Nitrosoamide Decomposition. A New Method for Selective Degradation of Amino-sugar-containing Compounds[1]

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Methyl glycoside derivatives of 2-acetamido-3,4,6-tri-0-acetyl-2-deoxy-D-glucopyranose and -D-mannopyranose, and a disaccharide derivative containing the former amide, have been N-nitrosated, and the decomposition of the β-glucoside nitrosoamides has been studied. The reaction of the nitrosoamides in aqueous solution to give 3,4,6-tri-O-acetyl-2,5-anhydro-D-mannose, a product of ring contraction, with concomitant release of the aglycone, affords a model for the selective cleavage of such glycosidic linkages in amino-sugar-containing compounds. The α-glycoside nitrosoamides were too labile to be isolated. The methyl β-glycoside nitrosoamide in chloroform solution underwent considerable denitrosation, and gave products of ring contraction and unrearranged pyranoside products.

ONE of the classical degradative methods used in studying the structures of oligosaccharides, polysaccharides, glycolipids, and glycopeptides is partial acidic hydrolysis. This depends on differences in the susceptibility of glycosidic linkages towards hydrolysis, and the yields of oligosaccharide products are often low. Methods for the selective cleavage of specific glycosidic linkages are clearly superior and give good yields of degradation products. Deamination with nitrous acid has been used in studies of amino-sugar-containing compounds with some success.† However, most amino-sugars occur in nature as N-acyl (usually N-acetyl) derivatives, and because early attempts at de-N-acetylation resulted in degradation [4],‡ we considered that a suitable alternative was the thermal decomposition of N-nitroso-N-acyl derivatives. Such reactions are considered to proceed via unstable diazonium ions to give products similar to those formed in deamination of the corresponding amine.

The most widely occurring amino-sugars are 2-amino-2-deoxy-D-glucose and -D-galactose, both of which exist in chair conformations that have equatorially attached amino-groups. Deamination of glycosides of these amino-sugars results [7,8] in a rearrangement which releases the aglycone. Similar cleavage of the glycosidic linkage should occur when N-nitroso-N-acyl derivatives [e.g. (1)] decompose in the presence of water. This paper reports the results obtained with four model compounds, namely methyl 3,4,6-tri-O-acetyl-2-deoxy-2-(N-nitrosoacetamido)-α- and -β-D-glucopyranoside and -α-D-mannopyranoside, and a disaccharide derivative, 1,2:3,4-di-O-

isopropylidene-6-O-[3,4,6-tri-O-acetyl-2-deoxy-2-(N-nitrosoacetamido)-β-D-glucopyranosyl]-α-D-galactopyranose.

Reaction of methyl 3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside at 0 °C with nitrosyl chloride in chloroform containing pyridine gave the yellow N-
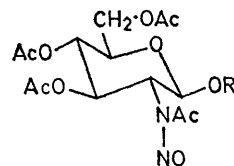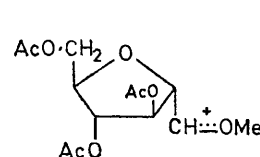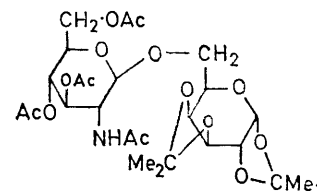

(1)
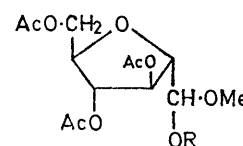
(2) R = Et
(3) R = Ac

(4)

(5)

nitroso-derivative (1), which was obtained crystalline in high yield.§ The structure was established by elemental analysis and u.v. and n.m.r. spectra. The nitrosamide was stable in the crystalline state at 0 °C for several

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† For discussion of the scope of such reactions see refs. 2 and 3.
‡ More recently it has been reported that hydrazinolysis, when catalysed by hydrazine sulphate, successfully removes N-acetyl groups.[5,6]
§ Nitrosation with dinitrogen tetroxide to give carbohydrate N-nitrosoamides has recently been reported.[9a,b]

[1] Preliminary report, J. W. Llewellyn and J. M. Williams, *Chem. Comm.*, 1971, 1386.
[2] N. M. K. Ng Ying Kin, J. M. Williams, and A. Horsington, *J. Chem. Soc. (C)*, 1971, 1578.
[3] J. M. Williams, *Adv. Carbohydrate Chem. Biochem.*, 1975, **31**, 9.

[4] Z. Yosizawa and T. Sato, *J. Biochem. (Japan)*, 1962, **51**, 233.
[5] Z. Yosizawa, T. Sato, and K. Schmid, *Biochim. Biophys. Acta*, 1966, **121**, 417.
[6] B. A. Dmitriev, Y. A. Knirel, and N. K. Kochetkov, *Carbohydrate Res.*, 1973, **29**, 451; 1973, **30**, 45.
[7] See *e.g.* H. E. Carter, S. Brooks, R. H. Gigg, D. R. Strobach, and T. Suami, *J. Biol. Chem.*, 1964, **239**, 743.
[8] E. V. Rao, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, 1966, **100**, 801.
[9] D. Horton and W. Loh, *Carbohydrate Res.*, 1974, (a) **36**, 121; (b) **38**, 189.

months, but decomposed slowly (in weeks) at 20—25 °C. The stability of the nitrosoamide in chloroform (containing 2% ethanol) was examined by following the decomposition of a 1.5% solution by u.v. measurements and by t.l.c. Reaction was complete in 25 days at 20—25 °C, three products being detected by t.l.c. and seven by g.l.c. The major product, methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside, which resulted from denitrosation, was isolated pure in 43% yield after chromatography on silica gel. Two products of ring contraction, namely 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose ethyl methyl acetal (2) and 1,3,4,6-tetra-*O*-acetyl-2,5-anhydro-D-mannose methyl hemiacetal (3), were identified by n.m.r.* and mass spectral data. The latter compound was obtained homogeneous in very low yield because little separation was achieved during column chromatography. Fractions containing different proportions of the two compounds were obtained, the total yield being *ca.* 30%. The ethyl methyl acetal was isolated from the mixture after deacetylation followed by removal of 2,5-anhydro-D-mannose [formed from (3)] by a strongly basic ion-exchange resin, and was characterised by n.m.r. and mass spectral analysis of the derived tribenzoate and triacetate. The ring-contracted compounds (2) and (3) presumably resulted from the reaction of the rearranged cation (4) with the nucleophiles present, namely acetate ions and ethanol.

The first fraction eluted from the silica gel column was shown by g.l.c. to contain mainly two compounds, one of which, methyl 3,4,6-tri-*O*-acetyl-2-chloro-2-deoxy-β-D-glucopyranoside, was obtained pure by fractional crystallisation. The formation of chlorine-containing products in the reactions of nitrosoamides in chlorinated solvents has been observed previously.[10] The second component of this fraction was tentatively identified as methyl 3,4,6-tri-*O*-acetyl-2-diazo-β-D-*arabino*-hexopyranoside, since impure material that crystallised from the mother liquor contained nitrogen and showed i.r. absorptions at 2120 and 1655 cm⁻¹.

In the more polar solvent, 50% aqueous acetone, the β-glucoside nitrosoamide (1) reacted more rapidly, 14 days at 20—25 °C being required for the formation of a mixture of products, in which unstable 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose predominated. This compound was characterised as the diethyl dithioacetal, the crystalline diethyl acetal, and the hydrate penta-acetate derivatives.[11] That ring contraction was not quantitative was shown by the isolation, after deacetylation and removal of 2,5-anhydro-D-mannose by a strongly basic ion-exchange resin, of a fraction (<10%) that contained predominantly methyl β-D-glucopyranoside. Methyl β-D-glycopyranoside tetra-acetate was also detected by g.l.c. after acetylation of the crude product. The same rearrangement to give 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-

mannose occurred when 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(*N*-nitrosoacetamido)-α- and -β-D-glucopyranose decomposed in aqueous acetone.[11] These nitrosoamides were also significantly less stable than the β-glucoside nitrosoamide.

The nitrosoamide derivative of the protected disaccharide (5), prepared by a standard Koenigs–Knorr reaction, was obtained in quantitative yield as a yellow syrup, and this was converted in 50% aqueous acetone at 20—25 °C in 9 days into a mixture that contained mainly 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose. The latter was isolated in 59% yield by chloroform extraction of the deacetylated product mixture, and was converted into the crystalline toluene-*p*-sulphonate. The former was characterised as the diethyl dithioacetal and diethyl acetal derivatives.

These results show that nitrosoamide decomposition in aqueous medium can be used to effect the cleavage of certain glycosidic linkages in amino-sugar-containing compounds. The reaction can, however, only be used when the *N*-nitrosoamide is sufficiently stable that it can be prepared and transferred to an aqueous medium prior to any decomposition. To assess the scope of the degradation method, we studied the *N*-nitrosation of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranoside and α-D-glucopyranoside. The nitrosation of both these glycosides was slower than that of the β-glucoside amides, and both nitrosoamides were too unstable to be isolated. The lower rate of nitrosation of the mannoside is presumably due to the axial orientation of the acetamido-group, and the lower rate for the α-glucoside parallels the relative rates of deamination of methyl 2-amino-2-deoxy-α- and -β-D-glucopyranoside, the α-anomer reacting more slowly. Two other α-glycoside derivatives of 2-(*N*-nitrosoacetamido)-2-deoxy-D-glucopyranose have also been reported [9b] to be unstable, but the generalisation [9b] that a *trans*-disposition of O-1 and the 2-(*N*-nitrosoacetamido)-group contributes to stabilisation of the nitrosoamide is not valid, as shown by the nitrosation of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranoside. Clearly, conformation is important as well as configuration. The nitrosations of the α-glycosides suggest that the use of nitrosoamide decomposition in aqueous medium may not be widely applicable. When this approach is not feasible, the degradation sequence can be modified by de-*N*-acetylation, using hydrazinolysis [5] or the action of triethyloxonium tetrafluoroborate [12] on a suitably protected derivative, followed by deamination.[6]

There are essentially two ways in which *N*-nitrosoamide decomposition can be applied to structure elucidation. Either the compound of interest (oligosaccharide, glycopeptide, *etc.*) can be nitrosated directly to give a polynitrite ester *N*-nitrosoamide, or the hydroxy-groups

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* The n.m.r. spectrum for each compound contained signals for both diastereoisomers.

[10] E. H. White, T. J. Ryan, and K. W. Field, *J. Amer. Chem. Soc.*, 1972, **94**, 1360.

[11] J. W. Llewellyn and J. M. Williams, *Carbohydrate Res.*, 1973, **28**, 339.

[12] S. Hanessian, *Tetrahedron Letters*, 1967, 1549.

can be protected, for example by acetylation or methylation, prior to nitrosation. Nitrite esters are more difficult to handle, but both approaches are currently being studied and applied to compounds of biochemical interest.

EXPERIMENTAL

General methods were as in ref. 13. G.l.c. columns were (A) 10% silicone rubber UCW 98 on silanised Chromosorb W (60—80 mesh), (B) 10% Carbowax 20M on Chromosorb W, (C) 10% butane-1,4-diol succinate on silanised Chromosorb W. Mass spectra were measured on an A.E.I. MS9 instrument.

Methyl-3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-(N-*nitrosoacetamido*)-β-D-*glucopyranoside*.—Methyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (722 mg) was suspended in anhydrous pyridine (0.5 ml) and cooled in an ice-bath. Cold ethanol-free chloroform (10 ml) containing nitrosyl chloride (1.52 g) was added, and the resulting brown solution was kept at 0—5 °C for 3.5 h; t.l.c. then showed reaction to be complete. The excess of nitrosyl chloride was removed by purging the solution with dry nitrogen at 0 °C for 1 h, the volume being maintained by addition of pure chloroform. The yellow solution was then washed sequentially with ice-cold 5M hydrochloric acid (2 × 5 ml), saturated aqueous sodium hydrogen carbonate (2 × 5 ml), and water (2 × 5 ml). The chloroform solution was dried (MgSO$_4$) and concentrated at 10 °C to give a gum which crystallised from ether yielding yellow needles of the *nitrosoamide* (729 mg, 93%), m.p. 87.5—88.5°; $[\alpha]_D$ −24° (CHCl$_3$); $\lambda_{max.}$ 428 (ε 86), 408 (83.7), and 393 nm (58.3); $\nu_{max.}$ (KBr) 1745 (C=O) and 1530 cm^{-1} (N=O); τ (CDCl$_3$) 4.44 (1H, q, J 9 and 10.5 Hz, H-3), 4.84 (1H, d, J 8 Hz, H-1), 4.92 (1H, q, J 9 and 10.5 Hz, H-4), 5.16 (1H, q, J 8 and 10.5 Hz, H-2), 5.69 (1H, q, H-6), 5.88 (1H, q, H-6′), 6.23 (1H, m, H-5), 6.63 (3H, s, OMe), 7.31 [3H, s, N(NO)Me], and 7.91, 7.99, 8.17 (each 3H, s, OCOMe) (Found: C, 46.3; H, 5.35; N, 7.2. C$_{15}$H$_{22}$NO$_{10}$ requires C, 46.15; H, 5.7; N, 7.2%).

6-O-(2-*Acetamido*-3,4,6-tri-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*)-1,2:3,4-*di*-O-*isopropylidene*-α-D-*galactopyranose*.—Anhydrous calcium sulphate (1 g) and finely powdered mercury(II) cyanide (1 g) were stirred in dry, ethanol-free chloroform at room temperature, in the dark, for 30 min, after which 1,2:3,4-di-O-isopropylidene-α-D-galactose (1 g) was added, followed by 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride (1.75 g). T.l.c. showed that reaction was complete after 2 days at room temperature. The mixture was diluted with chloroform and filtered, and the filtrate was washed in turn with saturated aqueous iron(II) sulphate (30 ml) and water (30 ml). The chloroform solution was dried (MgSO$_4$) and concentrated under reduced pressure to a syrup. Chromatography on silica gel (100 g; 200—300 mesh), with chloroform–ethyl acetate (1:1 v/v) as eluant, gave di-O-isopropylidenegalactose (0.423 g) and an impure fraction (0.848 g), m.p. 110—115°. Further chromatography of the latter fraction on silica gel (50 g) gave the *disaccharide* (0.628 g), m.p. 105—107° (needles from ether) and m.p. 117—119° (cubes from ether-pentane), and 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-

α-D-glucopyranose (0.194 g), m.p. and mixed m.p. 135.5—136.5°.

Both forms of the disaccharide had identical rotations, $[\alpha]_D^{22}$ −56.5° (CHCl$_3$), and spectra: $\nu_{max.}$ (KBr) 3340br (NH str.), 1750 (C=O, ester), 1675 (C=O; amide), and 1550 cm^{-1} (NH def.); τ (CDCl$_3$) 8.68 (6H, s, Me$_2$C), 8.55 and 8.49 (each 3H, s, Me$_2$C), 8.04, 7.99, 7.98, and 7.92 (each 3H, s, MeCO), 5.30 (1H, d, J 9.5Hz, H-1 of glucose), 4.48, (1H, d, J 5 Hz, H-1 of galactose), and 4.34 (1H, d, J 9 Hz, CONH); m/e 574 (7%) and 330 (29%) (Found: C, 52.7; H, 6.8; N, 2.4. C$_{26}$H$_{39}$NO$_{14}$ requires C, 53.0; H, 6.7; N, 2.4%). The yield of disaccharide was 37% based on 1,2:3,4-di-O-isopropylidene-α-D-galactose consumed

Disaccharide Nitrosoamide.—The disaccharide derivative (200 mg) from the preceding experiment was nitrosated by the procedure already described for the methyl glycoside amide [pyridine (0.1 ml) and nitrosyl chloride (500 mg), in ethanol-free chloroform (2 ml)]. Reaction was complete after 2 h at 0 °C, and the nitrosoamide was isolated as a yellow homogeneous (t.l.c.) gum (212 mg, 100%), $[\alpha]_D^{22}$ −54° (in CHCl$_3$); τ (CDCl$_3$) 8.71 (6H, s, Me$_2$C), 8.60 and 8.58 (each 3H, s, Me$_2$C), 8.18, 8.01, and 7.92 (each 3H, s, MeCO$_2$), and 7.30 [3H, s, N(NO)COMe]; $\nu_{max.}$ (film) 1755 and 1535 cm^{-1}; $\lambda_{max.}$ (CHCl$_3$) 423 (ε 69.6), 405 (69.0), and 390 nm (45.3).

Decomposition of Methyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-(N-*nitrosoacetamido*)-β-D-*glucopyranoside*. (a) *In chloroform–ethanol* (49 : 1). A solution of the nitrosoamide (1 g) in chloroform (65 ml) containing ethanol (2%) was kept for 25 days at room temperature.* The solution was then concentrated under reduced pressure to give a gum (992 mg). G.l.c. analysis [column (A) at 200 °C] gave four main peaks having retention times (t_R) relative to that of α-D-glucose penta-acetate of 0.57, 0.60, 0.70, and 1.65 (the latter predominating) and two minor peaks. The gum (943 mg) was chromatographed on silica gel (95 g; 200—300 mesh) with toluene–ether (2 : 1 v/v) as eluant. The first fraction eluted slowly crystallised (148 mg; m.p. 108—122°), and several recrystallisations from ether–pentane gave pure methyl 3,4,6-tri-O-acetyl-2-chloro-2-deoxy-β-D-glucopyranoside (26 mg), m.p. 149—151°, $[\alpha]_D^{22}$ +38° (in CHCl$_3$) {lit.,[14] m.p. 149—150°, $[\alpha]_D$ +40° (in CHCl$_3$)}, t_R [column (A) at 200 °C] 0.57.

Next eluted was a mixture of 1,3,4,6-tetra-O-acetyl-2,5-anhydro-D-mannose methyl hemiacetal (3) and 3,4,6-tri-acetyl-2,5-anhydro-D-mannose ethyl methyl acetal (235 mg), τ (CDCl$_3$) 8.79 and 8.77 (two t, CH$_2$CH$_3$), 7.91 and 7.87 (two s, CH$_3$CO), 6.59, 6.57, 6.52, and 6.50 (four s, OMe), 5.49 (two d, H-1 of triacetate diastereoisomers), 4.13 (two d, H-1 of tetra-acetate diastereoisomers); ratio of integrals for signals at τ 4.17 and 5.49 1.3 : 1. G.l.c. analysis gave two main peaks (t_R 0.60 and 0.70). Deacetylation of the mixture in methanol (40 ml) with sodium (19 mg) gave, after deionisation with Amberlite MB-3 resin, a syrup (81 mg). Reducing compounds were removed by passing an aqueous solution through Amberlite IRA 410 (OH$^-$) resin (10 g) (elution with CO$_2$-free water). Concentration of the eluate gave the crude syrupy methyl ethyl acetal, which was purified by preparative paper chromatography. The homogeneous acetal (36 mg) was then benzoylated with benzoyl chloride (0.3 ml) in pyridine (3 ml) at 0 °C to give, after extraction with

* Reaction at 40 °C reduced the time and gave a qualitatively similar product mixture.

[13] J. W. Llewellyn and J. M. Williams, *J.C.S. Perkin I*, 1973, 1997.
[14] R. U. Lemieux and B. Fraser-Reid, *Canad. J. Chem.*, 1964, **42**, 532.

chloroform, 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-mannose ethyl methyl acetal as a diastereoisomeric mixture (66 mg), τ (CDCl$_3$) 8.80 and 8.76 (3H, two t, J 7 Hz, CH$_2$CH_3), 6.53, 6.52 (3H, two s, OMe), 6.5—6.1 (2H, m, OCH_2·CH$_3$), 5.6—4.1 (7H, H-1—6), and 2.8—1.9 (15H, ArH); m/e 503† (<1%, M − OMe), 489† (<1%, M − OEt), 89 (100%, MeOCHOEt). Debenzoylation of the tribenzoate (52 mg) with methanolic sodium methoxide followed by acetylation with pyridine and acetic anhydride regenerated the diastereoisomeric acetals (2), obtained as a syrup (19 mg); τ (CDCl$_3$) 8.79, 8.77 (3H, two t, J 7 Hz, CH$_2$·CH_3), 7.91 (9H, s, MeCO), 6.59 and 6.57 (3H, two s, OMe), 6.4—6.2 (2H, m, CH_2CH$_3$), 6.0—5.7 (6H, m, CH_2CH$_3$ + H-2, -5, -6, and -6'), 5.49 (1H, two d, H-1), and 4.9 and 4.60 (each 1H, m, H-3 and -4); m/e 317† (<1%, M − OMe), 303† (<1%, M − OEt), 197 (1%), 183 (1%), and 89 (100% MeOCHOEt); t_R [column (A) at 200 °C] 0.60.

Next eluted was a small quantity (20 mg) of the methyl hemiacetal tetra-acetate (3) [uncontaminated with the acetal (2)]; τ (CDCl$_3$) 7.88 and 7.85 (two s, total 12H, MeCO), 6.52 and 6.50 (two s, total 3H, OMe), 6.0—5.7 (4H, m, H-2, -5, -6, and -6'), 4.86 (1H, t, J 3 Hz, H-3), 4.6 (1H, m, H-4), 4.13 (1H, two d, J 5 and 6 Hz, H-1); m/e 303† (<1%, M − OAc), 289† (<1%, M − CH$_2$OAc), 259 (1%, M − AcOCHOMe), and 43 (100%); t_R [column (A) at 200 °C] 0.70.

Elution with toluene–ethanol (2 : 1 v/v) gave methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside as a homogeneous syrup (396 mg), which crystallised. Recrystallisation from ether–pentane gave the amide (194 mg), identical (mixed m.p. and i.r. spectra) with an authentic sample. G.l.c. gave one peak, t_R 1.65 [column (A) at 200 °C].

(b) *In aqueous acetone.* A solution of the nitrosoamide (539 mg) in acetone–water (1 : 1 v/v; 25 ml) was kept at room temperature for 14 days. Concentration under reduced pressure at 20 °C gave a syrup from which acetic acid was removed at 0.01 mmHg. Use of sodium hydrogen carbonate to remove the acetic acid (from a solution in chloroform) resulted in decomposition of the major component, which also occurred spontaneously over a period of time (see also ref. 15). The product mixture (419 mg) contained at least six components (t.l.c.), but one predominated, namely 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose. This was in equilibrium with the 'hydrate' {the integral of the aldehyde proton signal [τ (CDCl$_3$) 0.31 (s)] was <1}. The major product was characterised by the formation of four derivatives.

(i) *Acetylation.* A sample (42 mg) of the crude product was dissolved in acetic anhydride (0.1 ml) containing perchloric acid (0.01 ml). After 48 h at room temperature iced water (1 ml) was added. After 2 h, extraction with chloroform (3 × 2 ml), followed by washing of the organic phase in turn with saturated aqueous sodium hydrogen carbonate and water, drying (MgSO$_4$), and concentration gave a syrup (44 mg) that contained mainly 1-*C*-acetoxy-1,3,4,6-tetra-*O*-acetyl-2,5-anhydro-D-mannitol, identified by comparison (t.l.c. and g.l.c.) with an authentic sample. The g.l.c. [column (C) at 200 °C] gave two peaks (*t*− 1.2 and 0.65, area ratio 21 : 1) corresponding to the penta-acetate and methyl β-D-glucopyranoside tetra-acetate respectively.

(ii) *Dithioacetal formation.* Ethanethiol (0.03 ml) and

† Confirmed by accurate mass measurement.

15 D. Horton and K. D. Philips, *Carbohydrate Res.*, 1973, **30**, 367.

boron trifluoride–diethyl ether complex (0.03 ml) were added to a solution of the crude product (44 mg) in ether (2 ml). After 1 h at room temperature, the solution was washed with saturated aqueous sodium hydrogen carbonate (5 ml), then with water (5 ml). The organic phase was dried (MgSO$_4$) and concentrated to a syrup (34 mg) that contained mainly 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose diethyl dithioacetal, identified by comparison (t.l.c., g.l.c., and n.m.r. and mass spectra) with an authentic sample.

(iii) *Acetal formation.* The crude product (43 mg) was converted into the diethyl acetal by the procedure previously described.[11] The crystalline 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose diethyl acetal (13 mg, 25%), m.p. 53—55°, obtained was identical with an authentic sample.

(iv) *Deacetylation.* The crude product (94 mg) was dissolved in dry methanol (14 ml) and sodium (8 mg) was added. Next day the solution was concentrated to a syrup, which was dissolved in water and neutralised with Amberlite MB-3 resin. Concentration gave a syrup, paper chromatography of which indicated that 2,5-anhydro-D-mannose was the main component, with three minor components, one corresponding to methyl β-D-glucopyranoside. The syrup in water was passed through a column of Amberlite IRA 410 (OH$^-$) resin (2.5 g), with carbon dioxide-free water as eluant. Concentration of the eluate gave a syrup (4.5 mg) shown by g.l.c. to contain mainly methyl β-D-glucopyranoside.

Decomposition of the Disaccharide Nitrosoamide in Aqueous Acetone.—The disaccharide nitrosoamide (188 mg) was dissolved in a mixture of AnalaR acetone (6 ml) and water (5 ml), and kept at room temperature for 9 days. Concentration under reduced pressure gave a syrup (151 mg). The 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose was characterised by the preparation of (i) the diethyl dithioacetal derivative, obtained as a homogeneous syrup (8%) after purification on thin-layer plates, and (ii) the crystalline diethyl acetal derivative (18%) as in the preceding experiment. The second major product was identified as follows.

The crude product (56.5 mg) was deacetylated in dry methanol (10 ml) containing sodium methoxide (20 mg) at room temperature overnight. Water (15 ml) was added and the solution extracted with chloroform (3 × 15 ml). The organic phase was washed with water (20 ml), dried (MgSO$_4$), and then concentrated to give syrupy 1,2 : 3,4-di-*O*-isopropylidene-α-D-galactose (18 mg, 59%), identical (n.m.r.) with an authentic sample. The derived toluene-*p*-sulphonate had m.p. and mixed m.p. 96—98°.

Methyl-2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-mannopyranoside.—Acetylation of either methyl 2-amino-2-deoxy-α-D-mannopyranoside[13] or the *N*-acetyl derivative[16] with acetic anhydride in pyridine gave a quantitative yield of the syrupy amide triacetate, $[\alpha]_D^{21}$ +49.5° (CHCl$_3$); $\nu_{max.}$ (film) 3310, 1740, 1660, and 1530 cm^{-1}; τ (CDCl$_3$) 8.02 (3H, s COMe), 7.97 (6H, s, COMe), 7.90 (3H, s, COMe), 6.2—5.6 (3H, m, H-5, -6, and -6'), 5.55—5.3 (2H, m, H-1 and -2), 4.92 (1H, t, J 10 Hz, H-4), 4.67 (1H, q, J 10 and 4 Hz, H-3), and 4.24 (1H, m, NH).

Nitrosation of Methyl 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside and -D-mannopyranoside.—When the α-D-glucoside and α-D-mannoside amides were nitrosated under the same conditions as those used for the β-D-glucoside, the reaction was much slower, 20—24 h being required for the disappearance of starting material. The yellow

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nitrosoamide intermediate was detected (by t.l.c.) only for the α-glucoside reaction, but no nitrosoamide remained when the amide had reacted completely. T.l.c. analysis [with toluene–ether (1 : 1) as solvent] showed components having R_F 0.38, 0.35, 0.31 (major), 0.27, 0.22, 0.17, 0.13, and 0.11 for the α-mannoside product, and R_F 0.31 and 0.27 (poorly resolved) for the α-glucoside product.