28. *Preparation and Configurative Relationships of Methylglucosaminides.*

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α- and β-Methylglucosaminides have been prepared from the corresponding *N*-carbobenzyloxy-derivatives and it is shown that the methylglucosaminide prepared by Irvine is the β-compound. Comparison of the rates of acid hydrolysis of the two glucosaminides shows that the α-compound has *cis*-configuration on C_1. In this series of compounds, it is shown that Hudson's two rules of optical superposition are closely obeyed; further, physical data lend strong support to the assumption that glucosamine has the same configuration as glucose.

It was shown by Moggridge and Neuberger (J., 1938, 745) that the methylglucosaminide of Irvine, McNicoll, and Hynd (J., 1911, **99**, 250) has a normal glycosidic structure. The mode of preparation and the value of the optical rotation of this compound suggest that it has the β-configuration. The preparation of the corresponding α-glycoside was also

desired both on theoretical grounds and because it would be useful in a projected study of the enzymic hydrolysis of glucosamine-containing polysaccharides.

Treatment of glucosamine hydrochloride with methyl-alcoholic hydrogen chloride leads to no glycoside formation; this is doubtless to be ascribed to repulsion of catalytically active hydrions by the charged amino-group, since *N*-acylated glucosamines are readily converted into glycosides by such treatment (cf. Moggridge and Neuberger, *loc. cit.*). If *N*-carbobenzyloxyglucosamine be used as starting material, therefore, it should be possible to obtain the desired glycoside by removal of the carbobenzyloxy-residue from the reaction product by reduction with hydrogen and palladium (cf. Bergmann and Zervas, *Ber.*, 1932, **65**, 1192).

N-Carbobenzyloxyglucosamine (Chargaff and Bovarnick, *J. Biol. Chem.*, 1937, **118**, 421) with warm methyl-alcoholic hydrogen chloride yields a *product* which, on catalytic reduction, gives a *methylglucosaminide* having $[\alpha]_D$ + 127° and therefore to be regarded as the α-form; on acetylation this gives an *N*-acetyl derivative identical with the *N*-acetyl methylglucosaminide of Moggridge and Neuberger (*loc. cit.*). When the glycoside synthesis is carried out at room temperature, however, the major product, on reduction, gives β-methylglucosaminide, $[\alpha]_D$ − 24°, identical with the compound of Irvine, McNicoll, and Hynd (*loc. cit.*); the latter can be acetylated to give N-*acetyl* β-*methylglucosaminide*, $[\alpha]_D$ − 43°. Comparison of the rates of hydrolysis of the methylglucosaminide of Irvine, McNicoll and Hynd (*loc. cit.*) and of the new glucosaminide described here indicates that both compounds almost certainly possess the same (pyranoside) ring structure.

Optical Superposition in Glucosamine Derivatives.—With the aid of the present observations together with data already available in the literature it is now possible to make a preliminary study of the applicability of Hudson's rules of optical superposition to glucosamine derivatives. The rules are already known to apply to glucosamine itself and to its penta-acetate; Table I includes the data for these compounds together with those supplied by the present work; for purposes of comparison, values for appropriate derivatives of glucose are also given.

TABLE I.

	α-Compounds.		β-Compounds.				
Substance.	$[\alpha]_D$.	Molecular rotation.	$[\alpha]_D$.	Molecular rotation.	Solvent.	2*A*.	2*B*.
d-Glucosamine hydrochloride	+100°	+21,550°	+20° (a)	+ 4,310°	H_2O	17,240	25,860
d-Glucose	111·1	20,016	+17·5	+ 3,150	,,	16,866	23,166
Glucosamine penta-acetate	93·5 (b)	36,400	+ 1·2 (b)	+ 467	$CHCl_3$	35,933	36,867
Glucose penta-acetate	101·6	39,600	+ 3·8	+ 1,480	,,	38,120	41,080
d-Methylglucosaminide hydrochloride	127	29,146	−24 (c)	− 5,508	H_2O	34,654	23,638
d-Methylglucosaminide hydrochloride + 1 equiv. NaOH	120	27,599	−28	− 6,369	,,	33,968	21,230
d-Methylglucoside	158·9	28,602	−34·2	− 6,156	,,	34,758	22,446
N-Acetylmethylglucosaminide	105 (d)	24,675	−43	−10,105	,,	34,780	14,570
N-Carbobenzyloxymethylglucosaminide	80	26,160	−38	−12,426	C_5H_5N	38,586	13,734
N-Acetyl trimethyl methylglucosaminide	104·3 (e)	28,890	−29 (e)	− 8,033	H_2O	36,923	20,857
N-Acetyl trimethyl methylglucosaminide	120 (e)	33,240	+19·6 (e)	+ 5,429	$CHCl_3$	27,811	38,669

(a) Hisamura and Kusuno, *J. Biochem. Japan*, 1938, **27**, 378. (b) Hudson and Dale, *J. Amer. Chem. Soc.*, 1916, **38**, 1431. (c) Irvine, McNicoll, and Hynd, *loc. cit.* (d) Moggridge and Neuberger, *loc. cit.* (e) Cutler, Haworth, and Peat, J., 1937, 1979.

The figures for the acylated glucosaminides show that the contribution of C_1 is not significantly affected by substitution at C_2 or by methylation of the remaining hydroxyl groups; it is true that the *N*-carbobenzyloxy-derivative shows some deviation, but this may well be due to the difference in solvent, the powerful effect of which is indicated by the comparative figures for *N*-acetyl trimethyl methylglucosaminide in water and in chloroform. It will be noted also that addition of alkali to the methylglucosaminide hydrochlorides, while altering the magnitude of their molecular rotations, does not affect the magnitude of 2*A*; the contribution of C_1 is thus not affected by changes in the remainder

of the molecule. Moreover it is apparent that in so far as the $2A$ values (contribution of C_1) are concerned the agreement between the methylglycosides of glucose and glucosamine is even closer than that between the free sugars and between the penta-acetates.

The last column of Table I shows that the second rule of Hudson (lack of effect of alterations at C_1 on contribution of the rest of the molecule) is also obeyed in so far as free glucosamine and its methyl glycosides are concerned and that the magnitude of $2B$ is of the same order as that for the corresponding derivatives of glucose. On the other hand, *N*-acylation causes marked diminution of the value of $2B$ unless it is accompanied by methylation, in which case $2B$ is little affected.

On the whole the agreement of the glucosamine derivatives with the rules of optical superposition which apply to those of glucose is sufficiently close to permit the deduction that all these derivatives belong to the same (pyranoside) ring system.

Configurations of α- and β-Methylglucosaminides.—The method depending on conductivity measurements in boric acid solution which has been used to determine the configurations of the α- and β-methylglucosides is not applicable to the glucosaminides; in the case of the latter, however, the problem can be attacked in a different way. From the work of Moggridge and Neuberger (*loc. cit.*) it can be deduced that the smaller the distance between the amino-group and the glycoside linkage the lower will be the acid hydrolysis constant for the latter; the glycoside which possesses the *cis*-configuration should therefore be the more resistant of the two to acid hydrolysis. For β-methylglucosaminide Moggridge and Neuberger (*loc. cit.*) found $k = 2{\cdot}08 \times 10^{-5}$, the hydrolysis being carried out with 2·5N-acid at 100°; for α-methylglucosaminide under the same conditions we now find $k = 4{\cdot}03 \times 10^{-6}$ (data in Table II). The ratio of these hydrolysis constants is 100 : 510 as compared with a ratio of 100 : 180 for those of α- and β-methylglucosides (Moelwyn-Hughes, *Trans. Faraday Soc.*, 1929, **25**, 503). Comparing the hydrolysis constants of α-methylglucoside and α-methylglucosaminide and using the formula given by Moggridge and Neuberger (*loc. cit.*), we obtain a value of 0·7 A. for the distance between the charge and the glycoside linkage in the α-glucosaminide; this contrasts with the value of 1·7 A. calculated by the above-mentioned authors for the corresponding distance in β-methylglucosaminide. It is therefore reasonable to conclude that, as in the case of the methylglucosides, the α- and β-methylglucosaminides have *cis*- and *trans*-configurations respectively.

Configurative Relationships of Glucosamine.—The configurative relationship which is at present assumed to exist between *d*-glucosamine and *d*-glucose has not yet been proved chemically, the arguments in its favour being based mainly on the indirect evidence of experiments with glucosamic acid (*Ann. Reports*, 1937, **34**, 290); the assumption is, however, strongly supported by the following points which emerge from the present work.

(a) α-Glycosides in general have the *d*-configuration with respect to C_1. Now the kinetic experiments discussed above show that the methoxyl group in α-methylglucosaminide is in the *cis*-position with respect to the C_2-amino-group; C_2 must therefore also have the *d*-configuration as in *d*-glucose.

(b) Glucosamine hydrochloride, its glycosides and derivatives resemble glucose and galactose and differ from mannose in obeying the rules of optical superposition; moreover, the absolute values of the respective contributions of C_1 and of the rest of the molecule to the molecular rotations are essentially the same for corresponding derivatives of glucose and glucosamine.

(c) In the natural *l*-amino-acids, decreasing ionisation of the amino-group, brought about by addition of alkali, is accompanied by increasing dextro- or decreasing lævo-rotation (Lutz and Jirgensons, *Ber.*, 1930, **63**, 448). The same phenomenon was observed for *l*-bases in general by Leithe (*Ber.*, 1930, **63**, 1498). The methylglucosaminide hydrochlorides show decreasing dextro- and increasing lævo-rotation on addition of alkali and thus fall into the *d*-series.

Even if the uncertainty of the last point, which involves the comparison of the changes of rotation with ionisation in two entirely different series of compounds, is admitted the cumulative evidence that *d*-glucosamine is configuratively related to *d*-glucose is thus very strong.

Experimental.

N-*Carbobenzyloxy-α-methylglucosaminide.*—A solution of *N*-carbobenzyloxyglucosamine (16 g.) in 1 l. of absolute methyl alcohol containing 0·7% of hydrogen chloride was kept at 40°, and the rotation observed at intervals: $[\alpha]_D = +22{\cdot}7°$ (24 hours); + 35° (55 hours); + 60° (115 hours); + 65° (163 hours). After 163 hours, the solution was neutralised with silver oxide, filtered, and evaporated to dryness under reduced pressure. After one recrystallisation from water, the substance had $[\alpha]_D$ + 80° (constant on further recrystallisation). The pure *glucosaminide* crystallised from water in plates and from *n*-propyl alcohol in needles, m. p. 154—155°, $[\alpha]_D$ + 80° in pyridine (Found: N, 4·3. $C_{15}H_{21}O_7N$ requires N, 4·3%). Yield, 6·68 g. The residue contained in the mother-liquor had $[\alpha]_D$ + 26°, which did not alter on repeated crystallisation, and kinetic measurements on this residue gave a hydrolysis constant similar to that of the pure glucosaminide, thus excluding the presence of furanoside in the mixture.

α-*Methylglucosaminide Hydrochloride.*—*N*-Carbobenzyloxy-α-methylglucosaminide (2 g.) in 30 c.c. of absolute ethyl alcohol was reduced with hydrogen in the presence of 1 equiv. of hydrogen chloride and 400 mg. of palladium-black. When carbon dioxide was no longer evolved, the solution was filtered and evaporated to dryness under reduced pressure. Yield, 98%. The residue crystallised from absolute alcohol in needles, m. p. 119°, $[\alpha]_D$ + 127° in water (Found: C, 36·25; H, 7·2; N, 6·0. $C_7H_{15}O_5N$,HCl requires C, 36·6; H, 7·0; N, 6·1%). On addition of 1 equiv. of sodium hydroxide, $[\alpha]_D$ in terms of the hydrochloride was 120°.

An aqueous solution (20 c.c.) containing α-methylglucosaminide hydrochloride (300 mg.) was neutralised with freshly precipitated silver oxide and filtered. Keten was passed in for 2 hours and the solution was then extracted five times with ether to remove polymerisation products of keten. The solution was evaporated to dryness under reduced pressure at 40—45°, and alcohol added and distilled off. The residue was dried in a vacuum and recrystallised from alcohol; yield, 50 mg. $[\alpha]_D$ + 104°, m. p. 188—189°, not depressed by the *N*-acetylmethylglucosaminide of Moggridge and Neuberger (*loc. cit.*), m. p. 189°, $[\alpha]_D$ + 105°.

N-*Carbobenzyloxy-β-methylglucosaminide.*—A solution of *N*-carbobenzyloxyglucosamine (10 g.) in 600 c.c. of methyl alcohol containing 0·7% of hydrogen chloride was left at room temperature, and the reaction followed polarimetrically. After 72 hours, when the rotation had become constant, the solution was neutralised and worked up as described for the α-compound; the main product crystallised from water in needles (a residue was obtained with $[\alpha]_D$ + 26°, which could not be further separated). Yield, 2 g. M.p. 166—168°, $[\alpha]_D$ − 38° in pyridine (Found: N, 4·15. $C_{15}H_{21}O_7N$ requires N, 4·3%). Catalytic reduction of this *compound* gave β-methylglucosaminide hydrochloride, identical with that prepared by Irvine, McNicoll, and Hynd (*loc. cit.*); it had $[\alpha]_D$ − 24°, and on addition of 1 equiv. of sodium hydroxide $[\alpha]_D$ in terms of the hydrochloride was − 28°.

N-*Acetyl-β-methylglucosaminide.*—An aqueous solution of β-methylglucosaminide hydrochloride (230 mg.) was neutralised with 1 c.c. of 2N-sodium hydroxide, and keten passed into it for 1 hour. 2N-Hydrochloric acid (0·5 c.c.) was added, and the solution evaporated to dryness under reduced pressure. The *acetyl* compound was extracted with alcohol, from which it crystallised in plates, m. p. 195—196°, $[\alpha]_D$ − 43° in water [Found: N, 5·9; $CH_3{\cdot}CO$ (Pregl and Soltys), 18·1. $C_9H_{17}O_6N$ requires N, 6·0; $CH_3{\cdot}CO$, 18·3%].

N-*Carbobenzyloxy Tetra-acetyl Glucosamine.*—Ice-cold aqueous solutions of tetra-acetyl glucosamine hydrochloride (7·7 g. in 50 c.c.) and sodium bicarbonate (4 g. in 50 c.c.) were mixed, and benzyl chloroformate (3·5 c.c., *d* 1·18) added in 0·5 c.c. portions during 1 hour with shaking; the solution was then left at 0° for 6 hours. The product which crystallised was washed with a small quantity of ice-cold water, ground with ether (yield, 65%), dried, and recrystallised from 30% methyl alcohol, forming needles, m. p. 150—151°, $[\alpha]_D$ + 21·5° in pyridine (Found: C, 34·9; H, 5·7; N, 2·9. $C_{22}H_{27}O_{11}N$ requires C, 34·1; H, 5·6; N, 2·9%). This *compound* was also obtained, in poor yields, by acetylating *N*-carbobenzyloxyglucosamine both with pyridine and acetic anhydride, and with sodium acetate and acetic anhydride.

Kinetic Measurements.—The method used was identical with that of Moggridge and Neuberger (*loc. cit.*) and the hydrolysis constants were calculated according to Guggenheim (*Phil. Mag.*, 1926, **2**, 540). Hydrolysis was done in 2·5N-hydrochloric acid at 100°. Time is given in hours, and *R* denotes the equivalent of reducing sugar in c.c. of N/75-thiosulphate; 2·98 mg. of substance were used in each reading.

TABLE II.

t	½	1	2	3	4	6	8	9	10	12	15	18
R	0·12	0·33	0·50	0·51	0·77	0·80	1·05	1·17	1·35	1·52	1·70	2·27
t	19·5	21	24	27	30	31·5	33	36	39	42	43	—
R	2·02	2·32	2·42	2·70	3·22	3·00	3·16	3·04	3·22	3·42	3·40	7·30 *

* Theoretical end-point.

From these figures $k = 4{\cdot}03 \times 10^{-6}$.

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