THE OXIDATION OF 2-ACETAMIDO-2-DEOXYALDOSES WITH AQUEOUS BROMINE. TWO DIASTEREOISOMERIC 2-ACETAMIDO-2,3-DIDEOXYHEX-2-ENONO-1,4-LACTONES FROM 2-ACETAMIDO-2-DEOXY-D-GLUCOSE, -MANNOSE, AND -GALACTOSE[1]

N. PRAVDIĆ

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

AND H. G. FLETCHER, JR.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland 20014 (U. S. A.)

(Received May 6th, 1971)

ABSTRACT

Oxidation of 2-acetamido-2-deoxy-D-mannose (**2**) by bromine in unbuffered aqueous solution leads to the isolation of 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**5**). Similar oxidation of 2-acetamido-2-deoxy-D-galactose (**3**) gives the known 2-acetamido-2-deoxy-D-galactono-1,4-lactone (**6**). However, 2-acetamido-2-deoxy-D-glucose (**1**) forms a complex mixture. Besides unchanged **1**, this mixture contains 2-amino-2-deoxy-D-gluconic acid (**8**), and chromatography shows the presence of 2-acetamido-2-deoxy-D-glucono-1,5-lactone (**7**), as well as of an acid that can be isolated as its dicyclohexylammonium salt; the available evidence indicates that this salt is that of 2-acetamido-2-deoxy-D-gluconic acid (**9**). Finally, a crystalline lactone having the spectral characteristics of 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**5**) was isolated. It seems probable that the epimerization involved in the formation of this lactone occurred under the influence of the dicyclohexylamine.

When the lactones **5** and **6** and the crude reaction mixture from the oxidation of **1** are treated with methanolic potassium hydroxide, each gives the same mixture of two substances. These are diastereoisomeric 2-acetamido-2,3-dideoxyhex-2-enono-1,4-lactones that are interconvertible under the alkaline conditions used in their preparation. 2-Acetamido-2-deoxy-D-mannono-1,4-lactone (**5**) is readily converted into its 5,6-*O*-isopropylidene derivative (**16**); attempted *p*-toluenesulfonylation of **16** causes a β elimination, to give 2-acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-D-*erythro*-hex-2-enono-1,4-lactone (**14**). On isopropylidenation, one of the unsaturated lactones also gives **14**; it is, therefore, represented by formula **11**. The other isomer is assigned the D-*threo* configuration as depicted in **12**.

INTRODUCTION

The transformation of aldoses into the corresponding aldonic acids, typically brought about through the action of halogens[2], platinum-catalyzed oxygen[3], or

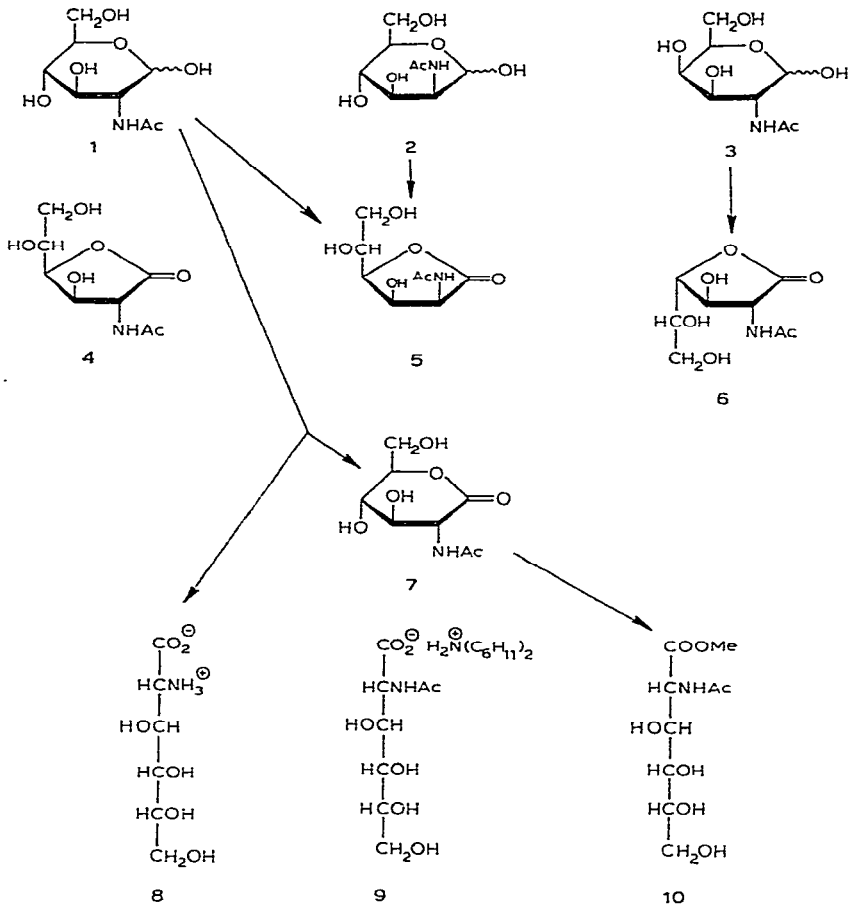
enzymes[4], is a long-established procedure in carbohydrate chemistry, but the analogous conversion of 2-acylamido-2-deoxyaldoses into 2-acylamido-2-deoxyaldonic acids has apparently attracted little investigation and, although a few 2-acylamido-2-deoxyaldonic acids have been reported[5], comparatively little is known of the chemistry of this class of compound. We have, therefore, undertaken an investigation of the oxidation of the three most accessible 2-acylamido-2-deoxyaldoses, namely, the 2-acetamido-2-deoxyhexoses of the D-glucose, D-mannose, and D-galactose series and, in this paper, describe the behavior of these three amino sugar derivatives with aqueous bromine. Characterization of some of the products of these oxidations revealed that they are markedly labile to alkali; an investigation of this aspect of their chemical properties formed a major part of the research reported here.

RESULTS

An unbuffered aqueous solution of 2-acetamido-2-deoxy-D-galactose (**3**, Scheme I) was treated with an excess of bromine for four days at room temperature. After removal of the unused bromine and of the hydrogen bromide that had been formed, the reaction mixture gave a crystalline product in 72% yield. The physical properties of this compound were in agreement with those reported[6,7] for 2-acetamido-2-deoxy-D-galactono-1,4-lactone (**6**), a compound that had been prepared by the earlier workers through the action of acetic anhydride and aqueous alkali on 2-amino-2-deoxy-D-galactonic acid. The i.r. absorption spectrum of the compound showed the carbonyl band in the range expected of a 1,4-lactone[8], supporting the assignment of ring size made earlier[6]; as will be discussed later, the present research afforded further evidence for the correctness of this assignment.

The oxidation of 2-acetamido-2-deoxy-D-mannose (**2**) with bromine water led to the isolation in 51% yield of a second crystalline lactone having a carbonyl absorption that indicated the presence of a five-membered ring. The product was assigned structure **5** and, in passing, it may be noted that the optical rotation of the compound ($[\alpha]_D$ +87.4°, water), as well as that of the corresponding D-*galacto* analog, **6**, ($[\alpha]_D$ −21.2°, water) conform to the lactone rule[9].

With bromine water, 2-acetamido-2-deoxy-D-glucose (**1**) appeared to behave in a manner that contrasted markedly with that of the two diastereoisomers just described. The reaction mixture produced was a complex one, and the only product isolable by direct crystallization was the well-known 2-amino-2-deoxy-D-gluconic acid[10] (**8**, 11% yield); although the high acidity of the oxidation mixture makes de-*N*-acetylation not unexpected, there is no evidence to indicate whether, in this case the *N*-acetyl group was removed before or after the oxidation. After removal of **8**, paper chromatography of the reaction mixture showed the presence of at least four components; one of these was unchanged **1**. Another component was an acidic substance, and a third had the chromatographic mobility shown by a sample of 2-acetamido-2-deoxy-D-glucono-1,5-lactone (**7**). This compound, recently reported in a patent[11] as a product of the acetylation of **8**, has been synthesized by an unequivocal route that will be described in the paper immediately following this one[12].



Scheme I

Treatment of the mixture in ethanolic solution with dicyclohexylamine afforded a crystalline product having the elemental composition of the dicyclohexylammonium salt of an acetamidodeoxyhexonic acid. The paper-chromatographic and electrophoretic behavior of the substance was that of a readily dissociable amine salt. An aqueous solution of the salt was decationized and then examined by paper chromatography; an acidic component, chromatographically identical with the one in the crude oxidation mixture from **1**, and a neutral component, indistinguishable from **7**, were detected. A sample of **7** was partially equilibrated in aqueous solution and then per(trimethylsilylated); g.l.c. revealed a series of components. That component having the longest retention time was chromatographically indistinguishable from the per(trimethylsilyl) derivative of the dicyclohexylammonium salt. Finally, a sample of the salt was hydrolyzed with strong acid to give a product that was chromatographically identical with **8**. On the basis of these pieces of evidence, we conclude that the salt has the expected D-*gluco* configuration (**9**).

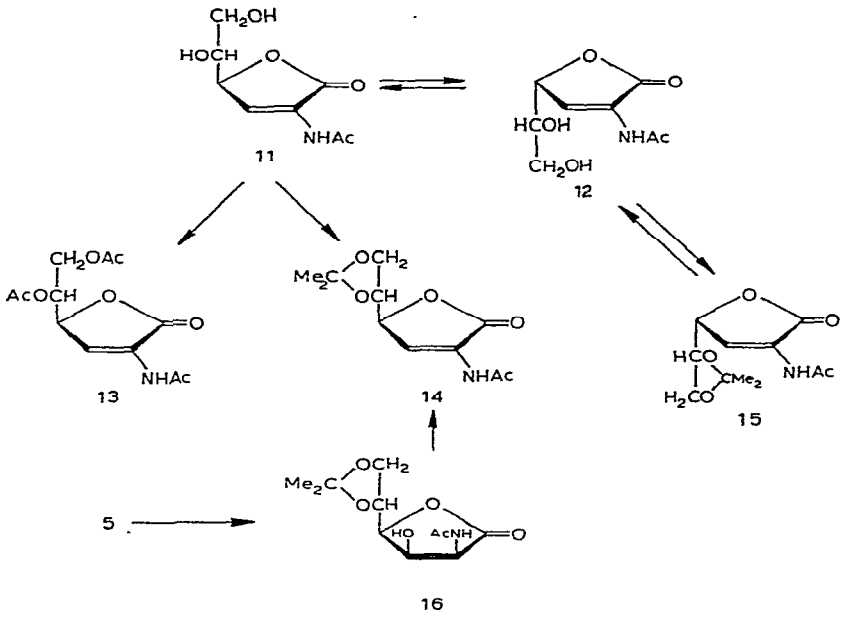
The solution from which **9** had been prepared yielded a crystalline lactone that had $[\alpha]_D$ +82.3° (water). This value, together with the melting point of the substance, suggested that it was impure 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**5**) and, indeed, the material had spectral characteristics (i.r. and n.m.r.) that were identical with those of **5**.

The mixture of products from the oxidation of 2-acetamido-2-deoxy-D-glucose (**1**) was found to react with methanol to give a crystalline substance; this proved to be methyl 2-acetamido-2-deoxy-D-gluconate[11,12] (**10**).

When 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**5**) was subjected to the action of methanolic potassium hydroxide, a mixture of two unsaturated compounds was formed; the same mixture was obtained (*a*) through similar treatment of 2-acetamido-2-deoxy-D-galactono-1,4-lactone (**6**) and (*b*) from the mixture of oxidation products from **1***. The spectral characteristics (i.r. and n.m.r.) of the two unsaturated products readily distinguished them from each other, and permitted estimation of the proportion of each in mixtures. However, only one of the two compounds could be obtained directly in pure crystalline form from the mixture. This substance had the elemental composition of a 2-acetamido-2,3-dideoxyhex-2-enonolactone (**11**, **12**), and the mass spectrum of its di-*O*-acetyl derivative showed it to have a five-membered ring (see Experimental section). On treatment with alkali, the compound gave a mixture similar to that from which it had been isolated. A mixture that had been enriched in the second unsaturated compound behaved in a similar manner, showing that the two substances were interconvertible.

The unsaturated lactone that had been isolated in pure form readily gave a crystalline isopropylidene derivative, and it was subsequently found that mixtures of the two unsaturated lactones could readily be separated after the two isomers had been converted into their isopropylidene derivatives. 2-Acetamido-2-deoxy-D-mannono-1,4-lactone (**5**), also, gave a crystalline isopropylidene derivative, and this may safely be assumed to have structure **16** (Scheme II). Treatment of **16** with *p*-toluenesulfonyl chloride in pyridine solution afforded 2-acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-D-*erythro*-hex-2-enono-1,4-lactone (**14**), and this compound proved to be identical with the isopropylidene derivative prepared from the unsaturated lactone. The first-isolated unsaturated lactone is, therefore, 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone (**11**). As there is no reason to question the configuration at C-5 in the compounds involved in these transformations, the second isopropylidene derivative, obtained from the mixture of unsaturated lactones, may be assigned the D-*threo* configuration (**15**); hydrolysis of the compound afforded 2-acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,4-lactone (**12**) in pure, crystalline form. This substance showed $[\alpha]_D$ −101.2° (water), and its D-*erythro* isomer (**11**) had $[\alpha]_D$ +41.1° (water); inasmuch as Hudson's rule[9] has been shown[13] to be applicable

*The reaction described here was first observed when the mixture from the oxidation of **1** was treated with an ether solution of diazomethane. In this case, it is presumed that the reaction was caused by traces of sodium hydroxide that had been carried over by entrainment in the codistillation normally used for the preparation of solutions of diazomethane in ether.



Scheme II

to unsaturated lactones closely related to these compounds, the signs of these optical rotations may be regarded as supporting the assigned configurations and ring sizes. As previously mentioned, the mass spectrum of the di-*O*-acetyl derivative of 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone (**13**) showed the presence of the five-membered ring; this evidence serves equally well, of course, to support the ring size of **5** and **6**.

DISCUSSION

The oxidation of 2-acetamido-2-deoxy-D-mannose (**2**) and -galactose (**3**) with unbuffered bromine water appears to be a relatively uncomplicated reaction, giving the corresponding 1,4-lactones (**5** and **6**) in moderately good yields. The oxidation of 2-acetamido-2-deoxy-D-glucose (**1**) may be equally simple, the heterogeneity of the reaction mixture being a consequence of the nature of the initial product formed. It seems likely that the equilibria that 2-acetamido-2-deoxyaldonic acids establish with their lactones in aqueous solution may be dependent upon configuration. In the mixture from the oxidation of **1**, chromatographic evidence certainly points to the presence of **7**, and it is not unreasonable to assume that this six-membered lactone provides some of the 2-acetamido-2-deoxy-D-gluconic acid that is isolated as its dicyclohexylammonium salt (**9**). The 1,5-lactone is also, probably, the source of the methyl 2-acetamido-2-deoxy-D-gluconate (**10**) that was obtained. The lactone that was isolated after exposure of the mixture to the action of dicyclohexylamine appeared to be an impure sample of the 1,4-lactone of the D-*manno* series (**5**). Doubtless, the

epimerization involved here was caused by the highly alkaline amine, but it is not necessary to assume that the as-yet-unknown D-*gluco* analog (**4**) is the species actually epimerized, for, as will be shown in the paper that immediately follows[12], 2-acetamido-2-deoxyhexonic acid derivatives that are incapable of forming 1,4-lactones may be epimerized through the action of amines. On the other hand, 2-acetamido-2-deoxy-D-glucono-1,4-lactone (**4**) is almost certainly present in the mixture prior to the addition of dicyclohexylamine, because this structure must be the source of compounds **11** and **12**. It is, of course, entirely possible that methanolic potassium hydroxide may rearrange **4** to **5** prior to the formation of **11** and **12**.

Let us now turn to a consideration of the steps that lead to the formation of **11** and **12**. The formation of the double bond is closely related to numerous β eliminations that have been observed to occur with serine and threonine derivatives. It has long been recognized that β eliminations from these hydroxy-amino acids are facilitated by substitution of both the carboxyl group and the amino group[14–18], conditions that are met by lactones **5** and **6**. In addition, β eliminations from serine have been found to be aided by replacement of the hydroxyl group by a leaving group such as *p*-tolylsulfonyloxy[19], diphenylphosphono-oxy[15], or even glycosyloxy[20–22]. By way of contrast, the five-membered lactones **5** and **6** undergo elimination without further substitution. Indeed, an attempt to insert a *p*-tolylsulfonyl group in **16** simply caused dehydration and, in a very closely related case, Isono and co-workers[23] have recently found that acetylation of 2-acetamido-2-deoxy-L-xylono-1,4-lactone with pyridine–acetic anhydride at room temperature causes the formation of 2-acetamido-5-*O*-acetyl-2,3-dideoxy-L-*glycero*-pent-2-enono-1,4-lactone. It is likely that the activation energy for such eliminations from five-membered rings is somewhat less than from acyclic analogs. Of the precise nature of the elimination we as yet know nothing; since the configuration at C-2 in the lactones **5** and **6** is obviously susceptible to change in the presence of alkali one cannot hope to throw light on the mechanistic details by seeking evidence for steric influences on the elimination.

The isomerization that takes place at C-4 may be regarded as the second step of the reaction, and as a normal consequence of the allylic activation of H-4.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Specific rotations were measured at 20–23°. Column chromatography was conducted on silica gel (0.05–0.20 mm; E. Merck, Darmstadt). Paper chromatography was performed on Whatman No.1 paper in the following systems: *A*, 55:30:11 isopropyl alcohol–hexane–water; *B*, 12:3:5 butyl alcohol–acetic acid–water; *C*, 80:5:15:1 acetonitrile–acetone–water–acetic acid; and *D*, 19:1 methanol–water. Systems *A*, *B*, and *D* were used in the descending, and *C* in the ascending mode. The components were located on the paper with alkaline silver nitrate, ninhydrin (for amino compounds), and Bromocresol Green (for acids). Electrophoresis was conducted at room temperature with a voltage gradient of 12 V/cm in pyridine–acetic acid, pH 6.5.

I.r. spectra were recorded with a Perkin–Elmer Model 137 i.r. spectrometer.

The p.m.r. spectra were recorded at 60 MHz, for compounds in the solvents specified, with a Varian A-60*A* spectrometer.

*The oxidation of 2-acetamido-2-deoxy-*D*-mannose* (**2**). *2-Acetamido-2-deoxy-*D*-mannono-1,4-lactone* (**5**). — A solution of the monohydrate of **2** (4.0 g) in water (80 ml) was treated with bromine (2.4 ml), and the mixture was kept in the dark overnight and then shaken for 3 days. The processing of the reaction mixture was conducted as described by von Euw and Reichstein[24]. The excess of bromine was removed *in vacuo* on a rotary evaporator without heating, and the solution was treated with silver carbonate. After filtration, the resulting solution was freed of silver ions with hydrogen sulfide, and the precipitate was removed by filtration through a layer of decolorizing carbon. The colorless filtrate was evaporated *in vacuo* (40° bath), and the syrupy residue was dried in a desiccator over phosphorus pentaoxide; crystallization was spontaneous. The product was triturated with methanol (10 ml), and removed by filtration: wt. 1.41 g, m.p. 164–167°. Upon concentration, the mother liquor yielded a second crop (0.47 g), raising the total yield to 51%. After recrystallization from methanol, the pure 2-acetamido-2-deoxy-D-mannono-1,4-lactone (**5**) showed m.p. 172–173° and $[\alpha]_D$ +87.4° (*c* 1.00, water); ν_{max}^{KBr} 3400 (OH), 3300 (NH), 1770 (C=O), and 1660 and 1550 cm^{-1} (amide); n.m.r. signals (methyl sulfoxide-d_6) at τ 1.92 (doublet, *J* 9.0 Hz, removed by D_2O exchange, NH), 4.40 (doublet, removed by D_2O exchange, OH), 5.02 (quartet, $J_{2,NH}$ 9.0, $J_{2,3}$ 4.5 Hz, H-2), 5.20 (doublet, OH), 5.40–6.60 (unresolved multiplets), and 8.04 (NAc).

Anal. Calc. for $C_8H_{13}NO_6$: C, 43.84; H, 5.98; N, 6.39. Found: C, 43.95; H, 5.98; N, 6.50.

*The oxidation of 2-acetamido-2-deoxy-*D*-galactose* (**3**). *2-Acetamido-2-deoxy-*D*-galactono-1,4-lactone* (**6**). — The lactone (**6**) was prepared from **3** in 72% yield by the procedure employed for the preparation of **5** from **2**. The pure lactone was found to have m.p. 162–166° and $[\alpha]_D$ −21.6° (*c* 1.09, water); lit. m.p. 165° (ref. 6) 168–169° (corr., dec.)[7], $[\alpha]_D^{22}$ −24° (*c* 1, water)[7]; ν_{max}^{KBr} 3350 (OH), 3250 (NH), 1780 (C=O), and 1650 and 1550 cm^{-1} (amide).

*The oxidation of 2-acetamido-2-deoxy-*D*-glucose* (**1**). — 2-Acetamido-2-deoxy-D-glucose (**1**, 4.0 g) was treated as described for the oxidation of **2**, and the product was dried in a desiccator. The colorless syrup was triturated with methanol (15 ml), and the mixture was stored overnight at room temperature; the crystals that deposited were removed by filtration, and washed with methanol: wt. 380 mg (11%). This product gave a positive test with ninhydrin, and its chromatographic behavior in systems *A* and *B*, as well as its i.r. spectrum, were indistinguishable from those of an authentic sample[10] of **8**.

The methanolic mother-liquor was evaporated *in vacuo*, and absolute ether was repeatedly added to and evaporated from the residue, to leave a hygroscopic foam (3.2 g) that was stable when stored in a desiccator; ν_{max}^{KBr} 1750 (C=O), 1660 and 1560 cm^{-1} (amide). Paper chromatography in systems *A*, *B*, *C*, with alkaline silver nitrate for detection, revealed at least four components; on the evidence of its mobility and characteristic yellow color with the reagent, one of these was identified as **1**.

The component that had the lowest rate of migration gave a yellow spot when sprayed with Bromocresol Green and was, therefore, an acid. The remaining two components were not reliably separated in systems *A* or *B*, but were clearly distinguishable from each other when chromatographed in system *C*; one of these two components had the same mobility as an authentic sample of **7**.

A portion (1.9 g) of the hygroscopic foam was dissolved in absolute ethanol (10 ml) without heating, and the solution was treated dropwise with dicyclohexylamine to pH 8. The mixture was kept for 2 days in a refrigerator, and the crystalline salt (**9**) that had formed was removed by filtration: wt. 362 mg, m.p. 187–188°. Absolute ether was added in small portions to the alkaline mother liquor, and the solution was cooled and scratched to hasten the formation of a second crop of crystals: wt. 875 mg, m.p. 148–150°; the i.r. spectrum (KBr) of this material showed strong carbonyl absorption at 1770 cm^{-1}. Chromatography in systems *A* and *B*, with alkaline silver nitrate solution for detection, showed this second crop to be a mixture of salt **9** with another compound that was neither **7** nor **1**; owing to the absorption observed at 1770 cm^{-1}, this compound was provisionally assumed to be a lactone.

The mother liquor was evaporated *in vacuo* to a yellow, semisolid residue (1.05 g) that was subjected to repeated chromatography on silica gel with 3:2 ethyl acetate–methanol. These operations yielded more of the crystalline salt (to make a total of 1.15 g), and gave 556 mg of the presumed lactone. The characterization of these two products will now be discussed separately.

*Dicyclohexylammonium 2-acetamido-2-deoxy-*D*-gluconate* (**9**). — The first crop of material (362 mg), obtained as just described, was recrystallized from ethanol to give 240 mg of crystals, m.p. 191–194° and $[\alpha]_D$ −2.9° (*c* 0.95, water); ν_{max}^{KBr} 3400 (OH), 3200 (NH), 1780 (C=O), and 1630 and 1560 cm^{-1} (amide).

Anal. Calc. for $C_{20}H_{38}N_2O_7$: C, 57.39; H, 9.15; N, 6.69. Found: C, 57.29; H, 8.93; N, 6.74.

Paper chromatography of the salt in solvent systems *A* and *C*, and spraying with Bromocresol Green, revealed two components. One migrated slowly and turned the indicator yellow; this was the acid. The other migrated more rapidly and made the indicator blue; this was dicyclohexylamine. The acid component was chromatographically identical with the acid noted in the crude oxidation product prior to the addition of the dicyclohexylamine.

An aqueous solution containing 50 mg of the salt was passed through a column (100 × 8 mm) of Dowex 50W-X8 (H^+) ion-exchange resin, and the column was eluted with water (~50 ml). The eluate (pH 2) was evaporated *in vacuo* (40° bath) to an unstable foam (wt. 30 mg, quantitative); chromatography in systems *A* and *C* revealed the acid that had been detected in the original, crude oxidation-product, as well as a component chromatographically indistinguishable from **7**.

A sample of the salt was subjected to electrophoresis and, after drying, the paper was sprayed with Bromocresol Green; the acid, appearing as a yellow spot, was found to have migrated 7.0 cm toward the cathode and the dicyclohexylamine (a blue spot) had migrated 8.7 cm toward the anode.

An authentic sample of 7 was dissolved in water, and the solution was stored overnight at room temperature; it was then lyophilized, and the residue was trimethylsilylated. A sample of the salt was also trimethylsilylated. Both trimethylsilyl derivatives were examined by g.l.c. at 150° with a column (6 ft × 0.25 in. o.d.) of 1% SE-30 on Gas-Chrom P, helium (~100 ml/min), and a flame ionization detector. The derivative from the salt showed a single component that cochromatographed with that component of longest retention-time derived from the authentic specimen of 7.

Finally, a sample of the salt was dissolved in 4M hydrochloric acid, and the solution was boiled for 45 min. Examination of the hydrolyzate by paper chromatography in systems *A*, *B*, and *D* revealed only one ninhydrin-positive component, and that was chromatographically indistinguishable from authentic[10] **8**.

Characterization of the lactone. — The mixture obtained as the second crop (875 mg, m.p. 148–150°) was powdered, triturated with methanol (8 ml), and kept for 1 h at room temperature; the solid was then separated from the solution by filtration; wt. 470 mg, m.p. 190–191°. The chromatographic behavior and i.r. spectrum of this material identified it as the pure dicyclohexylammonium salt **9**. The methanolic mother-liquor was evaporated *in vacuo* to a crystalline residue: wt. 390 mg, m.p. 137–141°. This was dissolved in warm methanol, and the solution was applied to a column of silica gel prepacked in 3:2 ethyl acetate–methanol. The column was eluted with the same solvent mixture, and fractions that contained homogeneous material were pooled, and evaporated, to give a crystalline product (250 mg) that was recrystallized from methanol–absolute ether: m.p. 141–143°, $[\alpha]_D$ +82.3° (*c* 0.98, water). On chromatography in systems *B* and *C*, the material appeared to be homogeneous, and was indistinguishable from **5**. The i.r. and n.m.r. spectra were superposable with those of an authentic sample of **5**.

*Methyl 2-acetamido-2-deoxy-*D*-gluconate* (**10**). — A solution of the crude oxidation product from **1** (500 mg) in methanol (2 ml) was stored for 3 weeks at room temperature. The prismatic crystals that slowly deposited during this period were removed by filtration: wt. 45 mg, m.p. 134–137°. Two further crops (94 mg, both having m.p. 142–144°) were obtained from the mother liquor; the i.r. spectra of the three crops were identical. The crops were combined, and recrystallized from methanol to give a product having m.p. 145–147° and $[\alpha]_D$ +12.4° (*c* 0.96, water). The i.r. and n.m.r. spectra of the product were identical with those of an authentic specimen of **10** prepared from methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-gluconate as described in the paper immediately following[12]; a mixed m.p. was undepressed.

Behavior of lactones with methanolic potassium hydroxide. — (*a*) *2-Acetamido-2-deoxy-*D*-mannono-1,4-lactone.* To a solution of **5** (920 mg) in methanol (50 ml), M methanolic potassium hydroxide was added dropwise to pH 7.5 (0.9 ml). The solution, now pale yellow, was kept for 2 h at room temperature, and then overnight at 0°. The progress of the reaction was monitored by t.l.c. with 9:1 ether–methanol and, even after 10 min, a component moving faster than **5** was detected. The solvent was removed *in vacuo*, and the residual syrup was chromatographed on a column of silica gel with 9:1 ether–methanol; 8-ml portions of eluate were collected. Fractions 9–63

contained the product, and these were pooled and evaporated to yield crystalline material: wt. 580 mg (69%); ν_{max}^{KBr} 3350 (OH), 3200 (NH), 1750 (C=O), 1680 (C=C), 1630 and 1540 (Amide I and II), 960, and 915 cm^{-1}. The last two absorption bands were of approximately equal intensity. After two careful, slow recrystallizations from methanol at room temperature, the crude product afforded **11**: m.p. 186–188°, $[\alpha]_D$ +41.1° (*c* 1.04, water); i.r. absorption (KBr) revealed all of the bands noted for the crude product, except that at 915 cm^{-1}; its n.m.r. spectrum (methyl sulfoxide-d_6) included signals at τ 0.05 (broad singlet, disappearing after D_2O exchange, NH), 2.50 (doublet, $J_{3,4}$ 2.0 Hz, vinyl proton, H-3), 4.75–5.0 (2 H, multiplet, collapsing on D_2O exchange to a 1-H quartet centered at 4.82, $J_{3,4}$ 2.0 Hz, $J_{4,5}$ 4.5 Hz, H-4), 5.27 (triplet, *J* 5.5 Hz, disappearing after D_2O exchange, primary OH), and 7.92 (NAc).

Anal. Calc. for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96; mol. wt. 201.18. Found: C, 47.65; H, 5.56; N, 7.17; mol. wt. 197*.

From the methanolic mother-liquor, a second crop of crystals was obtained: m.p. 150–157°, $[\alpha]_D$ −23.9° (*c* 1.04, water); i.r. bands at 960 and 915 cm^{-1}. The n.m.r. spectrum showed the doublet at τ 2.50 that is characteristic of **11**; in addition, it included a doublet at 2.58 (*J* 2.0 Hz) that was later found to be characteristic of lactone **12**. The relative intensities of the doublets at τ 2.50 and 2.58 suggested that the ratio of the *erythro* lactone **11** to the *threo* lactone **12** was ~2:3.

(*b*) *2-Acetamido-2-deoxy-*D*-galactono-1,4-lactone* (**6**). A solution of **6** (880 mg) in methanol (80 ml) was treated with M methanolic potassium hydroxide as described for **5**. After the solvent had been removed, the crude product was chromatographed on a column of silica gel (60 g) with 9:1 ether–methanol. Fractions containing the major product were pooled, and evaporated to give a crystalline residue: wt. 426 mg (53%). The i.r. and n.m.r. spectra indicated that this product was a mixture of **11** and **12** in approximately equal proportions.

(*c*) *The mixture from the oxidation of 2-acetamido-2-deoxy-*D*-glucose* (**1**). A sample (3.0 g) of the crude material from the bromine oxidation of **1** was dissolved in methanol (80 ml), and the solution was treated with M methanolic potassium hydroxide. The reaction mixture was processed as described in (*a*), the product being chromatographed with 6:1 ether–methanol to give a crystalline product (1.12 g). The i.r. spectrum of the material thus obtained was superposable on those obtained in (*a*) and (*b*); absorption bands at 915 and 960 cm^{-1} were of almost equal intensity. As the band at 915 cm^{-1} was later found to be shown only by **12**, and as the band at 960 cm^{-1} is prominent in the spectrum of **11** but extremely weak in **12**, it was evident that **11** and **12** were present in approximately equal quantities.

The interconversion of lactones **11** *and* **12**. — Prior to the isolation of **12** in pure form, the behavior, under alkaline conditions, of **11** and of a mixture rich in **12** was studied.

A sample of **11** (100 mg), showing $[\alpha]_D$ +40.2° in water, was dissolved in water;

*Determined in a Model 301A vapor pressure osmometer of Mechrolab, Inc., Mountain View, Calif., by Dr. W. C. Alford of the Section on Microanalytical Services and Instrumentation, NIAMD.

a few drops of methanolic potassium hydroxide were added to bring the pH to 7.5, and the solution was diluted with methanol to a volume of 10 ml. The optical rotation of the solution in a 1-dm tube was observed over a period of 48 h, and found to change from an initial value of +0.226° to a final (constant) rotation of +0.122°. The solution was evaporated *in vacuo*, and the residue was chromatographed on a column of silica gel (15 g) with 9:1 ether–methanol, to yield a crystalline product: wt. 80 mg, $[\alpha]_D$ −4.2° (*c* 0.57, water); the i.r. spectrum of the material showed absorption at 915 cm^{-1}, indicating the presence of **12**.

In another experiment, a mixture of lactones (10 mg) that showed $[\alpha]_D$ −91.7° (and was rich in **12**) was similarly treated with methanolic potassium hydroxide. The observed rotation was −0.382° immediately after the addition of the base; by 24 h, it had changed to −0.235°. Removal of the solvent gave crystals that showed a stronger band at 960 cm^{-1} than the starting material had shown.

2-Acetamido-5,6-di-O-*acetyl-2,3-dideoxy*-D-erythro-*hex-2-enono-1,4-lactone* (**13**). — A solution of **11** (123 mg) in a mixture of acetic anhydride (0.3 ml) and pyridine (1.5 ml) was kept overnight at room temperature, and then evaporated under high vacuum to a yellow syrup that was chromatographed on a column of silica gel (25 g) with 30:1 ether–methanol, 3-ml portions of eluate being collected. Fractions 20–27 contained the major product; they were pooled, and evaporated to give a syrup: wt. 117 mg (67%). Prior to analysis, the substance was rechromatographed on silica gel with the same solvent mixture. The colorless syrup showed $[\alpha]_D$ +58.1° (*c* 0.86, chloroform), and i.r. absorption (neat) at 3280 (NH), 1750 (C=O), 1720 (OAc), 1660 (C=C), and 1630 and 1540 cm^{-1} (Amide I and II). The n.m.r. spectrum of the compound (chloroform-*d*) included signals at τ 1.92 (broad singlet, NH), 2.51 (doublet, $J_{3,4}$ 1.5 Hz, H-3), 4.65–4.90 (multiplet, 2 H), 5.40–5.90 (multiplet, 2 H), 7.77 (NAc), 7.88 and 7.92 (OAc).

Anal. Calc. for $C_{12}H_{15}NO_7$: C, 50.53; H, 5.30; N, 4.91. Found: C, 50.58; H, 5.23; N, 4.89.

The mass spectrum of the compound included signals at *m/e* 225 (M-AcOH), *m/e* 183 (225-ketene), *m/e* 141 (183-ketene), and *m/e* 123 (183-AcOH). Although weak, a signal at *m/e* 145 (CH_2OAc–CHOAc–) confirmed the five-membered ring-structure; this signal, incidentally, was absent from the mass spectrum of 2-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone[12].

2-Acetamido-2,3-dideoxy-5,6-O-*isopropylidene*-D-erythro-*hex-2-enono-1,4-lactone* (**14**). — To a solution of **11** (100 mg) in anhydrous acetone (10 ml), anhydrous copper(II) sulfate (300 mg, dried at 110°) was added, and the suspension was stirred for 3 days at room temperature, the progress of the reaction being monitored by t.l.c. with ether. The solid was removed by filtration, and the filtrate was evaporated *in vacuo* to give a crystalline residue that was chromatographed on a column of silica gel (30 g) with 6:1:1 ether–acetone–hexane, 2-ml portions of eluate being collected, Fractions 18–25 were pooled, and evaporated, to give pure **14**: wt. 96 mg (80%), m.p. 124–125°, $[\alpha]_D$ −29.1° (*c* 1.01, chloroform); ν_{max}^{KBr} 3300 (NH), 1780 (C=O), 1710 (C=C), 1660 and 1550 (amide), and 1385 and 1380 cm^{-1} (Me_2C); n.m.r. signals (chloro-

form-*d*) at τ 2.15 (broad singlet, NH), 2.41 (doublet, *J* 2.0 Hz, H-3), 5.06 (quartet, *J* 2.0 and 7.1 Hz, H-4), 5.85–6.10 (multiplet, 3 H), 7.80 (NAc), and 8.55 and 8.67 (Me_2C).

Anal. Calc. for $C_{11}H_{15}NO_5$: C, 54.76; H, 6.27; N, 5.81. Found: C, 54.89; H, 6.03; N, 5.77.

*2-Acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-*D*-threo-hex-2-enono-1,4-lactone* (**15**). — A quantity (370 mg) of a mixture of **11** and **12** (~1:1, as judged by its n.m.r. spectrum) was dissolved in anhydrous acetone (40 ml), and anhydrous copper(II) sulfate (1.2 g, dried at 110°) was added. The suspension was stirred at room temperature for 3 days, the progress of the reaction being monitored by t.l.c. with 6:1:1 ether–acetone–hexane. At the conclusion of the reaction, two new compounds were detectable, and the quantity of starting material remaining appeared to be very small. The solid was removed by filtration, and washed with acetone. The filtrate and washings were combined, and evaporated *in vacuo* to a syrup that was chromatographed on a column of silica gel (60 g) with 6:1:1 ether–acetone–hexane and the collection of eluate in 5-ml portions. Fractions 17–27 contained the isomer having the *erythro* configuration (**14**): wt. 133 mg (30%), m.p. 124–125°, undepressed on admixture with a sample of **14** prepared from pure **11**. Fractions 28–33 (66 mg, 15%) were heterogeneous. Fractions 34–54 were pooled, and evaporated to give **15**: wt. 159 mg (36%), m.p. 156–158°, $[\alpha]_D$ −46.2° (*c* 0.81, chloroform); ν_{max}^{KBr} 3250 (NH), 1750 (C=O), 1700 (C=C), and 1650 and 1540 cm^{-1} (Amide I and II); n.m.r. signals (chloroform-*d*) at τ 1.81 (broad singlet, NH), 2.58 (doublet, *J* 2.0 Hz, H-3), 4.89 (quartet, *J* 2.0 and 4.2 Hz, H-4), 5.5–6.2 (multiplet, 3 H), 7.80 (NAc), 8.58 and 8.65 (Me_2C).

Anal. Calc. for $C_{11}H_{15}NO_5$: C, 54.76; H, 6.27; N, 5.81. Found: C, 54.82; H, 6.22; N, 5.61.

*2-Acetamido-2,3-dideoxy-*D*-threo-hex-2-enono-1,4-lactone* (**12**). — The isopropylidene derivative (**15**, 320 mg) was dissolved in methanol (12 ml), and 1% sulfuric acid (5 ml) was added. The mixture was kept at room temperature, and the progress of the hydrolysis was followed by t.l.c. in 6:1:1 ether–acetone–hexane. After 4 days, **15** was no longer detectable. Aqueous barium chloride (10%) was added, and the precipitate was removed by centrifugation. Upon evaporation *in vacuo*, the solution gave a crystalline residue that was recrystallized from methanol: wt. 135 mg (50%), m.p. 176–178° (depressed on admixture with **11**); the i.r. spectrum of the compound showed a strong band at 915 cm^{-1} and a weak one at 960 cm^{-1}. Successive recrystallization from methanol and then from ethanol failed to change the m.p., and gave pure **12**: $[\alpha]_D$ −101.2° (*c* 0.57, water); n.m.r. signals (methyl sulfoxide-d_6) at τ 0.07 (broad singlet, disappearing after D_2O exchange, NH), 2.58 (doublet, $J_{3,4}$ 2.0 Hz, H-3), 4.82 (triplet, *J* 2.0 Hz, H-4), 5.0 (doublet, *J* 5.5 Hz, disappearing after D_2O exchange, secondary hydroxyl), 5.19 (triplet, *J* 5.5 Hz, disappearing after D_2O exchange, primary hydroxyl), 6.3–6.6 (multiplet, 3 H), and 7.93 (NAc).

Anal. Calc. for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47.48; H, 5.64; N, 7.17.

*2-Acetamido-2-deoxy-5,6-*O*-isopropylidene-*D*-mannono-1,4-lactone* (**16**). — A suspension of **5** (657 mg) and anhydrous copper(II) sulfate (1.8 g) in absolute acetone (120 ml) was stirred at room temperature for 3 days, the progress of the condensation being monitored by t.l.c. in 15:1 ether–methanol. The solid was removed by filtration, and washed with acetone; evaporation *in vacuo* of the combined filtrate and washings afforded a crystalline product (755 mg, 97%). As recrystallization from either acetone or alcohol failed to eliminate traces of **5**, the product was chromatographed on a column of silica gel (30 g) with 4:1 ether–acetone to give a chromatographically homogeneous substance: wt. 530 mg (68%), m.p. 161–164°, $[\alpha]_D$ +75.8° (*c* 1.04, methanol); ν_{max}^{KBr} 3300 (OH), 3200 (NH), 1750 (C=O), 1650 and 1540 (amide), and 1380 and 1370 cm^{-1} (Me_2C); n.m.r. signals (acetone-d_6) at τ 7.05 (removed by D_2O exchange, OH), 7.97 (NAc), and 8.61 and 8.68 (Me_2C). The compound was stable over a period of 5 days when stored at room temperature in pyridine solution.

Anal. Calc. for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.68; H, 6.34; N, 5.38.

*2-Acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-*D*-*erythro*-hex-2-enono-1,4-lactone* (**14**) *from 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-*D*-mannono-1,4-lactone* (**16**). — To a solution of **16** (170 mg) in dry pyridine (4 ml) was added *p*-toluenesulfonyl chloride (0.5 g), and the solution was kept for 3 days at room temperature. Water was then added (to hydrolyze the excess of reagent) and the mixture was poured into water. The product was extracted with chloroform, and the extract was washed successively with 2M hydrochloric acid and water, dried (sodium sulfate), and evaporated *in vacuo*, to give a residue that was chromatographed on a column of silica gel (20 g) with 6:1:1 ether–acetone–hexane and the collection of eluate in 4-ml portions. Fractions 11–19 were pooled, and evaporated to yield 2-acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-*D*-*erythro*-hex-2-enono-1,4-lactone (**14**): wt. 70 mg (44%). The chromatographic behavior of the product, and its i.r. and n.m.r. spectra, were indistinguishable from those of a sample of **14** that had been prepared from **11**; a mixed m.p. was undepressed.

ACKNOWLEDGMENTS

This work was supported, in part, through Agreement No. 719810 under U. S. Public Law 480. We are indebted (at the "Rudjer Bošković" Institute) to Mr. B. Danilov for his skilful technical assistance, to Dr. O. Hadžija for microanalyses, and to Mrs. L. Tomić for n.m.r. spectra, as well as to Mr. V. Kramer of the "Jožef Stefan" Institute, Ljubljana, for recording mass spectra. Special thanks are due Mr. W. N. Jones, Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey, England, for the generous gift of a sample of **7** that had been prepared by the late Dr. N. M. Cross.

REFERENCES

1 For a preliminary report, see N. PRAVDIĆ AND H. G. FLETCHER, JR., *Carbohyd. Res.*, 12 (1970) 471.
2 J. W. GREEN, *Advan. Carbohyd. Chem.*, 3 (1948) 129.
3 K. HEYNS AND H. PAULSEN, *Advan. Carbohyd. Chem.*, 17 (1962) 169.
4 M. DIXON AND E. C. WEBB, *Enzymes*, 2nd ed., Academic Press, New York, N. Y., 1964, *passim.*
5 D. HORTON, *Advan. Carbohyd. Chem.*, 15 (1960) 159.
6 P. KARRER AND J. MAYER, *Helv. Chim. Acta*, 20 (1937) 407.
7 J. FINDLAY, G. A. LEVVY, AND C. A. MARSH, *Biochem. J.*, 69 (1958) 467.
8 S. A. BARKER, E. J. BOURNE, R. M. PINKARD, AND D. H. WHIFFEN, *Chem. Ind.* (London), (1958) 658.
9 C. S. HUDSON, *J. Amer. Chem. Soc.*, 32 (1910) 338; 61 (1939) 1525.
10 D. B. HOPE AND P. W. KENT, *J. Chem. Soc.*, (1955) 1831.
11 N. M. CROSS (Lilly Industries, Ltd.), Brit. Pat. 1,138,367 (Jan. 1, 1969); *Chem. Abstr.*, 70 (1969) 88204y.
12 N. PRAVDIĆ AND H. G. FLETCHER, JR., *Carbohyd. Res.*, 19 (1971) 253.
13 R. KUHN, D. WEISER, AND H. FISCHER, *Ann. Chem.*, 628 (1959) 215.
14 J. P. GREENSTEIN AND M. WINITZ, *Chemistry of the Amino Acids*, Wiley, 1961, p. 2214.
15 G. RILEY, J. H. TURNBULL, AND W. WILSON, *J. Chem. Soc.*, (1957) 1373.
16 J. MONTREUIL, M. MONSIGNY, AND M.-T. BUCHET, *Compt. Rend.*, 264 (1967) 2068.
17 V. A. DEREVITSKAYA, M. G. VAFINA, AND N. K. KOCHETKOV, *Carbohyd. Res.*, 3 (1967) 377.
18 A. BELLA AND I. DANISHEVSKY, *J. Biol. Chem.*, 243 (1968) 2660.
19 I. PHOTAKI, *J. Amer. Chem. Soc.*, 85 (1963) 1123.
20 S. HARBON, G. HERMAN, B. ROSSIGNOL, P. JOLLÈS, AND H. CLAUSER, *Biochem. Biophys. Res. Commun.*, 17 (1964) 57.
21 B. ANDERSON, P. HOFFMAN, AND K. MEYER, *J. Biol. Chem.*, 240 (1965) 156.
22 J. R. VERCELLOTTI, N. NIENABER, AND C. J. CHANG, *Carbohyd. Res.*, 13 (1970) 63, and references cited therein.
23 K. ISONO, K. ISAHI, AND S. SUZUKI, *J. Amer. Chem. Soc.*, 91 (1969) 7490.
24 J. VON EUW AND T. REICHSTEIN, *Helv. Chim. Acta*, 33 (1950) 485.