871. *Amino-sugars and Related Compounds. Part I. The Deamination of* D-*Glucosamine Hydrochloride.*

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Deamination of D-glucosamine hydrochloride with sodium nitrite and hydrochloric acid yields mainly chitose. Reduction of chitose affords crystalline 2 : 5-anhydro-D-mannitol the structure of which is proved, thus confirming the structure of chitose as 2 : 5-anhydro-D-mannose.

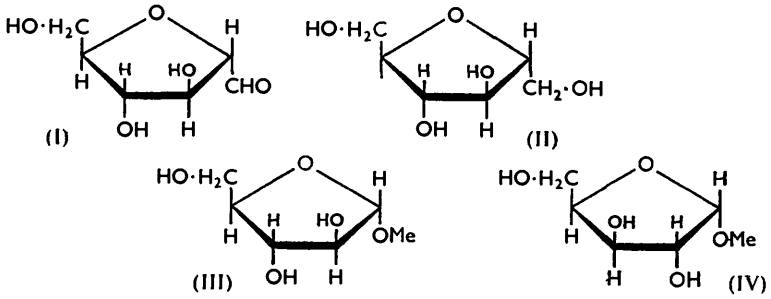
Chitose (2 : 5-anhydro-D-mannose) (I), the amorphous product obtained by the action of nitrous acid on D-glucosamine hydrochloride (2-amino-2-deoxy-D-glucose hydrochloride) has long been known.[1] The presence of the 2 : 5-anhydro-ring was inferred[2] when 5-acetoxymethylfuran-2-carboxylic acid was obtained on treatment of chitonic acid (2 : 5-anhydro-D-mannonic acid) with acetic anhydride and sodium acetate. The stereochemical relationship of chitose and chitonic acid has been substantiated recently.[3] Allocation of the D-mannose configuration to chitose followed from the demonstration,[4] by indirect methods, that " isosaccharic acid " (obtained from chitose or chitonic acid by oxidation with nitric acid) was 2 : 5-anhydro-D-mannosaccharic acid. We report herein evidence which substantiates the structure of chitose as 2 : 5-anhydro-D-mannose.

[1] Ledderhose, *Z. physiol. Chem.*, 1880, **4**, 139.
[2] Fischer and Andreae, *Ber.*, 1903, **36**, 2587.
[3] Grant, *New Zealand J. Sci. Technol.*, 1956, **37**, 509.
[4] Levene and La Forge, *J. Biol. Chem.*, 1915, **21**, 345, 351; Levene, *ibid.*, 1918, **36**, 89.

In connection with the deaminative degradation of ψ-heparin [5] it became essential to ascertain the precise course of the deamination reaction and as model compounds D-glucosamine hydrochloride and methyl α- and β-D-glucosaminide (methyl 2-amino-2-deoxy-α- and -β-D-glucopyranoside) have been studied. The results obtained with D-glucosamine hydrochloride are described herein.

D-Glucosamine hydrochloride was deaminated in the presence of sodium nitrite and hydrochloric acid. The course of the reaction could be followed conveniently by zone electrophoresis (ionophoresis *) on paper using an alkaline borate buffer.[6] Chitose was observed to be the main product of reaction along with traces of unidentified substances. D-Glucose, D-mannose, and D-arabinose, which have characteristic ionophoretic behaviour, appeared not to be formed (cf. Grant [3]). Deamination of *trans*-2-amino*cyclo*hexanol, in which the amino-group is predominantly equatorial, yields *cyclo*pentylmethanol in high yield.[7] There is a close analogy between this reaction and the deamination of D-glucosamine hydrochloride; thus, since chitose is the predominant product of the deamination of D-glucosamine hydrochloride, the amino-sugar must be present in the reaction solution as, or must react principally in, the pyranose form, the preferred conformation of which would have the amino-group in an equatorial position. The mechanism of deamination of D-glucosamine hydrochloride suggested by Peat [8] would therefore appear to be the most probable and would be expected to lead to inversion at $C_{(2)}$. The presence of a small amount of the furanose and acyclic forms in equilibrium with the pyranose form of D-glucosamine hydrochloride in solution might account for some or all of the trace products since on deamination they would be expected to give products other than chitose.

In aqueous solutions containing sodium carbonate, sodium hydrogen carbonate, pyridine, ammonia, or methyldi-*n*-octylamine, chitose was converted into a complex mixture containing a main component and traces of nine others. This behaviour, which is being investigated further, emphasises that alkaline conditions must be avoided in the preparation of chitose. It is unlikely that chitose is affected under the conditions of ionophoresis since it has been noted [9] that disaccharides (*e.g.*, nigerose) which are very sensitive to alkali are not decomposed during ionophoresis in a borate buffer at pH 10 although they undergo decomposition in this buffer when not adsorbed on paper.



Chitose is amorphous and relatively few crystalline derivatives are known; [3,8] however, reduction of chitose with sodium borohydride or preferably with hydrogen and Raney nickel gave a good yield of crystalline 2 : 5-anhydro-D-mannitol (II) : ionophoresis revealed the presence of other products also and it is possible that they may have originated by alkaline decomposition of chitose during reduction. Alkaline conditions develop in aqueous

* Subsequent references to ionophoresis imply the use of paper as the electrolyte support and a borate buffer (pH 10) as the electrolyte unless otherwise stated.

[5] Foster, Martlew, and Stacey, *Chem. and Ind.*, 1953, 825; Foster and Huggard, *Adv. Carbohydrate Chem.*, 1955, **10**, 335.

[6] Foster, Newton-Hearn, and Stacey, *J.*, 1956, 30, and references cited therein.

[7] McCasland, *J. Amer. Chem. Soc.*, 1951, **73**, 2293; cf. Curtin and Schmukler, *ibid.*, 1955, **77**, 1105.

[8] Peat, *Adv. Carbohydrate Chem.*, 1946, **2**, 37.

[9] Barker, Bourne, and O'Mant, unpublished data cited by Barker, Bourne, and Theander, *J.*, 1955, 4276.

solutions of sodium borohydride and it is of interest that the Raney nickel, prepared by the well known procedure,[10] gave an alkaline reaction when stirred with phenolphthalein although the aqueous washings from the same catalyst were neutral.

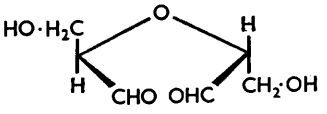
Ionophoresis of the furanoside derivatives shown in the Table indicates that vicinal *cis*- but not vicinal *trans*-hydroxyl and -hydroxymethyl groups in five-membered rings react strongly with borate ions. The low M_G value (0·04) of 2 : 5-anhydro-D-mannitol is consistent with the formula (II) which contains only *trans*-related vicinal hydroxyl and

*M_G values of some furan derivatives.**

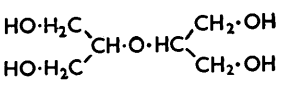
Derivative	M_G value	Derivative	M_G value
Methyl α-D- and α-L-arabofuranoside (III)	0·035	Methyl α-D-xylofuranoside (IV)	0·56
Methyl β-D- and α-L-arabofuranoside	0·035	Methyl β-D-xylofuranoside	0·33
		2 : 5-Anhydro-D-mannitol (II)	0·04

* Ionophoresis was carried out with the apparatus and technique described by Foster (*Chem. and Ind.*, 1952, 1050), on Whatman No. 3 paper in sodium borate (cf. ref. 6) (pH 10) for 2 hr. at a potential gradient of 22 v/cm. Detection was by alkaline silver solution (Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444).

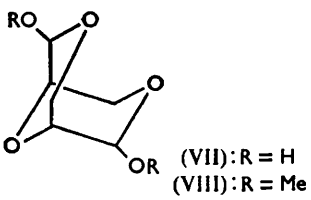
hydroxymethyl groups. Further data supporting the structure (II) for 2 : 5-anhydro-D-mannitol followed from periodate oxidation. The anhydro-derivative (II) rapidly consumed one mol. of periodate without the formation of formic acid or formaldehyde. The rapid uptake is, however, surprising, since the related compound 1 : 4-anhydro-D-threitol, which exhibits the expected behaviour of a vicinal *trans*-glycol, is only slowly oxidised by periodate.[11] The dialdehyde (V) thus obtained from 2 : 5-anhydro-D-mannitol was amorphous but migrated as a single zone in ionophoresis and was optically active. The



(V)



(VI)



(VII) : R = H
(VIII) : R = Me

latter property substantiates the mannose configuration at $C_{(2)}$ since oxidation of 2 : 5-anhydro-1 : 6-dibenzoyl-D-glucitol with lead tetra-acetate [12] gave an optically inactive dialdehyde. Aqueous solutions of the dialdehyde (V) showed no ultraviolet carbonyl absorption and gave no carbonyl wave on polarography, thus resembling the dialdehyde obtained [13] on periodate oxidation of methyl α-D-glucopyranoside. The latter dialdehyde, however, has one carbonyl group masked by intramolecular hemiacetal formation and the second hydrated. The dialdehyde (V) gave unsatisfactory analyses but from models it appears possible for both carbonyl groups to be involved in intramolecular hemiacetal formation to give a bicyclic system (VII). Each carbonyl-oxygen atom in (V) is separated from a hydroxyl-oxygen atom by 5 atoms and it is known that 5-hydroxypentanal exists predominantly in the hemiacetal form.[14] With methanolic hydrogen chloride the dialdehyde (V) gave a product which, on the basis of methoxyl content and non-reducing character, probably has structure (VIII). The formation of a methyl glycosidic but not a dimethyl acetal type of product in this reaction is strong evidence for the dihemiacetal structure (VII).

Reduction of the dialdehyde (V) with sodium borohydride gave the optically inactive ether (VI), isolated as tetra-acetate. This is also obtained, together with two isomeric

[10] Adkins and Pavlic, *J. Amer. Chem. Soc.*, 1947, **69**, 3039.
[11] Klosterman and Smith, *ibid.*, 1952, **74**, 5336.
[12] Hockett, Zief, and Goepp., *ibid.*, 1946, **68**, 935.
[13] Hurd, Baker, Holysz, and Saunders, *J. Org. Chem.*, 1953, **18**, 186.
[14] Hurd and Saunders, *J. Amer. Chem. Soc.*, 1952, **74**, 5324.

products, by condensation of two molecules of glycerol. Three such products are known [15] but their structures are undetermined.

It is clear that the reactions of 2 : 5-anhydro-D-mannitol described above can only be accommodated by the structure (II). Since it is most unlikely that any stereochemical change occurs in the reduction of chitose to 2 : 5-anhydro-D-mannitol the structure of chitose is substantiated.

Experimental

Deamination of D-*Glucosamine Hydrochloride.*—The following optimum conditions were established. A solution of D-glucosamine hydrochloride (10·8 g.) in concentrated hydrochloric acid (22·5 ml.) and water (977·5 ml.) was set aside at room temperature for 4—6 hr. (to reach mutarotational equilibrium), then treated with sodium nitrite (27·6 g.) in water (1 l.) at room temperature, and the deamination was followed polarimetrically. Optical rotation was constant after 30 min. and ionophoresis at this point with the apparatus and technique previously described [16] with an acetate buffer (pH 5·0) revealed the absence of D-glucosamine (ninhydrin).

The solution was aerated for 2 hr. under reduced pressure and then passed down a column (60 × 3·3 cm.) of cation-exchanger (Amberlite IR-120, H^+ form; 500 g.). The eluate (3·2 l.) was immediately freed from acid by shaking it several times with a 5% solution of methyldi-*n*-octylamine [17] in chloroform. Excess of amine was removed by further extractions with chloroform and finally with ether. The aqueous solution was concentrated to 250 ml. at <35° (bath)/12—15 mm. and used directly in the reductions. Ionophoresis in borate buffer (pH 10) of the solution after each stage in the purification revealed no change in the content of reducing sugars. In separate experiments chitose was isolated as a viscous syrup.

Reduction of 2 : 5-*Anhydro*-D-*mannose* (*Chitose*).—(*a*) The solution of chitose described above was reduced by hydrogen (40 atm.) in the presence of W-2 Raney nickel catalyst [10] (25 ml. of suspension). The temperature was raised to 95—100° during 4—5 hr. and the solution then left to cool overnight and filtered through carbon. The filtrate, when evaporated, gave a colourless syrup (7·7 g.), ionophoresis of which, and also paper chromatography [irrigation with the organic phase of butanol–ethanol–water–ammonia (40 : 10 : 49 : 1)], revealed traces of substances other than 2 : 5-anhydro-D-mannitol. The syrup crystallized when seeded and, after crystallisation from ethanol–ether and finally ethanol, 2 : 5-*anhydro*-D-*mannitol* (6·1 g.) was obtained having m. p. 100—101°, $[\alpha]_D^{20}$ +58·2° (*c*, 1·37 in H_2O) (Found: C, 44·0; H, 7·2. $C_6H_{12}O_5$ requires C, 43·9; H, 7·4%).

(*b*) A solution of chitose [from D-glucosamine hydrochloride (1·08 g.)] in water (60 ml.) was treated with sodium borohydride (0·24 g.). The solution gave a strongly alkaline reaction and, when a negative Benedict reaction was obtained, excess of sodium borohydride was decomposed with carbon dioxide and the solution evaporated to dryness at 40° (bath)/12—15 mm. The residue was extracted with ethanol (2 × 20—25 ml.), and the combined extracts were evaporated to a pale yellow syrup. Ionophoresis of this product revealed appreciable amounts of substances other than 2 : 5-anhydro-D-mannitol which latter could be isolated from the syrup in low yield as described in (*a*).

Behaviour of Chitose in Alkaline Media.—Sodium carbonate, sodium hydrogen carbonate, pyridine, and ammonia were severally added to solutions of chitose prepared as described above. Ionophoresis of the solutions after 24 hr. at room temperature showed some disappearance of chitose (M_G value 0·07) and the appearance of an unidentified product (M_G value 0·62). In preliminary attempts to isolate chitose, pyridine extracts of the sugar were concentrated during 30 min. at 40° (bath)/12—15 mm. Ionophoresis of the residue revealed a complex mixture. The main components were chitose and the product of M_G value 0·62; there were nine other products (M_G values: 0·33, 0·55, 0·70, 0·79, 0·82, 0·91, 0·99, 1·06, 1·12). Attempted fractionation on charcoal–celite and cellulose columns was unsuccessful.

Periodate Oxidation of 2 : 5-*Anhydro*-D-*mannitol.*—The consumption of oxidant at room temperature in aqueous 0·075M-sodium metaperiodate (50 ml.) containing 2 : 5-anhydro-D-mannitol (0·101 g.) was found by standard procedures (cf. Klosterman and Smith [11]) to be essentially complete after 15 min. (1·02 mols. of oxidant consumed). Neither formic acid nor formaldehyde was produced.

[15] Levene and Walti, *J. Biol. Chem.*, 1927, **75**, 325; 1928, **77**, 685; Lewis, *J. Soc. Chem. Ind.*, 1922, **41**, 99T; cf. Rayner, *ibid.*, 1922, **41**, 224T.
[16] Foster, *Chem. and Ind.*, 1952, 1050.
[17] Lester Smith and Page, *J. Soc. Chem. Ind.*, 1948, **67**, 48; cf. Hughes and Williamson, *Biochem. J.*, 1951, **48**, 487.

The dialdehyde (V) ($[\alpha]_D$ *ca.* +30° in water), isolated by evaporation and extraction of the residue with alcohol, strongly reduced Benedict's solution, decomposed on attempted distillation, gave a single, reducing component on ionophoresis,[16] showed no absorption in the range 230—350 mμ, and gave no carbonyl reduction wave on polarography.[18]

A solution of the crude dialdehyde (V), obtained after periodate oxidation of 2 : 5-anhydro-D-mannitol (2·4 g.) in 2% w/v methanolic hydrogen chloride (100 ml.), was boiled under reflux for 6 hr. then neutralised with silver carbonate and worked up in the usual way. Distillation of the residue gave 2 : 6-*dimethoxy*-3 : 7 : 9-*trioxo*bicyclo[3 : 3 : 1]*nonane* (VIII) (0·61 g.), b. p. 120—125° (bath)/0·02 mm., n^{20} 1·4538, $[\alpha]_D^{20}$ +62·72° (*c*, 1·12 in MeOH) (Found : MeO, 31·9, 32·3, 32·6. $C_8H_{12}O_5$ requires MeO, 32·6%). Acidic hydrolysis regenerated the dialdehyde (V).

Reduction of the Dialdehyde (V).—A solution of the dialdehyde [obtained from 2 : 5-anhydro-D-mannitol (1·0 g.) by essentially method (*b*)] in water (50 ml.) was treated with sodium borohydride (0·25 g.) at room temperature. The solution rapidly lost its optical activity and reducing power towards Benedict's solution. After 30 min. the excess of borohydride was decomposed by carbon dioxide, the solution evaporated to dryness, and the dried (*in vacuo* over P_2O_5) residue acetylated in pyridine (10 ml.) with acetic anhydride (4 ml.). Isolation in the usual way gave a syrupy product from which *di*-(2-*hydroxy*-1-*hydroxymethylethyl*) *ether* (VI) was obtained as a colourless, viscous liquid (0·7 g.), b. p. 190—200° (bath)/0·01—0·05 mm., n^{23} 1·4425 (Found : Ac, 51·2. $C_{14}H_{22}O_9$ requires Ac, 51·5%). The tetra-acetate was optically inactive.

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[18] Linstead, Elvidge, and Whalley, " A Course in Modern Techniques of Organic Chemistry," Butterworths. London, 1955, p. 161.