Carbohydrate Research, 26 (1973) 323–333

DICYCLOHEXYLAMMONIUM SALTS FOR THE ISOLATION AND CHARACTERIZATION OF ALDONIC ACIDS*

Emmanuel Zissis, Harry W. Diehl, Hewitt G. Fletcher, Jr.,
National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland 20014 (U. S. A.)

and Nevenka Pravdić
Department of Organic Chemistry and Biochemistry, "Rudjer Bošković" Institute, Zagreb, Croatia (Yugoslavia)

(Received May 19th, 1972; accepted for publication, June 15th, 1972)

ABSTRACT

Dicyclohexylammonium salts of aldonic acids may be prepared from aldonolactones, as well as from metal aldonates and the free acids. Although accompanied by decomposition, their melting points are usually sharp, and these salts appear to have some potential utility for the isolation and characterization of aldonic acids.

Dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate (**1**) has recently been described; in the course of the present investigation, it was converted into 2-acetamido-2-deoxy-D-glucose (**3**), confirming the configuration previously assigned to it. With aqueous dicyclohexylamine, 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**2**) gives **1**. The configuration of **2** was reconfirmed through reduction to 2-acetamido-2-deoxy-D-mannitol (**4**), and the optical rotations of this compound and its D-*gluco* isomer in acidified ammonium molybdate solution were found to be useful physical constants for distinguishing these alditols.

2-Acetamido-2-deoxy-D-galactono-1,4-lactone affords a crystalline dicyclohexylammonium salt of the corresponding acid, from which the lactone may be regenerated.

INTRODUCTION

Although cyclohexylamine and dicyclohexylamine became commercially available in the nineteen-thirties and were the subjects of a review[1] in 1937, their utility for the preparation of crystalline derivatives of organic acids was apparently first recognized by Harris and Sanderson[2] who, in 1948, separated the resin acids isolable from the turpentine of *Pinus palustris* as their salts with these bases. In the carbohydrate field, the use of cyclohexylamine for such purposes appears to have been restricted to the sugar phosphates, with which it frequently affords splendidly crystalline salts. Dicyclohexylamine, however, was recently found[3] to form a readily crystalline salt with 2-acetamido-2-deoxy-D-gluconic acid (**1**) and we, therefore, deemed it of interest to examine the properties of the dicyclohexylammonium salts of a few other carbohydrate-derived carboxylic acids.

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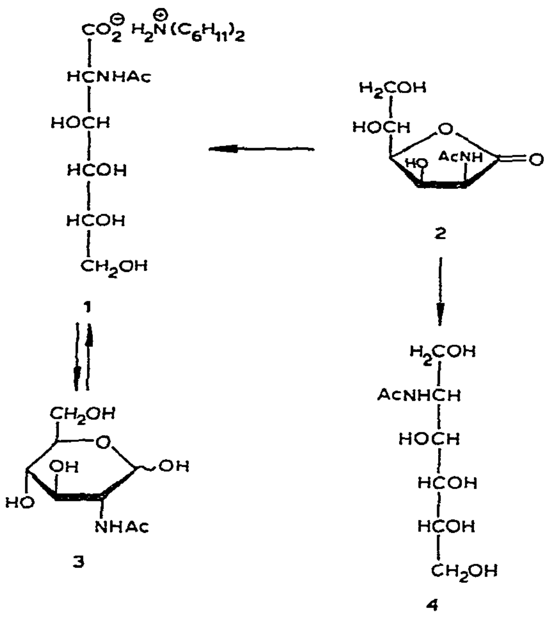
*Dedicated to Professor V. Deulofeu, in honor of his 70th birthday.

RESULTS AND DISCUSSION

When an aqueous solution of D-glucono-1,5-lactone was treated with an approximately equivalent quantity of dicyclohexylamine, the base slowly dissolved and the resulting solution yielded a crystalline product having the elemental composition of a dicyclohexylammonium hexonate; D-glucono-1,4-lactone served equally well for this purpose. In passing, it may be noted that the earlier work[3], already cited, included treatment of 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**2**) with dicyclohexylamine under nonaqueous conditions; neither then nor in the course of the present investigation was evidence for formation of amide detected.



Aldonic acids have most frequently been isolated as their lactones and as the salts of the alkaline-earth elements, particularly barium, calcium, and strontium, along with cadmium and zinc. As all of these elements form relatively insoluble oxalates, conversion of the metal salts of the aldonates into the corresponding dicyclohexylamine salts through the action of oxalic acid and dicyclohexylamine should constitute a relatively simple process, and this was shown to be true in the case of calcium D-gluconate. The attractive plan of carrying this reaction out as a simple metathesis under neutral conditions is thwarted by the fact (confirmed in the course of the present research) that the acid salt of oxalic acid with dicyclohexylamine is the only one accessible[4].

The dicyclohexylammonium salt of D-mannonic acid, prepared from D-mannono-1,4-lactone, was found to be a crystalline, high-melting solid, as were the corresponding salts of D-galactonic and lactobionic acids (see Table I). The latter salt was

TABLE I

MELTING POINT AND SPECIFIC ROTATION OF SOME DICYCLOHEXYLAMMONIUM ALDONATES

Dicyclohexylammonium	*M.p. (degrees)*	$[\alpha]_D^{20}$ *(degrees; in water)*
D-Gluconate	162–163	+6.82
D-Mannonate	144–145	−4.9
D-Galactonate	156	+1.8
Lactobionate	183–184	+16.6
Maltobionate	amorph.	+6.84
2-Acetamido-2-deoxy-D-gluconate	202–203	−2.8
2-Acetamido-2-deoxy-D-galactonate	174–175	−23.5

prepared in high yield from lactobiono-1,5-lactone, but could equally well be isolated subsequent to a conventional oxidation of lactose with aqueous bromine. Maltobionic acid gave a solid, but apparently amorphous, salt with dicyclohexylamine, and attempts to obtain dicyclohexylammonium cellobionate were unsuccessful. Galactaric acid gave solids having varying nitrogen content; it is probable that the low solubility of the acid presents special problems here.

The preparation of the dicyclohexylammonium salt of 2-acetamido-2-deoxy-D-gluconic acid from 2-acetamido-2-deoxy-D-glucose (3), described previously[3], was re-investigated in the course of the present research, and was so modified as to make the isolation of pure **1** somewhat more rapid and convenient. In further extending this series of salts, attention was turned to the preparation of the epimer of **1**, namely, dicyclohexylammonium 2-acetamido-2-deoxy-D-mannonate. 2-Acetamido-2-deoxy-D-mannono-1,4-lactone (**2**), a crystalline compound readily preparable through the bromine-water oxidation of 2-acetamido-2-deoxy-D-mannose[3], was treated in aqueous solution with dicyclohexylamine, to give, in 81% yield, a crystalline salt; however, this product proved to be identical with the salt previously prepared through the oxidation of 2-acetamido-2-deoxy-D-glucose (**3**). The salt was also made following the oxidation of 2-acetamido-2-deoxy-D-mannose but omitting the isolation of the lactone **2**, but, in this case, the yield was only 14%. Here, for the first time in the course of this investigation, it was apparent that an epimerization had occurred at some point in the reaction sequence. Although evidence adduced earlier[3] had appeared wholly adequate to justify assignment of the D-*gluco* configuration (**1**) to this salt, such evidence was based on the results of chromatography and electrophoresis. We, therefore, removed the dicyclohexylamine from an aqueous solution of the salt by using a cation-exchange resin, and concentrated the solution to a syrupy mixture of lactones which was then reduced with sodium amalgam in the presence of oxalate buffer; 2-acetamido-2-deoxy-D-glucose was subsequently isolated in 51% yield, and so the configuration of this salt as depicted in **1** may be regarded as confirmed.

Although the configuration of the lactone **2** appeared to be firmly established[3], it was of some interest to reconfirm this as well. Here, however, the buffered reduction with sodium amalgam appeared to fail completely; a similar difficulty was encountered in an attempt to reduce the known[3,5] 2-acetamido-2-deoxy-D-galactono-1,4-lactone, although, in this instance, chromatography showed that partial reduction had taken place after repeated exposure of the material to sodium amalgam. As Isono *et al.*[6] apparently encountered no difficulty in reducing 2-acetamido-2-deoxy-L-xylono-1,4-lactone to the corresponding aldose with oxalate-buffered sodium amalgam, these results are somewhat surprising. In contrast to sodium amalgam, sodium borohydride in cold, buffered solution, readily reduced **2**, giving 2-acetamido-2-deoxy-D-mannitol (**4**); as this is a known compound of undisputed configuration[7], the lactone is certainly represented by **2**.

In passing, we wish to point out that, although the melting point of **4**, reported as 138° (ref. 7) and 136–137° (ref. 8), readily distinguishes this alditol from the epimeric 2-acetamido-2-deoxy-D-glucitol [m.p. 153° (ref. 9), 156.2° (ref. 10), 152–154° (ref. 11), and 150–151° (ref. 12)], the specific rotations recorded for **4**, namely, $[\alpha]_D^{20}$ $-7.7°$ (ref. 7) and $[\alpha]_D^{22}$ $-8.6°$ (ref. 8), are too close to those of its epimer $\{[\alpha]_D^{18}$ $-11°$ (ref. 9) and $[\alpha]_D^{21}$ $-9 \pm 1°$ (ref. 12)$\}$ to be useful for identification purposes. Such low specific rotations are characteristic of the alditols, but Richtmyer and Hudson[13] showed that these values are greatly enhanced when measured in aqueous ammonium molybdate solution, and are still further augmented when the molybdate solution is acidified. We have found that the optical rotations of the two 2-acetamido-2-deoxyalditols under discussion are also markedly increased when measured in this way, and the values given in Table II are clearly suitable for distinguishing between these two compounds.

TABLE II

OPTICAL ROTATIONS OF TWO 2-ACETAMIDO-2-DEOXYHEXITOLS

Isomer	$[\alpha]_D^{20}$ *(degrees)*	
	in 5% molybdate	*in acidified molybdate*
D-*manno*	+46.7 (*c* 0.44)	+171 (*c* 0.35)
D-*gluco*	−18.4 (*c* 0.67)	+35.6 (*c* 0.53)

The oxidation of 2-acetamido-2-deoxy-D-galactose with unbuffered, aqueous bromine has been shown[3] to give the long-known 2-acetamido-2-deoxy-D-galactono-1,4-lactone[5] in 72% yield. We repeated this oxidation but, instead of isolating the lactone, we added dicyclohexylamine and thus obtained a crystalline salt having the elemental composition expected. That this product was, indeed, dicyclohexylammonium 2-acetamido-2-deoxy-D-galactonate (not the D-*talo* isomer) was shown by its reconversion into 2-acetamido-2-deoxy-D-galactono-1,4-lactone.

In conclusion, dicyclohexylamine appears to be a convenient reagent for the isolation and identification of non-nitrogenous aldonic acids but, although salts of this base have been recommended as useful derivatives for a variety of optically active *N*-substituted α-amino acids[14], epimerization by this strongly alkaline reagent presents a hazard (or a preparative asset) which should be kept in mind; a more detailed investigation of such epimerization is under way in the Bethesda laboratory.

EXPERIMENTAL

General methods. — Melting points given are equivalent to corrected values. The dicyclohexylamine employed was purchased from Aldrich Chemical Co. and was used without further purification. G.l.c. was performed with a Hewlett–Packard Research Chromatograph, Model No. 5750, with nitrogen (~90 ml/min) as the carrier gas, a column (6 ft × 0.25 in. OD) of 1% SE-30 on Gas-chrom P (Applied Science Laboratories, Inc.), and a flame-ionization detector. Tri-sil-Z (Pierce Chemical Co.) was used for trimethylsilylation. Paper chromatography was conducted with the solvent systems specified, components being located with alkaline silver nitrate.

Dicyclohexylammonium D-*gluconate.* — (*A*) *From* D-*glucono-1,5-lactone.* D-Glucono-1,5-lactone (Dawes Laboratories; 1.0 g) was warmed with water (1.0 ml) to ~40° to effect dissolution, and the solution was then kept overnight at room temperature. Dicyclohexylamine (1.0 g) was added, and the mixture was stirred until homogeneous. On cooling and scratching, material crystallized; absolute ethanol (2 ml) was stirred in, and the mixture was kept for 1 h at +5°. The product was removed by filtration, washed with cold absolute ethanol, and dried at room temperature; yield 70 mg (33%), m.p. 158–160°. After recrystallization from absolute ethanol (11.5 ml/g), the product was obtained as needles, m.p. 161–162°, $[\alpha]_D^{20}$ +6.8° (*c* 1.77, water).

Anal. Calc. for $C_{18}H_{35}NO_7$: C, 57.27; H, 9.35; N, 3.71. Found: C, 57.42; H, 9.07; N, 3.56.

(*B*) *From* D-*glucono-1,4-lactone.* D-Glucono-1,4-lactone[15] (0.5 g) was warmed with water (0.5 ml) to 35° until dissolution was complete. Dicyclohexylamine (0.53 ml) was added, and the mixture was stirred until the amine had dissolved (~2 h). On cooling the mixture in an ice-bath, material crystallized; absolute ethanol (1 ml) was stirred into the mixture, which was then kept for 2 h at +5°. The crystals were removed by filtration, washed with absolute ethanol, and dried; wt. 0.4 g (38%), m.p. 159–161°. After recrystallization from absolute ethanol, the product had m.p. 162–163° and $[\alpha]_D^{20}$ +5.8° (*c* 1.88, water).

(*C*) *From calcium* D-*gluconate.* A mixture of calcium D-gluconate monohydrate (594 mg) and dicyclohexylamine (0.54 ml) was warmed with water (3 ml) until the salt had dissolved. A solution of oxalic acid dihydrate (174 mg) in 95% ethanol (16 ml) was added, and the warm suspension was stirred. On filtration through a thin layer of Filter-cel, the mixture gave a clear, colorless filtrate which was evaporated *in vacuo* to a dry, crystalline mass (1.45 g). The crude product was dissolved in hot absolute ethanol (5 ml) and, after filtration, the solution was cooled, to yield needle-shaped crystals of the salt; wt. 0.58 g (58%), m.p. 161–162°, $[\alpha]_D^{20}$ +7.1° (*c* 1.5, water). A mixed m.p. with material prepared from D-glucono-1,5-lactone was undepressed.

Dicyclohexylammonium D-*mannonate.* — D-Mannono-1,4-lactone[16] (1.0 g) was warmed with water (1.0 ml) until it had dissolved. The solution was cooled, stirred with dicyclohexylamine (1.1 ml) for 7 h, and the mixture then stored for 2 days at room temperature. Isopropyl alcohol (2 ml) and acetone (6 ml) were added; crystallization, initiated by scratching, progressed rapidly. More of the same mixture of solvents (8 ml) was stirred into the mass, and the crystals were removed by filtration and dried at room temperature: wt. 1.5 g (71%). Recrystallized from absolute ethanol (10 ml/g), the product was obtained as very fine needles; wt. 1.3 g, m.p. 144–145°. A further recrystallization from this solvent failed to change the m.p., and gave a product having $[\alpha]_D^{20}$ −4.9° (*c* 3.5, water).

Anal. Calc. for $C_{18}H_{35}NO_7$: C, 57.27; H, 9.35: N, 3.71. Found: C, 57.12; H, 9.26; N, 3.59.

Dicyclohexylammonium lactobionate. — (*A*) *From lactobiono-1,5-lactone.* A solution of lactobiono-1,5-lactone (Sheffield Farms Co., Inc.; 1.0 g) in water (1.0 ml) was treated with dicyclohexylamine (1.1 ml), and the mixture was stirred for ~18 h. Isopropyl alcohol (5 ml) and acetone (3 ml) were added, and crystallization was allowed to progress overnight at room temperature. Absolute ethanol (3 ml) was then stirred into the mass, and the crystals were removed by filtration, washed with absolute ethanol (1 ml), and dried at room temperature; wt. 1.5 g (95%). After recrystallization from hot absolute ethanol (50 ml/g), the salt was obtained as fine needles, m.p. 184–185°. The product was recrystallized once more from the same solvent, and then dried for 2 h at 100°; m.p. 183–184°, $[\alpha]_D^{20}$ +16.6° (*c* 2.6, water).

Anal. Calc. for $C_{24}H_{45}NO_{12}$: C, 53.42; H, 8.41; N. 2.60. Found: C, 53.18; H, 8.53; N, 2.55.

(*B*) *From lactose.* For the oxidation of lactose, a modification of the method used by Antoniani[17] for the conversion of cellobiose into cellobionic acid was employed. A mixture of α-lactose monohydrate (12.0 g) and barium benzoate dihydrate (21.0 g) in water (500 ml) was cooled to 0° and stirred, while bromine (2.3 ml) was added. The reaction mixture, enclosed in a tightly stoppered vessel, was stirred for 2 h at 0° and then for 50 h at room temperature. The bromine remaining was then removed by aeration, and the precipitated benzoic acid (10.2 g) was removed by filtration. The filtrate was decationized by passage through a column (4 × 40 cm) of Amberlite IR-120 (H^+) ion-exchange resin, and the resin was washed with water until the effluent was neutral. The solution and washings were combined, washed with dichloromethane (3 × 50 ml) to remove the remaining benzoic acid, filtered through a thin layer of decolorizing carbon, and then treated with silver carbonate (12.5 g). After being stirred for 2.5 h at room temperature, the mixture was filtered, and the filtrate was treated with an excess of hydrogen sulfide, filtered through a layer of Filter-cel and decolorizing carbon, and evaporated *in vacuo* (bath temp. 32°) to a syrup which was diluted with water (11 ml) and treated with dicyclohexylamine (11.2 ml). The mixture was stirred overnight at room temperature, and was then diluted with isopropyl alcohol (50 ml) and with acetone (60 ml). Crystallization was spontaneous and was allowed to progress for 3 h at room temperature before the product was removed by filtration, washed with 1:1 isopropyl alcohol–acetone, and dried in the air; wt. 9.7 g, m.p. 183–184°, $[\alpha]_D^{20}$ +16.3° (*c* 2.9, water). Concentration of the mother liquor afforded a second crop (wt. 2.6 g, m.p. 182–184°), raising the total yield to 68%. For final purification, a solution of the product in water (1 ml/g) was filtered through a thin layer of decolorizing carbon and the filtrate was diluted with isopropyl alcohol (4 ml/g) and acetone (5 ml/g), nucleated, and kept overnight at room temperature, to give fine needles, m.p. 183–184°, $[\alpha]_D^{20}$ +17.9° (*c* 3.1, water).

Dicyclohexylammonium maltobionate. — A solution of lithium maltobionate trihydrate[18] (10.0 g) in cold water (75 ml) was passed through a column (50 ml) of Amberlite IR-120 (H^+) ion-exchange resin at +5°. The column was washed with cold water (500 ml), and the combined effluent and washings were evaporated *in vacuo* at room temperature to a syrup which was dissolved in a little water and the solution treated with dicyclohexylamine (10 ml). After several hours at room temperature, the mixture was diluted with acetone (100 ml), and the resulting precipitate was washed with acetone (75 ml) to give, after drying at room temperature, a free-flowing powder (wt. 12.9 g, 100%) that softened at ~75° and melted at 96–100°, although it was almost certainly amorphous; the salt showed $[\alpha]_D^{20}$ +6.8° (*c* 0.62, water).

Anal. Calc. for $C_{24}H_{45}NO_{12}$: C, 53.42; H, 8.41; N, 2.60. Found: C, 53.66; H, 8.62; N, 2.35.

*Dicyclohexylammonium 2-acetamido-2-deoxy-*D*-gluconate* (**1**). — (*A*) *From 2-acetamido-2-deoxy-*D*-glucose* (**3**). To a solution of 2-acetamido-2-deoxy-D-glucose (20.0 g, 90.4 mmoles) in water (400 ml) was added bromine (12.0 ml, 234 mmoles), and the mixture was stirred in the dark for 3 days at room temperature. The excess of bromine was removed by aeration, and the clear, colorless solution was stirred with silver carbonate (40 g) for 1 h at room temperature. The suspension was filtered through Filter-cel, the filtrate was saturated with hydrogen sulfide, the precipitated silver sulfide was removed by filtration on a bed of decolorizing carbon, the hydrogen sulfide in the filtrate was removed by aeration, and the solution was concentrated *in vacuo* (bath temp. 32°) to 50 ml. The solution was transferred to an Erlenmeyer flask with a total of 25 ml of water and then diluted with absolute ethanol (50 ml). Dicyclohexylamine (12 ml) was added, and the mixture was stirred until homogeneous. Crystallization began in a few minutes, and was allowed to proceed for 4 h at room temperature and then overnight at +5°. Removed by filtration, washed with 3:2 (v/v) ethanol–water, and dried at room temperature, the first crop of crude product amounted to 17.5 g. Two further crops were subsequently obtained from the mother liquor, raising the total yield of crude material to 23.7 g (63%). Combined crops of product from several such oxidations (wt. 30 g) were dissolved in warm water (150 ml), and the solution was filtered. Ethanol (500 ml) was added, and crystallization was allowed to proceed, first at room temperature, and then overnight at +5°. The crystals were removed by filtration, washed with ethanol, and dried at room temperature, affording 5.0 g of glistening, white scales which melted at ~230° (after preliminary darkening) and showed $[\alpha]_D^{20}$ $-3.2°$ (*c* 2.4, water). Recrystallization from boiling water gave a product having $[\alpha]_D^{20}$ $-3.7°$ (*c* 2.3, water) and an i.r. spectrum identical with that of an authentic specimen of 2-amino-2-deoxy-D-gluconic acid.

The mother liquor remaining after removal of the first crop (5.0 g) of crystals was evaporated *in vacuo* to a crystalline mass which was dissolved in water (100 ml). Ethanol (100 ml) was added, and the solution was kept overnight at $-5°$, to give pure **1**; 16.7 g (35%, based on 3), m.p. 202–203° (dec.), $[\alpha]_D^{20}$ $-2.8°$ (*c* 2.0, water). Repeated recrystallization from ethanol failed to change the optical rotation of the product significantly. The i.r. spectrum of the compound was identical with that of a specimen of **1** prepared as described earlier[3].

(*B*) *From 2-acetamido-2-deoxy-*D*-mannono-1,4-lactone* (**2**). 2-Acetamido-2-deoxy-D-mannono-1,4-lactone[3] (0.5 g), having m.p. 182–183°, $[\alpha]_D^{20}$ $+78.8°$ (*c* 2.0, water), was stirred with water (0.5 ml) and dicyclohexylamine (0.53 ml) at room temperature until the mixture became solid (several hours). Absolute ethanol (0.5 ml) was stirred into the mixture, which was then cooled to $+5°$. The product was removed by filtration, washed with ethanol, and dried; wt. 0.77 g (81%), m.p. 174–175.5° (dec.; sample introduced at 168°; ~4°/min). After recrystallization from hot ethanol, the product (0.30 g) had m.p. 204–205° (dec.) and $[\alpha]_D^{20}$ $-2.9°$ (*c* 1.3, water). A mixed m.p. with material prepared as described in *A* was undepressed; the i.r. absorption spectra of samples from the two sources were identical.

(*C*) *From 2-acetamido-2-deoxy-*D*-mannose.* 2-Acetamido-2-deoxy-D-mannose monohydrate (Pfanstiehl Laboratories, Inc.; 2.0 g) was oxidized with unbuffered bromine water, and the reaction mixture was processed as described earlier[3]. The syrupy product was dissolved in water (5 ml), dicyclohexylamine (1.25 ml) was added, and the mixture was stirred until dissolution was complete and then stored overnight at $-5°$. After concentration *in vacuo* to 4 ml, the solution deposited crystals; absolute ethanol (4 ml) was added, the mixture was cooled to $-5°$, and the crystals were filtered off; wt. 0.5 g (14%), m.p. 200–201°. Recrystallization from hot ethanol entailed little loss, and gave a product having m.p. 201–202° and $[\alpha]_D^{20}$ $-3.2°$ (*c* 1.7, water). A mixed m.p. with a sample of dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate prepared as described in *A* was undepressed; the i.r. absorption spectra of samples from the two sources were indistinguishable.

*2-Acetamido-2-deoxy-*D*-glucose* (**3**) *from dicyclohexylammonium 2-acetamido-2-deoxy-*D*-gluconate* (**1**). — A solution of **1** (5 g) in water (200 ml) was passed through a column (1 liter) of Amberlite IR-120 (H^+) ion-exchange resin, and the column was washed with 1.2 liters of water. The solution and washings were combined, and lyophilized to give a syrupy product (2.9 g). A solution of a sample (0.3 g) of this syrup in water (25 ml) was added to a mixture of oxalic acid dihydrate (4.41 g), sodium oxalate (4.26 g), and water (75 ml) precooled to 5–7°. With vigorous stirring and continued cooling, the mixture was treated with 5% sodium amalgam (15 g). After 35 min, the stirring and cooling were halted, and the supernatant liquor was separated from the mercury by decantation. The suspension was filtered, the residue was washed with water, and the pH of the combined filtrate and washings was raised to 5–5.5 by addition of M sodium hydroxide. The solution was then concentrated *in vacuo* until sodium oxalate began to crystallize; methanol (100 ml) was added, the mixture was chilled slightly, and the sodium oxalate was removed by filtration. It was washed with 85% aqueous methanol, and the filtrate and combined washings were evaporated *in vacuo* to a residue which was dissolved in water (20 ml). The solution was passed through columns of Dowex 50W X8 (20 ml) and Amberlite IR-54 (20 ml) ion-exchange resins, the first column being washed with 100 ml of water, and the second with 50 ml of water. Evaporation of the combined effluent and washings gave a crystalline mass (0.14 g, 51%) which was recrystallized from water–ethanol–ether to yield prismatic needles, m.p. 198–202° (dec.); $[\alpha]_D^{20}$ +73.1° (8 min)→ +40.7° (2.5 h) (*c* 0.5, water); lit[19]. m.p. 205°, $[\alpha]_D^{20}$ +64°→ +41° (water). After per(trimethylsilyl)ation, g.l.c. showed the product to be indistinguishable from authentic **3**, and clearly differentiated it from 2-acetamido-2-deoxy-D-mannose.

*2-Acetamido-2-deoxy-*D*-mannitol* (**4**) *from 2-acetamido-2-deoxy-*D*-mannono-1,4-lactone* (**2**). — A chilled solution of **2** (219 mg) in 0.5M boric acid (10 ml) was mixed with Amberlite IR-120 (H^+) ion-exchange resin (2 ml), and 0.3M sodium borohydride (10 ml) was added dropwise during 2–3 min. The mixture was stirred for 30 min, 10 ml of the sodium borohydride solution was added, and the suspension was stirred for 30 min, and kept overnight at +5°. The solution was decanted directly from the resin onto a column (15 ml) of Amberlite IR-120 (H^+) ion-exchange resin, and 100 ml of water was used to wash the resin and the column. The effluent and washings were combined and evaporated *in vacuo* to dryness, and methanol was thrice added and evaporated. At this point, paper chromatography with 12:2:5 butanol–acetic acid–water showed the presence of a single compound, not identical with the starting material. On being kept overnight in a refrigerator, the syrup crystallized spontaneously; a few drops of ethanol were added, and the product was removed by filtration and washed with cold ethanol; wt. 142 mg (64%). Recrystallized from hot ethanol, the product had m.p. 137–138.5°; lit. m.p. 136–137° (ref. 8), 138° (ref. 7). In 5% ammonium molybdate solution (prepared by dissolving 5 g of $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ in water and diluting to 100 ml), the 2-acetamido-2-deoxy-D-mannitol (**4**) showed $[\alpha]_D^{20}$ +46.7° (*c* 0.44); dilution of 8.0 ml of this rotation solution with 2.0 ml of 0.5M sulfuric acid gave a solution having an observed optical rotation corresponding to $[\alpha]_D^{20}$ +171° (*c* 0.35).

Another specimen of **4**, prepared through the reduction of 2-acetamido-2-deoxy-D-mannose with sodium borohydride, had m.p. 138–139° and the following specific rotations: $[\alpha]_D^{20}$ +46.8° (*c* 0.5 in 5% ammonium molybdate), +163° (*c* 0.4, acidified molybdate).

A sample of 2-acetamido-2-deoxy-D-glucitol, prepared several years ago in this laboratory by Dr. T. D. Inch, had m.p. 150–153° (lit.[5] m.p. 153°) and an acceptable elemental analysis; in 5% ammonium molybdate (*c* 0.67), it had $[\alpha]_D^{20}$ −18.4°, and, in acidified molybdate (*c* 0.53), $[\alpha]_D^{20}$ +35.6°.

*Dicyclohexylammonium 2-acetamido-2-deoxy-*D*-galactonate.* — 2-Acetamido-

2-deoxy-D-galactose (Pfanstiehl Laboratories, Inc.; 5 g) was oxidized with unbuffered aqueous bromine as described earlier[3]. The resulting solution was freed of bromine and bromide ions, and concentrated *in vacuo* to ~20 ml. Dicyclohexylamine (3 ml) was added, and the mixture was stirred at room temperature until clear. The solution was evaporated *in vacuo* to a thick syrup which was cooled to +5°, and nucleating crystals, obtained by diluting a sample of the solution with acetone, were added. After being kept for several days at +5°, the mixture was diluted with ethanol (25 ml); the crystals were removed by filtration, and washed with absolute ethanol; wt. 2.8 g (30%). The crude product was dissolved in hot ethanol, and the solution was treated with a little decolorizing carbon and filtered; crystallization was allowed to proceed at room temperature and then at +5°, to give 2.0 g of product which was recrystallized from ethanol in the same way, wt. 1.7 g, m.p. 174–175°, $[\alpha]_D^{20}$ −23.5° (*c* 1.5, water).

Anal. Calc. for $C_{20}H_{38}N_2O_7$: C, 57.40; H, 9.15; N, 6.69. Found: C, 57.12; H, 9.28; N, 6.54.

*2-Acetamido-2-deoxy-*D*-galactono-1,4-lactone from dicyclohexylammonium 2-acetamido-2-deoxy-*D*-galactonate.* — A solution of the salt (1.0 g) in water (10 ml) was passed through a column (25 ml) of Amberlite IR-120(H^+) ion-exchange resin, and the resin was washed with water (100 ml). On evaporation *in vacuo*, the combined solution and washings gave a syrup which was dissolved in methanol (25 ml). The methanol was removed *in vacuo*, and the residue was crystallized from isopropyl alcohol; wt. 0.31 g (59%). Recrystallized from hot absolute ethanol, the product had m.p. 173.5–174° and $[\alpha]_D^{20}$ −15.9° (*c* 1.7, water); its i.r. absorption spectrum was indistinguishable from that of an authentic sample of 2-acetamido-2-deoxy-D-galactono-1,4-lactone.

ACKNOWLEDGMENTS

We thank Dr. R. Schaffer of the National Bureau of Standards for nucleating crystals of lithium maltobionate trihydrate and of maltobiono-1,5-lactone. Elemental analyses were performed by the staff of the Section on Microanalytical Services and Instrumentation, NIAMDD.

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