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The Structure of N-Acetyl-*d*-glucosylamine

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In 1893 Lobry de Bruyn and Franchimont[1] obtained a crystalline reaction product, $C_6H_{13}O_5N$, m. p. 127–128°, $(\alpha)_D$ 19.4° (initial rotation in water), by the condensation of *d*-glucose with ammonia. When this condensation product is treated with ketene a monoacetyl derivative, $(\alpha)_D$ −22.4°, is obtained. It is the purpose of this communication to show that this monoacetyl compound, which was first prepared by Brigl and Keppler,[2] is a N-acetyl-*d*-glucopyranosylamine.

As the monoacetyl derivative, $(\alpha)_D$ −22.4°, can be prepared by the partial deacetylation of a pentaacetyl-*d*-glucosylamine[2] or, as in the present work, by the interaction of the glucose–ammonia condensation product with ketene, in hydroxylic solvents, it is evident that the monoacetyl compound $(\alpha)_D$ −22.4°, contains an acetoamino or an acetimino residue. On the basis of previous knowledge[3] it is certain that the nitrogen atom in the monoacetyl derivative, $(\alpha)_D$ −22.4°, is linked to carbon atom one of the glucose residue and it follows that this compound is one of twenty-one isomers. Upon acetylation, under conditions minimizing acetolysis, eleven of the possible isomers would form hexaacetates and ten pentaacetates. When the monoacetyl compound, $(\alpha)_D$ −22.4°, was acetylated with pyridine and acetic anhydride at 25° a pentaacetate[2] was obtained.

A further elimination of untenable structures can be made on the basis of the well-known studies of Malaprade[4] and of Jackson and Hudson,[5] *i. e.*, through oxidation with periodic acid. Six of the remaining ten isomeric structures upon reaction with periodic acid would consume two moles of oxidant per mole of hexose whereas the other four would require three moles. As we have found that one mole of the monoacetyl derivative, $(\alpha)_D$ −22.4°, consumes two moles of periodic acid (Table I), it is evident that this compound must possess one of the following structures: I, N-acetyl-α-*d*-glucopyranosylamine; II, N-acetyl-β-*d*-glucopyranosylamine; III, N-acetyl-α-*d*-glucofuranosylamine; IV, N-acetyl-β-*d*-glucofuranosylamine; V, N-acetyl-α-*d*-glucotetranosylamine; VI, N-acetyl-β-*d*-glucotetranosylamine.[6] Upon oxidation, first with periodic acid and then with bromine, structures V and VI would form monobasic acids, I and II dibasic acids, and III and IV tribasic acids.[5] We have found that, under the above conditions, the monoacetyl compound $(\alpha)_D$ −22.4°, forms a dibasic acid which was isolated as the barium and brucine salt. It therefore follows that the monoacetyl derivative, $(\alpha)_D$ −22.4°, *i. e.*, N-acetyl-*d*-glucosylamine, is either N-acetyl-α-*d*-glucopyranosylamine or N-acetyl-β-*d*-glucopyranosylamine. An unequivocal final choice can not be made at present but the optical rotation suggests that the N-acetyl-*d*-glucopyranosylamine, $(\alpha)_D$ −22.4°, is the β compound.

Experimental

N-Acetyl-*d*-glucosylamine.—Five grams of the glucose–ammonia condensation product, $(\alpha)_D$ 22.1° (initial rotation in water), m. p. 127–128°, prepared according to the directions given by Ling and Nanji,[7] in 165 ml. of 91% methanol was treated with ketene,[8] at 0°, until the solution gave a negative reaction with ninhydrin. Upon standing 5.2 g. (85%) of N-acetyl-*d*-glucosylamine crystallized from the reaction mixture. After two recrystallizations from aqueous acetone the compound, m. p. 255° (uncor.) with decompn., exhibited a specific rotation of $[\alpha]^{22}D$ −22.4° (c = 1.24% in water). Brigl and Keppler[2] report an initial rotation (eleven minutes) of $(\alpha)_D$ −22.0° and a final rotation (five hours) of $(\alpha)_D$ −23.0° for this compound. We did not observe any mutarotation.

Anal. Calcd. for $C_8H_{15}O_6N$ (221): C, 43.4; H, 6.8; N, 6.3. Found: C, 43.3; H, 6.9; N, 6.3.

Pentaacetyl-*d*-glucosylamine.—Five grams of N-acetyl-*d*-glucosylamine, 25 ml. of acetic anhydride, and 50 ml. of pyridine were shaken, at 25°, for forty-eight hours. The undissolved material (1.1 g.) was removed, an equal volume of chloroform added to the filtrate, the chloroform solution washed repeatedly with water and bicarbonate solution, and finally dried over sodium sulfate. The solution was then concentrated to 30 ml. and upon adding 85 ml. of ligroin crystals began to appear. After standing overnight

(1) C. A. Lobry de Bruyn and A. P. N. Franchimont, *Rec. trav. chim.*, **12**, 286 (1893).

(2) P. Brigl and H. Keppler, *Z. physiol. Chem.*, **180**, 38 (1929).

(3) (a) Tollens–Elsner, "Kurzes Handbuch der Kohlenhydrate," 4th ed., J. A. Barth, Leipzig, 1935; (b) Beilstein, "Handbuch der organischen Chemie," 4th ed., Vol. 31, J. Springer, Berlin, 1938.

(4) M. L. Malaprade, *Bull. soc. chim.*, [5] **1**, 833 (1934).

(5) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937).

(6) It is of interest to note that the single acyclic structure is eliminated by the acetylation experiment and by the quantitative oxidation with periodic acid. On the basis of this and other evidence[2] it appears that the monoacetyl derivative, $(\alpha)_D$ −22.4°, and the pentaacetyl derivative, $(\alpha)_D$ 17.7°, possess identical ring structures.

(7) A. R. Ling and D. R. Nanji, *J. Chem. Soc.*, **121**, 1682 (1922).

(8) M. Bergmann and F. Stern, *Ber.*, **63**, 437 (1930).

5 g. of crude pentaacetyl-*d*-glucosylamine was obtained. After recrystallization from chloroform and ligroin and then from ethanol the substance, m. p. 160–161° (uncor.), had a specific rotation of $[\alpha]^{22}D$ 17.7° (*c* = 0.76%, in chloroform). Brigl and Keppler[2] report a m. p. of 159–160° and a specific rotation of $(\alpha)D$ 16.2° (*c* 2.43%, in chloroform).

Anal. Calcd. for $C_{16}H_{23}O_{10}N$ (389): C, 49.3; H, 5.9; N, 3.6; O-acetyl,[9] 44.3. Found: C, 49.5; H, 5.8; N, 3.9; O-acetyl, 44.5.

Oxidation of N-Acetyl-*d*-glucosylamine with Periodic Acid.—Ten-ml. aliquots of a 0.1 *M* N-acetyl-*d*-glucosylamine solution were allowed to react, at 25°, with 30 ml. of a 0.1 *M* periodic acid solution. At suitable intervals the samples were neutralized, to a phenolphthalein end-point, with aqueous sodium carbonate and the excess periodic acid determined by titrating the iodine liberated from excess iodide, in the presence of a borax–boric acid buffer with standard arsenite solution.[10] The data so obtained are listed in Table I.

TABLE I

OXIDATION OF N-ACETYL-*d*-GLUCOSYLAMINE WITH PERIODIC ACID

Reaction time in hours	Mmoles HIO_4 consumed / Mmoles hexose taken
1	1.49
2	1.80
3	1.91
4	2.00
9	2.12
24	2.23
36	2.38

Barium Acetamino-D-hydroxymethyl Diglycolate.[5]—Six grams (0.027 mole) of N-acetyl-*d*-glucosylamine in 100 ml. of water was added to 18.5 g. (0.081 mole) of periodic acid in 100 ml. of water and the reaction mixture maintained at 25° for four and one-half hours. The solution was exactly neutralized with barium hydroxide, the precipitate removed and the filtrate evaporated to dryness *in vacuo*. The solid was extracted with absolute ethanol (100 ml.), the ethanol extract evaporated to dryness, the residue taken up in ethanol, the solution centrifuged and again evaporated to dryness. The residue was dissolved in 1 liter of water, 98 g. of barium carbonate and 6 ml. of bromine was added and the mixture stirred mechanically for thirty-six hours. After removing the excess bromine by aeration, bromide ion with silver carbonate, silver with hydrogen sulfide and hydrogen sulfide by aeration, the solution was concentrated, in the presence of barium carbonate, to 30 ml., centrifuged and the clear liquid poured into 300 ml. of absolute methanol. After twenty-four hours the precipitate was collected and dried *in vacuo* over sulfuric acid. Prior to analysis the substance was reprecipitated from aqueous methanol and dried *in vacuo* at 100°. The yield of barium salt, from three separate experiments, was I, 3.3 g.; II, 4.4 g.; and III, 3.9 g.

Anal. Calcd. for $C_7H_8O_7NBa$ (355.5): Ba, 38.65; N, 3.9. Found: I, Ba, 38.5, N, 3.5; II, Ba, 38.8; N, 3.5; III, Ba, 38.2; N, 3.6.

The above barium salts possessed a specific rotation of $[\alpha]^{22}D$ 24 ± 2° (*c* 2.5% in water), and all of the salts gave a positive reaction for glyceric acid when tested according to the procedure of Eegriwe[11] as modified by Rapoport.[12]

Brucine Acetamino-D-hydroxymethyl Diglycolate.[5]—Barium acetamino-D-hydroxymethyl diglycolate (2 g.) in 10 ml. of water was treated with 82% of the theoretical amount of brucine sulfate heptahydrate dissolved in the minimum quantity of hot water and the reaction mixture poured into 10 volumes of absolute ethanol. After twenty-four hours the precipitate was removed and the filtrate concentrated, *in vacuo*, to 10 ml. Upon standing the brucine salt crystallized from the solution. Prior to analysis the salt was recrystallized several times from aqueous ethanol and finally dried at 100° *in vacuo*.

Anal. Calcd. for $C_{53}H_{63}O_{15}N_5$ (1009.5): C, 63.0; H, 6.3; N, 6.9; OCH_3, 12.3. Found: C, 63.2; H, 6.2; N, 6.8; OCH_3, 12.2.

Summary

It has been shown that N-acetyl-*d*-glucosylamine, $(\alpha)_D$ −22.4°, is a pyranoside and it is suggested that this compound is N-acetyl-β-*d*-glucopyranosylamine.

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(9) (a) A. Kunz and C. S. Hudson, THIS JOURNAL, **48**, 1982 (1926); (b) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *ibid.*, **58**, 490 (1936).

(10) E. Müller and G. Wegelin, *Z. anal. Chem.*, **52**, 758 (1913).

(11) E. Eegriwe, *ibid.*, **95**, 323 (1933).

(12) S. Rapoport, *Biochem. Z.*, **289**, 406 (1937).