage and that, under the influence of the strongly basic amine, **3** is converted into the as yet uncharacterized 2-acetamido-2-deoxy-D-glucono-1,4-lactone (**10**) and that, in the presence of water, this hydrolyzes to give the salt **5**. In an attempt to confirm this view, **3** was treated with dicyclohexylamine in absolute alcohol. Chromatographic studies showed that **3** gradually disappeared; subsequently, two compounds, dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate (**5**) and 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone (**6**) were isolated. The latter compound, arising from a simple β-elimination, had been characterized in an earlier study[1]. This experiment provided no evidence that **3** had isomerized into **10**. However, it should be noted that no chromatographic system has yet been found that will reliably distinguish **3** from that component (in the mixture of lactones preparable from **5**) which is most probably 2-acetamido-2-deoxy-D-glucono-1,4-lactone (**10**).

The behavior of 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**3**) in aqueous



SCHEME II

dicyclohexylamine solution was now examined much more rigorously than had been done before[2]. The crystalline mass which formed rapidly and in high yield (92%) at room temperature was found to be made up of two components that could be separated by fractional crystallization from aqueous acetone. The less soluble fraction proved to be the salt (5) having the D-*gluco* configuration and the more soluble product was its D-*manno* isomer (4).

On the basis of the available evidence, it appears probable that **10** and **3** are indeed readily interconvertible under alkaline conditions and that, with water present, each lactone opens to give the corresponding salt.

The facile preparation of 2-acetamido-2-deoxy-D-mannonic acid induced us to explore this new class of compounds further. The oxidation of 2-acetamido-2-deoxy-D-glucose (7) with bromine in the presence of barium benzoate readily gave another crystalline 2-acetamido-2-deoxyhexonic acid, but the yield, 29%, was disappointing. Subsequently, the oxidation of **7** with bromine in the presence of cadmium carbonate was found to afford the acid in 69% yield. It may be noted in passing that

the insolubility of cadmium carbonate and of cadmium sulfide makes this latter oxidation procedure rather more attractive than that using barium benzoate as the oxidation mixture is simpler to work up. It is probable that the yield of **2** from **1** could be improved through use of the bromine–cadmium carbonate combination.

With aqueous dicyclohexylamine, the acid from **7** gave the salt **5**, showing that the acid was 2-acetamido-2-deoxy-D-gluconic acid (**8**), as expected. Indeed, turning matters around, the salt **5** proved to be a convenient precursor in the preparation of the acid. Exchange of the cation of **5** with silver was easily accomplished through the use of Amberlite IR-120 in the silver salt form; subsequent removal of the silver from the solution with hydrogen sulfide gave the acid **8** in 44% yield.

Ion-exchange removal of the dicyclohexylamine from an aqueous solution of **5**, followed by lactonization of the product, gave a syrup which chromatography clearly showed to consist of an acid and two lactones, presumably **8**, **9**, and **10**. Treated in absolute alcohol with dicyclohexylamine, this mixture gave **5** and **3**, confirming the observations made earlier[1].

Attempts to prepare 2-acetamido-2-deoxy-D-galactonic acid (**11**) through the oxidation of 2-acetamido-2-deoxy-D-galactose with bromine–barium benzoate or with bromine–cadmium carbonate were uniformly unsuccessful, giving in each case only 2-acetamido-2-deoxy-D-galactono-1,4-lactone[1,5] (**12**). However, conversion of dicyclohexylammonium 2-acetamido-2-deoxy-D-galactonate[2] into the corresponding silver salt through ion exchange, followed by removal of the silver with hydrogen sulfide, led to the isolation of a crystalline product having an elemental composition corresponding to a dihydrate of 2-acetamido-2-deoxy-D-galactonic acid (**11**). On drying *in vacuo* at 85°, the material sintered and lost weight corresponding to two mole equivalents of water; material so treated was recrystallized and found to be 2-acetamido-2-deoxy-D-galactono-1,4-lactone (**12**).

EXPERIMENTAL

General methods. — Melting points are equivalent to corrected values. Silica Gel GF, 250 μm (Analtech, Inc.) with the solvent systems specified was used for analytical t.l.c. Unless otherwise noted, components were detected by charring with 10% sulfuric acid. Whatman No. 1 paper was used for paper chromatography.

2-Acetamido-2-deoxy-D-mannonic acid (**2**). — 2-Acetamido-2-deoxy-D-mannose monohydrate (**1**, Pfanstiehl Labs., Inc., 6.0 g, 25.1 mmoles) was dissolved in water (400 ml) and barium benzoate dihydrate (15.78 g, 38 mmoles) and bromine (1.65 ml, 32.2 mmoles) were added to the solution. The mixture was stirred in the dark at room temperature, benzoic acid beginning to precipitate in 15–20 min. After 12 h, the mixture was extracted with dichloromethane (50 ml, 4 × 25 ml) and then kept *in vacuo* at room temperature on a rotating evaporator until free of bromine. The solution was then passed through a column (100 ml) of Amberlite IR-120(H^+), the resin being washed thereafter with 500 ml of water. Combined, the solution and washings were extracted with dichloromethane (4 × 25 ml) and then stirred for 1 h with silver carbonate (8 g). The solid material was removed by filtration on a bed of

Filter-cel, and the filtrate was treated with hydrogen sulfide. Silver sulfide was removed by filtration on a bed of decolorizing carbon overlaid with Filter-cel. Air was blown through the filtrate to remove the remaining hydrogen sulfide and the solution was filtered again through decolorizing carbon. It was then concentrated *in vacuo* (36–40° bath) with a high-efficiency evaporator equipped with a condenser cooled by Dry Ice. When nearly solvent-free, the crystalline mass was cooled to −5° and abs. ethanol (20 ml) was added. The mixture was kept for 1 h at −5° and the crystals were then collected on a filter and washed with abs. ethanol. Dried in the air, the product, pure **2** (3.3 g, 55%), had m.p. 173.5–174° (sample introduced at 169°, 2° per min, gas evolution, dec.) and $[\alpha]_D^{20}$ −19.0° (4 min, *c* 2.02, water). A dextro-mutarotation was observed but this was not seen to cease and, eventually, mycelia were observed in the solution. In saturated phenylmercuric acetate solution (*c*, 2.26), **2** showed $[\alpha]_D^{20}$ −19.8° (5 min)→ +57.3° (72 days, not constant); in acetic acid–water (1:1, *c* 2.15) it showed $[\alpha]_D^{20}$ −10.8° (5 min)→ +83.4° (5 days)→ +67.2° (12 days, not constant). Although **2** may be recrystallized from water, the process entails substantial loss and fails to change the m.p.

Anal. Calc. for $C_8H_{15}NO_7$: C, 40.51; H, 6.37; N, 5.91. Found: C, 40.53; H, 6.53; N, 5.62.

The i.r. spectrum of **2** (Nujol mull) showed absorption at 3424 (OH), 1709 (CO_2H), 1613, and 1563 cm^{-1} (amide). Attempts to obtain further crops of crystalline material from the mother liquor led, in one experiment, to the isolation of a small quantity (84.7 mg) of **3**, m.p. 167–168°, identified through its i.r. spectrum. In another experiment, the mother liquor yielded crystals (1.3 g, m.p. 135–142°) having i.r. and n.m.r. spectral features that clearly showed it to be a mixture. Fractional crystallization from 1,4-dioxane with the addition of an increasing proportion of pentane permitted the resolution of this mixture into two components. The n.m.r. spectrum (methyl sulfoxide-d_6, 100 MHz) of the more soluble component showed two downfield doublets, one of which (J_{NHCH} 8.4 Hz) was centered at τ 1.85 and clearly represented impure **3**. The less-soluble component had m.p. 158–164° and $[\alpha]_D^{20}$ +143° (8 min)→ +30.5° (5 days, constant) (*c* 0.51, water); i.r. absorption (Nujol mull) at 1725 (CO), 1634, and 1534 cm^{-1} (amide; n.m.r. signal (methyl sulfoxide-d_6, 100 MHz): doublet centered at τ 2.0, J_{NHCH} 8.0 Hz (the doublet centered at τ 1.85 and representing **3** was absent). Applied to paper, the compound gave a positive lactone test when sprayed with hydroxylamine–ferric chloride[6]. The compound had the elemental composition of an acetamido-deoxyhexonolactone but the quantity in hand was too small to permit unequivocal assignment of configuration.

Anal. Calc. for $C_8H_{13}NO_6$: C, 43.84; H, 5.98; N, 6.39. Found: C, 43.54; H, 6.11; N, 6.11.

*2-Acetamido-2-deoxy-*D*-mannono-1,4-lactone* (**3**) *from 2-acetamido-2-deoxy-*D*-mannonic acid* (**2**). — The acid (**2**, 300 mg) was dissolved in boiling glacial acetic acid (12 ml), the total time from addition to complete solution being 3.5 min. The hot solution was immediately evaporated *in vacuo* to a syrup that was dissolved in water. The solution was evaporated *in vacuo* and the residual syrup was crystallized by the

addition of isopropyl alcohol. Some pentane was added and the crystals were washed with isopropyl alcohol–pentane and dried; yield 214 mg (77%). Recrystallized thrice from isopropyl alcohol, the product had m.p. 167–171°, either alone or in admixture with an authentic sample of 2-acetamido-2-deoxy-D-mannono-1,4-lactone[1]; the i.r. absorption spectra of the two samples were identical. It should be emphasized that the duration of the reaction is critical here; exposure of the mixture to hot glacial acetic acid for a longer period than prescribed gives rise to the formation of by-products which, on t.l.c. (dichloromethane–methanol, 13:4), move more rapidly than **3**.

Dicyclohexylammonium 2-acetamido-2-deoxy-D-mannonate (**4**). — A sample of 2-acetamido-2-deoxy-D-mannonic acid (**2**, 500 mg, 2.11 mmoles) was neutralized with dicyclohexylamine (0.42 ml, 2.11 mmoles) in the following manner. To a stirred mixture of water (10 ml) and acetone (10 ml) a few mg of the **2** were added, followed by a few drops of a solution of the dicyclohexylamine in acetone (3 ml); repeated alternate additions were made in such a manner that the pH of the solution varied from 5 to 7. When the additions were complete, the solution (pH 6.2) was concentrated *in vacuo* to a crystalline mass. Recrystallized from aqueous acetone, the salt (800 mg, 91%) had m.p. 160–164° (gas evolution, dec.) and $[\alpha]_D^{20}$ +1.3° (*c* 3.5, water). It may also be recrystallized from ethanol–pentane or from ethanol–acetone. The fingerprint region of the i.r. spectrum of the salt (Nujol mull) showed characteristic absorption bands at 1057, 1036, and 891 cm^{-1}; these bands readily distinguish the compound from its D-*gluco* isomer[1], which has characteristic absorption at 1016, 974 and 885 cm^{-1}.

Anal. Calc. for $C_{20}H_{38}N_2O_7$: C, 57.40; H, 9.15; N, 6.69. Found: C, 57.25; H, 9.04; N, 6.61.

2-Acetamido-2-deoxy-D-mannono-1,4-lactone (**3**) *from* **4**. — A solution of **4** (0.6 g) in water (25 ml) was passed through a column (120 ml) of Amberlite IR-120 (H^+). The column was washed with water (200 ml) and the combined eluate and washings were concentrated *in vacuo* to a crystalline residue (0.3 g) which was dissolved on the steam bath in glacial acetic acid (12 ml) over the course of 15 min. Concentration of the solution *in vacuo* gave a slightly colored syrup that crystallized spontaneously. The product was triturated with isopropyl alcohol, removed by filtration, washed with isopropyl alcohol–pentane and dried: yield 211 mg (67%); recrystallized thrice from isopropyl alcohol, it did not depress the m.p. of **3** and gave an i.r. spectrum identical with that of an authentic sample of **3**.

Behavior of dicyclohexylammonium 2-acetamido-2-deoxy-D-mannonate (**4**) (*and of* **2**) *with aqueous dicyclohexylamine.* — The salt (**4**, 0.5 g) was dissolved in water (1 ml), dicyclohexylamine (0.8 ml) was added to the solution, and the mixture was stirred overnight at room temperature. The dicyclohexylamine was largely removed with a pipette and the aqueous layer was then washed several times with ether; evaporated *in vacuo*, the aqueous layer afforded a crystalline residue (440 mg). Recrystallization from aqueous acetone gave three crops: 253 mg (m.p. 163–168°), 128 mg (m.p. 157–164°), and 25 mg (m.p. 152–158°). The i.r. spectra of these crops did not differ significantly from that of pure **4**.

A sample of **2** (250 mg) was similarly treated with water (1 ml) and dicyclo-

hexylamine (2 ml), giving two crops of crystalline product (wt. 323 mg, 73%); the i.r. spectrum of each crop showed it to be **4**.

*Behavior of 2-acetamido-2-deoxy-*D*-mannono-1,4-lactone* (**3**) *with dicyclohexylamine.* — (*a*) *In absolute ethanol.* The lactone (**3**, 113 mg) was dissolved in warm abs. ethanol (8 ml); the solution was cooled to room temperature, treated with three drops of dicyclohexylamine, and the reaction mixture was kept for 2 days at room temperature. A minute deposit of fine needles was removed by filtration and washed with a few drops of ethanol; these had m.p. 165–170° but were not further investigated. A portion (3 ml) of the filtrate was cooled, diluted with ether and pentane, and seeded with the aforementioned needles to give 14 mg of crystalline material that was indistinguishable by t.l.c. (dichloromethane–methanol, 13:4) from **3**.

The remainder of the filtrate was stored at room temperature and was examined by paper chromatography with 1-butanol–acetic acid–water (12:2:5), using silver nitrate for development. In addition to a component migrating at the same rate as **3**, there was a faster-moving as well as a slower-moving component. After 19 days, crystals were observed in the solution and were separated by decantation; these represented the slower-moving component, which was identified through its i.r. spectrum as dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate (**5**). The decanted solution, diluted with pentane, deposited a second crop of crystals which represented the faster-moving component of the mixture as shown by t.l.c. (dichloromethane–methanol, 13:4). This product had m.p. 170–177°; its chromatographic behavior and i.r. spectrum were indistinguishable from those of 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone (**6**) which has recently been described[1].

Twenty-four days after the reaction had been initiated, **3** could no longer be detected by t.l.c. of the solution.

(*b*) *In aqueous dicyclohexylamine.* 2-Acetamido-2-deoxy-D-mannono-1,4-lactone (**3**, 500 mg) was dissolved in water (0.6 ml), dicyclohexylamine (0.53 ml) was added and the mixture was stirred at room temperature. In ~15 min crystals began to form and, within 7 h, the reaction mixture became unstirrable. On the following day the mass was chilled, the crystals were removed by filtration and washed successively with cold 90% ethanol and then abs. ethanol to give a first crop: wt. 490 mg, m.p. 161–166° (dec., melt not clear). The mother liquor yielded a second crop [wt. 238 mg, 161–166° (dec., melt not clear)] and, finally, a third crop: wt. 156 mg, m.p. 155–165° (total yield 92%). Fractional crystallization of the first crop gave 154 mg of dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate (**5**) and 200 mg of its D-*manno* isomer (**4**); 126 mg of the latter compound was recovered through fractional crystallization of the second crop; the third crop was not further investigated. The separation of these isomers depends upon the relative insolubility in aqueous acetone of **5** as compared with **4**; identification in each case was made through the characteristic i.r. absorption bands of the two salts that were cited earlier in this paper.

*2-Acetamido-2-deoxy-*D*-gluconic acid* (**8**). — (*a*) *From 2-acetamido-2-deoxy-*D*-glucose* (**7**). 2-Acetamido-2-deoxy-D-glucose (**7**, 5.0 g, 22.6 mmoles) was dissolved in

water (340 ml) and to the solution were added cadmium carbonate (12 g, 70 mmoles) and bromine (1.4 ml, 27.4 mmoles). The reaction mixture was stirred in a closed 500-ml flask overnight at room temperature. The remaining cadmium carbonate was removed by filtration and the bromine was removed from the filtrate by aeration. Silver carbonate (18 g) was added and the suspension was stirred for 0.5 h; after filtration on a bed of Filter-cel, the solution was treated with hydrogen sulfide and refiltered through a bed (~3 mm) of Darco-X overlayered with Filter-cel. Aeration removed the remaining hydrogen sulfide in the filtrate and the solution was refiltered through a bed of Darco-X–Filter-cel. On concentration *in vacuo*, the solution afforded a crystalline mass; abs. ethanol was worked into this, the mixture was cooled, and the crystals were removed by filtration: yield 3.7 g (69%). As first prepared, the 2-acetamido-2-deoxy-D-gluconic acid (**8**) was obtained from aqueous ethanol as prismatic crystals of m.p. 145–146° (sample introduced at 138°, 2°/min, dec., gas evolution) and $[\alpha]_D^{20}$ −2.5° (4 min) → +31.2° (20 h) → +43.2° (19 days) (*c* 1.35, water); i.r. absorption (Nujol mull): 1715 (CO_2H), 1613, and 1570 cm^{-1} (amide).

Anal. Calc. for $C_8H_{15}NO_7$: C, 40.51; H, 6.37; N, 5.91. Found: C, 40.22; H, 6.63; N, 6.15.

In a subsequent preparation, the product was obtained from aqueous ethanol solution with m.p. 162–163° (sample introduced at 138°, 2°/min, dec., gas evolution) and $[\alpha]_D^{20}$ −2.9° (5 min) → +31.3° (18 h) → +43.7° (19 days) (*c* 1.4, water).

Anal. Found: C, 40.78; H, 6.53; N, 5.78.

The i.r. spectrum showed absorption at 1693 (CO_2H), 1612, and 1556 cm^{-1} (amide). Recrystallization of the lower-melting form from aqueous alcohol gave, with appropriate seeding, the higher-melting form.

(*b*) *From dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate* (**5**). A solution of silver nitrate (100 g) in water (800 ml) was passed through a column of Amberlite IR-120 (H^+) (120 ml) and the resin was then washed with distilled water until silver ions ceased to emerge. A solution of **5** (1.0 g) in water (20 ml) was passed through the column of resin, which was subsequently washed with water (250 ml). The combined effluent and washings were concentrated *in vacuo* (30° bath) to a volume of 30 ml and then treated with hydrogen sulfide. The silver sulfide was removed by filtration on a thin layer of decolorizing carbon and the filtrate was freed of hydrogen sulfide by aeration; it was then evaporated *in vacuo* (28–30° bath) to a syrup to which abs. ethanol (15 ml) was added. The turbid solution was filtered through a very small quantity of decolorizing carbon; on scratching the walls of the glass container, the clear, colorless filtrate began to deposit crystals. The mixture was kept overnight at room temperature and the crystals were then removed by filtration, washed with abs. ethanol and dried: yield 250 mg (44%), m.p. 162–163°; the i.r. spectrum of the product was indistinguishable from that of **8**, prepared as described already in (*a*).

Compound **5** *from* **8**. — 2-Acetamido-2-deoxy-D-gluconic acid (**8**, 200 mg of the lower-melting dimorph) was dissolved at room temperature in the minimum quantity of water (3 ml). Dicyclohexylamine (0.168 ml) was added and the mixture was stirred overnight. Abs. ethanol (6 ml) was added and the solution was chilled.

After 4 h, the crystalline product was removed, washed with alcohol and dried: yield 0.15 g (43%), m.p. 206–102°; its i.r. spectrum clearly identified it as **5**.

*Behavior of crude, mixed 2-acetamido-2-deoxy-*D*-gluconolactones* (**9** *and* **10**) *with dicyclohexylamine in absolute ethanol.* — An amorphous mixture of 2-acetamido-2-deoxy-D-gluconolactones was prepared through the decationization of **5** as described recently[2]. Chromatography of a sample of this syrup on paper with 1-butanol–acetic acid–water (12:2:5), followed by spraying with silver nitrate and treatment with alkali, revealed the presence of three components; only two of these appeared when a similar chromatogram was sprayed with alkaline hydroxylamine and then with ferric chloride[6]. Spraying with Bromocresol Green[1] showed the third component of the mixture to be an acid. These observations are consistent with the view that the syrup was a mixture of **8**, **9**, and **10**. In order to maximize the lactone content of the syrup, a sample (~20 mg) was heated with glacial acetic acid for about 3 min. The solution was evaporated *in vacuo* to a syrup that was dissolved in abs. alcohol. The solution was evaporated *in vacuo* and the treatment with ethanol was repeated. The residual syrup was finally dissolved in abs. ethanol (4 ml) and two drops of dicyclohexylamine were added. After 6 h, the reaction mixture was concentrated *in vacuo* to a residue that was washed with ether. The residue was then dissolved in water (2 drops) and acetone was added. Clusters of prismatic needles were formed: wt. 7 mg, m.p. 180–190°. This product showed the chromatographic behavior and had the i.r. spectrum of dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate (**5**). The syrupy residue from the mother liquor crystallized in part and was diluted with ethanol and pentane; the crystals were washed with ethanol–pentane: wt. 3 mg, m.p. 150–165°. The i.r. spectrum of this second crop was identical with that of 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**3**).

*2-Acetamido-2-deoxy-*D*-galactonic acid* (**11**). — Through a column of Amberlite IR-120(H^+) (120 ml) was passed a solution of silver nitrate (100 g) in water (700 ml). The column was washed with distilled water until the effluent was free of silver ions. A solution of dicyclohexylammonium 2-acetamido-2-deoxy-D-galactonate[2] (1.0 g) in water (40 ml) was passed down the column and the resin was washed with water (100 ml). The combined effluent and washings were concentrated *in vacuo* (30° bath) to a volume of ~25 ml and the solution was then treated with hydrogen sulfide. After filtration through layers of Filter-cel and Darco-X, the solution was aerated to remove hydrogen sulfide and then refiltered. The solution was lyophilized to give a partially crystalline residue. Abs. ethanol (3 ml) was added at room temperature and a small amount of crystalline **11** dihydrate (70 mg) was removed by filtration. The filtrate was concentrated *in vacuo* to a crystalline mass which was chilled and treated with abs. ethanol (2 ml). Removed by filtration, washed with abs. ethanol and dried at room temperature, the crystals of **11** dihydrate (0.2 g, total yield 44%) had m.p. 102–103° and $[\alpha]_D^{20}$ −33.8° (2 min) → −16.0° (3 days, constant) (*c* 1.56, water): i.r. absorption at 1721 (CO_2H), 1616, and 1560 cm^{-1} (amide).

Anal. Calc. for $C_8H_{15}NO_7 \cdot H_2O$: C, 37.65; H, 6.71; N, 5.49; H_2O (2 moles), 14.12. Found: C, 37.42; H, 6.60; N, 5.45; wt. loss on drying *in vacuo* at 85°, 14.16.

*2-Acetamido-2-deoxy-*D*-galactono-1,4-lactone* (**12**) *from* **11**. — A sample of the monohydrate of **11** was dried *in vacuo* at 85° to constant weight.

Anal. Calc. for an acetamidodeoxyhexonolactone ($C_8H_{13}NO_6$): C, 43.84; H, 5.98; N, 6.39. Found: C, 43.72; H, 6.27; N, 5.91.

Another sample of **11** dihydrate (100 mg) was similarly dried. The pale-brown mass, which appeared to be a resolidified melt, was dissolved in water (1 ml) and the solution was filtered through a very small quantity of decolorizing carbon. It was then concentrated at room temperature with a stream of air and the crystalline residue was cooled to +5°. Abs. ethanol (3 ml) was stirred in and the mixture was stored overnight at +5°. Removed by filtration, washed with abs. ethanol, and dried at room temperature, the product (70 mg, 82%) had m.p. 174–175°; its i.r. spectrum and chromatographic behavior (t.l.c. with chloroform–methanol, 1:1) were indistinguishable from those of an authentic sample[1] of **12**.

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