2-ACETAMIDO-3,6-ANHYDRO-2-DEOXY-D-HEXOSES: PRODUCTS OF THE ALKALINE DEGRADATION OF 2-ACETAMIDO-2-DEOXY-D-HEXOSES

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ABSTRACT

The formation of 2-acetamido-3,6-anhydro-2-deoxy-D-hexoses during the alkaline degradation of 2-acetamido-2-deoxy-D-hexoses is reported for the first time. Thus, 2-acetamido-3,6-anhydro-2-deoxy-D-glucose and -D-mannose are formed from 2-acetamido-2-deoxy-D-glucose or -D-mannose. The structure of 2-acetamido-3,6-anhydro-2-deoxy-D-mannose has been elucidated, and certain derivatives have been obtained. The 3,6-anhydro derivative formed during the alkaline degradation of 2-acetamido-2-deoxy-D-galactose is different from those formed from the D-*gluco* and D-*manno* analogues. This allows identification of a 2-acetamido-2-deoxy-D-hexose that decomposes during the alkaline treatment of glycoproteins in an amino acid analyzer.

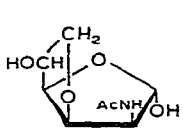
INTRODUCTION

Amongst the products of alkaline degradation of 2-acetamido-2-deoxy-D-hexoses, only three compounds have been found so far which give a Morgan–Elson reaction with *p*-dimethylaminobenzaldehyde. The structure 3-acetamido-5-(1,2-dihydroxyethyl)furan has been firmly assigned to one of these compounds, and the others have been identified as a 2-acetamido-2-deoxy-hex-2-enose and a 2-acetamido-2-deoxy-hex-3-enose, but not unequivocally[1]. We now report on the formation of 2-acetamido-3,6-anhydro-2-deoxy-D-hexoses during the alkaline degradation of 2-acetamido-2-deoxy-D-hexoses.

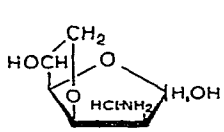
RESULTS AND DISCUSSION

2-Acetamido-3,6-anhydro-2-deoxy-α-D-mannofuranose (**1**) was isolated in ~10% yield by chromatography on silica gel after 2-acetamido-2-deoxy-D-glucose had been treated with 50mM sodium carbonate at 70°, for 2.5 h, in a nitrogen atmosphere. Under the standard conditions of the Morgan–Elson reaction (100°, 4 min), the formation of 2-acetamido-3,6-anhydro-2-deoxyhexoses was also observed. Compound **1** reacted with aniline hydrogen phthalate but only slightly with the Schiff reagent. It was reduced with sodium borohydride with a significant change in

optical rotation (+154°→+60°). On periodate oxidation, **1** consumed 1 mol. of oxidant without releasing formaldehyde, and its i.r. spectrum showed bonds at 1640 and 1550 (amide group), 878 and 825 cm^{-1} (3,6-anhydrohexofuranose[2]).



1



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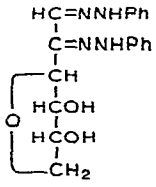

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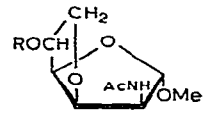


5 R=H
6 R=Me
7 R=Ac

The presence of the 3,6-anhydro ring and the absence of configurational changes at C-3, C-4, and C-5 were proved by the conversion of **1** into 3,6-anhydro-D-*arabino*-hexose phenylosazone after *N*-deacetylation with 2M hydrochloric acid (100°, 2 h). 2-Amino-3,6-anhydro-2-deoxy-D-mannofuranose hydrochloride (**2**) was obtained crystalline in high yield.

The elution time of **2** in the amino acid analyzer is different from that of 2-amino-3,6-anhydro-2-deoxy-D-glucose[2] (**4**). The *manno* configuration of **1** was further indicated by n.m.r. spectroscopy of certain derivatives. Treatment of **1** with methanol in the presence of Dowex-50 (H^+) resin at 20° for 5 h gave the methyl glycoside **5** in ~90% yield. The mass spectrum of **5** was consistent with the structure assigned and contained a weak peak for the molecular ion *m/e* 217 (0.4% of base peak), and peaks at *m/e* 199 ($M^+ - H_2O$, 0.75%), 185 ($M^+ - CH_3OH$, 41%), 158 ($M^+ - CH_3CONH_2$, 100%), and 128 ($M^+ - CH_3CONH_2 - CH_2O$, 59%). The increase in specific rotation (+154°→+197°) associated with the transformation **1**→**5** indicates that the latter compound is the α-D anomer. The n.m.r. spectrum of **5**, in deuteriopyridine, showed singlets of τ 6.78 and 8.10, corresponding to the protons of one methoxyl and one acetyl group, respectively.

The furanose structure of **5** was proved as follows. Treatment of **5** with methyl iodide in the presence of silver oxide gave a chromatographically homogeneous 5-*O*-methyl derivative **6** which, on mild hydrolysis with acid, lost the glycosidic substituent to afford a product giving an intense Morgan–Elson reaction consistent[3] with the location of the methoxyl group at C-5.

The acetate **7** of the methyl glycoside **5** gave an n.m.r. spectrum (CCl_4) which exhibited singlets at τ 7.97, 8.10 (acetyl groups), and 6.72 (methoxyl), and a doublet at 5.26 ($J_{1,2}$ 1.7 Hz). The last signal, which is assigned to H-1, is associated with a coupling constant that is consistent with a *trans* disposition of H-1 and H-2 (*cf.* Ref. 4).

Collectively, the above data unequivocally establish the structure of **1** as 2-acetamido-3,6-anhydro-2-deoxy-D-mannose. No mutarotation was observed for **1**; both **1** and **5** were obtained as α-D anomers. This observation is in agreement with the known[5,6] high stability of the anomers of 3,6-anhydro-D-glucose, -D-galactose, and -D-mannose derivatives having a *trans* disposition of substituents at C-1 and C-2.

Examination (amino acid analyzer) of the reaction mixture obtained by the treatment of 2-acetamido-2-deoxy-D-glucose with 50 mM sodium carbonate revealed the presence of another anhydro sugar, the formation of which was faster than that of **1**. It was identified as 2-amino-3,6-anhydro-2-deoxy-D-glucose (**4**) on the basis of a comparison with the authentic compound obtained from the known cyclic carbamate **8**. The m.p. of **8** was in accord with literature data but the $[\alpha]_D$ was different (+46.4°, *cf.* lit.[2] +26°). The assigned structure was, however, supported by mass-spectral data. In addition to a weak peak for the molecular ion (*m/e* 201, 9.5%), the mass spectrum of **8** showed an intense peak *m/e* 141 ($M^+ - HCOOCH_3$, 5%) and peaks at 103 (100%) and 98 (25%) arising by splitting of the molecule into two fragments during rearrangement (*cf.* Ref. 7) as shown in Scheme 1.

Acid hydrolysis of the carbamate **8** yielded a product [presumably 2-amino-

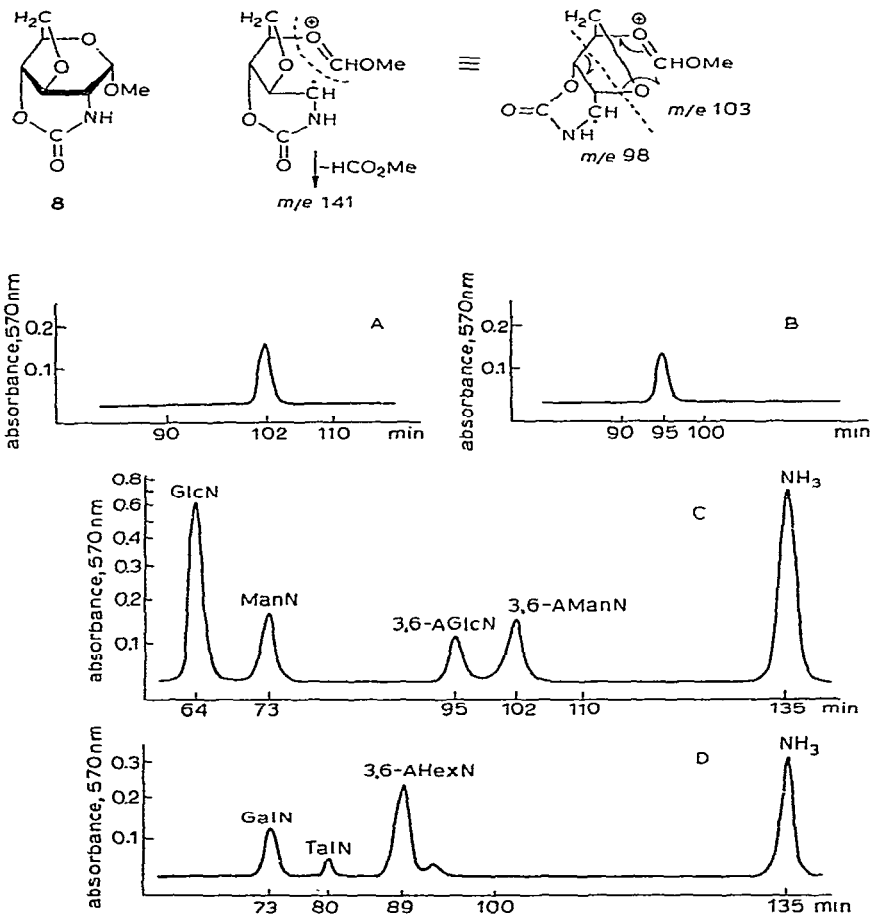
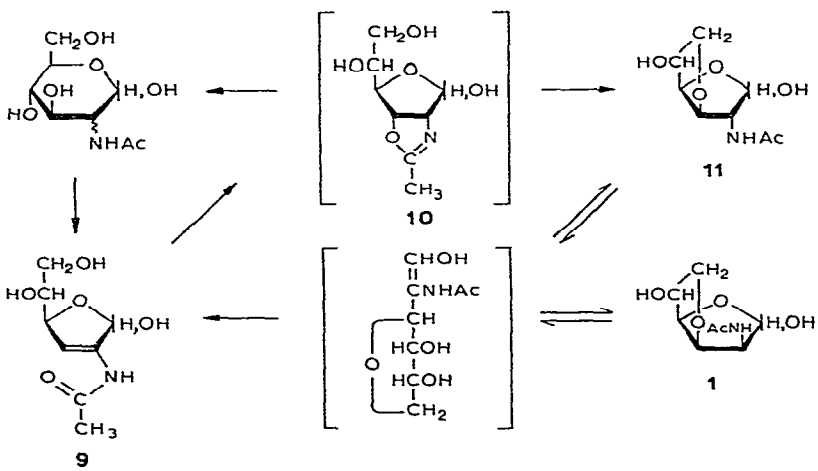


Fig. 1. *A*, 2-Amino-3,6-anhydro-2-deoxy-D-mannose (**2**). *B*, 2-Amino-3,6-anhydro-2-deoxy-D-glucose (**4**). Hydrolysates of alkali-degraded (50mM Na_2CO_3, 2.5 h, 70°) 2-acetamido-2-deoxy-D-glucose or -D-mannose (*C*) and 2-acetamido-2-deoxy-D-galactose (*D*).

3,6-anhydro-2-deoxy-D-glucose hydrochloride (4)] which, in the amino acid analyzer, gave a single peak identical to that produced by the product formed on alkaline degradation of 2-acetamido-2-deoxy-D-glucose (Fig. 1).

Thus, during the alkaline degradation of 2-acetamido-2-deoxy-D-glucose two epimeric 2-acetamido-3,6-anhydro-2-deoxyhexoses are formed in addition to the known chromogens. The ratio of the *gluco* and *manno* isomers depends on the duration of the alkaline treatment of 2-acetamido-2-deoxy-D-glucose; the respective yields (determined with the amino acid analyzer) with time were as follows: 7 min, 3% and traces, 15 min, 3.4 and 1.4%, 30 min, 9.2 and 4.8%, 1 h, 10.6 and 7.2%, 2 h, 14.2 and 11.3%, 4 h, 6.7 and 7%. After 2 h, ~20% of 2-acetamido-2-deoxy-D-glucose and -D-mannose (equilibrium ratio 3:1) was found in the reaction mixture.



Starting with 2-acetamido-2-deoxy-D-glucose, a possible pathway for formation of the 3,6-anhydro derivative involves attack of the *N*-acetyl carbonyl group on a double bond of one of chromogens, 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enose (**9**), to give the oxazoline **10**. Intramolecular attack of HO-6 on C-3 in **10** then affords 2-acetamido-3,6-anhydro-2-deoxy-D-glucose (**11**) (*cf.* Refs. 8 and 9). The *gluco* isomer **11** then undergoes partial epimerization to give the *manno* isomer **1**. Simultaneously, the chromogen **9** would be formed. This view is supported by the fact that, on heating a solution of **1** in aqueous sodium carbonate a Morgan–Elson reaction develops which is approximately twice as intense as that for 2-acetamido-2-deoxy-D-glucose. Moreover, **1** is converted into the *gluco* isomer **11**, and small amounts of 2-acetamido-2-deoxy-D-glucose and -D-mannose are formed (Table I).

The formation of 3,6-anhydro derivatives was also observed for other 2-acetamido-2-deoxyhexoses. Treatment of 2-acetamido-2-deoxy-D-mannose with 50mM sodium carbonate gave a mixture of **1** and **11**, their ratio being the same as that observed in the degradation of 2-acetamido-2-deoxy-D-glucose. Under the conditions used, 2-acetamido-2-deoxy-D-mannose rapidly epimerizes to form 2-acetamido-2-

TABLE I

DATA[a] ON THE ALKALINE DEGRADATION OF 2-ACETAMIDO-3,6-ANHYDRO-2-DEOXY-D-MANNOSE (1) IN 50mM SODIUM CARBONATE AT 70°

Treatment time (min)	*AM:AG*[b]	*AM+AG* (%)	*GlcN:ManN*	*GlcN+ManN* (%)
3	7:1	81.5		0
5	2.7:1	72.5		
15	1.4:1	54.7		
30	1.3:1	50.8	2.6:1	5.2
60	1.3:1	46.5	3:1	7.9
240	1.4:1	29.0	3:1	8.9

[a]Amino sugars were determined, using an amino acid analyser, after hydrolysis with 2M hydrochloric acid at 100° for 2 h. [b]AM = 2-acetamido-3,6-anhydro-2-deoxy-D-mannose, AG = 2-acetamido-3,6-anhydro-2-deoxy-D-glucose.

deoxy-D-glucose (equilibrium ratio 1:3). A compound, apparently a 3,6-anhydro derivative isomeric to **2** and **4**, was formed on alkaline degradation of 2-acetamido-2-deoxy-D-galactose and could be detected by means of the amino acid analyzer. Although its structure has not been finally established, it is important to point out that the elution time is different from those of **2** and **4** (Fig. 1).

The reaction reported herein is a new transformation of 2-acetamido-2-deoxy-D-hexoses which appears to have analytical value and may serve as a useful procedure for the establishment of carbohydrate chain structure[10]. The alkaline degradation of many glycoproteins is known to proceed with the destruction of their component 2-acetamido-2-deoxy-D-hexoses. Previously, it was possible only to estimate the chromogens formed in the degradation; 2-acetamido-2-deoxy-D-hexoses could not be differentiated. Since the 3,6-anhydro derivatives of 2-amino-2-deoxy-D-hexoses are easily distinguishable in the amino acid analyzer, it is now possible to identify 2-acetamido-2-deoxyhexoses which are degraded upon alkaline treatment of glycoproteins. This approach is being used in structural studies of blood-group substances.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) on silica gel was performed with chloroform–methanol, 4:1 (*A*) and 9:1 (*B*). Electrophoresis was performed in a pyridine–acetate buffer (pH 4.5) at 30 volts/cm for 60 min. Aniline hydrogen phthalate, silver nitrate, ninhydrin, *p*-dimethylaminobenzaldehyde (DAB), periodate–cuprate, and sulphuric acid were used as detection reagents.

N.m.r. spectra were recorded with a DA-60-IL 60 MHz instrument (internal reference, hexamethyldisiloxane) and mass spectra with a MX-1303 spectrometer with ion-source temperature of 50–60°. Analyses were performed on an amino acid analyser 6020 A (Czechoslovakia) with a column (30 × 0.8 cm) of Aminex SB (blend Q-60) and elution at 52° with a standard citrate–hydrochloric acid buffer, pH 5.28 (0.35M Na^+), at 40 ml/h.

2-Acetamido-3,6-anhydro-2-deoxy-α-D-mannofuranose (**1**). — 2-Acetamido-2-deoxy-D-glucopyranose (15 g) dissolved in 50mM sodium carbonate (1.5 l) was heated at 70° for 2.5 h in a nitrogen atmosphere. The solution was then neutralized with Dowex-50 (H^+) resin, filtered, and evaporated *in vacuo*. The product mixture was eluted from a column of silica gel by using a gradient of chloroform → chloroform–methanol (9:1). Fractions were monitored by t.l.c. (solvent *A*). Fractions containing a component with R_F 0.64, which was oxidized by periodate and gave no appreciable colour with DAB, were collected and evaporated. The product (1.6 g, 11%) was recrystallized from methanol to give **1**, m.p. 184–185°, $[\alpha]_D^{20}$ +154° (*c* 1, water), R_F 0.28 (solvent *B*) (Found: C, 47.46; H, 6.36; N, 6.88. $C_8H_{13}NO_5$ calc.: C, 47.28; H, 6.45; N, 6.89%). One mol. of sodium metaperiodate was consumed by **1** (spectrophotometry at 305 nm). The oxidation was complete in 30 min, and formaldehyde was not liberated.

Hydrochloride of 2-amino-3,6-anhydro-2-deoxy-D-mannose (**2**). — Compound **1** (130 mg) was hydrolyzed with 2M hydrochloric acid at 100° for 2 h. The hydrolyzate was evaporated, and the product (100 mg) was recrystallized from aqueous ethanol to give **2**, m.p. 184–186°, $[\alpha]_D^{20}$ +56° (*c* 0.5, water) (Found: C, 36.49; H, 5.95; Cl, 17.67. $C_6H_{12}ClNO_4$ calc: C, 36.47; H, 6.12; Cl, 17.94%). I.r. data: bands at 3000, 2520, 1980, 1600, and 1520 cm^{-1} which are characteristic of the NH_3^+ group. Compound **2** gave a single peak in the amino acid analyzer, with an elution time of 102 min (Fig. 1).

A solution of **2** (73 mg) in water (1.3 ml) and acetic acid (0.13 ml), containing phenylhydrazine (0.15 g) and sodium acetate (0.23 g), was heated at 95° for 1.5 h. The precipitate was collected and crystallized from methanol (2 ml) and water (20 ml). The product (30 mg), after recrystallization from 40% ethanol, had m.p. 173–175° alone and in admixture with 3,6-anhydro-D-*arabino*-hexose phenylosazone[11] (m.p. 174°) obtained from 3,6-anhydro-D-glucose.

Methyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannofuranoside (**5**). — A mixture of **1** (80 mg), Dowex-50 (H^+) resin (230 mg), and methanol (2.4 ml) was stirred at room temperature for 5 h, and then filtered and evaporated. The product (77.2 mg, 90%) was chromatographically homogeneous (t.l.c., solvents *A* and *B*) and, after recrystallization, **5** was obtained having m.p. 174–175°, $[\alpha]_D^{20}$ +197° (*c* 1, water), R_F 0.44 (solvent *B*) (Found: C, 49.81; H, 6.98; N, 6.43. $C_9H_{15}NO_5$ calc.: C, 49.77; H, 6.95; N, 6.45%).

Methyl 2-acetamido-3,6-anhydro-2-deoxy-5-O-methyl- (**6**) *and -5-O-acetyl-α-D-mannofuranoside* (**7**). — Methyl iodide (920 mg) was added to **5** (10 mg) at 45°, and silver oxide (75 mg) was added, with stirring, every 30 min during 5 h. Heating was continued for another 6 h. The silver oxide was separated and thoroughly extracted with hot acetone. The combined extract and reaction solution was evaporated, and the residue was subjected to preparative t.l.c. (solvent *B*). The bond of **6** (R_F 0.68) was eluted with acetone to give material (7 mg) having $[\alpha]_D^{20}$ +235° (*c* 0.28, methanol). The mass spectrum showed peaks at *m/e* 199 ($M^+ - CH_3OH$, 2.6%), 172 ($M^+ - CH_3CONH_2$, 61.7%), 142 ($M^+ - CH_3CONH_2 - CH_2O$, 100%).

After hydrolysis of **6** with 2M hydrochloric acid for 2 h, a product giving a

characteristic peak (125 min) in the amino acid analyzer was obtained. No peak at 102 min, corresponding to **2**, was observed.

Dowex-50 (H^+) resin (10 mg) was added to a solution of **6** (2.5 mg) in aqueous acetone (0.5 ml) at 50°. The hydrolysis was monitored by t.l.c. (solvent *B*). Only one substance (R_F 0.45) was found in the hydrolyzate after 22 h. The resin was collected and washed with aqueous acetone, and the combined filtrate and washings were evaporated. The residue (1.5 mg) was dissolved in aqueous acetone (1 ml). An aliquot (0.1 ml) was evaporated, and the residue was treated with 50 mM sodium carbonate (1 ml) at 100° for 4 min. Then 1 ml of a 1% solution of DAB in 50% acetic acid and 0.2 ml of conc. hydrochloric acid were added. The absorbance at 540nm was measured after 10–15 min; **6**, 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-glucose, and **1** were treated under similar conditions. Compound **6** and 2-acetamido-2-deoxy-3,4-di-*O*-methyl-D-glucose did not produce any colour, and the absorbance values for **1** and **6** were similar.

Compound **5** (23 mg) was treated with acetic anhydride (0.1 ml) and pyridine (0.13 ml) in the usual manner to give **7** as a chromatographically homogeneous (t.l.c., solvents *A* and *B*) syrup having $[\alpha]_D^{20}$ +135° (*c* 0.46, carbon tetrachloride) and R_F 0.71 (solvent *B*). The mass spectrum showed intense peaks at *m/e* 200 ($M^+ - CH_3CONH_2$, 64%) and 170 ($M^+ - CH_3CONH_2 - CH_2O$, 57%).

Alkaline degradation and subsequent analysis using the amino acid analyzer. — Samples (0.5 mg) of 2-acetamido-2-deoxy-D-hexose or **1** were treated with 0.5 ml of 50mM sodium carbonate at 70° in a nitrogen atmosphere, for periods of 3 min to 4 h. 4M Hydrochloric acid (0.5 ml) was then added, and the mixture was kept for 2 h. The hydrolyzate was evaporated, and 0.5 ml of water was added. A sample (0.2 ml) was then analyzed in the amino acid analyzer.

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