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5. *A New Chemical Proof of the Cyclic Structure of Glucosaminides.*

By Albert Neuberger.

N-Acetyl-α-methylglucosaminide was methylated with methyl sulphate and alkali to give *N*-acetyl 3 : 4 : 6-trimethyl α-methylglucosaminide, which on acid hydrolysis yielded 3 : 4 : 6-trimethyl glucosamine hydrochloride, thus proving the pyranoside structure of both the α- and the β-methylglycosides of glucosamine and *N*-acetylglucosamine. 3 : 4 : 6-Trimethyl glucosamine hydrochloride on treatment with two equivalents of naphthalene-1-sulphonchloroamide gave 2 : 3 : 5-*trimethyl arabofuranose*; when three equivalents of the oxidising agent were used, 2 : 3 : 5-trimethyl arabononitrile was obtained. As a by-product of the latter reaction, a substance was obtained to which the structure of an imino-acid lactone is assigned.

The hydrolysis constants of α-methylglucosaminide hydrochloride, α- and β-*N*-acetylmethylglucosaminides (Moggridge and Neuberger, J., 1938, 745; Neuberger and Pitt Rivers, J., 1939, 123), and β-methylglucosaminide hydrochloride (Irvine and Hynd, J., 1911, **99**, 250) indicate that all these compounds have pyranoside structures; this physical evidence has been supplemented by direct chemical proof.

N-Acetyl-α-methylglucosaminide (I) was methylated with methyl sulphate and alkali to give *N*-acetyl trimethyl α-methylglucosaminide (II) and this compound was hydrolysed by acid to a trimethyl glucosamine hydrochloride (III). (II) and (III) are identical with the compounds obtained by Cutler, Haworth, and Peat (J., 1937, 1979) from β-methylglucosaminide. The structure of (III) as a 3 : 4 : 6-trimethyl compound has already been definitely established by synthesis from 4 : 6-dimethyl 2 : 3-anhydrohexose (Haworth, Lake, and Peat, J., 1939, 271), whence it follows that (I) also must be a pyranoside; since α- and β-methylglucosaminide hydrochloride can be converted into the corresponding *N*-acetyl compounds under conditions which exclude change of the ring structure (Neuberger and Pitt Rivers, *loc. cit.*), the four known methylglucosaminides all possess pyranoside structures.

The structure of (III) was also demonstrated by a different method, which consists in the conversion of the hexose into a pentose derivative of known structure, and may be of general applicability for the elucidation of the structures of 2-aminohexoses.

Dakin (*Biochem. J.*, 1917, **11**, 79) found that α-amino-acids are oxidised by chloramine-T to either the next lower aldehyde or the next lower nitrile, depending on whether one or two equivalents of oxidising agent are used. This reaction was applied to glucosamic acid by Herbst (*J. Biol. Chem.*, 1937, **119**, 85) and this author has also shown that glucosamine itself can be directly oxidised to *d*-arabinose if two equivalents of chloramine-T are used, without an isolation of the intermediate glucosamic acid. An attempt was made to apply this method to compound (III), but it was found that the *p*-toluenesulphonamide formed during the reaction did not completely separate even from concentrated aqueous solution at 0°, and, since it is also soluble in chloroform, it cannot be easily separated from the main reaction products. In later experiments, therefore, the sodium salt of naphthalene-1-

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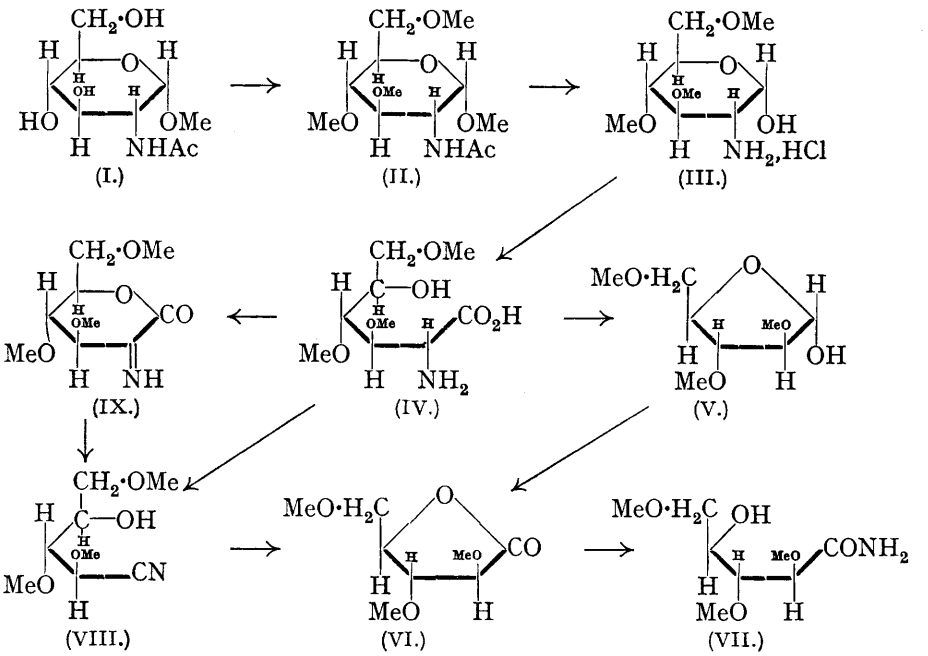
sulphonchloroamide was used; this compound is sufficiently soluble in water to be used for the oxidation, and the product, naphthalene-1-sulphonamide, is practically insoluble in water and can therefore be removed quantitatively before the chloroform extraction is carried out.

When two equivalents of oxidising agent were added very slowly at p_H 7·5, (III) was converted mainly into 2 : 3 : 5-*trimethyl* d-*arabofuranose* (V), presumably being first oxidised to 3 : 4 : 6-trimethyl glucosamic acid (IV). The pentose derivative was characterised by oxidation to the crystalline 2 : 3 : 5-trimethyl *d*-arabonolactone (VI) and further to 2 : 3 : 5-trimethyl *d*-arabonamide (VII). If three equivalents of oxidising agent were added at once and the reaction was carried out at p_H 5·0, the product consisted mainly of 2 : 3 : 5-trimethyl *d*-arabononitrile (VIII), which was converted into (VI) and ultimately into (VII). This series of reactions provides further evidence for the pyranoside structure of the known glucosaminides.



In the experiments at the lower p_H a by-product was obtained which is considered to be an imino-acid lactone and to have the structure (IX). The *substance* behaves as a lactone on titration with alkali and requires the calculated amount of sodium hydroxide for neutralisation. The nitrogen is quantitatively liberated as ammonia on heating with 2N-acid, and alkali liberates ammonia much more slowly. A solution of (IX) reduced Fehling's solution on prolonged boiling, but after acid hydrolysis and neutralisation instantaneous reduction took place. It has been often assumed that imino-acids are intermediate products in the oxidation of amino-acids by various agents. It seems likely, therefore, that in this case the imino-acid is first formed from (IV), which can be either stabilised as a lactone, or hydrolysed to the aldehyde (V), or, if more oxidising agent is present, further oxidised to the nitrile (VIII).

Experimental.

N-Acetyl 3 : 4 : 6-*Trimethyl* α-*Methylglucosaminide*.—*N*-Acetyl-α-methylglucosaminide (4·1 g.) (Moggridge and Neuberger, *loc. cit.*) was dissolved in water (100 ml.) and methyl sulphate (100 ml.) and 40% sodium hydroxide solution (120 ml.) were added during 6 hours with mechanical stirring; the temperature was kept at 30° during the first hour, allowed to rise to 50° during the second hour, and thereafter maintained at 50°. The solution was left overnight and

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then extracted with chloroform; the combined extracts were concentrated to small bulk, and the product crystallised by addition of light petroleum. Yield, 70%. The product was obtained in needles, m. p. 151°, $[\alpha]_D$ + 102° (water) (Found: C, 52·0; H, 8·25; N, 5·0. Calc. for $C_{12}H_{23}O_6N$: C, 52·0; H, 8·3; N, 5·05%). Cutler, Haworth, and Peat (*loc. cit.*) give m. p. 150° and $[\alpha]_D$ (water) + 104·3°.

3 : 4 : 6-*Trimethyl Glucosamine Hydrochloride.*—A solution of the preceding compound (7 g.) in 3·5N-hydrochloric acid (125 ml.) was refluxed for 2 hours, treated with charcoal, filtered, and concentrated in a vacuum. Most of the syrup thus obtained crystallised in a desiccator. It was triturated with alcohol (15 ml.) and then with anhydrous ether (35 ml.) and, after filtration (yield, 70%), recrystallised from alcohol; m. p. 215° (decomp.). The initial rotation of different samples varied from $[\alpha]_D$ + 115° (water) to + 52°, but the equilibrium rotations (after 18 hours) were identical, $[\alpha]_D$ (water) being 99—100°. The substance was strongly reducing, and yielded the whole of its nitrogen on 3 minutes' shaking in the apparatus of Van Slyke (Found: C, 41·8; H, 7·70; N, 5·4. Calc. for $C_9H_{20}O_5NCl$: C, 41·9; H, 7·8; N, 5·4%). A sample of trimethyl glucosamine hydrochloride prepared from *N*-acetyl 3 : 4 : 6-trimethyl-β-methylglucosaminide (Cutler, Haworth, and Peat, *loc. cit.*) had properties identical with those of the compound described and a mixed sample showed no depression of m. p.

N-*Benzoyl* 3 : 4 : 6-*Trimethyl Glucosamine.*—To a solution of 3 : 4 : 6-trimethyl glucosamine hydrochloride (6·5 g.) in water which was kept at 5°, benzoyl chloride (3·5 ml.) and sodium bicarbonate (4·6 g.) were added in ten portions during 1 hour. A crystalline precipitate soon formed and after 2 hours the mixture was extracted with chloroform and the combined extracts were washed with aqueous bicarbonate, dried, and evaporated to small bulk. The product crystallised for the most part and the mother-liquor gave a further crop on addition of light petroleum (b. p. 80—100°). Yield, 75%. The *substance* was recrystallised from chloroform and light petroleum and had m. p. 213°; $[\alpha]_D$ (initial) + 124°; after 48 hours, + 105° (wet pyridine). It reduced Fehling's solution on boiling (Found: N, 4·1. $C_{16}H_{23}O_6N$ requires N, 4·3%). The substance was very refractory to oxidation, unchanged material being recovered after standing for 6 days in a solution of dioxan–water (1·1) containing an excess of bromine, or on treatment with potassium permanganate in acetone.

Oxidation of 3 : 4 : 6-*Trimethyl Glucosamine Hydrochloride to* 2 : 3 : 5-*Trimethyl Arabinose.*—(1) *Oxidation at* p_H 7·5. To a solution of 3 : 4 : 6-trimethyl glucosamine hydrochloride (2·5 g.) in water (50 ml.) containing 0·75 g. of sodium bicarbonate, exactly 2 equivs. of sodium naphthalene-1-sulphonchloroamide in 250 ml. of water were added in twenty portions during 5 hours. The mixture was left overnight in the incubator, acidified to p_H 5·0 with acetic acid, and the precipitate filtered off after standing for several hours in the ice-chest. The solution was concentrated to small bulk under reduced pressure and a small precipitate which formed was filtered off and discarded. The solution was now extracted with chloroform, the chloroform solution evaporated to dryness, and the residue distilled. The main fraction, b. p. 80—83° (bath temp.)/0·01 mm., was an oil which reduced Fehling's solution strongly on warming, and gave figures on analysis indicating that it was slightly impure 2 : 3 : 5-*trimethyl* d-*arabofuranose* (Found: C, 49·2; H, 8·05. $C_8H_{16}O_5$ requires C, 50·0; H, 8·3%). $[\alpha]_D$ + 38° (methyl alcohol). Yield, 30%.

2 : 3 : 5-Trimethyl *d*-arabofuranose (0·5 g.) was oxidised with an excess of bromine, and the resulting 2 : 3 : 5-trimethyl arabonolactone distilled in a vacuum, b. p. 90° (bath temp.)/0·05 mm. The solid was recrystallised from ligroin; it had m. p. 33°, $[\alpha]_D$ (initial in water) + 44°, (after 400 hours) + 26°, and properties identical with those of the compound described by Avery, Haworth, and Hirst (J., 1927, 2317). For further characterisation it was converted by treatment with methyl-alcoholic ammonia into the amide, m. p. 137·5°, $[\alpha]_D$ — 14·2° (Found: C, 46·8; H, 8·2; N, 6·5. Calc.: C, 46·5; H, 8·2; N, 6·7%), identical with that described by Haworth, Peat, and Whetstone (J., 1938, 1975).

(2) *Oxidation at* p_H 5·0. 3 : 4 : 6-Trimethyl glucosamine hydrochloride (2·05 g.) was dissolved in a small amount of water containing 3 equivs. of sodium acetate and 2 equivs. of acetic acid, an aqueous solution of sodium naphthalene-1-sulphonchloroamide (3 equivs.) added, and the mixture left overnight at room temperature. Iodometric titration indicated that about 2·5 equivs. of the chloroamide had been used up. The mixture was then left for several hours at 0°, the precipitate of naphthalene-1-sulphonamide filtered off, and the solution evaporated in a vacuum to small bulk and left in the ice-chest. The crystalline precipitate that formed was filtered off, dried, and dissolved in chloroform, the solution filtered and evaporated to dryness, and the residue dissolved in ether. On slow evaporation large prisms appeared (450 mg.), m. p. 86·5°, $[\alpha]_D$ — 40° (in chloroform) (Found: C, 50·2; H, 7·2; N, 6·65. $C_9H_{15}O_5N$ requires

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C, 49·8; H, 6·95; N, 6·45%). The *substance* behaved as a lactone on titration with alkali; 14 mg. required 3·1 ml. of 0·02N-sodium hydroxide for neutralisation, with phenolphthalein as indicator (calc. for $C_9H_{15}O_5N$, 3·22 ml.). 43·4 Mg., dissolved in 5 ml. of 2N-hydrochloric acid, were heated for 1 hour on a boiling water-bath. The solution, after extraction with chloroform, was concentrated, and the residue crystallised from alcohol and ether. It amounted to 9·8 mg. and analysis showed it to be ammonium chloride (Found : N, 26·2. Calc. : N, 26·15%). Ammonia was given off too, but more slowly, on heating a solution of the substance with N-potassium hydroxide on the water-bath, but not in the cold.

The aqueous solution which had been freed from the imino-acid lactone was extracted exhaustively with chloroform, the combined extracts evaporated to dryness, and the residue distilled. The oil obtained, b. p. 65—70° (bath temp.)/0·02 mm. (Found : N, 6·4. Calc. for the nitrile, 7·4%), was redistilled; it then crystallised in ice–salt and had m. p. about 2° (Found : N, 6·8%). It was converted into the lactone by hydrolysis with 5N-hydrochloric acid and heating to 100° in a vacuum. The product was shown to be 2 : 3 : 5-trimethyl *d*-arabonolactone by m. p., rotation, and analysis. The lactone was further characterised by conversion into the amide, which was identical with 2 : 3 : 5-trimethyl arabonamide, described above.

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