522. *Amino-sugars and Related Compounds. Part VII.* 2-Amino-2-deoxy-1,3,4,5-tetra-O-methyl-D-glucitol, 2-Amino-2-deoxy-L-threitol and Certain Derivatives thereof.*

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Reaction sequences for the linkage-analysis of disaccharides in which an amino-sugar derivative constitutes the reducing moiety are suggested. The synthesis of 2-amino-2-deoxy-1,3,4,5-tetra-*O*-methyl-D-glucitol and 2-amino-2-deoxy-L-threitol and certain of their derivatives is described. Standard carbohydrate methods are employed to obtain the D-glucitol compound, but the synthesis of the L-threitol derivative involves the use, novel in the carbohydrate field, of the *O*-methyl ether grouping as a blocking agent and its removal with boron trichloride.

The application of classical methylation techniques in the linkage-analysis of disaccharides (and oligosaccharides) where 2-acetamido-2-deoxy-D-glucose (or other *N*-acetylated amino-sugar) is the reducing moiety is complicated by the alkali-sensitivity of the reducing components.[1] Initial attempts to overcome this limitation by using mild reaction conditions failed. For example, treatment of 2-acetamido-2-deoxy-D-glucose with dimethyl sulphate at room temperature and pH 8·46 did not effect glycosidation. Although glycosidation of amino-sugar derivatives can be accomplished with diazomethane,[2] the method has not been widely used.

As a first alternative approach to the problem, the disaccharide may be reduced and, after methylation, the tetra-*O*-methylated 2-amino-2-deoxy-D-glucitol derivative (or analogous compound) isolated and characterised. A similar reaction sequence has been applied [1,3] to oligosaccharides in which the amino-sugar component was not the reducing moiety. The feasibility of the method is indicated by the facts that 2-acetamido-2-deoxy-D-glucose and the *N*-acetylated chitosaccharides are smoothly reduced by sodium borohydride [4] apparently without the incursion of side reactions and that 2-acetamido-2-deoxy-D-glucitol may be completely methylated by a single treatment with methyl iodide and silver oxide in dimethyl formamide,[5] to yield 2-acetamido-2-deoxy-1,3,4,5,6-penta-*O*-methyl-D-glucitol. As reference compounds for the proposed alternative method a series of 2-amino-2-deoxytetra-*O*-methyl-D-glucitol derivatives are required and we now describe the synthesis of 2-amino-2-deoxy-1,3,4,5-tetra-*O*-methyl-D-glucitol and certain of its derivatives.

Treatment of 2-acetamido-2-deoxy-D-glucose with triphenylmethyl chloride in pyridine gave a 6-*O*-trityl derivative which was smoothly reduced to 2-acetamido-2-deoxy-6-*O*-trityl-D-glucitol by ethanolic sodium borohydride. The location of the trityl group was indicated when the compound reduced 1·9 mol. of periodate with the release of 0·66 mol. of formic acid and proved by the isolation of 2-hydroxyethyl triphenylmethyl ether after reduction of the periodate oxidation products with sodium borohydride. The same ether and ethylene bistriphenylmethyl ether were formed when ethane-1,2-diol was treated with 1 mol. of triphenylmethyl chloride in pyridine. Methylation [5] of 2-acetamido-2-deoxy-6-*O*-trityl-D-glucitol gave the tetra-*O*-methyl derivative from which the *O*-trityl group was cleaved by hot aqueous acetic acid to afford, after acetylation, 2-acetamido-6-*O*-acetyl-2-deoxy-1,3,4,5-tetra-*O*-methyl-D-glucitol. Acid-hydrolysis of this compound

* Part VI, *Acta Chem. Scand.*, 1959, **13**, 281.

[1] Foster and Horton, *Adv. Carbohydrate Chem.*, 1959, **14**, 213.
[2] Kuhn and Baer, *Chem. Ber.*, 1953, **86**, 724; see also Neeman, Caserio, Roberts, and Johnson, *Tetrahedron*, 1959, **6**, 36.
[3] *E.g.*, Kuhn and Baer, *Chem. Ber.*, 1956, **89**, 504.
[4] Barker, Foster, Stacey, and Webber, *J.*, 1958, 2218.
[5] Kuhn, Trischmann, and Löw, *Angew. Chem.*, 1955, **67**, 32.

gave syrupy 2-amino-2-deoxy-1,3,4,5-tetra-*O*-methyl-D-glucitol hydrochloride. Characterisation of this compound would be best effected by selective *N*-acetylation [6] and tritylation.

By a second approach, an indication of the linkage position may be obtained normally from the periodate oxidation pattern of the reduced disaccharide. The presence of the 2-acetamido-2-deoxy-group limits attack by periodate and hence simplifies the oxidation pattern. Moreover, serious overoxidation [7] of the 2-acetamido-2-deoxy-D-glucitol moiety cannot occur since malondialdehyde derivatives are not formed. This behaviour has been observed with 2-acetamido-2-deoxy-D-glucitol, di-*N*-acetylchitobi-itol, and tri-*N*-acetylchitotri-itol.[4] Further, and importantly, reduction and then acidic hydrolysis of the periodate oxidation products yield, from the 2-acetamido-2-deoxy-D-glucitol moiety, a non-reducing fragment whose size is characteristic of the location of the interglycosidic linkage. Thus for 1→3, 1→4, and 1→6 (and 1→5) linked disaccharides the respective relevant products are 2-amino-2-deoxy-L-threitol, 2-amino-2-deoxy-D-xylitol, and 2-aminopropane-1,3-diol. Isolation and characterisation of these products provides a definitive structural method. 2-Aminopropane-1,3-diol has been characterised,[8] but the tetritol and pentitol derivatives are unknown although 2-amino-2-deoxy-D-xylose has been described.[9] We now report a synthesis of 2-amino-2-deoxy-L-threitol.

2-Acetamido-2-deoxy-L-threose (and subsequently the threitol derivative by reduction) cannot be obtained by graded periodate oxidation [10] of 2-acetamido-2-deoxy-D-glucitol since the reagent preferentially attacks vicinal *threo*-diol groupings,[10] thereby yielding 2-acetamido-2-deoxy-L-glyceraldehyde. Graded periodate oxidation of 2-acetamido-2-deoxy-D-galactitol should afford 2-acetamido-2-deoxy-L-threose, but the low yields to be expected [10] and the inaccessibility of the galactitol derivative largely deprive the method of value. Alternatively, the threitol derivative may be synthesised by periodate oxidation of a suitable 3-*O*-substituted 2-acylamido-2-deoxy-D-glucitol followed by reduction and then the removal of the protecting groups. Synthesis of a 3-*O*-benzyl derivative was first examined.

Treatment of a benzene solution of methyl 4,6-*O*-benzylidene-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside [11] successively with sodium and benzyl bromide, followed by acidic hydrolysis of the product under conditions normally employed for glycoside hydrolysis (3N-hydrochloric acid at 95—100° for 3—4 hr.), did not yield any reducing amino-sugar derivative. A similar result was obtained when the benzyl bromide was omitted, whereas direct acid-hydrolysis of the benzylidene derivative yielded 2-amino-2-deoxy-D-glucose. An explanation for this result is not readily apparent although ingress of moisture during the reaction with sodium would result in rapid cleavage of the benzyloxycarbonylamino-group,[11] yielding a free amino-compound strongly resistant to acid-hydrolysis.[12] Alternatively *N*-benzylation may have occurred, again yielding an acid-resistant product; Jeanloz and Jeanloz [13] have observed such a reaction during *O*-benzylation of methyl 2-acetamido-2-deoxy-6-*O*-trityl-α-D-glucopyranoside.

The observation [14] that *O*-methyl sugar derivatives are readily demethylated by boron trichloride at low temperature suggests the possibility, novel in the carbohydrate field, of employing the methyl ether group as a blocking agent generally in suitable carbohydrate syntheses and particularly in the synthesis of 2-amino-2-deoxy-L-threitol. Preliminary experiments showed that 2-amino-2-deoxy-D-glucose hydrochloride was

[6] Inoue, Onodera, Kitaoka, and Kirii, *Bull. Inst. Chem. Res. Kyoto Univ.*, 1955, **33**, 270; *Chem. Abs.*, 1956, **50**, 10,656.
[7] Bose, Foster, and Stephens, *J.*, 1959, 3314.
[8] Foster, Horton, and Stacey, *J.*, 1958, 1890.
[9] Wolfrom and Anno, *J. Amer. Chem. Soc.*, 1953, **75**, 1038.
[10] Schwarz, *J.*, 1957, 276.
[11] Foster, Stacey, and Vardheim, *Acta Chem. Scand.*, 1959, **13**, 281.
[12] Foster, Horton, and Stacey, *J.*, 1957, 81.
[13] Jeanloz and Jeanloz, *Chimia*, 1953, **7**, 233.
[14] Allen, Bonner, Bourne, and Saville, *Chem. and Ind.*, 1958, 630.

unaffected by boron trichloride and that its 3-*O*-methyl derivative was smoothly demethylated, apparently without the incursion of side reactions. Some decomposition occurred in addition to de-*N*-acetylation, when 2-acetamido-2-deoxy-D-glucose was treated with the reagent.

2-Acetamido-2-deoxy-3-*O*-methyl-D-glucitol was synthesised and degraded to 2-amino-2-deoxy-L-threitol as follows. Treatment of 2-acetamido-2-deoxy-D-glucose with boiling 2% methanolic hydrogen chloride for 3 hr. resulted in glycosidation but also 24% of de-*N*-acetylation. Methyl 2-acetamido-2-deoxy-αβ-D-glucopyranoside was subsequently isolated in poor yield (20%). When Amberlite I.R.-120 (H^+ form) was used as glycosidation catalyst,[15] the de-*N*-acetylated product was adsorbed on the resin and a much improved yield (72%) of methyl 2-acetamido-2-deoxy-αβ-D-glucopyranoside resulted. This glycoside, which contains 10—15% of the β-anomer, readily condensed with benzaldehyde, by the Gerhardt method,[16] to yield methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside. It is of interest that, whilst methyl-α-D-glucopyranoside and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside have similar $[M]_D$ values (+307° and +308° respectively in water), the $[M]_D$ values for the 4,6-*O*-benzylidene derivatives are quite different (+329° and +104° respectively in chloroform), that for the amino-sugar derivative being unexpectedly low.

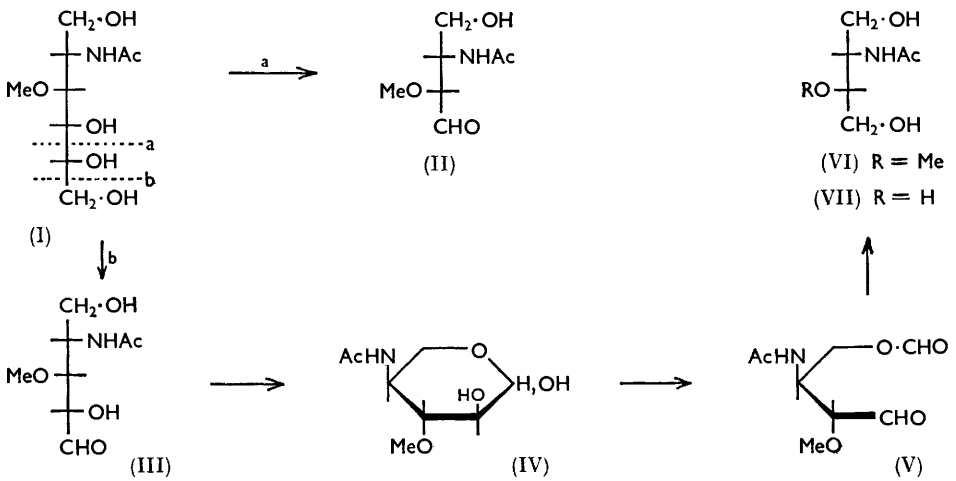
Repeated treatment of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside with methyl iodide–silver oxide failed to achieve complete methylation since acid-hydrolysis of the product invariably yielded a mixture of 2-amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride and the parent amino-sugar. Treatment of homogeneous 2-amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride with acid did not effect any demethylation. Similar results were obtained when methyl 4,6-*O*-benzylidene-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside was methylated with methyl iodide–silver oxide and when methyl iodide–silver oxide in dimethylformamide was used, although an improved yield of product was obtained with the latter reagent. The resistance of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside and its 2-benzyloxycarbonylamino-analogue to methylation by the latter reagent contrasts with the ease of methylation of the acyclic amino-sugar derivatives described above. Homogeneous 2-amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride could only be isolated by paper column chromatography. Methylation of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside is reported [17] to proceed smoothly in dioxan, but experimental details have not been published. It is of interest that crystalline samples of 2-amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride obtained from several sources all contained small amounts of the parent amino-sugar.

Selective *N*-acetylation [6] of 2-amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride gave 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose which crystallised in the α-form as indicated [18] by $\nu_{max.}$ at 842 $cm.^{-1}$ and the downward mutarotation on dissolution in water. The *N*-acetate was smoothly reduced by sodium borohydride to syrupy 2-acetamido-2-deoxy-3-*O*-methyl-D-glucitol (I) characterised as a tetra-(*p*-phenylazobenzoate). Whereas the $[\alpha]_D$ value of the reaction solution obtained after reduction of 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose did not change significantly on acidification with acetic acid, that of the solution obtained after reduction of 2-acetamido-2-deoxy-D-glucose changed from −48·4° to −15·7°. Analogous results were obtained with D-glucose ($[\alpha]_D$ +6·0° ⟶ −0·9° on acidification) and its 3-*O*-methyl derivative ($[\alpha]_D$ +11·4° before and after acidification). The effect is undoubtedly due to borate-complex formation,[19] and the importance of

[15] Zilliken, Rose, Braun, and György, *Arch. Biochem. Biophys.*, **54**, 392.
[16] Gerhardt, G.P. 253,083/1910; *Chem. Zentr.*, 1912, **83**, **11**, 1955; Hill, Whelen, and Hibbert, *J. Amer. Chem. Soc.*, 1928, **50**, 2235.
[17] Roth and Pigman, Abs. Papers, Amer. Chem. Soc. Meeting, Atlantic City, September, 1959; Jeanloz, *Adv. Carbohydrate Chem.*, 1958, **13**, 192.
[18] Cf. Barker, Bourne, Stacey, and Whiffen, *J.*, 1954, 171.
[19] Isbell, Brewster, Holt, and Frush, *J. Res. Nat. Bur. Stand.*, 1948, **40**, 129.

the 3-hydroxyl group in the above examples strongly suggests that borate ions form complexes preferentially with vicinal *threo*-diol groups (cf. Frahn and Mills [20]). A parallel is thus provided with the reaction of acetone [21] and periodate [10] with polyhydric alcohols.

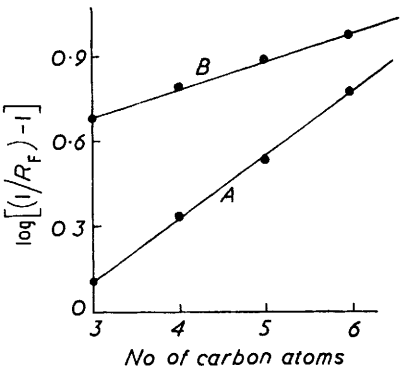
Under conditions where 2-acetamido-2-deoxy-D-glucitol rapidly consumed 3·0 mol. of periodate yielding 1·0 mol. of formaldehyde and 1·8 mol. of formic acid, 2-acetamido-2-deoxy-3-*O*-methyl-D-glucitol consumed oxidant initially rapidly and subsequently relatively slowly, finally reducing 1·9 mol. of oxidant and releasing 1·0 mol. of formaldehyde but only 0·2 mol. of formic acid as determined by direct titration with standard alkali. Addition of an excess of standard alkali and back-titration with acid revealed a true formic acid production of 0·9 mol. These results may be explained as follows. Initial oxidation of 2-acetamido-2-deoxy-3-*O*-methyl-D-glucitol (I) at position a yields 3-acetamido-3-deoxy-2-*O*-methyl-L-threose (II). However, alternative initial attack at b gives 4-acetamido-4-deoxy-3-*O*-methyl-*aldehydo*-L-xylose (III) which would rapidly change to the pyranose form (IV), thereafter consuming periodate to afford 3-acetamido-3-deoxy-4-*O*-formyl-3-*O*-methyl-L-threose (V), the ester moiety of which would be stable in the weakly acid periodate solution but sensitive to alkali (cf. the results obtained by Hough *et al.*[22] on periodate oxidation of 3-*O*-methyl-D-glucose and 3-*O*-methyl-D-glucitol). The initial release of 0·2 mol. of formic acid indicates that 20% of 2-acetamido-2-deoxy-3-*O*-methyl-D-glucitol (I) is attacked by periodate initially at a; the initial rapid uptake of oxidant corresponds to cleavage at a or b, and the subsequent slower rate of periodate consumption corresponds to attack of the pyranose structure (IV). These and the preceding results amplify Schwarz's finding [11] that the susceptibility of acyclic vicinal diols to attack by periodate follows the sequence *threo* > terminal > *erythro*.



Simultaneous reduction and saponification of 3-acetamido-3-deoxy-4-*O*-formyl-3-*O*-methyl-L-threose (V) with sodium borohydride yielded crystalline 2-acetamido-2-deoxy-3-*O*-methyl-L-threitol (VI), which was characterised as its bis-(*p*-phenylazobenzoate). Acid-hydrolysis of the methyl ether (VI) gave 2-amino-2-deoxy-3-*O*-methyl-L-threitol hydrochloride which rapidly consumed 1·0 mol. of periodate, releasing 1·1 mol. of formaldehyde. The methyl ether group in the latter compound was smoothly cleaved by boron trichloride, affording syrupy 2-amino-2-deoxy-L-threitol hydrochloride which was isolated as its crystalline *N*-acetyl derivative (VII). 2-Amino-2-deoxy-L-threitol hydrochloride rapidly consumed 2·8 mol. of periodate, releasing 1·8 mol. of formaldehyde.

[20] Frahn and Mills, *Austral. J. Chem.*, 1959, **12**, 65.
[21] Barker and Bourne, *Adv. Carbohydrate Chem.*, 1952, **7**, 137.
[22] Hough, Taylor, Thomas, and Woods, *J.*, 1958, 1212; cf. Cantley, Hough, and Pittet, *Chem. and Ind.*, 1959, 1253.

A plot [23] of $\log_{10}[(1/R_F) - 1]$, where R_F is the paper chromatographic mobility observed on downward irrigation with the organic phase of butanol–ethanol–water (4 : 1 : 5), against the number of carbon atoms for the series glycerol, erythritol, D-arabitol, and D-glucitol gave a straight line (see Figure). Although the R_F values for the 2-amino-2-deoxy-analogues were much lower a straight line was observed (see Figure) for the hydrochlorides of the series 2-amino-2-deoxyglycerol, 2-amino-2-deoxy-L-threitol, 2-amino-2-deoxy-D-xylitol [24] and 2-amino-2-deoxy-D-glucitol.



Paper chromatographic behaviour [23] *of polyhydric alcohols (A) and their 2-amino-2-deoxy-derivatives (B), of series* C_3 = *glycerol,* C_4 = *erythritol,* C_5 = *arabitol, xylitol, and* C_6 = *glucitol.*

Experimental

Wherever possible, optical rotations were measured in 2 dm. tubes. Paper chromatography was performed on Whatman No. 1 paper by downward irrigation of the organic phase of butanol–ethanol–water (4 : 1 : 5), and detection was by aniline hydrogen phthalate,[25] ninhydrin, and silver nitrate [26] in the appropriate cases.

*Attempted Methylation of 2-Acetamido-2-deoxy-*D*-glucose.*—The following is a typical experiment. Carbon dioxide was passed through a solution of sodium carbonate (0·106 g., 1·0 mmole) and 2-acetamido-2-deoxy-D-glucose (100 mg., 0·45 mmole) in water (11 ml.) at 0° until a constant pH (8·46) was obtained. Dimethyl sulphate (0·110 g., 0·81 mmole) was added during 1 hr. and the mixture was then allowed to attain room temperature. There was no change in reducing power of the solution during 4 hr.

*Methylation of 2-Acetamido-2-deoxy-*D*-glucitol.*—A solution of 2-acetamido-2-deoxy-D-glucitol (0·501 g., m. p. 152—154°) in dry, purified [27] dimethylformamide (20 ml.) and methyl iodide (3 ml.) was treated [5] with dry silver oxide (3 g.) in portions during 30 min. with continuous shaking. After being shaken overnight, the mixture was centrifuged and the residue was washed with chloroform. The combined washings and supernatant liquid were dried ($MgSO_4$) and concentrated, and the residue was distilled, to yield *2-acetamido-2-deoxy-1,3,4,5,6-penta-O-methyl-*D*-glucitol* (0·427 g., 66%), b. p. 180—190° (bath)/0·2 mm., n^{20} 1·4550 (Found: C, 53·5; H, 9·4; N, 4·9. $C_{13}H_{27}NO_6$ requires C, 53·3; H, 9·2; N, 4·8%). Hydrolysis of the product (112 mg.) with 2N-hydrochloric acid at 100° for 2 hr. gave a product which appeared homogeneous on paper chromatography and detection with silver nitrate but did not react with ninhydrin.

*2-Acetamido-2-deoxy-6-O-trityl-*D*-glucose.*—A solution of 2-acetamido-2-deoxy-D-glucose (3·18 g.) in dry pyridine (20 ml.) was treated with a solution of triphenylmethyl chloride (4·05 g.) in pyridine (20 ml.) at room temperature for 6 days. The mixture was then poured into water (500 ml.), and the product was extracted with chloroform (3 × 100 ml.). Evaporation of the combined and dried ($MgSO_4$) extracts and recrystallisation of the residue gave *2-acetamido-2-deoxy-6-O-trityl-*D*-glucose* (4·3 g., 64%), m. p. 130°, $[\alpha]_D$ +33° (*c* 0·4 in EtOH), $[M]_D$ +152° (Found: C, 70·0; H, 6·5; N, 3·1. $C_{27}H_{29}NO_6$ requires C, 70·0; H, 6·3; N, 3·0%). From the

[23] Cf. French and Wild, *J. Amer. Chem. Soc.*, 1953, **75**, 2612.
[24] Featherstone, Foster, and Webber, unpublished work.
[25] Partridge, *Nature*, 1949, **164**, 443.
[26] Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.
[27] Leader and Grovely, *J. Amer. Chem. Soc.*, 1951, **73**, 5731.

mother-liquors an unidentified product (0·2 g.), m. p. 169—170°, was also isolated (Found: C, 70·2; H, 6·1; N, 3·0%).

2-*Acetamido*-2-*deoxy*-6-O-*trityl*-D-*glucitol*.—Sodium borohydride (0·15 g.) was added to a solution of 2-acetamido-2-deoxy-6-*O*-trityl-D-glucose (1·3 g.) in ethanol (150 ml.), and the mixture was stored at room temperature for 12 hr. After acidification with dilute acetic acid the mixture was diluted with water (200 ml.), and the precipitate collected, washed with water, and recrystallised from aqueous ethanol, to give the *product* (1·27 g., 97%), m. p. 76—78°, $[\alpha]_D$ −2° (*c* 0·5 in EtOH), $[M]_D$ −9° (Found: C, 70·0; H, 7·0; N, 2·7. $C_{27}H_{31}NO_6$ requires C, 69·7; H, 6·7; N, 3·0%).

A solution of the product (50 mg.) in ethanol (40 ml.) was treated with aqueous 0·25M-sodium metaperiodate (5 ml.) and water (5 ml.). The consumption of periodate was followed by a standard procedure;[28] after 3 hr., 1·9 mol. of oxidant had been reduced and 0·66 mol. of formic acid released.

In a parallel experiment the product (20 mg.) was oxidised as above for 3 hr. and the solution was then treated with ethane-1,2-diol (0·1 g.) followed by sodium borohydride (10 mg.). After 20 min. the solution was acidified with dilute acetic acid and concentrated, and the precipitate recrystallised from aqueous ethanol to yield 2-*hydroxyethyl triphenylmethyl ether* (10 mg., 63%), m. p. 93—95° alone and 92—93° in admixture with the authentic material described below (Found: C, 83·8; H, 6·5. $C_{21}H_{20}O_2$ requires C, 82·9; H, 6·6%).

O-*Triphenylmethyl Derivatives of Ethane*-1,2-*diol*.—A solution of ethane-1,2-diol (0·31 g., 5 mmoles) and triphenylmethyl chloride (1·39 g., 5 mmoles) in dry pyridine (20 ml.) was boiled under reflux for 20 min., cooled, and poured into water. The precipitate was collected, washed with water, and crystallised from aqueous ethanol, to yield *ethylene bistriphenylmethyl ether* (0·95 g.), m. p. 187° (Found: C, 88·0; H, 6·6. $C_{40}H_{34}O_2$ requires C, 87·9; H, 6·2%).

From the mother-liquors 2-hydroxyethyl triphenylmethyl ether (0·108 g.), m. p. 94—96°, was isolated (Found: C, 83·3; H, 6·6%).

2-*Acetamido*-6-O-*acetyl*-2-*deoxy*-1,3,4,5-*tetra*-O-*methyl*-D-*glucitol*.—2-Acetamido-2-deoxy-6-*O*-trityl-D-glucitol was methylated essentially as described above with methyl iodide (10 ml.), silver oxide (10 g.), and dimethylformamide (30 ml.). The resulting 2-*acetamido*-2-*deoxy*-1,3,4,5-*tetra*-O-*methyl*-6-O-*trityl*-D-*glucitol* (1·12 g., theoretical yield) was dried over P_2O_5 at 110°/0·01 mm.; it could not be distilled (Found: C, 70·7; H, 7·5; N, 3·4; OMe, 23·8. $C_{31}H_{39}NO_6$ requires C, 71·4; H, 7·5; N, 2·7; OMe, 23·8%).

A solution of this compound (0·67 g.) in 60% aqueous acetic acid (25 ml.) was heated at 100° for 1 hr., then cooled and diluted with water. Triphenylmethanol (0·312 g., 96%; m. p. 157—158°) was removed and the filtrate concentrated, to yield syrupy 2-acetamido-2-deoxy-1,3,4,5-tetra-*O*-methyl-D-glucitol (0·29 g., 83%) which was treated with acetic anhydride (0·5 ml.) and pyridine (10 ml.) at room temperature for 34 hr. Water (30 ml.) was then added and after 3 hr. the solution was extracted with chloroform. The combined and dried ($MgSO_4$) extracts were concentrated and the residue was distilled, to yield 2-*acetamido*-6-O-*acetyl*-2-*deoxy*-1,3,4,5-*tetra*-O-*methyl*-D-*glucitol* (0·278 g., 68%), b. p. 160° (bath)/0·02 mm., n^{20} 1·4562, $[\alpha]_D^{19}$ +9·5° (*c* 2·3 in EtOH), $[M]_D$ +30° (Found: C, 52·1; H, 8·4; N, 4·3. $C_{14}H_{27}NO_7$ requires C, 52·3; H, 8·4; N, 4·4%).

A solution of this compound (126 mg.) in 2·3N-hydrochloric acid (20 ml.) was heated at 100° for 1 hr. and then concentrated to yield, presumably, 2-amino-2-deoxy-1,3,4,5-tetra-*O*-methyl-D-glucitol hydrochloride (95 mg., 90%), $[\alpha]_D^{20}$ +5° (*c* 0·7 in EtOH), $[M]_D$ +19°, after drying at 100°/0·02 mm. The product, which failed to crystallise, appeared homogeneous on paper chromatography and detection with silver nitrate; it did not react with ninhydrin. When a sample was dried at 140°/0·02 mm., partial decomposition occurred.

Methyl 2-*Acetamido*-2-*deoxy*-αβ-D-*glucopyranoside*.—(*a*) 2-Acetamido-2-deoxy-D-glucose[6] (2·56 g.; m. p. 205°; $[\alpha]_D$ +41° in H_2O) was added to 2% methanolic hydrogen chloride (75 ml.), and the mixture was boiled under reflux. Dissolution occurred in 11 min. and the reaction was then followed polarimetrically; α_D +2·8° ⟶ +6·2° (final constant value) in 3 hr. The solution was treated with lead carbonate and filtered; the filtrate was evaporated at 40° (bath)/12—15 mm. and the residue (2·69 g.) recrystallised from ethanol to yield a product (0·152 g., 5·8%), m. p. 188—189°. Paper chromatographic examination of the mother-liquor revealed an appreciable amount of free amino-compounds with R_F values identical with those of 2-amino-2-deoxy-D-glucose and methyl 2-amino-2-deoxy-D-glucopyranoside. (In a parallel

[28] Jackson, *Org. Reactions*, 1944, **2**, 341.

experiment titration with standard sodium hydroxide indicated 24·3% of de-*N*-acetylation.) Concentration of the mother liquor gave a further yield (1·43 g.) of product, m. p. 150—158°, which was found to be heterogeneous by paper chromatography. Recrystallisation from ethanol yielded methyl 2-acetamido-2-deoxy-αβ-D-glucopyranoside (0·385 g., 14·3%), m. p. 187—189°, $[\alpha]_D$ +106·9° (*c* 1·12 in water), $[M]_D$ +251°. Moggridge and Neuberger [29] give m. p. 189°, $[\alpha]_D$ +105° in water for this compound, which was shown by Kuhn, Zilliken, and Gauhe [30] to contain 10—15% of the β-anomer.

(*b*) A mixture of 2-acetamido-2-deoxy-D-glucose (5·2 g.), Amberlite I.R.-120 (15 g.; H^+ form) and methanol (154 ml.) was boiled under reflux for 4 hr. (cf. Zilliken *et al.*[15]); dissolution was complete in 12 min. The mixture was filtered, the resin was washed with methanol (40 ml.), and the combined filtrate and washings were evaporated at 40° (bath)/12—15 mm., yielding the product (4·02 g., 72·4%), m. p. 184—188°, $[\alpha]_D$ +104·6° (*c* 1·2 in H_2O) and +121° (*c* 1·0 in MeOH).

Methyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside.—Methyl 2-acetamido-2-deoxy-αβ-D-glucopyranoside (5·9 g.; m. p. 184—188°) and freshly distilled benzaldehyde (45 ml.) were heated at 140—145° for 4 hr. and a stream of carbon dioxide was passed through the mixture; [16] the mixture became homogeneous after 10 min. It was then poured into light petroleum (400 ml.; b. p. 60—80°) and after 1 hr. the product (8·48 g.) was collected and washed with light petroleum (b. p. 60—80°). A solution of the crude product in chloroform (*ca.* 100 ml.) was decolorised with charcoal and concentrated until a saturated solution was obtained. On storage overnight at −20° methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (5·4 g., 64%) separated with m. p. 258—259°, $[\alpha]_D$ +33·4° (*c* 0·75 in $CHCl_3$), $[M]_D$ +108°. A further yield of product (1·61 g., 19%), m. p. 255—256°, $[\alpha]_D$ +26·8° (*c* 0·76 in $CHCl_3$), was obtained by concentration of the mother-liquors. Neuberger [31] gives m. p. 255°, $[\alpha]_D$ +19° in $CHCl_3$, for this compound prepared by a different method.

The above reaction was repeated several times and in certain cases extensive decomposition occurred. The cause was not determined.

Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (0·8 g.; m. p. 257—258°; $[\alpha]_D$ +36° in $CHCl_3$) was treated with 10·4N-acetic acid (20 ml.) at 60—70° for 0·5 hr. The mixture was diluted with water (*ca.* 50 ml.) and, after extraction with light petroleum (b. p. 60—80°), concentrated at 40° (bath)/~12 mm., and water was distilled from the residue. Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside thus obtained had $[\alpha]_D$ +130·3° (*c* 0·76 in water). Kuhn, Zilliken, and Gauhe [30] record $[\alpha]_D$ +131° in water for the α-glucoside purified through the tri-*O*-acetate.

Methylation of Methyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside.—(*a*) A boiling solution of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (4·84 g.) in methyl iodide (60 ml.) was treated portionwise with dry silver oxide (26 g.) during 2 hr. After a further 4 hr. the mixture was filtered and the residue washed with methyl iodide (20 ml.) and chloroform (50 ml.). Concentration of the combined filtrate and washings gave a product (1·28 g., 26%), m. p. 279—283°, $[\alpha]_D$ +41·2° (*c* 0·7 in $CHCl_3$). Continuous extraction of the residue gave a further yield of product (3·61 g., 74%), m. p. 279—283°. Neuberger [31] gives m. p. 277—279°, $[\alpha]_D$ +39° in chloroform, for methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-α-D-glucopyranoside.

The foregoing product (50 mg.) was treated with 2·5N-hydrochloric acid (3 ml.) at 95—100° for 4 hr. The hydrolysate was diluted with water (7 ml.), and extracted with light petroleum (b. p. 60—80°) to remove benzaldehyde and then twice with a chloroform solution of methyl di-n-octylamine (5 ml.; 5% v/v) to remove acid. The solution was decolorised with charcoal, concentrated, and examined by paper chromatography. Detection with ninhydrin and aniline hydrogen phthalate revealed a mixture of 2-amino-2-deoxy-D-glucose and its 3-*O*-methyl ether. Similar acid-treatment of chromatographically homogeneous 2-amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride with acid yielded no de-*O*-methylated product.

(*b*) A solution of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (1 g.) in purified [27] dimethylformamide (40 ml.) and methyl iodide (5 ml.) was treated at room temperature with silver oxide (5 g.) portionwise during 1 hr. (cf. Kuhn *et al.*[5]) and the mixture then shaken overnight. Concentration of the filtered solution gave a product (0·638 g., 63%), m. p.

[29] Moggridge and Neuberger, *J.*, 1938, 745.
[30] Kuhn, Zilliken, and Gauhe, *Chem. Ber.*, 1953, **86**, 466.
[31] Neuberger, *J.*, 1941, 50.

281—285°, $[\alpha]_D$ +40·8° (*c* 0·11 in $CHCl_3$). Continuous extraction of the insoluble material with chloroform gave a further yield of product (0·375 g., 36·8%), m. p. 281—285°. Acid-hydrolysis of each fraction and paper chromatographic analysis of the hydrolysate as in (*a*) revealed a mixture of 2-amino-2-deoxy-D-glucose and its 3-*O*-methyl ether.

A mixture (10 g.) of 2-amino-2-deoxy-D-glucose hydrochloride and its 3-*O*-methyl ether was separated on a Gryksbo spiral filter-paper column by elution with the organic phase of a butanol–ethanol–water (4 : 1 : 5) solvent system. The 3-*O*-methyl ether emerged first and the chromatographically homogeneous product (1·68 g.) obtained after recrystallisation from methanol–acetone at −20° had an indefinite m. p., $[\alpha]_D$ +93·1° (equilibrium) (*c* 1·1 in H_2O), $[M]_D$ +214° (Found: C, 37·0; H, 6·75; Cl, 14·7. Calc. for $C_7H_{16}ClNO_5$: C, 36·6; H, 7·0; Cl, 15·5%). Neuberger [31] gives m. p. 215° (decomp.), $[\alpha]_D$ +91·3° in H_2O.

Boron Trichloride Experiments.—(*a*) 2-Amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride (53 mg.) was suspended in dichloromethane (3 ml.) and cooled to −70°. Boron trichloride (*ca.* 2 ml.) was distilled into the mixture which was then stored under anhydrous conditions at −70° for 1 hr. with occasional shaking, becoming homogeneous. After being kept overnight at room temperature, the mixture was concentrated at 50°, the residue was treated with methanol (10 ml.) and water (0·5 ml.), and the solution again concentrated at 40° (bath)/12—15 mm. The process was repeated twice to remove boric acid. The residue (38 mg.) was examined chromatographically and found to contain 2-amino-2-deoxy-D-glucose together with traces of fast-moving substances. Crystallisation of the residue from aqueous acetone gave 2-amino-2-deoxy-D-glucose hydrochloride (23 mg.) which had $[\alpha]_D$ +70·6° (*c* 0·85 in H_2O) and an infrared spectrum (KCl disc) indistinguishable from that of authentic material.

(*b*) Treatment of 2-acetamido-2-deoxy-D-glucose with boron trichloride as in (*a*) resulted in de-*N*-acetylation (assessed chromatographically). Likewise, after treatment of 2-amino-2-deoxy-D-glucose hydrochloride (100 mg.) with boron trichloride crystalline unchanged material (70 mg.) was recovered.

2-*Acetamido*-2-*deoxy*-3-O-*methyl*-D-*glucose*.—A solution of 2-amino-2-deoxy-3-*O*-methyl-D-glucose hydrochloride (0·8 g.) in methanol (10 ml.) was treated with sodium methoxide obtained from sodium (83 mg.) and methanol (2·6 ml.). After 20 min., precipitated sodium chloride was removed, acetic anhydride (0·45 ml.) was added to the filtrate, and the mixture stored at 0° overnight. The mixture was evaporated and a solution of the residue (0·86 g.) in water (*ca.*, 70 ml.) was freed from unchanged amino-sugar by passage down a column of Amberlite IR-120 resin (H^+ form). The eluate was neutralised [32] with methyl di-n-octylamine and concentrated. The infrared spectrum (KCl disc) of the residue showed absorptions at 1590 $cm.^{-1}$ (*N*-acetyl group) and at 824 and 888 $cm.^{-1}$ indicative [18] of α- and β-configuration at the glycosidic centre. Recrystallisation of the residue gave 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose (0·156 g., 19·6%), m. p. 195—196°, $[\alpha]_D$ +31·9° (equilibrium) (*c* 2·76 in H_2O), $[M]_D$ +75° (Found: C, 46·1; H, 7·4. Calc. for $C_9H_{17}NO_6$: C, 45·95; H, 7·2%). Jeanloz [33] gives m. p. 195—198°, $[\alpha]_D$ +33° in water, but no preparative details.

Sodium Borohydride Reductions.—(*a*) A solution of 2-acetamido-2-deoxy-D-glucose (0·48 g.) in water (*ca.* 12 ml.) was stored for 2 hr. to reach mutarotational equilibrium, then treated with sodium borohydride (0·22 g.) in water (*ca.* 5 ml.), and the volume was adjusted to 50 ml. {$[\alpha]_D \longrightarrow$ −48·4° (final constant value) during 60 min.; after treatment of the solution with acetic acid (3·5 ml.), $[\alpha]_D \longrightarrow$ −15·7°}. 2-Acetamido-2-deoxy-D-glucitol, m. p. 153°, was subsequently isolated.

(*b*) Under similar conditions, on reduction of (1) D-glucose there was observed $[\alpha]_D \longrightarrow$ +6·0° (final constant value) and $\longrightarrow$ −0·9° on acidification, and of (2) 3-*O*-methyl-D-glucose $[\alpha]_D \longrightarrow$ +11·4° (final constant value), essentially unchanged on acidification.

(*c*) A solution of 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose (0·48 g.) in water (*ca.* 12 ml.) was treated after 6 hr. with a solution of sodium borohydride (0·22 g.) in water (*ca.* 5 ml.) at room temperature and the volume rapidly adjusted to 25 ml.; $[\alpha]_D$ reached −19·3° (final constant value) within 1 hr. Destruction of the excess of borohydride by addition of 10% acetic acid (*ca.* 3 ml.) did not significantly change $[\alpha]_D$. The mixture was processed as in (*a*), to yield 2-acetamido-2-deoxy-3-*O*-methyl-D-glucitol (0·485 g.) as a colourless syrup, $[\alpha]_D$ +6·8° (*c* 2·2 in H_2O), $[M]_D$ +16°, which appeared homogeneous on paper chromatography (R_F 0·31). It failed to yield a crystalline *O*-benzylidene derivative when the Gerhardt method [16] was used

[32] Lester Smith and Page, *J. Soc. Chem. Ind.*, 1948, **67**, 48.
[33] Jeanloz, *Adv. Carbohydrate Chem.*, 1958, **13**, 189.

but gave [34] a crystalline *tetrakis*-(p-*phenylazobenzoate*), m. p. 254—257° (from chloroform–ethanol) (Found: N, 11·2. $C_{61}H_{51}N_9O_{10}$ requires N, 11·8%).

Periodate oxidation of 2-*Acetamido*-2-*deoxy*-3-O-*methyl*-D-*glucitol*.—(*a*) A solution of the syrupy 3-*O*-methyl ether (44·5 mg.) in water (5 ml.) was treated with 0·2M-sodium metaperiodate (10 ml.), and the volume was rapidly adjusted to 50 ml. The consumption of oxidant was followed by Jackson's method; [28] formic acid liberation was determined by direct titration with 0·00954N-sodium hydroxide, and formaldehyde by the chromotropic acid method.[35] Periodate was initially consumed rapidly and subsequently more slowly; 1·9 mol. of oxidant (final constant value) was reduced after 80 min. After 22 hr., 0·2 mol. of formic acid and 1·04 mol. of formaldehyde had been released.

(*b*) The periodate oxidation was repeated as in (*a*) and, after 2 hr., 0·06N-sodium hydroxide (4 ml.) was added to an aliquot part (5 ml.), the volume was adjusted to 50 ml. and the excess of alkali was determined with 0·009N-hydrochloric acid after intervals of 1 hr. and 5 hr. A value of 0·91 mol. of formic acid was obtained. (Formate esters are rapidly hydrolysed in alkaline solution.)

(*c*) A solution of the 3-*O*-methyl ether (1 g.) in water (20 ml.) was treated with a solution of 0·22M-sodium metaperiodate (80 ml.) for 4 hr. at room temperature and then overnight at 0°. The pH of the mixture was adjusted to 6 and dilute aqueous barium chloride was added until no further precipitation occurred. Insoluble material was removed and sodium borohydride (0·5 g.) was added to the filtrate. After storage for 12 hr. at room temperature excess of reductant was destroyed by the addition of 10% acetic acid (*ca.* 8 ml.). After dilution to 700 ml., the solution was de-ionised by means of Amberlite resins IRA-400 (700 ml.; HO^- form) and IR-120 (700 ml.; H^+ form) and freeze-dried, to yield 2-*acetamido*-2-*deoxy*-3-O-*methyl*-L-*threitol hemihydrate* (0·59 g.) as plates, m. p. 87—91°, $[\alpha]_D$ −12·8° (*c* 1·2 in H_2O), $[M]_D$ −24° (Found: C, 45·45; H, 8·3. $C_7H_{15}NO_4,\frac{1}{2}H_2O$ requires C, 45·2; H, 8·6%). The tetritol derivative readily gave [34] a *bis*-(p-*phenylazobenzoate*), m. p. 165—167° (from chloroform–ethanol) (Found: C, 67·1; H, 5·4; N, 11·2. $C_{33}H_{31}N_5O_6$ requires C, 66·8; H, 5·2; N, 11·8%).

2-*Acetamido*-2-*deoxy*-L-*threitol*.—A solution of 2-acetamido-2-deoxy-3-*O*-methyl-L-threitol (0·5 g.) in 2N-hydrochloric acid (10 ml.) was kept at 95—100° for 2 hr. After dilution with water (10 ml.) the hydrolysate was neutralised with a 5% solution of methyl di-n-octylamine in chloroform.[32] 0·1N-Hydrochloric acid (1 ml.) was then added to the aqueous solution which was concentrated at 50° (bath)/12—15 mm., and the residue was dried *in vacuo* (P_2O_5), to yield syrupy 2-amino-2-deoxy-3-*O*-methyl-L-threitol hydrochloride (0·48 g.). The product appeared homogeneous on chromatography and ionophoresis, and on oxidation [28] with an excess of periodate (13 mol.) it rapidly consumed 1·0 mol. of oxidant, releasing [35] 1·1 mol. of formaldehyde.

A suspension of 2-amino-2-deoxy-3-*O*-methyl-L-threitol hydrochloride (0·43 g.) in methylene chloride (*ca.* 5 ml.) was treated with freshly distilled boron trichloride (*ca.* 15 ml.) at −70° for 3 hr. After storage at room temperature overnight, excess of reagent and solvent was distilled off and methanol (3 × 20 ml.) evaporated from the residue to remove boric acid. 2-Amino-2-deoxy-L-threitol hydrochloride (0·41 g.) thus obtained had $[\alpha]_D$ +6·3° (*c* 3·0 in H_2O), $[M]_D$ +10°, R_F 0·14, and contained traces of an unidentified product. Oxidation of the compound with an excess of periodate (24 mol.) resulted in the rapid consumption of 2·8 mol. of oxidant with the liberation of 1·8 mol. of formaldehyde.

A solution of 2-amino-2-deoxy-L-threitol hydrochloride (0·12 g.) in dry methanol (1·5 ml.) was treated with a solution of sodium methoxide [from sodium (17·4 mg.) in methanol (1·05 ml.)] for 30 min. at room temperature. Precipitated sodium chloride was removed and the filtrate was treated with acetic anhydride (0·085 ml.) and then stored at 0° for 12 hr. Electrophoresis of the mixture revealed a small amount of free amine. The solution was then de-ionised by using Amberlite resins IRA-400 (HO^- form) and IR-120 (H^+ form) and freeze-dried, to yield 2-acetamido-2-deoxy-L-threitol (0·795 g.), m. p. 90—90·5°, $[\alpha]_D$ −42° ± 4° (*c* 1·0 in H_2O; 0·5 dm. tube), $[M]_D$ −68° (Found: C, 44·0; H, 8·1. $C_6H_{13}NO_4$ requires C, 44·2; H, 8·0%), $\nu_{max.}$ (KCl disc) 1543 and 1653 cm.$^{-1}$ characteristic of *N*-acetyl.

Paper-chromatographic Behaviour of the Polyhydric Alcohols and their 2-*Amino*-2-*deoxy-analogues*.—When the standard solvent system described above was used the following R_F values were observed: glycerol 0·43, erythritol 0·315, D-arabitol 0·225, D-glucitol 0·145,

[34] Cf. Brimacombe, Foster, and Haines, preceding paper; Woolfolk, Beach, and McPherson, *J. Org. Chem.*, 1955, **10**, 391.

[35] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.

2-amino-2-deoxyglycerol hydrochloride 0·17, 2-amino-2-deoxy-L-threitol hydrochloride 0·14, 2-amino-2-deoxy-L-xylitol hydrochloride [24] 0·12, 2-amino-2-deoxy-D-glucitol hydrochloride 0·097.

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