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# THE SYNTHESIS AND DECOMPOSITION OF 1,3,4,6-TETRA-O-ACETYL-2-DEOXY-2-(*N*-NITROSO)ACETAMIDO-α- AND β-D-GLUCOPYRANOSE*

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ABSTRACT

The synthesis of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(*N*-nitroso)acetamido-α- and β-D-glucopyranose is described. Decomposition of the α-nitrosoamide in chloroform containing 2% of ethanol at room temperature afforded β-D-glucopyranose pentaacetate and ethyl β-D-glucopyranoside tetraacetate as major products, the former predominating. Reaction in 1:5 (*v*/*v*) acetic acid–acetic anhydride containing sodium acetate also gave β-D-glucose pentaacetate as major product, together with 1,1,3,4,6-penta-*O*-acetyl-2,5-anhydro-D-mannose aldehydrol. Decompositions of both α and β-nitrosoamides in 1:1 (*v*/*v*) acetone–water gave mainly 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose and its aldehydrol form. The synthesis, from 2,5-anhydro-D-mannose, of four new derivatives is also reported.

## INTRODUCTION

As part of a study[1] of the deamination of carbohydrate amines, we have examined the thermal decomposition of *N*-nitroso-*N*-acetyl derivatives. Such reactions can proceed via unstable diazonium ions to give products similar to those obtained in deamination of the corresponding amines[2]. We have examined peracetylated compounds for two reasons; first, per-*O*-acetylated carbohydrate amines and inosamines have been used in deamination studies to minimise rearrangements[3], and second, peracetylated compounds allow a wide range of solvents to be used. We report herein the preparation and mode of decomposition of the α- and β-anomers of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(*N*-nitroso)acetamido-D-glucopyranose (**1** and **2**); the products have been characterized by comparison with reference compounds prepared from 2,5-anhydro-D-mannose. The preparation of other *N*-(nitroso)-acetamido sugar derivatives has been described briefly in a preliminary report[1a].

## RESULTS

*Synthesis and decomposition of nitrosoamides.* — 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α- and β-D-glucopyranose were nitrosated at 0° by nitrosyl chloride in

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

ethanol-free chloroform containing pyridine. Both nitrosoamides were obtained as golden yellow syrups containing traces of amide starting material; they could not be crystallised. The u.v. spectrum of the α-nitrosoamide (**1**) exhibited characteristic[4a] fine structure ($\lambda_{max}$ 424, 405, and 389 nm); further characterisation was afforded by n.m.r.[4a] [singlet at $\tau$ 7.33 due to −N(NO)COMe] and i.r. [C=O stretching absorption of −N(NO)COMe at 1710 $cm^{-1}$] spectra. The compound was relatively labile, some 25% of decomposition having occurred after 72 h at 0°, as indicated by the absorbance at 424 nm. The β-nitrosoamide (**2**) was more stable than the α-anomer, and was similarly characterised by u.v. ($\lambda_{max}$ 426, 407, 391 nm) and n.m.r. (singlet at $\tau$ 7.33) spectra.

A solution of the α-nitrosoamide (**1**) in chloroform containing 2% of ethanol was kept at room temperature and the reaction was monitored by spectrophotometry and t.l.c. After 12 days, no nitrosoamide remained and the solution was colourless. Evaporation of the solvents gave a syrup that was shown by t.l.c. and g.l.c. analysis to contain two major and several minor products. The major products corresponded to β-D-glucose pentaacetate (**3**, R = Ac) and ethyl β-D-glucopyranoside tetraacetate (**3**, R = Et), and the n.m.r. spectrum of the crude product displayed the superimposed spectra of β-D-glucose pentaacetate and ethyl glucoside tetraacetate in a ratio of approximately 2.5:1. α-D-Glucose pentaacetate was not detected. Crystallisation of the crude product from chloroform–petroleum ether gave β-D-glucose pentaacetate, identical with an authentic sample, and a further quantity (total yield 39%) was obtained by column chromatography on silica gel. A pure sample of ethyl β-D-glucopyranoside tetraacetate was obtained in 3% yield by preparative t.l.c., which gave a poor recovery. Further characterization of the products was obtained by sodium methoxide-catalysed deacetylation in methanol, which gave glucose and ethyl β-D-glucopyranoside, identified by g.l.c. analysis of the trimethylsilyl ethers (ratio of peak areas was 2.5:1 for α+β-glucose:ethyl glucoside), together with several minor products, one of which was identified (by g.l.c. and paper chromatography) as 2-acetamido-2-deoxy-D-glucose. Neither 2,5-anhydro-D-mannose nor D-mannose was detected.

The decomposition of the β-nitrosoamide (**2**) in chloroform was not studied in detail, but it was shown that β-D-glucopyranose pentaacetate is not the major product.

A solution of the α-nitrosoamide (**1**) in 5:1 (*v*/*v*) acetic anhydride–acetic acid containing 4% of sodium acetate was kept for 3 days at ambient temperature, when decomposition was complete. The syrupy product, obtained after extraction with chloroform, was shown by t.l.c. and g.l.c. analysis to contain mainly β-D-glucopyranose pentaacetate, smaller proportions of 2,5-anhydro-D-mannose aldehydrol pentaacetate (**4**, see later), α-D-glucopyranose pentaacetate, and 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose, and two minor components. β-D-Glucose pentaacetate, although the major product, could not be isolated in good yield because it had physical properties very similar to those of the aldehydrol pentaacetate **4**. Thus, column chromatography on silica gel gave a fraction that was homogeneous on t.l.c.

analysis with several solvent systems, but which was shown by g.l.c. and n.m.r. analysis to be a mixture of β-D-glucose pentaacetate (H-1 doublet at τ 4.30) and the aldehydrol pentaacetate (H-1 doublet at τ 3.303), with the former predominating. Deacetylation of an aliquot of the crude product mixture gave (paper chromatogram) mainly glucose, small amounts of 2-acetamido-2-deoxyglucose, and 2,5-anhydro-mannose, and traces of other compounds. The chromatographic detection of a component behaving as 2,5-anhydromannose provided further support for the identification of the aldehydrol pentaacetate.

When solutions of the α- and β-nitrosoamides in 50% aqueous acetone were kept at ambient temperature, reaction was complete after 3 and 7 days, respectively, for the two anomers. The major product, 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose[1b], was unstable, and neither extraction with chloroform (washing with sodium hydrogen carbonate) nor chromatography on silica gel could be used without causing secondary reactions. On deacetylation, the crude product gave mainly 2,5-anhydro-D-mannose, together with small amounts of glucose and 2-acetamido-2-deoxyglucose, as detected by paper chromatography. The aldehyde signal (at τ 0.31) in the n.m.r. spectrum of the crude product was relatively weak, presumably because 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose exists predominantly in the aldehydrol form[4b] (**5**). Identification of the major product from the α-nitrosoamide was confirmed by the formation of the pentaacetate (**4**), diethyl dithioacetal (**6**), and diethyl acetal (**7**) derivatives, which were compared with authentic samples prepared from 2,5-anhydro-D-mannose. In addition, deacetylation followed by reduction with sodium borohydride gave a product that contained mainly 2,5-anhydromannitol, together with glucitol and other minor components. The β-nitrosoamide product was similarly converted into the diethyl acetal and dithioacetal derivatives.

*2,5-Anhydro-D-mannose derivatives as reference compounds.* — Crude 2,5-anhydro-D-mannose, obtained by deamination of 2-amino-2-deoxy-D-glucose hydrochloride[5], was converted into the diethyl acetal by the action of ethanolic hydrogen chloride. The diethyl acetal, obtained pure in 48% yield after preparative paper chromatography, gave a satisfactory n.m.r. spectrum, and consumed one equivalent of sodium metaperiodate. Acetylation gave a crystalline triacetate (**7**), the structure of which was supported by n.m.r. and mass spectra. The latter spectrum displayed ions corresponding to loss of OEt and OEt+HOAc from the molecular ion, and also the stable ion, $(EtOCHOEt)^+$.

2,5-Anhydro-D-mannose diethyl dithioacetal[6] was converted into the triacetate **6**, which was readily characterised by g.l.c., n.m.r., and mass spectrometry. The mass spectrum showed, among other peaks, a molecular ion, accurate mass-measurement of which gave the correct formula, together with ions resulting from loss of HOAc, HOAc+SEt, and $CH(SEt)_2$; the strongest peak corresponded to $(EtSCHSEt)^+$.

Perchloric acid-catalysed acetylation of crude 2,5-anhydro-D-mannose gave a mixture, which upon fractionation on silica gel, yielded pure syrupy 1,1,3,4,6-penta-*O*-acetyl-2,5-anhydro-D-mannose aldehydrol (29%), the structure (**4**) of which was established by the n.m.r. and mass spectra. The n.m.r. spectrum indicated five acetyl

groups; and H-1, being deshielded by two neighbouring acetoxyl groups, resonated at a characteristic low field[7] ($\tau$ 3.03). The mass spectrum showed major ions corresponding to loss of OAc, $CH_2OAc$, and $(AcO)_2CH$.

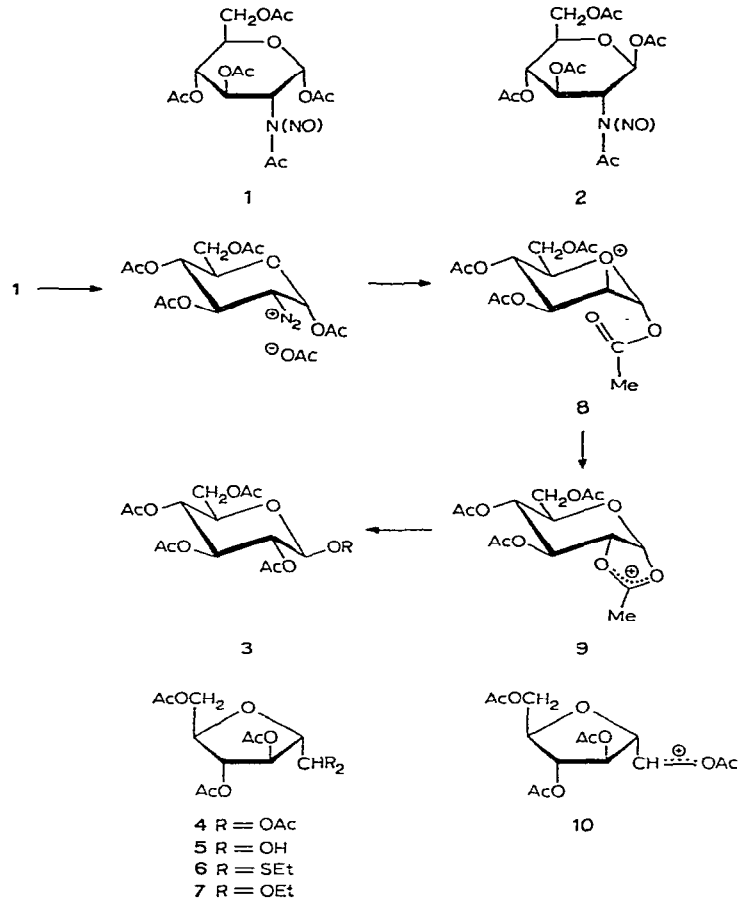
These derivatives (**4**, **6**, and **7**) were assigned the *manno* configuration, but the *gluco* configuration cannot strictly be excluded, as no derivative was isolated in >48% yield and the reaction conditions could cause epimerisation at C-2. The value of $J_{2,3}$ (4 Hz) obtained from the n.m.r. spectrum of the diethyl acetal **7** did not allow the configuration to be deduced. However, there is no obvious reason why the epimerisation at C-2, if it occurred, should give ⩾48% of the gluco isomer (diethyl acetal), in which the substituents at C-2 and C-3 have the less favourable *cis* relationship.

## DISCUSSION

The $\beta$-configuration of the major products formed from the $\alpha$-nitrosoamide (**1**) in chloroform can be accounted for by the mechanism outlined in Scheme I. The nitrosoamide is presumed to rearrange to the diazo ester, heterolysis of which gives a diazonium acetate ion-pair. Heterolysis of the diazonium ion occurs with participation of the ring-oxygen atom to give the bicyclic oxonium ion (**8**). A second neighbouring-group participation then converts the ion into a cyclic acetoxonium ion (**9**), which undergoes nucleophilic attack at C-1 by counter-ion (acetate) and solvent (ethanol) to give $\beta$-D-glucose pentaacetate and ethyl $\beta$-D-glucopyranoside tetraacetate*. The intermediate acetoxonium ion **9** would be expected, on the basis of King and Allbutt's results[9], to undergo diaxial ring-opening in chloroform solution to give $\alpha$-D-mannopyranose derivatives. That such a reaction does not occur to a significant extent however, can be attributed to the greater electrophilic character of the anomeric carbon atom rather than C-2 in the acetoxonium ion. The absence of a ring-contracted product shows that the formation of the ion **10** is less favoured than the above pathway, but the reaction course clearly depends on the solvent, because ring contraction occurs to a small extent in acetic anhydride–acetic acid, and is the major reaction in aqueous acetone. The profound influence of solvent on the course of deamination of amino sugar derivatives has been noted by others[10].

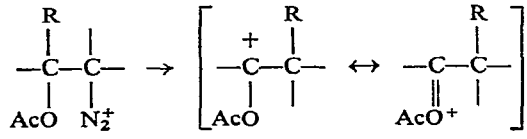
Reaction of the $\alpha$-nitrosoamide in acetic anhydride–acetic acid again gave $\beta$-D-glucose pentaacetate as the major product. 1,1,3,4,6-Penta-*O*-acetyl-2,5-anhydro-D-mannose aldehydrol was formed by reaction of acetate ion with the ion **10**, which resulted from ring-contraction. $\alpha$-D-Glucose pentaacetate, a minor product tentatively identified by g.l.c., could arise either from the oxonium ion **8** or from the acetoxonium ion **9** by a *cis* ring-opening. Examples of the latter were reported recently[11] for reactions in a similar solvent at 100°.

*An alternative mechanism involves an intermediate diazoalkane, protonation of which could give the *manno*-diazonium ion, from which the acetoxonium ion **9** could be formed directly. The diazoalkane pathway occurs most commonly in the reactions of nitrosoamides derived from primary carbinamines in non-polar solvents[8].




Scheme 1

*O*-Acetylation prior to deamination has been used to minimize rearrangements in deaminations of amino sugars and inosamines[3]. Presumably the replacement of OH by OAc decreases the mesomeric stabilization of the rearranged cation, and thus



the rearrangement is less favorable. However, ring-contraction was the major pathway in the reactions of both α- and β-nitrosamides in aqueous acetone, leading to 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose, derived from cation **10**. A related glycoside nitrosoamide underwent a similar rearrangement[16].

Amide starting material was detected in each product mixture, both before and after deacetylation; this arose from trace contamination of the nitrosoamides and also possibly from some denitrosation.

These results show that the decomposition products of the nitrosoamides depend markedly on the nature of the solvent, and that the use of acetic esters does not necessarily minimize rearrangements in such reactions.

## EXPERIMENTAL

*General.* — Melting points were measured on a Kofler hot stage and are uncorrected. N.m.r. spectra were recorded on a Varian HA-100 spectrometer; tetramethylsilane was used as internal reference in deuteriochloroform and pyridine solutions, and 2-methyl-2-propanol in $D_2O$ solution, unless stated otherwise. The apparent coupling constants (Hz) are the directly observed line-spacings. Mass spectra were recorded on an A.E.I. MS-9 mass spectrometer, and optical rotation measurements were made with a Perkin–Elmer Model 141 polarimeter. Comparisons of materials with authentic compounds were made wherever possible by mixed m.p. determination, i.r., n.m.r., u.v., and mass spectrometry, paper, thin-layer, and gas–liquid chromatography. Solutions in organic solvents were dried with anhydrous magnesium sulphate and concentrated under diminished pressure unless stated otherwise.

*Chromatographic methods.* — T.l.c. was performed on Kieselgel-G (Merck) plates (0.25 mm thick) unless stated otherwise. The following solvent systems were used: (*A*) 1:1 (*v/v*), benzene–ether, (*B*) 6:24:1 (*v/v*), benzene–ethyl acetate–acetone, (*C*) 4:1 (*v/v*), benzene–ethyl acetate, (*D*) 1:1 (*v/v*) chloroform–ethyl acetate. Spots were detected by (*i*) spraying with 5% *v/v* sulphuric acid in ethanol and heating to 120°, or (*ii*) exposure of the plate to a saturated atmosphere of iodine vapour. Analytical and preparative paper chromatography utilized the descending technique, on Whatman No. 1 and No. 3 papers, respectively, in a 4:1:5 (*v/v*) 1-butanol–ethanol–water system. Spots were located by silver nitrate[12] and *p*-anisidine hydrochloride[13] spray reagents.

G.l.c. was performed with an F and M 810 chromatograph the recorder being equipped with a disc integrator and automatic attenuator. Glass columns (240 cm × 0.4 cm i.d.) were used packed with silanised Chromosorb W (60–80 mesh) coated with (*A*) 3.4% silicone rubber U.C.W. 98, (*B*) 10% silicone rubber U.C.W. 98, (*C*) 10% Carbowax 20M (the support in this case was unsilanised), or (*D*) 10% butan-1,4-diol succinate.

The carrier gas (argon) flow-rate was 50 ml/min; the column temperature was 180° unless otherwise stated, and the injection port temperature 240°. The compounds were detected by flame ionization.

*1,3,4,6-Tetra*-O-*acetyl-2-deoxy-2*-(N-*nitroso*)*acetamido*-α-D-*glucopyranose* (**1**). — 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[14] (389 mg, 1 mmole) was dissolved in anhydrous pyridine (0.25 ml, 3.1 mmole) and the solution was cooled to 0°. Nitrosyl chloride (450 mg, 6.9 mmole) in dry, ethanol-free chloroform (5 ml) was added. The resulting solution was kept for 4 h at 0° after which time t.l.c. (system *D*) indicated the reaction to be complete. The solution was purged of

excess nitrosyl chloride by passage of dry nitrogen for 1 h at 0°. Dilution of the now golden solution with dry chloroform (10 ml) was followed by washing at 0° with M HCl (2 × 5 ml) and then with de-ionized water (10 ml). The organic phase was dried at 0°, concentrated at 9–10°, and evacuated under high vacuum for an hour at 0° to afford the nitrosoamide **1** as a golden yellow syrup (394 mg, 94%), $\nu_{max}$ 1750 and 1710 $cm^{-1}$; $\lambda_{max}^{CHCl_3}$ 424, 405, and 389 nm; $\tau$ ($CDCl_3$) 3.67 (q, *J* 9 and 11 Hz, H-3?), 4.12 (d, *J* 3 Hz, H-1), 7.33 [s, N(NO)COMe]; the remainder of the spectrum did not show correct integrals because decomposition occurred during the measurements (at probe temperature of 29°); the first spectrum measured showed weak signals, some assigned to β-D-glucose pentaacetate, which increased in intensity with time. T.l.c. (system *D*) revealed a slight trace of original amide in the product.

*1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(N-nitroso)acetamido-β-D-glucopyranose* (**2**). — 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose[15] (319 mg, 0.87 mmole) was dissolved in anhydrous pyridine (0.25 ml, 3.1 mmole) and cooled to 0°. Nitrosation with nitrosyl chloride (400 mg, 6.1 mmole) in dry, ethanol-free chloroform (7 ml), purging with nitrogen, and washing and drying of the resulting solution, were performed as described for the α-anomer, to yield the nitrosoamide as a yellow syrup (336 mg, 97.5%), $\lambda_{max}^{CHCl_3}$ 426, 407, 391 nm, ε 88, 85.6, 53.5; $\tau$ ($CDCl_3$) 3.68 (1H, d, *J* 9 Hz, H-1), 4.36 (1H, t, *J* 10 Hz), 4.96 (2H, m), 5.6–6.0 (2H, m, H-6 + H-6'), 6.15 (1H, m, H-5), 7.33 [3H, s, N(NO)COMe], 7.91, 8.00, 8.16 (each 3H, s, COMe). T.l.c. (system *A*) revealed a trace of original amide.

*Decomposition of* **1** *in chloroform solution.* — A solution of the α-nitrosoamide **1** (780 mg, 1.36 mmole) in chloroform containing 2% of ethanol as stabilizer (24 ml) was kept at room temperature (20 ±2°). Decomposition was monitored by u.v. spectrophotometry and t.l.c. (systems *A* and *C*), and was complete after 12 days. The solution was concentrated to a syrup (688 mg), g.l.c. analysis of which (column *B* at 200°) indicated five components, two major ones having retention times (relative to α-D-glucose pentaacetate, $R_G$) of 0.85 and 1.0. The n.m.r. spectrum ($CDCl_3$) showed characteristic signals at $\tau$ 8.81 (triplet, *J* 7 Hz), 5.53 (doublet, *J* 8 Hz) and 4.34 (doublet, *J* 7 Hz). Crystallization from ether–pentane gave a solid, recrystallization of which from chloroform–petroleum ether (60–80°) gave β-D-glucopyranose pentaacetate (203 mg), m.p. 131–132°, identical with an authentic sample. Chromatography of the mother liquor (380 mg) on silica gel (200–300 mesh, 40 g), eluting with 2:1 (*v*/*v*), benzene–ether afforded further crystalline β-pentaacetate (83 mg). Elution of the column with methanol yielded impure 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (27 mg), $\nu_{max}$ (film) 1750, 1660 $cm^{-1}$.

Crystalline ethyl β-D-glucopyranoside tetraacetate (16 mg), m.p. 103–105°, identical with an authentic sample, was isolated by preparative t.l.c. of the product (624 mg) obtained from a further sample of the nitrosoamide (729 mg).

Deacetylation of the crude product (260 mg) with sodium (7 mg) in anhydrous methanol (16 ml) gave, after deionization and removal of solvent, a syrup (132 mg) that was shown by paper chromatography and g.l.c.[16] analysis to contain mainly glucose and ethyl β-D-glucopyranoside. Fractionation of an aliquot (100 mg) of

the syrup on paper gave pure glucose (14 mg) and ethyl β-D-glucopyranoside (12.5 mg), characterized by g.l.c.[16].

*Decomposition of the α-nitrosoamide* (**1**) *in acetic acid–acetic anhydride containing sodium acetate.* — Compound **1** (403 mg, 0.97 mmole) was dissolved in acetic anhydride (5 ml). Glacial acetic acid (1 ml) and anhydrous sodium acetate (0.25 g) were added, and the solution was kept for 3 days at ~20°, after which time t.l.c. (system *D*) indicated decomposition to be complete. The solution was then poured into iced water (50 ml) and kept at ~20° for a further 3 h. The aqueous solution was extracted with chloroform (3 × 30 ml), and the organic phase washed with saturated sodium hydrogen carbonate solution (2 × 50 ml) and water (2 × 50 ml). After drying and concentration to a syrup, acetic acid was removed under high vacuum during several h. The product (245 mg), when analysed by g.l.c. (column *D* at 200°) gave peaks corresponding to β-D-glucopyranose pentaacetate (major component, $R_G$ 1.22), 1,1,3,4,6-penta-*O*-acetyl-2,5-anhydro-D-mannose aldehydrol (shoulder, $R_G$ 1.15), α-D-glucopyranose pentaacetate, and two other minor components ($R_G$ 0.64 and 0.20).

Chromatography of the mixture (191 mg) on silica gel (200–300 mesh, 24 g), with 3:1 (*v*/*v*) benzene–ether as solvent, afforded a syrup (62.5 mg) that was homogenous by t.l.c. but which was shown by its n.m.r. spectrum ($CDCl_3$) to be a mixture. The gum was subsequently crystallized to give β-D-glucopyranose pentaacetate (12 mg), m.p. 129–130°, identical with an authentic sample.

Deacetylation of the product mixture (24 mg) with sodium (3 mg) in methanol (4 ml) in the usual way yielded a syrup (8 mg). Paper chromatography showed mainly D-glucose, together with components migrating as 2,5-anhydro-D-mannose and 2-acetamido-2-deoxy-D-glucose.

*Decomposition of the α-nitrosoamide* **1** *in acetone–water 1:1* (v/v). — Nitrosoamide **1** (403 mg) was dissolved in 1:1 (*v*/*v*) acetone–water (15 ml), and the solution was kept at room temperature. The reaction was monitored by t.l.c. (system *D*) and was complete after 3 days. The solution was concentrated, and the last traces of solvent and acetic acid were removed under high vacuum for several h at ~20°. The resulting colourless syrup (311 mg) was resolved by t.l.c. (system *D*) into one major and three minor components.

*Treatment with 2% ethanolic HCl.* An aliquot of the product mixture (52 mg) was dissolved in abs. ethanol (3 ml) and acetyl chloride (0.04 ml) was added. The solution was refluxed with the exclusion of moisture for 2 h, cooled, and neutralised with lead carbonate. Removal of the solids and solvent afforded a syrup (27 mg, 72% based on nitrosoamide), which contained one major product (paper chromatography, g.l.c.[16]). Preparative paper-chromatography afforded the diethyl acetal as a homogeneous syrup (6 mg), which corresponded to authentic 2,5-anhydro-D-mannose diethyl acetal ($R_F$ 0.8 on paper, retention time relative to α-D-glucose on g.l.c.[16] analysis: 0.59 on column *B*, 1.39 on column *C*). The syrup was then acetylated in dry pyridine (0.05 ml) and acetic anhydride (0.05 ml) at ~20° to give the syrupy triacetate (10 mg), which was identified by comparison with an authentic sample by t.l.c., g.l.c. ($R_G$ 0.70, column *B* at 200°), and mass spectrometry.

*Treatment with ethanethiol.* An aliquot of the product (54 mg) was dissolved in ether (2 ml), and ethanethiol (0.04 ml) and boron trifluoride diethyl etherate (0.05 ml) were added. The solution was kept for 3 h, at room temperature, and was washed with saturated sodium hydrogen carbonate solution (2 × 5 ml) and then with water (2 × 5 ml). It was dried and concentrated to a syrup (26 mg) that was shown by t.l.c. (system *A*), g.l.c. (column *B* at 200°), and n.m.r. spectrum to be mainly the diethyl dithioacetal triacetate **6**.

*Acetylation.* An aliquot of the product (211 mg) was dissolved in acetic anhydride (1 ml), and perchloric acid (0.01 ml) was added. The mixture was kept for 2 days at 20° and then poured into water (40 ml), left for several h, and then extracted with chloroform (3 × 30 ml). The organic phase was washed with saturated sodium hydrogen carbonate solution (2 × 20 ml) and water (2 × 20 ml). After drying, concentration yielded a syrup (171 mg) that was shown by g.l.c. (column *D* at 200°) to contain mainly 1,1,3,4,6-penta-*O*-acetyl-2,5-anhydro-D-mannose aldehydrol ($R_G$ 1.15) and a second component having $R_G$ 1.0.

*Deacetylation.* An aliquot of crude product (29.5 mg) was dissolved in abs. methanol (4 ml) and sodium (3 mg) was added. The solution was kept overnight at ~20° after which time the methanol was removed under diminished pressure, and the residual gum was de-ionized in water with Amberlite MB-3 resin. Concentration of the aqueous solution gave a syrup (10 mg), which on paper chromatograms showed three components corresponding to 2,5-anhydro-D-mannose, 2-acetamido-2-deoxy-D-glucose, and D-glucose.

*Reduction.* The deacylated product-mixture (227 mg), prepared as before was dissolved in distilled water (10 ml), and sodium borohydride (75 mg) was slowly added. When the vigorous effervescence had subsided the mixture was de-ionized with Amberlite MB-3 resin and concentrated to a syrup. The syrup was dissolved in methanol and the solvent was then evaporated. This process was repeated to afford a colourless syrup (91 mg). T.l.c. [1-butanol–ethanol–water (4:1:5, organic phase) on microgranular cellulose C.T. (Reeve Angel Scientific)] and g.l.c.[16] analysis indicated 2,5-anhydro-D-mannitol to be the major product. Glucitol and other unidentified compounds were also present.

*Decomposition of β-nitrosoamide* **2** *in chloroform.* — The β-nitrosoamide (36 mg) was dissolved in chloroform (2 ml) and kept for 10 days at room temperature, when decomposition was complete (t.l.c., system *A*). Concentration of the solution afforded a syrup (20 mg) that by t.l.c. (system *A*) contained four products, the major one of which did not correspond to α- or β-D-glucose pentaacetate.

*Decomposition of β-nitrosoamide in 1:1* (v/v) *acetone–water.* — The β-nitrosoamide (**2**, 320 mg) was dissolved in 1:1 (*v/v*) acetone–water (15 ml), and the solution was kept for 7.5 days at ambient temperature. T.l.c. (system *D*) indicated decomposition to be complete, and concentration afforded a mobile syrup. The last traces of solvent were removed under high vacuum to give a colourless syrup (209 mg). T.l.c. (system *D*) showed one major and two minor products present.

*Treatment with 2% ethanolic HCl.* An aliquot of the product (44 mg) was

dissolved in dry ethanol (2 ml) and acetyl chloride (0.03 ml) was added. The reaction was conducted as described above for the α-anomer, to give a syrup (20 mg, 52.5% based on nitrosoamide) that contained mainly 2,5-anhydro-D-mannose diethyl acetal (paper chromatography and g.l.c.[16]).

Preparative paper-chromatography yielded the pure acetal (8 mg), and acetylation with acetic anhydride (0.04 ml) in pyridine (0.05 ml) at room temperature gave the triacetate (9 mg), identified by comparison with an authentic sample by t.l.c. (system *D*), g.l.c. (column *B* at 200°), and mass spectrometry.

*Treatment with ethanethiol.* An aliquot of the product (42 mg) was treated with ethanethiol (0.05 ml) and boron trifluoride diethyl etherate (0.05 ml) in dry ether (2 ml) as described for the α-nitrosoamide. The resultant pale-yellow syrup (29 mg, 45% based on nitrosoamide) was largely the diethyl dithioacetal triacetate **6**, as indicated by t.l.c. (system *A*), g.l.c. (column *B* at 200°), and n.m.r. spectrum.

*Deacetylation.* The crude product (18 mg) was treated with sodium (3 mg) in anhydrous methanol (4 ml) as described already for the α-anomer. The resulting mixture was shown by paper chromatography to contain 2,5-anhydro-D-mannose, 2-acetamido-2-deoxy-D-glucose, and D-glucose.

*1,1,3,4,6-Penta-*O*-acetyl-2,5-anhydro-*D*-mannose aldehydrol* (**4**). — Crude 2,5-anhydro-D-mannose (prepared by nitrous acid deamination of 2-amino-2-deoxy-α-D-glucopyranose[5], 176.5 mg) was dissolved in acetic anhydride (1.7 ml), and perchloric acid (0.02 ml) was added. The solution was kept for 3 days at 0°, iced water (6 ml) was then added, and the mixture was kept for 3 h at ambient temperature. Extraction with chloroform (3 × 30 ml) gave a colourless syrup (353 mg) that was fractionated on a column of silica gel (46 g). Elution with 3:1 (*v*/*v*) benzene–ether yielded the pentaacetate **4** as a colourless syrup (110 mg, 29%), $[\alpha]_D^{22}$ +34° ($CHCl_3$); τ ($CDCl_3$) 3.03 (1H, d, *J* 6 Hz, H-1), 4.69 (1H, t, *J* 5 Hz), 4.87 (1H, m), 5.7–5.9 (4H, m, H-2, H-6, H-6′+impurity), 6.2–6.4 (1H, m, H-5), 7.89–7.91 (15H, 4s, COMe); *m/e* 331* (0.5%), 317* (0.3%), 289 (0.5%), 259 (2.5%), 229 (0.5%), 215 (1%), 200 (4%), 169 (1%), 168 (1%), 157 (2%), 140 (3%), 139 (3%), 126 (3%), 115 (2%), 109 (2%), 103 (1%), 98 (15%), 97 (7%), 43 (100%). G.l.c. analysis gave one peak, $R_G$ 1.15 (column *D* at 200°).

*2,5-Anhydro-*D*-mannose diethyl acetal.* — Impure 2,5-anhydro-D-mannose[5] (184 mg) was dissolved in abs. ethanol (5 ml) and acetyl chloride (0.1 ml) was added. The solution was refluxed with the exclusion of moisture for 4 h, and upon cooling was neutralized with lead carbonate. Filtration and concentration gave a brown syrup containing at least two products by paper chromatography. Preparative paper-chromatography and elution of the band of highest $R_F$ value yielded the diethyl acetal as a colourless syrup (115 mg, 48%), $[\alpha]_D^{22}$ +28° (ethanol) after concentration of the eluate. This was shown to be homogeneous by paper chromatography ($R_F$ 0.8) and g.l.c.[16] (retention time relative to α-D-glucopyranose: 0.59 on column *B*, 1.39 on column *C*); n.m.r. spectrum (pyridine after $D_2O$ exchange): τ, 8.83, 8.81 (each 3H, t, *J*,

*Accurate mass measurements gave correct compositions.

7 Hz, $CH_2CH_3$), 6.1–6.5 (4*H*, m), 5.7–6.0 (2*H*, m), 5.35–5.6 (2*H*, m), and 4.9–5.3 (3H, m). Periodate oxidation with 7.5 mg of the acetal and 0.015M sodium metaperiodate gave an uptake of periodate, determined spectrophotometrically[17], of one equivalent after 3 h at 35°.

*3,4,6-Tri-*O*-acetyl-2,5-anhydro-*D*-mannose diethyl acetal* (**7**). — 2,5-Anhydro-D-mannose diethyl acetal (55.6 mg), prepared as before, was dissolved in pyridine (0.3 ml), cooled to 0°, and acetic anhydride (0.25 ml) was added. The solution was kept for 24 h at ~20°, and iced water was added. The aqueous solution, after 3 h at 20° was extracted with chloroform (3 × 2 ml), and the organic phase was washed with 5M HCl (2 × 2 ml), saturated sodium hydrogen carbonate solution (3 × 2 ml), and then water (3 × 2 ml). Drying and concentration yielded the syrupy triacetate*, which crystallized on standing. Recrystallization from aqueous ethanol at 0° gave colourless needles (26 mg, 30%), m.p. 54–56°, $[\alpha]_D^{20}$ +34° ($CHCl_3$); $\tau$ ($CDCl_3$) 4.59 (1H, q, *J* 4.0 and 3.0 Hz, H-3), 4.88 (1H, m, H-4), 5.42 (1H, d, *J* 5.5 Hz, H-1), 5.75 (2H, m, H-6, H-6'), 5.91 (1H, q, *J* 4.0 and 5.5 Hz, H-2), 6.2–6.6 (4H, m, $CH_2CH_3$), 7.91 (3H, s, COMe), 7.92 (6H, s, COMe), 8.77 and 8.79 (each 3H, t, *J* 7 Hz, $CH_2CH_3$), assignments confirmed by spin decoupling; *m/e* 317 (0.7%), 303 (0.2%), 259 (0.3%), 257 (1.5%), 229 (0.5%), 215 (1.5%), 197 (3.5%), 155 (5%), 103 (100%), 75 (34%), 47 (32%). G.l.c. analysis gave one peak, $R_G$ 0.70 (column *B* at 200°).

*Anal.* Calc. for $C_{16}H_{26}O_9$: C, 53.0; H, 7.24. Found: C, 53.3; H, 7.06.

*3,4,6-Tri-*O*-acetyl-2,5-anhydro-*D*-mannose diethyl dithioacetal* (**6**). — 2,5-Anhydro-D-mannose diethyl dithioacetal was prepared by a method based on that of Grant[6]. Crude 2,5-anhydro-D-mannose (360 mg) was dissolved in concentrated HCl (1.6 ml), cooled to 0°, and ethanethiol (1.25 ml) was added. The solution was kept at 0° for 30 min, iced water (10 ml) was added, and the solution was kept for a further 15 min at ~20° before extracting with ethyl acetate (3 × 10 ml). Concentration of the organic phase afforded a yellow-brown gum (216 mg, 36%), $[\alpha]_D^{22}$ +51° (acetone) (lit.[6] $[\alpha]_D^{15}$ +60.4°). Paper chromatography showed the crude product to be homogeneous. The crude diethyl dithioacetal (216 mg) was acetylated in pyridine–acetic anhydride for 24 h at 0°. Conventional processing by chloroform extraction gave a straw-coloured syrup (272 mg, 85%), $[\alpha]_D^{22}$ +44° (chloroform), which was shown to be homogeneous by t.l.c. (System *A*) and g.l.c. ($R_G$ 2.29, column *B* at 200°); $\tau$ ($CDCl_3$) 4.44 (1H, q, *J* 3.5 and 4.5 Hz), 4.84 (1H, t, *J* 3.5 Hz), 5.65–5.85 (3H, m, H-6, H-6', H-2), 5.96 (1H, d, *J* 6 Hz, H-1), 6.2–6.4 (1H, m, H-5), 7.29 (4H, q, *J* 7 Hz, $CH_2CH_3$), 7.89 (9H, s, COMe), 8.74 (6H, t, *J* 7 Hz, $CH_2CH_3$); *m/e* 394† (3%), 273 (2%), 259 (5%), 231 (1%), 213 (9%), 189 (2%), 171 (17%), 139 (7%), 137 (10%), 135 (100%).

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*When first prepared, the acetal triacetate was a syrup, and all comparisons were made with the pure syrup. The syrup crystallized on prolonged standing at 0–5°.

†Accurate mass measurement gave the correct molecular formula.

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