401. *The Reaction of 2-Acetamido-2-deoxy-D-glucose with Ethanethiol and Hydrochloric Acid.*

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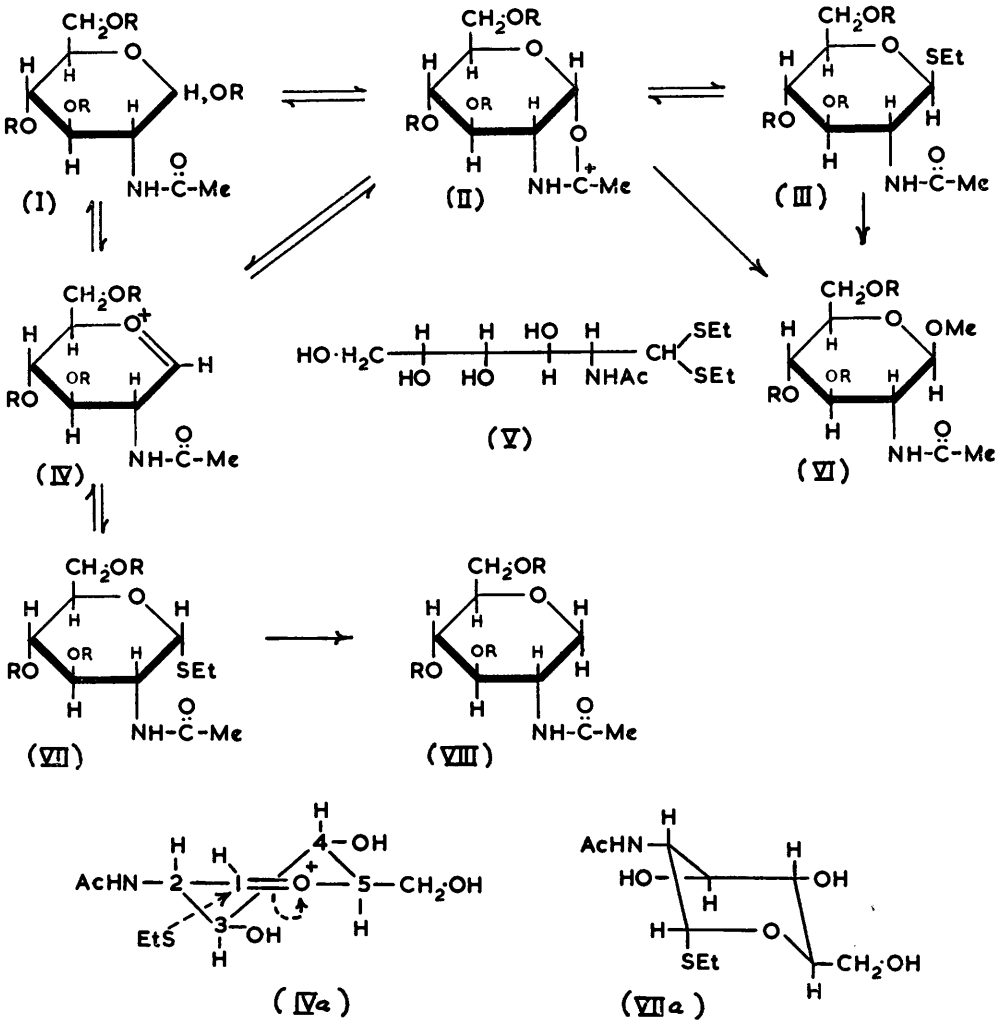
The products obtained from the condensation of 2-acetamido-2-deoxy-D-glucose with ethanethiol in the presence of hydrochloric acid at room temperature have been separated by chromatography on cellulose, and characterised as 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal, 2-acetamido-2-deoxy-α-D-glucopyranosylthioethane, 2-acetamido-2-deoxy-β-D-glucopyranosylthioethane, and 2-amino-2-deoxy-D-glucose hydrochloride.

2-ACETAMIDO-2-DEOXY-D-GLUCOSE DIETHYL DITHIOACETAL (V) was required for oxidation to the diethylsulphonyl derivative in order to compare the properties of the latter with those of similar derivatives prepared from pentose and hexose diethyl dithioacetals.[1] 2-Acetamido-2-deoxy-D-glucose (I; R = H) (*N*-acetyl-D-glucosamine) was prepared from 2-amino-2-deoxy-D-glucose (D-glucosamine) hydrochloride by an improved method,[2] involving acetylation to the penta-acetate (I; R = Ac), followed by de-*O*-acetylation with alcoholic ammonia (cf. White,[3] Kuhn, and Haben,[4] and Roseman and Ludowieg [5]). 2-Acetamido-2-deoxy-D-glucose (I; R = H), ethanethiol, and hydrochloric acid were allowed to react for 24 hr. at room temperature, and subsequent examination [1] of the products on paper chromatograms revealed at least five components. Separation of the mixture by chromatography on a column of cellulose [6] afforded five crystalline materials, of which three might be expected, namely, unchanged 2-acetamido-2-deoxy-D-glucose (I; R = H) (12%), 2-amino-2-deoxy-D-glucose hydrochloride (29%) (formed by de-*N*-acetylation [7,8] of the former), and 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal (V) (24%). The last was obtained by Wolfrom and Anno [9] in 81% yield by carrying out the reaction at 0° for 24 hr.

Analysis of the two other (unknown) crystalline materials (*X* and *Y*) revealed that they were isomers ($C_{10}H_{19}O_5SN$) each containing one *N*-acetyl and one ethylthio-function. The absence of a coloration with *p*-anisidine hydrochloride on paper chromatograms suggested that the ethylthio-radical in both was probably located at $C_{(1)}$. On acetylation they afforded crystalline tri-*O*-acetyl derivatives, thus suggesting the presence of a ring structure involving one of the hydroxyl groups. This conclusion was supported by periodate oxidation experiments in which no formaldehyde was detected. Reductive de-sulphurisation [10] of the compound (*X*) ($[\alpha]_D$ +175°) with Raney nickel in ethanol gave crystalline 2-acetamido-1 : 5-anhydro-2-deoxy-D-glucitol (VIII; R = H) which was characterised by the formation of a crystalline triacetate (VIII; R = Ac) and by its rapid consumption of 1 mol. of metaperiodate without simultaneous liberation of formic acid or formaldehyde. The compound (*X*) was thus identified as a 2-acetamido-2-deoxy-D-glucopyranosylthioethane (*e.g.*, VII; R = H). The compound (*Y*) ($[\alpha]_D$ −55°) was similar since it formed a crystalline triphenylmethyl derivative, and gave no formaldehyde on oxidation with metaperiodate, indicating, respectively, a free hydroxyl group at $C_{(6)}$ but none at $C_{(5)}$. Reductive desulphurisation of the compound (*Y*) using either fresh or aged Raney nickel gave none of the deoxy-compound (VIII; R = H), but only 2-acetamido-2-deoxy-D-glucose (I; R = H). In one case only has this reaction been reported to cause desulphurisation of a glycosylthioethane to the corresponding reducing sugar, namely, the reaction of tetra-acetylstreptobiosaminosylthioethane diethyl dithioacetal with aged Raney nickel

[1] Hough and Taylor, *J.*, 1955, 1212, 3544.
[2] Kent and Whitehouse, " Biochemistry of the Aminosugars," Butterworths Scientific Publications, London, 1955.
[3] White, *J.*, 1940, 428.
[4] Kuhn and Haber, *Chem. Ber.*, 1953, **86**, 722.
[5] Roseman and Ludowieg, *J. Amer. Chem. Soc.*, 1954, **76**, 301.
[6] Hough, Jones, and Wadman, *J.*, 1949, 2511; 1950, 1702.
[7] Moggridge and Neuberger, *J.*, 1938, 745.
[8] Levene, *J. Biol. Chem.*, 1941, **137**, 29.
[9] Wolfrom and Anno, *J. Amer. Chem. Soc.*, 1952, **74**, 6150.
[10] Fletcher and Hudson, *ibid.*, 1947, **69**, 921.

to give tetra-acetyldeoxydihydrostreptobiosamine.[11] Clearly the two compounds (*X* and *Y*) were the α- and the β-isomer of 2-acetamido-2-deoxy-D-glucopyranosylthioethane (VII and III; R = H), and application of Hudson's [12,13] rules of isorotation indicated the configurations at the anomeric carbon atom. Compound (*Y*) had a molecular rotation of −14,600° (A + B; where A is the molecular rotation due to the asymmetric $C_{(1)}$ and B is that due to the rest of the molecule) and by using a value of +10,200° for B, derived



from the molecular rotations of α- and β-isomers of methyl 2-acetamido-2-deoxy-D-glucopyranoside,[14] $A_{SEt}(H_2O)$ was found to be −24,800°, thus indicating the β-configuration (III; R = H). Compound *X* was recognised as the α-isomer (VII; R = H) since it showed a molecular rotation of +46,300° which gave $A_{SEt}(H_2O)$ +36,100°. Wolfrom, Olin, and Polglase [15] obtained $A_{SEt}(CHCl_3)$ + 35,500° for 2-acetamido-2-deoxy-α-D-glucofuranosylthioethane.

[11] Brink, Kuehl, Flynn, and Folkers, *J. Amer. Chem. Soc.*, 1948, **70**, 2085.
[12] Hudson, *ibid.*, 1909, **31**, 66.
[13] Neuberger and Pitt-Rivers, *J.*, 1939, 122.
[14] Zilliken, Rose, Braun, and György, *Arch. Biochem. Biophys.*, 1955, **54**, 392.
[15] Wolfrom, Olin, and Polglase, *J. Amer. Chem. Soc.*, 1950, **72**, 1724.

Methanolysis [16] of 2-acetamido-3 : 4 : 6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylthioethane (III; R = Ac) with mercuric chloride in methanol containing cadmium carbonate yielded methyl 2-acetamido-3 : 4 : 6-tri-*O*-acetyl-2-deoxy-β-D-glucoside (VI; R = Ac), thus confirming the assignment of the pyranose structure to the original thioethane derivative (III; R = H). Retention of configuration at $C_{(1)}$ is probably the consequence of participation by the neighbouring acetamido-group, giving rise to the cyclic carbonium ion (II; R = Ac) and thus facilitating the methanolysis. De-*O*-acetylation of this β-glucoside (VI; R = Ac) with alcoholic ammonia gave rise to (mainly) methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (VI; R = H) which was identical with an authentic specimen [14] kindly provided by Professor György. A little of the α-D-glucoside derivative was also isolated and presumably it arose by inversion during the deacetylation reaction. The α-D-glucopyranosylthioethane derivative (VII; R = Ac) was resistant to methanolysis under the above conditions, and this failure to react is undoubtedly due to the difference in stereochemistry at $C_{(1)}$ since in this case the thioethyl group is in the axial position, preventing participitation by the neighbouring equatorial acetamido-group (see VII*a*). This difference in reactivity of the α- and the β-isomer (VII; III; R = Ac) is also illustrated in the above Raney nickel desulphurisation experiments.

The isolated 2-acetamido-3 : 4 : 6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylthioethane (III; R = Ac) was identical with that synthesised by mercaptolysis [17] of 2-acetamido-1 : 3 : 4 : 6-tetra-*O*-acetyl-2-deoxy-α-D-glucose (I; R = Ac) with ethanethiol and zinc chloride. This reaction proceeded with inversion at $C_{(1)}$, probably as the result of cleavage of the $C_{(1)}$-to-acetoxy-bond with the formation of the oxonium ion (IV; R = Ac). This ion would be stabilised as the cyclic carbonium ion (II; R = Ac) by neighbouring-group participation and would lead exclusively to the β-configuration. This mechanism was proposed by Lemieux and Brice [17] for a similar mercaptolysis of β-D-mannopyranose penta-acetate which proceeded with inversion, whereas with α-D-mannopyranose penta-acetate retention of configuration was observed, suggesting the participation of the neighbouring acetoxy-group. Winstein and Heck [18] have given evidence for such ion-pair intermediates in various rearrangements and in general they are associated with processes involving neighbouring-group participation.

Acid-catalysed reactions of glycosides at the anomeric $C_{(1)}$ have been interpreted by conformational analysis [19,20,21] and by assuming the occurrence of oxonium-ion intermediates. Likewise acid-catalysed anomerisations of acetylated monosaccharides have been satisfactorily explained,[21,22] although here neighbouring-group participation also plays a large part. Such intermediates probably account for the fact that, when 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal (V) and the α- and the β-isomer of 2-acetamido-2-deoxy-D-glucopyranosylthioethane (VII and III; R = H) were separately treated with ethanethiol and hydrochloric acid at room temperature for 24 hr., they each yielded a mixture of the three, as indicated by paper chromatography, thus suggesting that in this reaction they are in equilibrium one with another (*i.e.*, VII $\rightleftharpoons$ V $\rightleftharpoons$ III). On treatment with hydrochloric acid alone, each compound was hydrolysed, giving a mixture of 2-amino-2-deoxy-D-glucose and its *N*-acetyl derivative.

Of the two isomeric 2-acetamido-2-deoxy-D-glucopyranosylthioethanes isolated, the β-isomer predominated, probably because the rate-limiting step in the reaction of 2-acetamido-2-deoxy-D-glucose is the dissociation of the hydroxyl group from $C_{(1)}$. This process is facilitated by participation of the neighbouring acetamido-group with the formation of the cyclic carbonium ion (II; R = H), which can only give rise to the β-isomer. The α-isomer was conceivably formed by a less favoured process, involving the intermediary oxonium ion (IV; R = H), and conformational analysis [20] leads to the prediction that the most likely route would be through the half-chair form (IV*a*). Attack by the ethylthio-ion

[16] Baker, *Canad. J. Chem.*, 1955, **33**, 1102.
[17] Lemieux and Brice, *ibid.*, 1955, **33**, 109.
[18] Winstein and Heck, *J. Amer. Chem. Soc.*, 1952, **74**, 5584.
[19] Foster and Overend, *Chem. and Ind.*, 1955, 566.
[20] Edward, *ibid.*, 1955, 1102.
[21] Huber, *Helv. Chim. Acta*, 1955, **38**, 1224.
[22] Lemieux, Brice, and Huber, *Canad. J. Chem.*, 1955, **33**, 134, 149.

could then occur either from beneath the plane of the ring, giving the α-isomer (VII*a*), in which all the functional groups except the ethylthio-group would be equatorial, or from above to give the β-isomer; but the latter process is less likely since it would lead to a conformation in which all the substituent groups are axial.

Jeanloz and Forchielli [23] oxidised 2-amino-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-glucose, and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside with sodium metaperiodate in the dark at 5° and pH 4·5, and obtained theoretical results for the consumption of periodate, the side reactions being at a minimum under these conditions. In the work described herein, oxidations were carried out with unbuffered sodium metaperiodate in the dark at room temperature so that the acid liberated could be titrated. In the case of 2-acetamido-2-deoxy-D-glucose (I; R = H) and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, one mol. of oxidant was rapidly consumed, followed by over-oxidation which was rather fast in the former case, but much slower in the latter. These results suggest that 2-acetamido-2-deoxy-D-glucose is oxidised in the pyranose ring form and substantiate the conclusion of previous workers [24,25] that an α-acetamido-β-hydroxy-structure is relatively stable to periodate oxidation. The α-and the β-isomers of 2-acetamido-2-deoxy-D-glucopyranosylthioethane each rapidly consumed about two mols. of oxidant, the former faster than the latter, with the formation of only a trace of acid, followed by a slow over-oxidation. Clearly the introduction of an ethylthio-group has caused the consumption of an extra mol. of periodate over the theoretical and subsequent experiments have lead us to the conclusion that structures containing alkylthio-groups on carbon atoms adjacent to carbon atoms substituted by hydroxyl, amino, or acetamido, are attacked by periodate with disruption of the carbon–carbon bond. 1 : 1-Di(ethylthio)-ethan-2-ol readily consumed one mole of periodate with the simultaneous liberation of one mol. of formaldehyde. This was followed by a slow over-oxidation, presumably of the sulphur atoms. Under the same conditions, D-galactose diethyl dithioacetal rapidly consumed 5 mols. of periodate with the formation of 4 equiv. of acid. On the other hand, 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal readily consumed six mols. of periodate with the simultaneous liberation of one mol. of formaldehyde and three equiv. of acid. These abnormal results call for further investigation before any other generalisations can be made.

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper by the descending method with (i) butan-1-ol–ethanol–water (40 : 11 : 19 v/v); (ii) butan-1-ol–pyridine–water (10 : 3 : 3 v/v), or (iii) ethyl acetate–acetic acid–water (9 : 2 : 2 v/v) as mobile phase. The separated substances were detected with (*a*) 4% ammonical silver nitrate reagent, (*b*) *p*-anisidine hydrochloride [6] in butan-1-ol–ethanol–water, or (*c*) ninhydrin in butan-1-ol. Solutions were evaporated under reduced pressure. Optical rotations are at *ca.* 20° and, unless otherwise stated, in water.

*2-Acetamido-*1 : 3 : 4 - 6-*tetra*-O-*acetyl-2-deoxy-*α-D-*glucose.*—A stirred mixture of 2-amino-2-deoxy-D-glucose hydrochloride (13 g.) (prepared from crab shells which were kindly provided by Mac Fisheries Ltd.), dry pyridine (100 g.), and acetic anhydride (100 ml.) was heated at 95—100° for 5 hr.; solution was then complete and the mixture was poured into ice-water (*ca.* 1 l.), the acetylated product was extracted with chloroform (3 × 100 ml.), and the combined extracts were washed successively with 2N-hydrochloric acid (2 × 100 ml.), 2N-sodium hydrogen carbonate solution (2 × 100 ml.), and water, and dried ($MgSO_4$). Subsequent concentration gave a brown syrup which was decolorised (charcoal) in hot methanol; evaporation of this solution yielded a pale yellow syrup (19·0 g., 78%) which crystallised. Recrystallisation from ether gave needles, m. p. 138°, $[\alpha]_D$ +91° (*c* 2·0 in $CHCl_3$) (Found: C, 49·5; H, 5·7; N, 3·5. Calc. for $C_{16}H_{23}O_{10}N$: C, 49·4; H, 5·9; N, 3·6%). Hudson and Dale [26] record m. p. 139—140°, $[\alpha]_D$ +93° (in $CHCl_3$).

*2-Acetamido-2-deoxy-*α-D-*glucose.*—Dry ammonia gas was bubbled for 30 min. through an ice-cold solution of 2-acetamido-1 : 3 : 4 : 6-tetra-*O*-acetyl-2-deoxy-α-D-glucose (18 g.) in

[23] Jeanloz and Forchielli, *J. Biol. Chem.*, 1951, **188**, 361.
[24] Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1939, **61**, 1615.
[25] Neuberger, *J.*, 1941, 47.
[26] Hudson and Dale, *J. Amer. Chem. Soc.*, 1916, **38**, 1431.

methanol (50 ml.), and the solution stored at room temperature for 1 hr. Subsequent concentration gave a pale yellow syrup (10 g., 98%) which crystallised on trituration with acetone. After recrystallisation from methanol–ether, the 2-acetamido-2-deoxy-α-D-glucose had m. p. 200°, $[\alpha]_D$ +78° (initial) (*c* 2·34) (Found: C, 43·4; H, 6·9; N, 6·5; Ac, 19·0. Calc. for $C_8H_{15}O_6N$: C, 43·4; H, 6·8; N, 6·3; Ac, 19·5%). White[2] records m. p. 196°, $[\alpha]_D$ +75° (initial).

Reaction of 2-Acetamido-2-deoxy-α-D-glucose with Ethanethiol and Hydrochloric Acid and Separation of the Products.—A mixture of 2-acetamido-2-deoxy-D-glucose (15 g.), concentrated hydrochloric acid (25 ml.), and ethanethiol (20 ml.) was shaken until a homogeneous solution was obtained. After 24 hr., the solution was diluted with methanol (200 ml.) and neutralised with lead carbonate, and the insoluble lead salts were filtered off and washed with hot methanol (200 ml.). Concentration of the combined filtrate and washings yielded a pale yellow syrup (12 g.) which when examined on paper chromatograms with spray (*a*) was observed to contain at least four products [R_F 0·67, 0·48, 0·42, 0·06; solvent (i)]. The syrup was fractionated on a column of cellulose with solvent (i) as mobile phase. Four fractions were obtained.

Fraction 1 gave crystals (5·4 g., 24·3%) of 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal. This, on recrystallisation from methanol–ether, afforded needles with m. p. 132°, $[\alpha]_D$ −29·3° (*c* 1·5) (Found: C, 44·1; H, 7·7; S, 19·8; N, 4·4; Ac, 12·7. Calc. for $C_{12}H_{25}O_5NS_2$: C, 44·0; H, 7·6; S, 19·6; N, 4·3; Ac, 13·3%). Wolfrom and Anno[9] record m. p. 129·5—130·5°, $[\alpha]_D$ −35° and Kent[27] found m. p. 121—122°, $[\alpha]_D$ −24°.

Fraction 2 gave *2-acetamido-2-deoxy-α-D-glucopyranosylthioethane* (1·7 g., 9·5%) which on recrystallisation from methanol–ether had m. p. 228°, $[\alpha]_D$ +175° (*c* 0·8) (Found: C, 45·1; H, 7·2; S, 12·2; N, 5·1; Ac, 15·9. $C_{10}H_{19}O_5NS$ requires C, 45·3; H, 7·2; S, 12·1; N, 5·3; Ac, 16·2%).

Fraction 3 gave needles of *2-acetamido-2-deoxy-β-D-glucopyranosylthioethane* (3·0 g., 16·7%) which on recrystallisation from methanol had m. p. 215°, $[\alpha]_D$ −55° (*c* 1·0) (Found: C, 45·0; H, 7·3; S, 11·9; N, 5·2; Ac, 16·7%).

Fraction 4 gave crystals (4·0 g., 28·8%) of 2-amino-2-deoxy-D-glucose hydrochloride.

2-Acetamido-3:4:6-tri-O-acetyl-2-deoxy-α-D-glucopyranosylthioethane.—A mixture of 2-acetamido-2-deoxy-α-D-glucopyranosylthioethane (fraction 2) (0·1 g.), dry pyridine (2 ml.), and acetic anhydride (2 ml.) was set aside at room temperature for 24 hr. and then poured into ice water (*ca.* 20 ml.). The product was worked up as above and subsequent concentration of the chloroform solution gave the *acetate* as needles (0·11 g.) which after recrystallisation from ether had m. p. 73°, $[\alpha]_D$ +151·5° (*c* 2·79 in MeOH) (Found: C, 49·0; H, 6·3; S, 7·85; N, 3·7. $C_{16}H_{25}O_8NS$ requires C, 49·1; H, 6·4; S, 8·2; N, 3·6%).

2-Acetamido-1:5-anhydro-2-deoxy-D-glucitol.—A mixture of 2-acetamido-2-deoxy-α-D-glucopyranosylthioethane (fraction 2) (0·2 g.) and fresh Raney nickel (1 g.) suspended in absolute ethanol (25 ml.) was heated under reflux for 4 hr. After filtration and concentration a crystalline product (0·085 g.) was obtained which on recrystallisation from methanol–ether gave the *glucitol* as plates, m. p. 190° (Found: C, 46·6; H, 7·2; N, 6·6. $C_8H_{15}O_5N$ requires C, 46·8; H, 7·3; N, 6·8%).

2-Acetamido-3:4:6-tri-O-acetyl-1:5-anhydro-2-deoxy-D-glucitol (36 mg.) was obtained from the triol (40 mg.) as above, and after recrystallisation from ether had m. p. 164° (Found: C, 51·0; H, 6·5; N, 4·1. $C_{14}H_{21}O_8N$ requires C, 50·7; H, 6·3; N, 4·2%).

2-Acetamido-2-deoxy-6-O-triphenylmethyl-β-D-glucopyranosylthioethane.—To a solution of 2-acetamido-2-deoxy-β-D-glucopyranosylthioethane (fraction 3) (0·1 g.) in dry pyridine (5 ml.) at 0° was added triphenylchloromethane (0·2 g.). After being kept overnight at room temperature the crystals (0·077 g.) of triphenylmethanol hydrochloride[26] were filtered off and the filtrate was poured into ice-water (20 ml.). The oil which separated was washed several times with water and then dissolved in methanol (50 ml.). A small amount (0·06 g.) of triphenylmethanol was filtered off, and, on cooling, the filtrate yielded crystals of *2-acetamido-2-deoxy-6-O-triphenylmethyl-β-D-glucopyranosylthioethane* which were collected and dried on a porous tile. After recrystallisation from methanol, they had m. p. 95° (Found: C, 68·7; H, 6·3; S, 6·8; N, 2·4. $C_{19}H_{33}O_5NS$ requires C, 68·6; H, 6·5; S, 6·3; N, 2·8%).

2-Acetamido-3:4:6-tri-O-acetyl-2-deoxy-β-D-glucopyranosylthioethane.—(1) 2-Acetamido-2-deoxy-β-D-glucopyranosylthioethane (fraction 3) (1·0 g.), as above, yielded the *acetate* (1·1 g.) which after recrystallisation from ether had m. p. 181°, $[\alpha]_D$ −38·0° (*c* 1·00 in MeOH) (Found: C, 49·2; H, 6·2; S, 7·7; N, 3·7. $C_{16}H_{25}O_8NS$ requires C, 49·1; H, 6·4; S, 8·2; N, 3·6%).

[27] Kent, *Research*, 1950, **3**, 427.

The m. p. was not depressed on admixture with authentic 2-acetamido-3 : 4 : 6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylthioethane obtained by synthesis as follows.

(2) A mixture of 2-acetamido-1 : 3 : 4 : 6-tetra-*O*-acetyl-2-deoxy-α-D-glucose (2·5 g.) and anhydrous zinc chloride (1·5 g.) in ethanethiol (10 ml.) was shaken at 0° until dissolution was complete (24 hr.). This solution was poured with stirring into a saturated solution of sodium hydrogen carbonate (100 ml.), and the acetate extracted with chloroform (3 × 10 ml.). The combined chloroform extracts were washed with water and dried ($MgSO_4$). Subsequent concentration gave needles (1·0 g.) which after two recrystallisations from ether had m. p. 181°, $[\alpha]_D$ −35·4° (*c* 1·4 in $CHCl_3$) (Found : C, 48·8; H, 6·2; S, 8·0; N, 3·7%).

Methyl 2-Acetamido-3 : 4 : 6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside.—A mixture of 2-acetamido-3 : 4 : 6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylthioethane (1·0 g.), cadmium carbonate (5·0 g.), and mercuric chloride (2·0 g.) in methanol (50 ml.) was heated under reflux with stirring for 4 hr. and then set aside overnight at room temperature. Insoluble material was filtered off and washed with hot methanol (2 × 50 ml.), and the combined filtrate and washings were concentrated to dryness. The residue was dissolved in chloroform (50 ml.), washed with saturated potassium iodide solution (2 × 25 ml.) and then with distilled water (25 ml.), and dried ($MgSO_4$). Subsequent concentration gave crystals (0·5 g.) which after recrystallisation from ether had m. p. 160°, $[\alpha]_D$ −21° (*c* 2·7 in MeOH) (Found : C, 49·7; H, 6·4; N, 3·7; OMe, 8·1. Calc. for $C_{15}H_{23}O_9N$: C, 49·9; H, 6·4; N, 3·9; OMe, 8·6%). Moggridge and Neuberger [7] record m. p. 159°, $[\alpha]_D$ −21° (in MeOH).

Methyl 2-Acetamido-2-deoxy-β-D-glucopyranoside and Methyl 2-Acetamido-2-deoxy-α-D-glucopyranoside.—Dry ammonia gas was passed for 30 min. through an ice-cold solution of methyl 2-acetamido-3 : 4 : 6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (0·4 g.) in dry methanol (25 ml.). The solution was kept at room temperature for 1 hr. and then concentrated to a pale yellow syrup which showed two components on the paper chromatogram [R_F 0·39 and 0·35; solvent (i)] with spray (*a*). The two components were separated by chromatography on a sheet of filter paper (18·25″ × 22·5″) with solvent (i) and extracted from the appropriate parts of the chromatogram with hot methanol, and the methanol extracts were concentrated. The component (R_F 0·39) crystallised (0·20 g.) and after two recrystallisations from methanol–ether yielded methyl 2-acetamido-2-deoxy-β-D-glucopyranoside,[14] m. p. and mixed m. p. 200°, $[\alpha]_D$ −41·7° (*c* 1·2) (Found : C, 46·0; H, 7·2; N, 5·8; OMe, 14·0. Calc. for $C_9H_{17}O_6N$: C, 46·0; H, 7·2; N, 6·0; OMe, 13·2%). Neuberger and Pitt-Rivers [13] record m. p. 195—196°, $[\alpha]_D$ −43°. Zilliken, Rose, Braun, and György [14] record m. p. 204—205°, $[\alpha]_D$ −44·3°. The other component (R_F 0·35) was methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (0·036 g.) which after two recrystallisations from methanol–ether had $[\alpha]_D$ +118° (*c* 0·9), m. p. 189° undepressed on admixture with authentic specimen obtained from 2-acetamido-2-deoxy-D-glucose by Zilliken, Rose, Braun, and György's method [14] (they record m. p. 187—188°, $[\alpha]_D$ +131·5°). Neuberger and Pitt-Rivers [13] record m. p. 188—189°, $[\alpha]_D$ +104°.

Attempted methanolysis [16] of 2-acetamido-3 : 4 : 6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosylthioethane as above resulted in a 100% recovery of the starting material.

Reductive Desulphurisation of 2-Acetamido-2-deoxy-β-D-glucopyranosylthioethane (Fraction 3).—2-Acetamido-2-deoxy-β-D-glucopyranosylthioethane (0·25 g.) was treated with fresh Raney nickel as described above, yielding a crystalline product (0·1 g.), which when examined on paper chromatograms was observed to contain none of the 2-acetamido-1 : 5-anhydro-2-deoxy-D-glucitol. Recrystallisation from methanol–ether gave needles with m. p. 198°, undepressed on admixture with authentic 2-acetamido-2-deoxy-D-glucose and indistinguishable from it on paper chromatograms. An aged specimen of Raney nickel gave the same result.

1 : 1-*Di(ethylthio)ethan-2-ol.*—Glycollaldehyde (1·0 g.) was treated with ethanethiol as above, yielding an oily *product* (1·6 g.) which was twice distilled, b. p. 240° (bath-temp.)/15 mm. (Found : C, 43·9; H, 8·6. $C_6H_{14}OS_2$ requires C, 43·4; H, 8·4%).

Periodate Oxidation Experiments.—In each case, a mixture of *ca.* 0·3M-sodium periodate (2 ml.) and the compound (25—30 mg. accurately weighed) was made up to 25 ml. with water and stored in the dark. Under these conditions, iodine was not liberated, but if larger quantities of either the compound or sodium metaperiodate were used, then iodine was formed (cf. Bonner and Drisko [29]). A blank containing none of the compound was worked concurrently. At intervals, the periodate uptake was estimated by transferring samples (2 ml.) from the oxidation mixture and the blank into mixtures of phosphate buffer (pH 6·98; 25 ml.) and 20% potassium iodide solution (1 ml.), and the liberated iodine titrated with 0·01N-sodium thiosulphate solution,

[28] Helferich, Moog, and Jünger, *Ber.*, 1925, **58**, 872.
[29] Bonner and Drisko, *J. Amer. Chem. Soc.*, 1951, **73**, 3699.

starch being used as indicator.[30] Acid was determined [31] by taking samples (2 ml.) from the oxidation mixture and from the blank, adding ethylene glycol (1 ml.), and after 10 min. titrating it with 0·01N-sodium hydroxide (methyl-red, screened with methylene-blue). Formaldehyde was determined colorimetrically with chromotropic acid according to O'Dea and Gibbon's method,[32] D-glucose being used as standard. The results are given in the following Table.

Results of periodate oxidation experiments.

Compound		½hr.	1 hr.	2 hr.	3 hr.	4 hr.	9 hr.	CH_2O *
2-Acetamido-2-deoxy-D-glucose	Uptake *	0·30	0·50	0·82	1·05	1·17	1·60	
	Acid *	0·10	0·17	0·43	0·50	0·55	0·80	
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside	Uptake	0·89	0·95	1·05	1·15	1·15	1·3	Nil
	Acid	0·00	0·00	0·00	0·00	0·00	0·10	
2-Acetamido-2-deoxy-α-D-glucopyranosylthio-ethane	Uptake	0·95	1·20	1·40	1·60	1·75	2·20	Nil
	Acid	0·10	0·20	0·20	0·20	0·20	0·40	
2-Acetamido-2-deoxy-β-D-glucopyranosylthio-ethane	Uptake	1·60	1·70	1·85	2·05	2·10	2·40	Nil
	Acid	0·10	0·20	0·20	0·20	0·25	0·40	
1 : 1-Di(ethylthio)ethan-2-ol	Uptake	—	1·17	1·35	1·40	1·42	1·63	1·15
	Acid	—	0·00	0·00	0·00	0·00	0·00	
D-Galactose diethyl dithioacetal	Uptake	5·15	5·15	5·15	5·4	5·65	5·75	
	Acid	3·82	3·85	3·95	3·95	4·05	4·12	
D-Galactose diethyl dithioacetal penta-acetate	Uptake	—	0·45	0·57	0·59	0·70	0·75	
1 : 5-Anhydro-2-acetamido-2-deoxy-D-glucitol	Uptake	0·60	0·90	1·10	1·15	1·15	1·20	Nil
	Acid	0·00	0·00	0·00	0·00	0·00	0·00	
2-Acetamido-2-deoxy-D-glucose diethyl dithio-acetal	Uptake	5·95	6·02	6·20	6·20	6·20	6·30	1·03
	Acid	3·05	3·20	3·20	3·20	3·20	3·20	

* Moles/mole of compound oxidized.

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[30] Neumüller and Vasseur, *Arkiv. Kemi*, 1953, **5**, 235.
[31] Halsall, Hirst, and Jones, *J.*, 1947, 1427.
[32] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.