Carbohydrate Research, 36 (1974) 167–180

THE OXIDATION OF PARTIALLY PROTECTED 2-ACETAMIDO-2-DEOXYPYRANOSES WITH SILVER CARBONATE ON CELITE

NEVENKA PRAVDIĆ, BORIS DANILOV,

Department of Organic Chemistry and Biochemistry, "Rudjer Bošković" Institute, 41000 Zagreb, Croatia (Yugoslavia)

AND 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.)

(Received April 8th, 1974; accepted April 24th, 1974)

ABSTRACT

Silver carbonate on Celite (the Fetizon reagent) was shown to be selective as an oxidizing agent, and convenient for the preparation of various aldonolactones. Whereas substituted aldoses having the 1-hydroxyl group free were readily converted into the corresponding lactones, partially protected 2-acetamido-2-deoxypyranoses having more than one free hydroxyl group were selectively oxidized at C-1. The oxidation was carrried out in boiling benzene or 1,4-dioxane. A series of partially protected 2-acetamido-2-deoxy-1,5-aldonolactones [2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-mannono-1,5-lactone (**13**), 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucono-1,5-lactone (**15**), 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucono-1,5-lactone (**18**), 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-mannono-1,5-lactone (**20**), 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-mannono-1,5-lactone (**24**), and 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-glucono-1,5-lactone (**25**)] was thus prepared; for these, the oxidation was accompanied by two side-reactions: (*a*) an elimination (dehydration) that gave the unsaturated lactones [2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**12**), 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-D-*erythro*-hex-2-enono-1,5-lactone (**17**), and 2-acetamido-2,3-dideoxy-4-*O*-methyl-D-*erythro*-hex-2-enono-1,5-lactone (**23**)], and (*b*) partial *gluco*-to-*manno* epimerization occurring during the oxidation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranose (**14**), 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucopyranose (**16**), and 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-glucopyranose (**22**).

The free unsaturated lactone, 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**26**), was obtained on hydrolysis of the isopropylidene group in lactone **17**.

*Deceased October 19th, 1973.

INTRODUCTION

Present knowledge[1] concerning the action of aldonolactones as inhibitors of glycosidases involves some doubts regarding the inhibitory activity of aldono-1,4-lactones in general. Our recent studies have shown[2] that 2-acetamido-2-deoxy-β-D-glucosidase (β-*N*-acetylglucosaminidase) is inhibited by 2-acetamido-2-deoxy-D-glucono-1,4-lactone. However, in order to acquire a full understanding of the dependence of the inhibitory effect on the ring size of the lactone, the need arose for certain substituted aldonolactones locked in either the pyranoid or the furanoid form.

For the preparation of substituted aldonolactones, silver carbonate on Celite, the so-called Fetizon reagent[3], proved to be a convenient oxidant; this reagent, primarily employed for the oxidation of alcohols, has found some application in carbohydrate chemistry, the first papers introducing its use into this field indicating its high selectivity, *e.g.*, selective oxidation of the allylic hydroxyl group[4] and of the 1-hydroxyl group of aldoses[5]. Nevertheless, some limitations in the applicability of the Fetizon reagent were encountered, as compounds having the 2-hydroxyl group free[6], including 2-ketoses[7], or compounds having exocyclic glycol groupings[8] underwent degradation in the course of the oxidation.

The ultimate aim of this study was to examine the use of the Fetizon reagent in the oxidation of a series of partially protected 2-acetamido-2-deoxypyranoses. However, we first undertook an investigation of the synthesis of fully protected lactones, compounds already accessible by other methods. Oxidation with the Fetizon reagent afforded, in each case, a single product, and the method was found to be superior. In contrast, action of the Fetizon reagent on the 2-acetamido sugars afforded several products; their isolation and characterization are reported.

RESULTS AND DISCUSSION

On treatment with an excess of dry silver carbonate on Celite in boiling, anhydrous benzene, an aldose fully substituted except at O-1 afforded, as the sole product, the corresponding lactone in good yield. The following substituted aldoses or D-glucuronic esters having the 1-hydroxyl free were thus oxidized: 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose[9] (**1**), 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose[10] (**2**), benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate[11] (**3**), 2,3,5-tri-*O*-benzyl-D-arabinofuranose (**4**), and methyl 2,3,5-tri-*O*-methyl-D-glucofuranuronate[12] (**5**). The corresponding lactones (**6**–**10**) were readily purified, either by crystallization or by chromatography on silica gel. The data are summarized in Table I.

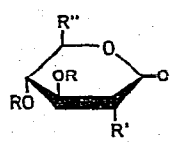
The lactones **6**, **7**, and **9** had been prepared earlier by applying methyl sulfoxide–acetic anhydride as the oxidant[13–15]; however, the procedure described here is in all instances superior. Some difficulties had been encountered[13] in the process of separation of the lactone **6** from the sulfur-containing byproducts, and the lactone was not obtained directly in the pure form. Although the oxidation of **2** with methyl sulfoxide[14] proceeded smoothly and gave the lactone **7** in high yield, the Fetizon reagent still

TABLE I

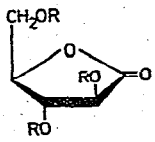
SUBSTITUTED LACTONES OBTAINED THROUGH THE OXIDATION OF THE CORRESPONDING ALDOSES OR URONIC ESTERS HAVING THE 1-HYDROXYL GROUP FREE

Number	*Lactone obtained*	*Duration of reaction*	*Solvent for chromatography (t.l.c., column)*	*Purification*	*Yield (%)*	*M.p. (degrees)*	$[\alpha]_D$ *(degrees) (solvent)*
6	2,3,4,6-Tetra-*O*-benzyl-D-glucono-1,5-lactone	5 h	*A*	chromatography on SiO_2	72	syrup[a]	81.3 ($CHCl_3$)
7	2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactone	3 days		crystallization (EtOH)	61	141–143[b]	
8	2,3,4-Tri-*O*-benzyl-D-glucaro-1,5-lactone 6-benzyl ester	4 days	*B*	chromatography on SiO_2	42[c]	syrup[d]	45.2 ($CHCl_3$)
9	2,3,5-Tri-*O*-benzyl-D-arabinono-1,4-lactone	3 h	*A*	crystallization (cyclohexane)	91	67–68[e]	
10	2,3,5-Tri-*O*-methyl-D-glucaro-1,4-lactone 6-methyl ester	20 h	*C*	crystallization (ether)	64	74–76[f]	−14.5 (H_2O)

[a]Ref. 13: $[\alpha]_D$ +79.9° ($CHCl_3$); Calc. for $C_{34}H_{34}O_6$: C, 75.82; H, 6.36. Found: C, 75.95; H, 6.34. [b]Ref. 14: m.p. 141–142°; the i.r. spectra of the two samples were superposable. [c]Compound 3 was recovered (16%). [d]Calc. for $C_{34}H_{32}O_7$: C, 73.89; H, 5.84. Found: C, 73.63; H, 5.78. [e]Ref. 15: m.p. 67°; the mixed m.p. with an authentic sample was undepressed. [f]Ref. 16: m.p. 78–79°, $[\alpha]_D$ −10° (H_2O).

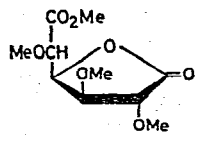


6 R'= OR R''= CH_2OR
7 R'= NHAc R''= CH_2OR
8 R'= OR R''= CO_2R



9

R= benzyl



10

had an advantage, because, if lactone **7** is to be used for the preparation of 2-acetamido-2-deoxy-D-glucono-1,5-lactone through catalytic debenzylation of **7**, the methyl sulfoxide reagent is certainly not the best choice, as it could be the source of poisoning of the catalyst. The oxidation of **4** with the Fetizon reagent is fast, and the lactone **9** is obtained in excellent yield. It should be noted that, in the course of this investigation, the reaction **4**→**9** was performed routinely in order to test the quality of freshly prepared batches of silver carbonate on Celite, and to check whether the catalyst had changed on prolonged storage.

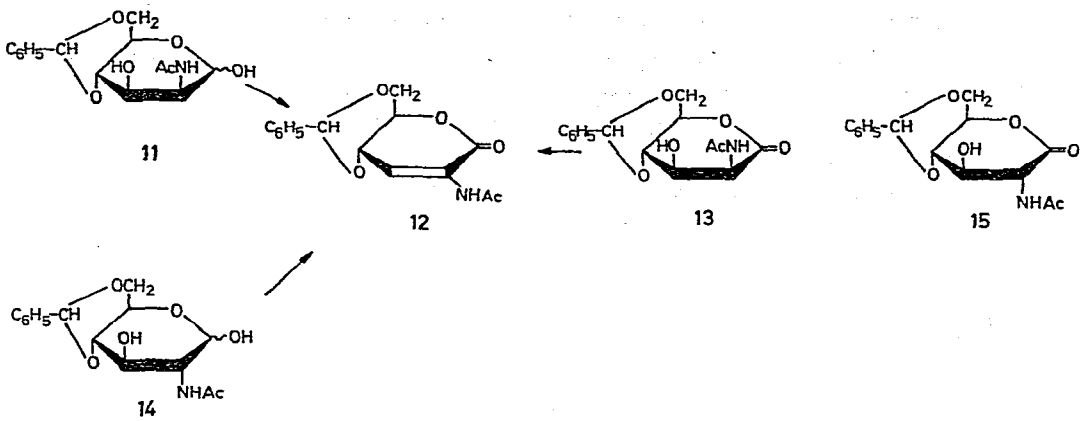
In order to ascertain whether the Fetizon reagent, under the conditions described earlier in this paper, might cause some effects on the primary or secondary hydroxyl groups of carbohydrate derivatives, experiments with model compounds were performed. 2,4:3,5-Di-*O*-methylene-L-iditol[17], a compound having two primary hydroxyl groups free, was subjected to the action of silver carbonate on Celite in boiling 1,4-dioxane, and was recovered unchanged in almost quantitative yield. 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose, a compound having a free hydroxyl group at C-3, was treated with a large proportion of the Fetizon reagent in boiling benzene; this diacetal, also, proved to be wholly immune to the action of the oxidant, and was recovered unchanged.

With this background, we next turned our attention to the oxidation of partially protected 2-acetamido-2-deoxypyranoses having more than one hydroxyl group free. The results obtained with 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-mannopyranose[18] (**11**) will be described first. Treatment of **11** with silver carbonate on Celite in boiling 1,4-dioxane for 24 hours gave a mixture that could be largely resolved by repeated chromatography on columns of silica gel. The first compound to emerge from the column was isolated in crystalline form; its nuclear magnetic resonance (n.m.r.) spectrum clearly identified it as an unsaturated compound, and its physical properties were in agreement with those for the known[19] 2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**12**). The major product of the reaction (27%) was 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-mannono-1,5-lactone (**13**). The oxidation was not complete, however, and ~10% of the starting aldose **11** was recovered.

The saturated lactone **13** was deliberately converted into the lactone **12** through the β-elimination that occurred on treatment with *p*-toluenesulfonyl chloride in pyridine. Catalytic hydrogenolysis of the benzylidene group from **13** in a nonaqueous

solvent afforded (after purification on silica gel) crystalline material which proved to be identical with authentic 2-acetamido-2-deoxy-D-mannono-1,4-lactone[20]. Normally[14], in the course of catalytic hydrogenolysis conducted in the absence of water, the lactone retains its original ring-size. It appears, then, that the 1,5-lactone **13** first gave 2-acetamido-2-deoxy-D-mannono-1,5-lactone, and that this was then transformed into the corresponding 1,4-lactone, most probably during the chromatography on silica gel.

The oxidation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranose[21] (**14**) with the Fetizon reagent gave three products. One of these, obtained in 25% yield, proved to be the same unsaturated lactone (**12**) encountered in the oxidation of **11**. The second product, isolated in 14% yield, was found to be 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-mannono-1,5-lactone (**13**), and a minor product was the isomeric 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucono-1,5-lactone (**15**).

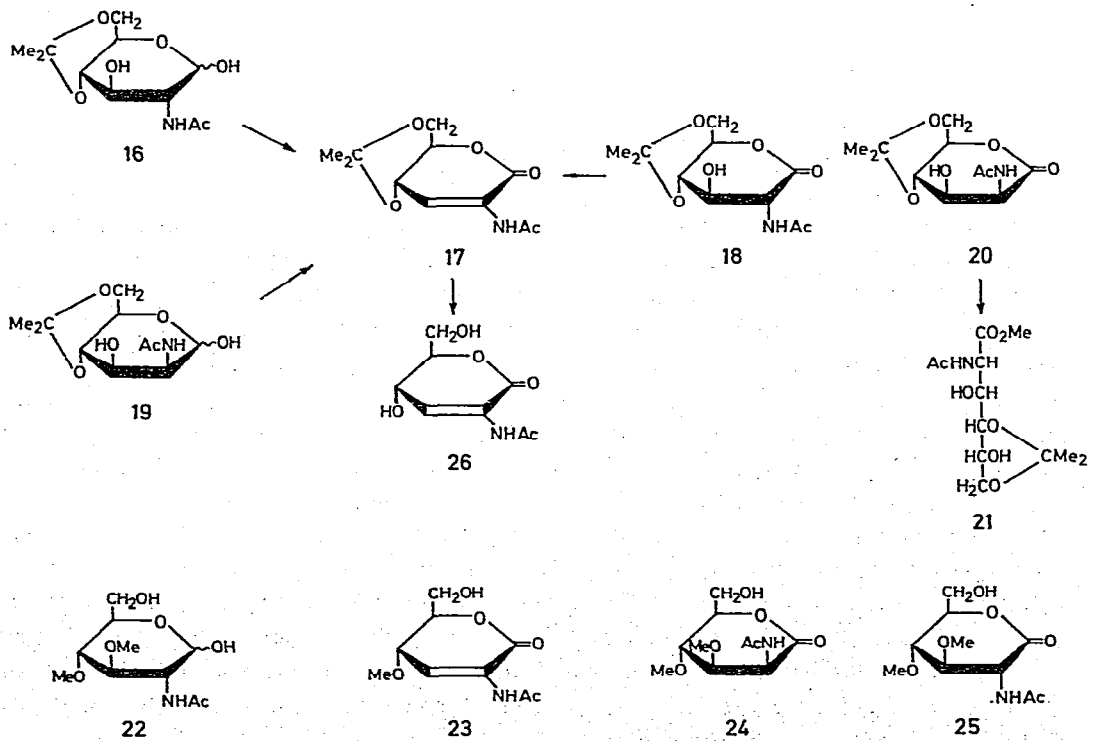


Our studies on oxidation with the Fetizon reagent were further extended to two 4,6-isopropylidene acetals of 2-acetamido-2-deoxyhexoses of the D-*gluco* and D-*manno* series that had recently been prepared[22]. 2-Acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucopyranose (**16**) gave several products; three lactones were isolated. The first was obtained in crystalline form; its infrared (i.r.) spectrum clearly showed it to be an unsaturated acetamido lactone, and its n.m.r. spectrum was consistent with structure **17**, tentatively ascribed to it. Two further products were isomeric: (*a*) the major one, isolated in 22% yield, appeared to be identical with a sample prepared[2] through the action of 2,2-dimethoxypropane on 2-acetamido-2-deoxy-D-glucono-1,5-lactone; therefore, the structure represented by **18**, namely, 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucono-1,5-lactone, was assigned to it. (*b*) The minor product, isolated in 4% yield, was found to be identical with the lactone obtained as the major product from 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-mannopyranose (**19**), *i.e.*, 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-mannono-1,5-lactone (**20**). With methanol at room temperature, compound **20** afforded crystalline methyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-mannonate (**21**).

The saturated lactone **18** was treated with *p*-toluenesulfonyl chloride in pyridine, to give 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-D-*erythro*-hex-2-enono-1,5-lactone (**17**), which proved to be the same unsaturated lactone as that isolated from among the products formed in the oxidation of **16** and **19**.

Treatment of 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-glucopyranose[23] (**22**) with the Fetizon reagent gave, as expected, one unsaturated and two saturated lactones. The n.m.r.-spectral characteristics of the unsaturated compound were fully consistent with the structure depicted in **23**, namely, 2-acetamido-2,3-dideoxy-4-*O*-methyl-D-*erythro*-hex-2-enono-1,5-lactone. In this case, neither of the two saturated lactones, prepared by a different, unequivocal route which might prove its structure, was available. However, in the oxidations of **11**, **14**, **16**, and **19**, discussed earlier, a certain regularity in the sequence of emergence of the products from columns of silica gel had been noticed: the *gluco* lactone was preceded by the *manno* derivative. In addition, the n.m.r. spectrum of the compound first eluted shows an *N*-acetyl signal at lower field than that of the saturated lactone eluted second. Therefore, we have tentatively assigned the structure of 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-mannono-1,5-lactone (**24**) to the first, and of 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-glucono-1,5-lactone (**25**) to the second saturated product.

The free unsaturated lactone, 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**26**) was prepared from **17**, by hydrolysis of the isopropylidene group with



a dry, ion-exchange resin in the H^+ form in 2-methoxyethanol[2]; lactone **26**, obtained in crystalline form after purification on silica gel, had an i.r. spectrum that clearly showed the presence of a 1,5-lactone ring.

Two types of reactions accompanying the oxidation of partially protected 2-acetamido-2-deoxypyranoses appear to merit special comment. (*a*) The first is the formation of the unsaturated lactones **12**, **17**, and **23** in the oxidations of **11** or **14** (or both), **16** or **19** (or both), and **22**, respectively. In monitoring the course of these oxidations by thin-layer chromatography (t.l.c.), the presence of the saturated lactones was observed prior to detection of the unsaturated ones. The formation of the double bond in these compounds seems to be caused by dehydration. Although it is known[24] that elimination reactions are aided by replacement of the hydroxyl group by a leaving group, we had reported[20] that some five-membered 2-acetamido-2-deoxy-aldonolactones undergo elimination without further substitution, and we have now provided several examples of occurrence of the elimination with six-membered lactones. It seems probable that the activation energy for such eliminations from the lactones, regardless of their ring-size, is less than that for eliminations from acyclic compounds.

(*b*) Secondly, attention is drawn to the *gluco*-to-*manno* epimerization that occurs to some extent in the course of the oxidation of compounds **14**, **16**, and **22**. Epimerization in the opposite direction, *i.e.*, *manno*-to-*gluco*, was also observed, but to a much lower degree. In earlier papers[14,20], we reported on the epimerization taking place under the influence of such amines as dicyclohexylamine or dimethylamine. Oxidation with a suspension of silver carbonate on Celite in an inert solvent, employed in the present study, is a heterogeneous reaction, and the fact that, even under such conditions, some 2-acetamido-2-deoxypyranose derivatives may be epimerized indicates that the alkalinity of silver carbonate is sufficient to bring about the inversion of the configuration at C-2. The consequence of these two side-reactions accompanying the oxidation with silver carbonate is that the saturated lactone having the D-*gluco* configuration is the product that could only be isolated in very low yield.

The results of this investigation indicate that the lactone derivatives are highly susceptible to a variety of structural changes, and, therefore, considerable attention should be given to avoiding oversimplified interpretations of processes in which lactones take part.

EXPERIMENTAL

General methods. — Specific rotations were measured at 20–24°. T.l.c. was conducted on silica gel (E. Merck) in the solvent system specified; the components were detected by spraying with 10% sulfuric acid and heating. Column chromatography was performed on silica gel (E. Merck) of 0.05- to 0.20-mm particle size, with the following solvent-systems, all ratios being v/v: *A*, 7:1 benzene–ether; *B*, 1:1 ether–hexane; *C*, 5:5:1 ether–benzene–methanol; *D*, 1:1:2 ether–chloroform–acetone; *E*, 6:1:1 ether–benzene–hexane; *F*, 2:1 ether–acetone; *G*, 80:5:15:1 acetonitrile–acetone–water–acetic acid; *H*, 1:1 chloroform–acetone; and *I*, 4:1 ether–hexane.

I.r. spectra were recorded on a Perkin–Elmer Model 137 spectrometer; the n.m.r. spectra were recorded at 60 MHz with a Varian A-60*A* spectrometer, for compounds in the solvents specified, with tetramethylsilane as the internal standard.

Silver carbonate on Celite was prepared according to the procedure of Fetizon *et al.*[25].

General procedure for the oxidation of substituted, free 1-hydroxylic compounds **1–5** *to the lactones* **6–10**. — To a solution of the free 1-hydroxylic compound (0.5 g) in anhydrous benzene (10–100 ml) was added dry silver carbonate on Celite (3 g). The suspension was boiled under reflux for a period of 3 h to 4 days, the progress of the reaction being monitored by t.l.c. with the solvents specified in Table I; normally, the lactone formed moves slightly faster than the corresponding starting compound. On completion of the oxidation, the hot mixture was filtered through a layer of Celite, the inorganic material was washed several times with warm benzene, and the filtrate and washings were combined and evaporated *in vacuo*; the crude product was purified either by crystallization, or by chromatography on columns of silica gel. The duration of the reactions, and the data for lactones **6–10**, are summarized in Table I. In the infrared spectrum, lactones **6–10** showed carbonyl absorption in the region 1780–1760 cm^{-1}.

*2-Acetamido-4,6-O-benzylidene-2-deoxy-*D*-mannono-1,5-lactone* (**13**). — To a solution of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-mannopyranose[18] (800 mg) in anhydrous 1,4-dioxane (180 ml) was added dry silver carbonate on Celite (8.4 g), the mixture was boiled under reflux overnight, and the progress of the reaction was monitored by t.l.c. in solvent *D*. At the end of the reaction, the spot for the starting compound was barely detectable, and that of the major component was faster moving.

The hot suspension was filtered through a layer of Celite, the precipitate was thoroughly washed with warm 1,4-dioxane, the filtrate and washings were combined, and evaporated *in vacuo*, and the residue was chromatographed on a column of silica gel (65 g) with solvent *D*; fractions (8 ml) were collected.

Fractions 10 and 11 contained partly crystalline material, which was triturated with anhydrous ether to yield pure crystals. The i.r. spectrum and chromatographic behavior in several solvent-systems identified this product as 2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**12**), described more fully later in this paper.

Fractions 14–19 contained crystalline lactone **13** (213 mg, 27%), which was recrystallized from absolute acetone: m.p. 200–202°, $[\alpha]_D$ +100.5° (*c* 0.26, acetone); ν_{max}^{KBr} 3550 (OH), 3320 (NH), 1760 (C=O), 1650 and 1550 (Amides I and II), 1480, 1170, 1070, 950, and 750 cm^{-1}; n.m.r. data (in Me_2SO-d_6): τ 2.02,(doublet, *J* 7.6 Hz, removed by D_2O exchange, NH), 2.53 (aromatic, 5 H), 3.93 (doublet, *J* 5.8 Hz, removed by D_2O exchange, OH), 4.26 (singlet, C_6H_5–C*H*), 4.78 (triplet, *J* 7.6 Hz, collapsed to a doublet by D_2O exchange, H-2), 5.3–6.4 (multiplet, unresolved, 5 H), and 8.03 (NAc).

Anal. Calc. for $C_{15}H_{17}NO_6$: C, 58.63; H, 5.58; N, 4.56. Found: C, 58.66; H, 5.81; N, 4.81.

Fraction 20 was a mixture (18 mg), and fractions 34–49 contained unreacted **11** (67 mg, 8%).

Removal of the benzylidene group from **13**. — A solution of **13** (270 mg) in 2-methoxyethanol (20 ml) was added to a suspension of freshly prepared palladium (from 150 mg of $PdCl_2$) in 2-methoxyethanol. The suspension was shaken with hydrogen overnight, the catalyst was removed, the filtrate was concentrated, and the product was chromatographed on a column of silica gel with acetone. Homogeneous fractions were pooled and evaporated, to yield a crystalline product (124 mg, 64%) which crystallized from methanol. Its i.r. spectrum and chromatographic behavior in several solvent-systems identified it as 2-acetamido-2-deoxy-D-mannono-1,4-lactone; a m.m.p. with an authentic sample[20] was undepressed.

*Oxidation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-*D*-glucopyranose* (**14**). — 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranose[21] (800 mg) was dissolved in warm, anhydrous 1,4-dioxane (190 ml), and to the solution was added dry silver carbonate on Celite (8.4 g). The mixture was mechanically stirred and boiled under reflux for 48 h, the progress of the reaction being monitored by t.l.c. (solvent *D*). The hot suspension was processed as already described, and the crude product was chromatographed on a column of silica gel (65 g) with solvent *D*, 10-ml portions of eluate being collected.

Fractions 16–18 contained a partly crystalline material (190 mg, 25%) which was twice rechromatographed on silica gel, first with solvent *E*, and then with ether, to yield pure product. Its chromatographic behavior and n.m.r. and i.r. spectra identified it as **12**.

Fractions 22–24 were pooled and evaporated, to yield crystalline 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-mannono-1,5-lactone (**13**) (110 mg, 14%), which crystallized from acetone; its i.r. spectrum and chromatographic behavior were indistinguishable from those of an authentic sample of **13**.

Fraction 25 (23 mg) was a mixture, and fractions 26–35 afforded the third product (41 mg, 5%) as a syrup. Material obtained from several batches was combined, and it was rechromatographed twice on silica gel with solvent *D*. Pure 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucono-1,5-lactone (**15**) was obtained as a friable glass: $[\alpha]_D$ −32.1° (*c* 0.50, acetone); ν_{max}^{KBr} 3500 (OH), 3350 (NH), 1730 (C=O), and 1640 and 1540 cm^{-1} (Amides I and II); n.m.r. data (in Me_2SO-d_6): τ 2.08 (doublet, *J* 7.8 Hz, removed by D_2O exchange, NH), 4.58 (singlet, C_6H_5–C*H*), 5.18 (doublet, *J* 9.0 Hz, removed by D_2O exchange, OH), 5.45 (quartet, *J* 6.1 and 7.8 Hz, collapsed to a doublet, *J* 6.1 Hz after D_2O exchange, H-2), and 8.22 (NAc).

Anal. Calc. for $C_{15}H_{17}NO_6$: C, 58.63; H, 5.58; N, 4.56. Found: C, 58.78; H, 5.73; N, 4.58.

Fractions 45–70 contained the starting compound **14** (80 mg, 10%).

*Oxidation of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-*D*-glucopyranose* (**16**). — To a solution of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucopyranose[22] (800 mg) in anhydrous 1,4-dioxane (200 ml) was added dry silver carbonate on Celite (8.4 g). The mixture was boiled under reflux overnight, and was then processed as

already described. As revealed by t.l.c. in solvent *D*, the crude product contained at least three components. It was dissolved in acetone (1 ml), and the solution was applied to a column of silica gel (70 g) prepacked in solvent *D*. The column was eluted with solvent *D*, 6-ml portions of eluate being collected.

Fractions 9–11 afforded a yellowish oil (158 mg) which was combined with material obtained from several such batches; this was rechromatographed with solvent *E*, to give partly crystalline product. Its n.m.r. spectrum showed it to be a mixture of two components. When the mixture was triturated with a few drops of acetone and anhydrous ether, crystals of 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-D-*erythro*-hex-2-enono-1,5-lactone (**17**) separated. The chromatographic behavior and i.r. spectrum of this compound were identical with those of a sample of **17** that will be described more fully in a later section.

Fractions 19–22 contained 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-mannono-1,5-lactone (**20**) (32 mg, 4%). Material from several batches was combined and rechromatographed twice on silica gel with solvent *D*; prior to analysis; it was chromatographed with solvent *F*, to yield the product in the form of a stable foam. The chromatographic behavior and i.r. and n.m.r. spectra were identical with those of a sample of the lactone **20** prepared from compound **19**.

Anal. Calc. for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.85; H, 6.77; N, 5.41.

Fractions 23 and 24 contained a mixture of **20** and **18**. Fractions 25–36 were collected and evaporated, to give crystalline 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucono-1,5-lactone (**18**) (174 mg, 22%). Recrystallized from absolute acetone, compound **18** had m.p. 162–164° and $[\alpha]_D$ +133.5° (*c* 0.50, acetone); lit.[2] m.p. 148–150°, $[\alpha]_D$ +130.2° (acetone) for a sample not recrystallized. Its chromatographic behavior and i.r. spectrum were indistinguishable from those of a sample prepared by a different route[2]. N.m.r. data (in acetone-d_6): τ 1.80 (doublet, $J \sim 8$ Hz, NH), 5.18 (OH), 6.0–6.7 (multiplet, unresolved, 6 H), 8.08 (NAc), and 8.48 and 8.66 (CMe_2).

On prolonged storage at room temperature, the sample was found to have become heterogeneous; t.l.c. in solvent *G* revealed the presence of several spots.

When the reaction in boiling 1,4-dioxane was conducted for 10 days, the major product was the unsaturated lactone **17**, isolated in 25% yield.

*Oxidation of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-*D*-mannopyranose* (**19**). — A suspension of **19** (ref. 22, 380 mg) and dry silver carbonate on Celite (4.0 g) in anhydrous 1,4-dioxane (80 ml) was boiled under reflux overnight. The crude product was chromatographed on a column of silica gel (25 g) with solvent *D*, 5-ml fractions being collected.

Fractions 9 and 10 contained an oil (19 mg) that, in several solvent-systems, was chromatographically indistinguishable from a sample of **17**.

After evaporation, fractions 15–18 afforded 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-mannono-1,5-lactone (**20**) (112 mg, 30%). Prior to analysis, it was rechromatographed with solvent *D*, isolated, and the product triturated with ether,

to give a stable foam, $[\alpha]_D$ +125.5° (*c* 1.01, acetone); ν_{max}^{KBr} 3450 (OH and NH), 1770 (C=O), 1660 and 1540 (Amides I and II), 1380, 1270, 1200, 1170, 1080, 945, 860, and 755 cm^{-1}; n.m.r. data (in acetone-d_6): τ 2.82 (doublet, *J* ~7 Hz, removed by D_2O exchange, NH), 4.90 (triplet, *J* 7.0 Hz, collapsed to a doublet by D_2O exchange, H-2), 4.92 (doublet, removed by D_2O exchange, OH), 5.4–6.5 (unresolved multiplet, 5 H), 8.00 (NAc), and 8.49 and 8.64 (CMe_2).

Anal. Calc. for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.99; H, 6.87; N, 5.65.

Fractions 20–25 gave a crystalline product (16 mg, 4%); its i.r. spectrum and chromatographic behavior identified it as the *gluco* lactone **18**.

The column was further eluted with acetone, to give the starting compound **19** (72 mg, 19%).

*Methyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-*D*-mannonate* (**21**) *from* **20**. — A solution of **20** (80 mg) in methanol (5 ml) was kept for 10 days at room temperature, and the progress of the reaction was monitored by t.l.c. (solvent *D*); at the end of the reaction, the presence of **20** was evident. The solvent was removed *in vacuo*, the residue was chromatographed on a column of silica gel (10 g) with solvent *D*, and fractions containing homogeneous material were evaporated to give **21** in crystalline form (58 mg, 64%). Prior to analysis, it was rechromatographed with the same solvent-mixture: m.p. 136–138°, $[\alpha]_D$ −32.0° (*c* 0.8, acetone); ν_{max}^{KBr} 3550 (OH), 3400 (NH), 1750 (C=O), and 1660 and 1560 cm^{-1} (Amides I and II); n.m.r. data (in acetone-d_6): τ 2.7 (broad signal, NH), 5.26 (quartet, H-2), 6.34 (OCH_3), 8.07 (NAc), and 8.58 and 8.67 (CMe_2). The compound was stable at room temperature.

Anal. Calc. for $C_{12}H_{21}NO_7$: C, 49.48; H, 7.27; N, 4.81. Found: C, 49.60; H, 7.35; N, 5.01.

*Oxidation of 2-acetamido-2-deoxy-3,4-di-*O*-methyl-*D*-glucopyranose* (**22**). — To a warm solution of **22** (ref. 23, 195 mg) in anhydrous 1,4-dioxane (30 ml) was added dry silver carbonate on Celite (2.0 g); the mixture was boiled under reflux for 8 h, and then processed as already described. The crude product was chromatographed on a column of silica gel (25 g) with solvent *D*, 4-ml fractions being collected.

Fractions 12–18 afforded crystalline 2-acetamido-2,3-dideoxy-4-*O*-methyl-D-*erythro*-hex-2-enono-1,5-lactone (**23**) (30 mg, 18%). Material from several batches was combined and crystallized from acetone–ether; m.p. 142–144°, $[\alpha]_D$ +96.0° (*c* 0.90, acetone). In t.l.c., the compound gave a positive test with fluorescein–bromine[26]; ν_{max}^{KBr} 3400 (OH), 3320 (NH), 1730 (C=O), 1660 and 1530 (Amides I and II), and 1640 cm^{-1} (C=C); n.m.r. data (in acetone-d_6): τ 1.56 (broad singlet, removed by D_2O exchange, NH), 2.44 (doublet, *J* 3.8 Hz, H-3), 5.4–5.9 (unresolved 3-proton multiplet; after D_2O exchange, this collapsed to a 2-proton multiplet, H-4, H-5, and OH), 6.22 (unresolved, 2-proton signal; after D_2O exchange, this appeared as a doublet, *J* 4.1 Hz, 2 H-6), 6.61 (OCH_3), and 7.90 (NAc).

Anal. Calc. for $C_9H_{13}NO_5$: C, 50.23; H, 6.09; N, 6.51. Found: C, 50.25; H, 6.34; N, 6.66.

Fractions 23–34 contained a mixture of two products (65 mg, 34%); this was

rechromatographed on silica gel with solvent *H*, to give partly crystalline 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-mannono-1,5-lactone (**24**), $[\alpha]_D$ +213.1° (*c* 0.50, acetone); ν_{max}^{KBr} 3500–3400 (OH and NH), 1760 (C=O), and 1650 and 1560 cm^{-1} (Amides I and II); n.m.r. data (in acetone-d_6): τ ~2.7 (broad signal, NH), 6.53 and 6.60 (OCH_3, 6 H), and 8.01 (NAc).

Anal. Calc. for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.93; N, 5.67. Found: C, 48.54; H, 6.96; N, 5.78.

From further fractions, the second compound was obtained as an unstable foam, $[\alpha]_D$ +130.5° (*c* 0.57, acetone); n.m.r. data (in acetone-d_6): τ 2.2 (broad signal, NH), 6.46 and 6.49 (OCH_3, 6 H), and 8.07 (NAc). The compound was tentatively assigned the structure of 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-glucono-1,5-lactone (**25**).

Anal. Calc. for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.93; N, 5.67. Found: C, 47.94; H, 7.59.

*2-Acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-*D*-erythro-hex-2-enono-1,5-lactone* (**17**). — To a precooled solution of **18** (355 mg) in dry pyridine (4 ml) was added *p*-toluenesulfonyl chloride (1.1 g), and the mixture was kept at room temperature for 18 h, the progress of the reaction being monitored by t.l.c. with solvents *E* and *I*. At the end of the reaction, the presence of two faster-moving products was evident; the starting compound **18** was barely visible.

Water was then added, the mixture was poured onto crushed ice, and the product was extracted with chloroform; the extract was successively washed with 2M hydrochloric acid, saturated sodium hydrogen carbonate solution, and water, dried (sodium sulfate), and evaporated *in vacuo*. The residue was chromatographed on a column of silica gel (40 g) with solvent *I*, 5-ml fractions being collected. Fractions 15–20 contained a compound, found to be unstable, which was not identified.

Fractions 22–36, which were chromatographically homogeneous and gave a positive test for unsaturation, were pooled, and evaporated to give compound **17** in crystalline form (100 mg, 30%). Recrystallized from acetone–ether, m.p. 162–163°, $[\alpha]_D$ −45.6° (*c* 0.75, $CHCl_3$); ν_{max}^{KBr} 3450 (NH), 1730 (C=O), 1710 (C=C), and 1640 and 1540 (Amides I and II); n.m.r. data (in $CDCl_3$): τ 2.29 (broad singlet, NH), 2.43 (doublet, $J_{3,4}$ 2.1 Hz, H-3), 5.28 (pair of doublets, $J_{3,4}$ 2.1 Hz, $J_{4,5}$ 11.2 Hz, H-4), 5.6–6.3 (multiplet, unresolved, 3 H), 7.90 (NAc), and 8.49 and 8.57 (CMe_2).

Anal. Calc. for $C_{11}H_{15}NO_5$: C, 54.76; H, 6.27; N, 5.81. Found: C, 54.58; H, 6.03; N, 5.93.

*2-Acetamido-4,6-*O*-benzylidene-2,3-dideoxy-*D*-erythro-hex-2-enono-1,5-lactone* (**12**) was prepared by elimination from **13**, in a reaction analogous to that described for the synthesis of **17**. The crude product was purified by chromatography on silica gel with ether, and was then crystallized from methanol, m.p. 189–190°, $[\alpha]_D$ −32.9° ($CHCl_3$); its n.m.r. and i.r. spectra were in accordance with the structure, and identical to those reported recently[20] {lit.[20] m.p. 193–194°, $[\alpha]_D$ −32.2° ($CHCl_3$)}.

*2-Acetamido-2,3-dideoxy-*D*-erythro-hex-2-enono-1,5-lactone* (**26**). — To a solution of **17** (280 mg) in 2-methoxyethanol (18 ml) was added dry Dowex-50 X-8 (H^+)

ion-exchange resin (2.8 g), and the mixture was kept at room temperature with occasional stirring. The progress of the reaction was monitored by t.l.c. with solvent *H*. The first product to appear was 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**26**). As the reaction progressed further, a zone of a second product, slightly faster-moving than that of **26**, could be detected. The intensity of the zone of **26** decreased, and, when the reaction was conducted overnight, the major product was the faster-moving, unidentified compound.

The reaction was, therefore, terminated after 3 h, the resin was filtered off and washed several times with acetone, and the filtrate and washings were combined and evaporated *in vacuo*. The crude product was chromatographed on a column of silica gel (28 g) with solvent *H*, 4-ml portions of eluate being collected.

From fractions 9–11, unreacted **17** was recovered (42 mg, 15%). Fractions 16–26 afforded degradation product (unidentified) in crystalline form (93 mg).

Fractions 28–40 contained homogeneous material; they were pooled, and evaporated to give a crystalline product (68 mg, 34%) which gave a positive test with fluorescein–bromine[26]. It was recrystallized from acetone, affording pure **26**: m.p. 130–132°, $[\alpha]_D$ +29.8° (*c* 0.70, acetone); ν_{max}^{KBr} 3500 (OH), 3400 (NH), 1730 (C=O), 1660 and 1540 (Amides I and II), and 1580 cm^{-1} (C=C); n.m.r. data (in acetone-d_6): τ 1.66 (broad singlet, removed by D_2O exchange, NH), 2.57 (doublet, $J_{3,4}$ 3.4 Hz, H-3), 5.2–6.3 (unresolved, 6 H), and 7.91 (NAc); after D_2O exchange, τ 5.34 (pair of doublets, $J_{3,4}$ 3.4, $J_{4,5}$ 8.0 Hz, H-4), 5.63 (sextet, $J_{4,5}$ 8.0, $J_{5,6}$ 4.2 Hz, H-5), and 6.18 (doublet, $J_{5,6}$ 4.2 Hz, 2 H-6).

Anal. Calc. for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47.47; H, 5.80; N, 7.10.

ACKNOWLEDGMENTS

This work was supported, in part, by a P.L. 480 grant from the National Institutes of Health, Bethesda, Md., Agreement No. 02-010-1.

We are indebted to Professor Derek Horton (Columbus, Ohio) for providing us with the M.S. Thesis mentioned in ref. 19, and to Professor Roger W. Jeanloz (Boston, Mass.) for kindly sending us a description of his preparation of compound **11** prior to its publication. We thank Dr. O. Hadžija for microanalyses, and Mrs. L. Tomić, B.Sc., for recording the n.m.r. spectra.

REFERENCES

1 G. A. LEVVY AND S. M. SNAITH, *Advan. Enzymol.*, 36 (1972) 151–181, and references cited therein.
2 N. PRAVDIĆ, E. ZISSIS, M. POKORNY, AND H. G. FLETCHER, JR., *Carbohyd. Res.*, 32 (1974) 115–126.
3 M. FETIZON AND M. GOLFIER, *Compt. Rend., C*, 267 (1968) 900–903.
4 J. M. J. TRONCHET, J. TRONCHET, AND A. BIRKHAEUSER, *Helv. Chim. Acta*, 53 (1970) 1489–1490.
5 S. MORGENLIE, *Acta Chem. Scand.*, 25 (1971) 1154–1155; 26 (1972) 2518–2522.
6 S. MORGENLIE, *Acta Chem. Scand.*, 25 (1971) 2773–2774; 26 (1972) 1709–1710.
7 S. MORGENLIE, *Acta Chem. Scand.*, 26 (1972) 2146–2147; 27 (1973) 1557–1564.
8 S. MORGENLIE, *Acta Chem. Scand.*, 27 (1973) 2217–2218.

9 T. D. PERRINE, C. P. J. GLAUDEMANS, R. K. NESS, J. KYLE, AND H. G. FLETCHER, JR., *J. Org. Chem.*, 32 (1967) 664–669.
10 R. HARRISON AND H. G. FLETCHER, JR., *J. Org. Chem.*, 30 (1965) 2317–2321.
11 N. PRAVDIĆ AND D. KEGLEVIĆ, *Tetrahedron*, 21 (1965) 1897–1901.
12 G. ROGLIĆ AND D. KEGLEVIĆ, *Croat. Chem. Acta*, 44 (1972) 229–242.
13 H. KUZUHARA AND H. G. FLETCHER, JR., *J. Org. Chem.*, 32 (1967) 2531–2534.
14 N. PRAVDIĆ AND H. G. FLETCHER, JR., *Carbohyd. Res.*, 19 (1971) 353–364.
15 Y. RABINSOHN AND H. G. FLETCHER, JR., *J. Org. Chem.*, 32 (1967) 3452–3457.
16 F. SMITH, *J. Chem. Soc.*, (1944) 584–587.
17 R. M. HANN AND C. S. HUDSON, *J. Amer. Chem. Soc.*, 67 (1945) 602–605.
18 A. M. STEPHEN AND R. W. JEANLOZ, *Fed. Proc.*, 25 (1966) 409.
19 J. K. THOMSON, M. S. Thesis, Ohio State University, 1969, p. 36.
20 N. PRAVDIĆ AND H. G. FLETCHER, JR., *Carbohyd. Res.*, 19 (1971) 339–352.
21 W. ROTH AND W. PIGMAN, *J. Amer. Chem. Soc.*, 82 (1960) 4608–4611.
22 A. HASEGAWA AND H. G. FLETCHER, JR., *Carbohyd. Res.*, 29 (1973) 209–222.
23 R. W. JEANLOZ, *J. Amer. Chem. Soc.*, 74 (1952) 4597–4599.
24 J. R. VERCELLOTTI, N. NIENABER, AND C. J. CHANG, *Carbohyd. Res.*, 13 (1970) 63–74, and references cited therein.
25 V. BALOGH, M. FETIZON, AND M. GOLFIER, *Angew. Chem.*, 81 (1969) 423–424.
26 E. STAHL (Ed.), *Thin-Layer Chromatography*, Springer Verlag, 1969, p. 877.