384. *Amino-sugars and Related Compounds. Part III.* Acid Reversion of 2-Acetamido-2-deoxy-D-glucose (N-Acetyl-D-glucosamine).*

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Prolonged exposure of 2-acetamido-2-deoxy-D-glucose to moist hydrogen chloride vapour causes the formation of a series of oligosaccharides (reversion). Two disaccharides have been isolated from the mixture. Evidence is presented which indicates their structures to be 2-acetamido-6-*O*-(2-acetamido-2-deoxy-α- and -β-D-glucopyranosyl)-2-deoxy-D-glucose.

The polymerisation (reversion) of D-glucose under certain acidic conditions to yield oligosaccharides has been well studied. Reversion may occur in acidic aqueous solutions [1] or on prolonged exposure to moist hydrogen chloride vapour.[2] The latter method has been adopted in these investigations; it has also been successfully applied to D-xylose [3] and L-rhamnose.[4]

In preliminary experiments it was observed that 2-amino-2-deoxy-D-glucose hydrochloride was unaffected by prolonged exposure to moist hydrogen chloride vapour. This is probably due to the positive charge acquired by the amino-group in acid media which electrostatically shields the glycosidic centre and prevents the approach of hydrions. Thus, under normal reaction conditions, 2-amino-2-deoxy-D-glucose hydrochloride resists glycosidation when treated with methanolic hydrogen chloride [5] and methyl 2-amino-2-deoxy-D-glucopyranoside is hydrolysed very slowly by aqueous acid.[5] The whole amino-sugar molecule appears to be electrostatically shielded by the $^{-}NH_3^{+}$ group, since 2-amino-2-deoxy-D-glucose hydrochloride is not incorporated into the oligosaccharides produced when a mixture of D-galactose and the amino-sugar derivative is exposed to moist hydrogen chloride vapour.[4]

Exposure of 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) to moist hydrogen chloride vapour for four weeks gave a series of oligosaccharides from which two of the lower members *A* and *B* were separated and purified by chromatography on charcoal–Celite. Evidence is presented which indicates that *A* is 2-acetamido-6-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-2-deoxy-D-glucose and that *B* is 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose.

The behaviour of *A* and *B* in charcoal–Celite and paper chromatography was similar to that of di-*N*-acetylchitobiose [6] and indicated them to be disaccharides; this was substantiated by elemental analysis and proved by hypoiodite oxidation. Both *A* and *B* were isolated crystalline and both exhibited slight downward mutarotation on dissolution in water.

A and *B* responded to the Morgan–Elson test [7] for *N*-acylamino-sugars and gave colours with intensities equivalent to 127% and 136% respectively of that given by an equimolar quantity of 2-acetamido-2-deoxy-D-glucose. These observations indicate that the glycosidic linkage in both *A* and *B* is 1 ⟶ 6 since Jeanloz and Trémège [8] have found that whereas 2-acetamido-2-deoxy-6-*O*-methyl-D-glucose yields a colour equivalent to 160% of that given by an equimolar amount of 2-acetamido-2-deoxy-D-glucose, the 3-*O*- and 4-*O*-methyl derivatives produce respectively 100% and 3% of the colour. Related results have been obtained by Kuhn, Baer, and Gauhe [9] with certain disaccharides.

* Part II, *J.*, 1957, 81.

[1] Thompson, Anno, Wolfrom, and Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309.
[2] Ricketts, *J.*, 1954, 4031.
[3] Bishop, *Canad. J. Chem.*, 1956, **34**, 1255.
[4] Foster and Horton, unpublished results.
[5] Moggridge and Neuberger, *J.*, 1938, 745; cf. Foster and Stacey, *Adv. Carbohydrate Chem.*, 1952, **7**, 247.
[6] Barker, Foster, Stacey, and Webber, *Chem. and Ind.*, 1957, 208.
[7] Morgan and Elson, *Biochem. J.*, 1934, **28**, 988.
[8] Jeanloz and Trémège, *Fed. Proc.*, 1956, **15**, 282.
[9] Kuhn, Baer, and Gauhe, *Chem. Ber.*, 1954, **87**, 1138, 1553.

Caution must be exercised in the interpretation of the reaction of *N*-acetylated amino-sugar derivatives with periodate since an adequate explanation of the reaction of 2-acetamido-2-deoxy-D-glucose itself is lacking. However it may reasonably be inferred that, since *A* consumes 5·95 mols. of oxidant, yielding 3·6 mols. formic acid but no formaldehyde, the non-reducing moiety is not a furanoside and the glycosidic linkage is possibly 1⟶6.

More reliable information on the position of the glycosidic linkage in *A* and *B* was obtained by periodate oxidation of their reduced derivatives. With sodium borohydride *A* and *B* readily yielded 2-acetamido-6-*O*-(2-acetamido-2-deoxy-α- and -β-D-glucopyranosyl)-2-deoxy-D-glucitol, (*A'*) and (*B'*) respectively. Both *A'* and *B'* rapidly (5 min.) consumed approximately 2 mols. of periodate with the concomitant release of approximately 1 mol. of formic acid, and slowly ($t_{\frac{1}{2}}$, 3—4 hr.) consumed a further mol. of oxidant. Formaldehyde was not liberated in the oxidation of *A'* and to an insignificant extent (<0·02 mol.) in the case of *B'*. These results, which are consistent with the structures allocated to *A'* and *B'*, indicate a rapid reaction of the acyclic moieties with periodate and a slow cleavage of the pyranoside moieties. This difference in reactivity would be expected from the behaviour of the model compounds 2-acetamido-2-deoxy-D-glucitol, which consumed 3 mols. of periodate within 5 min. with the concomitant release of formic acid (2 mols.) and formaldehyde (1 mol.), and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside which slowly ($t_{\frac{1}{2}}$ *ca.* 2 hr.) consumed 1 mol. of oxidant. Over-oxidation was not observed with the non-reducing amino-sugar derivatives. Reduction of the product, presumably α-acetamido-α-deoxy-L-glyceraldehyde, obtained by periodate oxidation of 2-acetamido-2-deoxy-D-glucitol, gave 2-acetamidopropane-1 : 3-diol; acidic hydrolysis of this compound gave the amino-diol hydrochloride.

TABLE 1. *Optical rotation data for A', B', and related compounds.*

Compound	$[M]_D$ [a]	Compound	$[M]_D$ [a]	2A [b]	2B [b]
A'	+328°	*B'*	−94°	422	234
Me 2-acetamido-2-deoxy-α-D-gluco-pyranoside [10]	+308	Me 2-acetamido-2-deoxy-β-D-gluco-pyranoside [11]	−104	412	204
Isomaltitol [12]	+306	Gentiobiitol [13]	−82	388	224

[a] Rotations determined in water. [b] Calc. by Hudson's method.[14] 2A = contribution of $C_{(1)}$; 2B = contribution of remainder of molecule.

Attempts to prove the molecular weight of compound *A* by determination of the 2-amino-2-deoxy-D-glucose liberated on acidic hydrolysis of the reduced derivative *A'* were unsuccessful. Under normal conditions of acidic hydrolysis (2N-acid at 100° for 3—4 hr.), 32% release of the amino-sugar was observed (calc. 51%), which did not significantly increase on prolonged hydrolysis, probably because hydrolysis yielded, in addition to 2-amino-2-deoxy-D-glucose, an acid-resistant product (presumably de-*N*-acetylated *A'*). This phenomenon has been described in detail in Part II.

Evidence which indicates the configuration of the linkages in *A* and *B* to be respectively α and β was obtained from several sources.

The optical rotation data in Table 1 illustrate the relation of *A'* and *B'* to the anomeric methyl 2-acetamido-2-deoxy-D-glucopyranosides and to the D-glucose derivatives isomaltitol and gentiobiitol. Application of Hudson's isorotation rules to *A'* and *B'* gave 2A and 2B values closely similar to those obtained for the related compounds in Table 1, again emphasising the structural relation.

The frequencies (cm.$^{-1}$) of the infrared absorption in the range 750—1000 cm.$^{-1}$ for *A*, *B*, and related compounds are shown in Table 2. The type 2a absorption at *ca.* 840 cm.$^{-1}$

[10] Kuhn, Zilliken, and Gauhe, *Chem. Ber.*, 1953, **86**, 466.
[11] Kuhn and Baer, *ibid.*, p. 724.
[12] Wolfrom, Thompson, O'Neill, and Galkowski, *J. Amer. Chem. Soc.*, 1952, **74**, 1062.
[13] Wolfrom and Gardner, *ibid.*, 1943, **65**, 750.
[14] Hudson, *ibid.*, 1909, **31**, 66.

which is indicative [15] of α-glucopyranosides is shown by methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, *A*, and *A'*, but not by the corresponding isomeric compounds methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, *B*, and *B'*. Since *A'* contains only one glycosidic centre the absorption at 843 cm.$^{-1}$ substantiates the allocation of an α-linkage to both *A* and *A'*. No inference can be drawn from the absorption of *B'* at 900 cm.$^{-1}$; although it falls near to the range (891 ± 7 cm.$^{-1}$) of the type 2b absorption indicative [15] of β-glucopyranosidic linkages, 2-acetamido-2-deoxy-D-glucitol absorbs in this range (892 cm.$^{-1}$). The absence of absorption at *ca.* 840 cm.$^{-1}$ in *B'* may be taken as negative evidence for the presence of a β-linkage.

It has been observed [16] that whilst glycosidic derivatives of 2-acetamido-2-deoxy-α-D-glucopyranose are ineffective as growth factors for *Lactobacillus bifidus* var. *Penn.*, the β-anomers are frequently active. Consistently with these observations, *A* was found [17] to be completely inactive; apparently the organism is unable to hydrolyse the disaccharide since 2-acetamido-2-deoxy-D-glucose has weak growth-factor activity. On the other

TABLE 2. *Frequencies (cm.$^{-1}$) of infrared absorptions (750—1000 cm.$^{-1}$) of* A, B, *and related compounds.*

Me 2-acetamido-2-deoxy-α-D-glucopyranoside [a]	951s	925w	896s	857w	840m	778w	765w, 758m
Me 2-acetamido-2-deoxy-β-D-glucopyranoside [a]	—	935m	896m, 860m	—	—	—	—
A	973s	—	919m	861w	844m, 836w	776s	—
B	961m	945m	892w	860vw	—	778w	—
A'	964m	924m	892m	860sh	834m	775sh	750sh
B'	955m	—	900m	—	—	—	—
2-Acetamido-2-deoxy-D-glucitol	966m	939m	892m	876s	—	—	761w

[a] Data taken from ref. 15. w = weak; m = medium; s = strong; v = very; sh = shoulder.

hand, *B* showed activity at quite a high level (200 μg./unit). For comparison one of the most active growth factors so far discovered for *L. bifidus* var. *Penn.*, 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucose,[16] has an activity of 80 μg./unit.

The evidence presented above indicates that the predominant disaccharides formed in the acid reversion of 2-acetamido-2-deoxy-D-glucose are linked α1⟶6 and β1⟶6. In their work on the acid reversion of D-glucose, using the aqueous acid method, Wolfrom *et al.*[1] found that isomaltose (α1⟶6 link) and gentiobiose (β1⟶6 link) were formed in approximately equal amounts but far in excess of any other disaccharide. Ricketts and Rowe [18] observed that the polymer produced on reversion of D-glucose contained predominantly 1⟶6 links.

EXPERIMENTAL

Unless otherwise stated, chromatography was performed on Whatman No. 1 paper by irrigation with the organic phase of a butanol–ethanol–water–ammonia (40 : 10 : 49 : 1) solvent system; R_F values refer to this system. Reducing sugars were detected with aniline hydrogen phthalate,[19] non-reducing carbohydrates with the silver nitrate–sodium hydroxide reagent,[20] and compounds containing free amino-groups with ninhydrin.

Acid Reversion of 2-Acetamido-2-deoxy-D-glucose.—2-Acetamido-2-deoxy-D-glucose (10 g.) was exposed to moist hydrogen chloride [2] for 4 weeks. Paper chromatography of the product revealed a series of reducing oligosaccharides (of which the lower members *A*, *B*, *C*, and *D* had R_F values 0·11, 0·08, 0·06, and 0·04 respectively), together with 2-amino-2-deoxy-D-glucose and its *N*-acetyl derivative. Negligible amounts of ninhydrin-positive material with R_F value

[15] Barker, Bourne, Stacey, and Whiffen, *J.*, 1954, 171.
[16] Zilliken, Smith, Rose, and György, *J. Biol. Chem.*, 1954, **208**, 299; 1955, **217**, 79.
[17] György, Norris, and Rose, *Arch. Biochem. Biophys.*, 1954, **48**, 193.
[18] Ricketts and Rowe, *J.*, 1955, 3809.
[19] Partridge, *Nature*, 1949, **164**, 443.
[20] Trevelyan, Procter, and Harrison, *ibid.*, 1950, **166**, 444.

less than that of 2-amino-2-deoxy-D-glucose was detected. The oligosaccharides produced on acidic hydrolysis of chitosan [6] react strongly with ninhydrin. *A* and *C* gave an identical characteristic reddish-brown colour with aniline hydrogen phthalate; *B* and *D* gave an identical grey-brown colour.

Fractionation of the saccharide mixture on a charcoal–Celite column [21] (5·5 × 30 cm.), using gradient elution [22] with aqueous ethanol (0 ⟶ 35% in 9 l.), led to the isolation of *A* (0·733 g.) and *B* (0·303 g.) which appeared homogeneous by paper chromatography. Fractions which contained 2-acetamido-2-deoxy-D-glucose, *A*, and *C* were strongly dextrorotatory, but those containing *B* and *D* had negligible optical activity. *B* emerged from the column before *A*, and *D* before *C*. In separate experiments it was shown that saccharides other than *A*, *B*, *C*, and *D*, and presumably of higher molecular weight, were present. None of these was isolated pure.

Crystallisation of *A* from ethanol occurred during 8 months and after recrystallisation from the same solvent 2-acetamido-6-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-2-deoxy-D-glucose was obtained, having m. p. 215°, $[M]_D^{13}$ +593° (initial, by extrapolation) ⟶ +564° (45 min.) ⟶ +530° (equilibrium, 24 hr.) (*c* 1·0 in H_2O), M_G value [23] 0·19 (borate buffer,[24] pH 10) (Found: C, 45·2; H, 6·5; N, 6·6. $C_{16}H_{28}O_{11}N_2$ requires C, 45·3; H, 6·6; N, 6·6%).

After crystallisation from ethanol–ether 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (*B*) was obtained, with m. p. 200° (decomp.), $[M]_D^{15·5}$ +51° (initial, by extrapolation) ⟶ +43° (45 min.) ⟶ +27° (equilibrium, 24 hr.) (*c* 1·2 in H_2O), M_G 0·18 (Found: C, 45·1; H, 6·9; N, 6·5%).

Both *A* and *B* were very hygroscopic and on acidic hydrolysis *A* gave 2-amino-2-deoxy-D-glucose (which was isolated as the crystalline hydrochloride), and a second product (R_F 0·05), presumably the corresponding de-*N*-acetylated disaccharide (see Part II [25]).

Hypoiodite Oxidation of A *and* B.—Both *A* and *B* were oxidised by hypoiodite at 2° under essentially the conditions described by Jeanloz and Forchielli; [26] 2-acetamido-2-deoxy-D-glucose was used as control. Reaction was complete within 12 hr., although *B* was oxidised more slowly than was *A* or the control. On the assumption of one reducing group per saccharide molecule the following mol. wts. were obtained: *A*, 435; *B*, 420 (Calc. for $C_{16}H_{28}O_{11}N_2$: 424) [control, 221 (Calc. for $C_8H_{15}O_6N$: 221)].

Behaviour of A *and* B *in the Morgan–Elson* [7] *Test.*—Aliquot parts (1 ml.) of 0·01% aqueous solutions of *A* and *B* were introduced separately into special graduated tubes [27] and treated successively with sodium carbonate and *p*-dimethylaminobenzaldehyde according to the procedure of Aminoff, Morgan, and Watkins; [28] 2-acetamido-2-deoxy-D-glucose was used as control. The absorptions of the coloured derivatives were measured with a "Spekker" photoelectric absorptiometer (H760, Hilger and Watts Ltd.), in 1 cm. cells with Ilford No. 605 filters (maximal transmission at 550 mμ). *A* and *B* gave, respectively, colour intensities equivalent to 127% and 136% of that produced by an equimolar amount of the control. Under the same conditions 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (di-*N*-acetylchitobiose [6]) gave 3% of the colour produced by an equimolar amount of 2-acetamido-2-deoxy-D-glucose. The absorption spectra of the coloured derivatives formed from *A*, *B*, and 2-acetamido-2-deoxy-D-glucose were closely similar in form in the range 460—640 mμ.

Reduction of A *and* B.—A solution of *A* (282 mg.) in water (20 ml.) was treated with sodium borohydride (42·2 mg.) in water (5 ml.). After 3 hr. the solution was acidified with dilute acetic acid and de-ionised with Amberlite resins IR-120 (H^+) and IRA-400 (HO^-). Evaporation of the ion-free solution gave a syrup which crystallised from 90% ethanol, to give 2-*acetamido*-6-O-(2-*acetamido*-2-*deoxy*-α-D-*glucopyranosyl*)-2-*deoxy*-D-*glucitol hydrate* (*A'*) (136 mg., 48%), m. p. 116° (evolution of gas), $[M]_D$ +328° (*c* 0·1 in H_2O), M_G 0·54 (borate buffer,[24] pH 10) (Found: C, 43·5; H, 7·3; N, 6·4. $C_{16}H_{30}O_{11}N_2,H_2O$ requires C, 43·2; H, 7·2; N, 6·3%). *A'* which was extremely hygroscopic could be dried to constant weight at 46° *in vacuo* over

[21] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.
[22] Lindberg and Wickberg, *Acta Chem. Scand.*, 1954, **8**, 569.
[23] Foster, *J.*, 1953, 982.
[24] Foster, Newton-Hearn, and Stacey, *J.*, 1956, 30.
[25] Foster, Horton, and Stacey, *J.*, 1957, 81.
[26] Jeanloz and Forchielli, *J. Biol. Chem.*, 1951, **188**, 361.
[27] Belcher, Nutten, and Sambrook, *Analyst*, 1954, **79**, 201.
[28] Aminoff, Morgan, and Watkins, *Biochem. J.*, 1952, **51**, 379.

P_2O_5. Elevation of the temperature to 135° caused a 4·2% loss in weight (calc. loss 4·1% for a monohydrate).

Compound *B* (64·6 mg.) was reduced to give, after crystallisation from alcohol, 2-*acetamido*-6-O-(2-*acetamido* 2-*deoxy*-β-D-*glucopyranosyl*)-2-*deoxy*-D-*glucitol* (*B'*) (22·7 mg., 35%), m. p. 201—202°, $[M]_D$ −94° (*c* 2·1 in H_2O), M_G 0·57 (Found: C, 45·2; H, 7·3; N, 6·2. $C_{16}H_{30}O_{11}N_2$ requires C, 45·1; H, 7·0; N, 6·6%).

Acidic Hydrolysis of 2-*Acetamido*-6-O-(2-*acetamido*-2-*deoxy*-α-D-*glucopyranosyl*)-2-*deoxy*-D-*glucitol* (A').—A solution of *A'* (1 mg.) in 2N-hydrochloric acid (1 ml.) was heated at 100°, then neutralised with N-sodium carbonate, and the volume made up to 10 ml. 2-Amino-2-deoxy-D-glucose was determined in aliquot parts (1 ml.) of this solution by the procedure given in detail in Part II.[25] Maximum release of amino-sugar was 32% (calc., 51%) which occurred within 4 hr., and did not significantly increase after a further 4 hr.

Periodate Oxidations.—(a) 2-*Acetamido*-6-O-(2-*acetamido*-2-*deoxy*-α-D-*glucopyranosyl*)-2-*deoxy*-D-*glucose* (A). A solution containing sodium metaperiodate (1·25 millimoles) and *A* (20 mg.) in water (50 ml.) was stored at room temperature in the dark. The consumption of periodate was followed in the usual way and found to be complete at 5·95 mols. (mean of 2 results) after 72 hr. After 88 hr., 3·6 mols. of formic acid had been formed, which rose to 3·87 after 167 hr. No formaldehyde was liberated (dimedone).

(b) 2-*Acetamido*-6-O-(2-*acetamido*-2-*deoxy*-α-D-*glucopyranosyl*)-2-*deoxy*-D-*glucitol* (A'). *A'* (30 mg.), when treated with periodate as in (a), consumed 1·95 mols. of oxidant within 5 min. and released 1·04 mols. of formic acid during 1 hr. After 24 hr. 3·01 mols. of oxidant had been consumed with no further increase in the formic acid production. Formaldehyde could not be detected (dimedone). No over-oxidation was observed after 24 hr.

Paper chromatography of the products revealed a component [R_F 0·63; organic phase of butanol–water–acetic acid (4 : 5 : 1)] with behaviour identical with that of α-acetamido-α-deoxy-L-glyceraldehyde

(c) 2-*Acetamido*-6-*O*-(2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*)-2-*deoxy*-D-*glucitol* (B'). *B'* (15·5 mg.), when treated with periodate as in (a), rapidly consumed 1·9 mols. of oxidant with the release of 0·81 mol. of formic acid. After 24 hr. 3·1 mols. of oxidant had been consumed, and 0·86 mol. of formic acid produced, with a trace of formaldehyde. In separate experiments the formaldehyde produced was <0·02 mol. as determined by the chromotropic acid method.[29]

(d) 2-*Acetamido*-2-*deoxy*-D-*glucitol*. A solution of 2-acetamido-2-deoxy-D-glucitol (2·1 g., 9·6 millimoles) was oxidised with 0·9N-periodic acid (56 ml., 50 millimoles) at room temperature for 15 min. After neutralisation with barium carbonate and centrifugation the supernatant liquid was hydrogenated in the presence of Raney nickel (W2) at 100°/75 atm. Catalyst was removed and the filtrate de-ionised with Amberlite resins IR-120 (H^+) and IRA-400 (HO^-). Evaporation of the solution and recrystallisation (twice) of the residue (1·08 g., 70%) from methanol–ethyl acetate gave 2-*acetamidopropane*-1 : 3-*diol*, m. p. 89—90° (Found: C, 45·1; H, 8·3; N, 10·7. $C_5H_{11}O_3N$ requires C, 45·1; H, 8·3; N, 10·5%).

In a parallel experiment, paper chromatography of the supernatant liquid before hydrogenation, with the organic phase of butanol–water–acetic acid (4 : 5 : 1), showed a single component (R_F 0·63), presumably α-acetamido-α-deoxy-L-glyceraldehyde.

Hydrolysis of 2-acetamidopropane-1 : 3-diol (0·255 g.) in N-hydrochloric acid (30 ml.) at 100° for 2 hr., followed by evaporation of the solution and recrystallisation of the residue from ethanol–ethyl acetate, gave 2-*aminopropane*-1 : 3-*diol hydrochloride* (0·208 g., 87%), m. p. 104° (Found: N, 10·5; Cl, 27·8. $C_3H_{10}O_2NCl$ requires N, 11·0; Cl, 27·8%).

Infrared Spectra.—The spectra were determined by the KCl or KBr disc method.

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