

*Acyclic Derivatives of Amino-sugars.*

By M. W. WHITEHOUSE, P. W. KENT, and C. A. PASTERNAK.

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Preparation of D-glucosamine diethyl mercaptal hydrochloride and N-acyl derivatives of the amine is described. Penta-acetyl-D-glucosamine diethyl mercaptal and mercuric chloride in aqueous acetone gave the *aldehydo*-sugar. The validity of certain diagnostic colour reactions is discussed. Analogous acyclic derivatives have been obtained from D-galactosamine.

SERIES of acyclic compounds of D-glucosamine and D-galactosamine have been prepared from the appropriate diethyl mercaptals in order to determine to what extent acyclic 2-amino-2-deoxy-hexoses are detectable in natural products by the usual amino-sugar tests. Since some of the results reported in our preliminary communication (Kent, *Research*, 1950, **3**, 427) have been disputed by Wolfrom and Anno (*J. Amer. Chem. Soc.*, 1952, **74**, 6150), we now give a fuller account and extend our description to derivatives of galactosamine.

Desulphurisation of penta-acetyl-D-glucosamine diethyl mercaptal (Wolfrom, *J. Amer. Chem. Soc.*, 1929, **51**, 2188) gave *aldehydo*-penta-acetyl-D-glucosamine, which was separated chromatographically on a powdered cellulose column (Hough, Hirst, and Jones, *J.*, 1949, 250). A solution of the *aldehydo*-sugar in an acetate buffer (pH 3.34; 0.02 mg. of sugar per ml.) had an absorption band at 265 m $\mu$  similar to that of *aldehydo*-penta-O-acetyl-glucose and -galactose under the same conditions (Pascu and Hillier, *J. Amer. Chem. Soc.*, 1948, **70**, 523). The *aldehydo*-sugar strongly reduced Fehling's solution, restored the colour to Schiff's reagent, gave an Elson-Morgan colour reaction, and formed a crystalline phenylhydrazone.

These mercaptals could be detected on paper chromatograms by the use of iodine vapour (Brante, *Nature*, 1949, **163**, 651; Greenway, Kent, and Whitehouse, *Research*, 1953, **6**, 6S). D-Glucosamine diethyl mercaptal hydrochloride gave the ninhydrin reaction and *aldehydo*-penta-O-acetyl-D-glucosamine was detected with aniline hydrogen phthalate (Partridge, *Nature*, 1949, **164**, 443). Acyclic derivatives of these types are not normally detected by the Molisch, Elson-Morgan, or Dische-Borenfreund colour tests.

## EXPERIMENTAL

Chromatography was performed on Whatman No. 1 paper in *n*-butanol-ethanol-water (4 : 1 : 5).

*2-Amino-2-deoxy-D-glucose Diethyl Mercaptal Hydrochloride* (D-Glucosamine Diethyl Mercaptal Hydrochloride).—D-Glucosamine hydrochloride (10 g.) was shaken with fuming hydrochloric acid (80 ml., saturated at 0°) and ethanethiol (30 ml.) overnight or until completely dissolved. Ethanol (70 ml.) and lead carbonate were added and the neutral filtrate was concentrated to small bulk. The product (70%), when recrystallised from aqueous alcohol, had m. p. 75–76°,  $[\alpha]_D^{20}$   $-18.5^\circ$  (*c*, 0.2 in H<sub>2</sub>O),  $R_F$  0.72 (Found: C, 37.05; H, 7.4; N, 4.1; S, 19.6. Calc. for C<sub>10</sub>H<sub>24</sub>O<sub>4</sub>NS<sub>2</sub>Cl: C, 37.2; H, 7.45; N, 4.35; S, 19.9%).

*2-Acetamido-2-deoxy-D-glucose Diethyl Mercaptal*.—(i) The mercaptal hydrochloride (8 g.) in dry methanol (50 ml.) and acetic anhydride (6 g.) was shaken with powdered silver acetate (6.5 g.) at room temperature for 3 hr. and finally boiled for 5 min. The filtrate was concentrated under reduced pressure to a syrup which crystallised readily. The product, recrystallised from ethanol-benzene, had m. p. 129°.

(ii) The hydrochloride (5 g.) in absolute ethanol (70 ml.) was treated with sodium (0.35 g.). Excess of keten (2 g.) (Herriot, *J. Gen. Physiol.*, 1934, **18**, 69) was passed into the filtered solution. The product recrystallised from ethanol-light petroleum as white plates, m. p. 122–123°,  $[\alpha]_D^{19}$   $-37^\circ$  (*c*, 0.96 in H<sub>2</sub>O),  $R_F$  0.81 (Found: C, 44.0; H, 7.6; N, 4.2; S, 19.2. Calc. for C<sub>12</sub>H<sub>25</sub>O<sub>5</sub>NS<sub>2</sub>: C, 44.0; H, 7.6; N, 4.3; S, 19.6%).

*N-Benzoyloxycarbonyl-D-glucosamine Diethyl Mercaptal*.—D-Glucosamine diethyl mercaptal hydrochloride (5 g.) in water (50 ml.) containing sodium hydrogen carbonate (3.5 g.) was treated with benzyl chloroformate (3.5 ml.). *N-Benzoyloxycarbonyl-D-glucosamine diethyl mercaptal*

(6.8 g.), which separated at 0°, recrystallised from aqueous alcohol or benzene as white plates, m. p. 94°,  $[\alpha]_D^{25} - 28^\circ$  (*c.* 0.29 in EtOH),  $R_F$  0.90 (Found: C, 51.2; H, 7.0; N, 3.4; S, 15.5.  $C_{18}H_{29}O_6NS_2$  requires C, 51.4; H, 6.9; N, 3.3; S, 15.3%).

*Pentamethyl-D-glucosamine Diethyl Mercaptal*.—The mercaptal hydrochloride (3.2 g.) in dioxan (50 ml.) was treated with sodium chloride (6 g. in 15 ml. of water) and methyl sulphate (3.8 ml.). After addition of solid carbon dioxide, the mixture was heated to the b. p., neutralised with sulphuric acid, and extracted repeatedly with chloroform. The extract of methyl derivative was dried ( $MgSO_4$ ) and concentrated to a syrup,  $[\alpha]_D^{25} - 15.8^\circ$  (*c.* 0.4 in  $CHCl_3$ ) (Found: OMe, 34.6.  $C_{15}H_{23}O_4NS_2$  requires OMe, 34.9%).

*N-Benzoyl-D-glucosamine Diethyl Mercaptal*.—Glucosamine diethyl mercaptal hydrochloride (0.5 g.) was treated with redistilled pyridine (5 ml.) and benzoyl chloride (0.5 ml.) and after 12 hr. poured into ice-water. The precipitate of *N-benzoyl-D-glucosamine diethyl mercaptal*, washed with water and recrystallised from aqueous ethanol, had m. p. 130—131°,  $[\alpha]_D^{25} - 18^\circ$  (*c.* 0.23 in EtOH),  $R_F$  0.98 (Found: C, 51.9; H, 6.9; N, 3.5; S, 15.9.  $C_{17}H_{27}O_5NS_2$  requires C, 52.4; H, 6.9; N, 3.6; S, 16.4%).

*Attempted O-Benzoylation of Glucosamine Diethyl Mercaptal*.—Treatment of the diethyl mercaptal hydrochloride with excess of benzoyl chloride yielded a product, m. p. 79—80° [C, 62.2; H, 5.25; N, 1.8; S, 9.2. Calc. for  $C_{45}H_{43}O_9NS_2$  (the pentabenzoyl mercaptal): C, 67.2; H, 5.3; N, 1.7; S, 7.95%]. Repeated crystallisation yielded a product, m. p. 129—130° alone or mixed with *N-benzoylglucosamine diethyl mercaptal*.

*N-Acetyl-tetra-O-benzoylglucosamine Diethyl Mercaptal*.—*N*-Acetylglucosamine diethyl mercaptal (0.5 g.) was dissolved in redistilled pyridine containing benzoyl chloride (1.2 ml.) and after 24 hr. poured into ice-water. The *penta-acyl* derivative, recrystallised from aqueous alcohol, had m. p. 141—143°,  $[\alpha]_D^{25} + 5^\circ$  (*c.* 0.5 in  $COMe_2$ ) (Found: C, 64.1; H, 5.6; N, 1.7; S, 9.2.  $C_{40}H_{41}O_9NS_2$  requires C, 64.6; H, 5.5; N, 1.9; S, 8.6%).

*Penta-acetyl-D-glucosamine Diethyl Mercaptal*.—*N*-Acetylglucosamine diethyl mercaptal (2 g.) was treated with acetic anhydride (5 ml.) in pyridine (20 ml.) and after 24 hr. poured into ice-water. The mixture was extracted with chloroform, and the extracts were washed repeatedly with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution, and water before being dried ( $Na_2CO_3$ ). Evaporation gave a syrup which crystallised from ethanol-light petroleum (b. p. 60—80°). The mercaptal, crystallised from chloroform-light petroleum, had m. p. 177°,  $[\alpha]_D^{20} - 36.5^\circ$  (*c.* 0.5 in  $CHCl_3$ ),  $R_F$  0.95 (Found: C, 48.5; H, 6.7; N, 2.8.  $C_{20}H_{33}O_9NS_2$  requires C, 48.5; H, 6.7; N, 2.8%). Wolfrom, Lemieux, and Olin (*J. Amer. Chem. Soc.*, 1949, 71, 2870) reported m. p. 126—127°,  $[\alpha]_D^{25} - 32^\circ$  (in  $CHCl_3$ ), which Wolfrom and Anno (*ibid.*, 1952, 74, 6150) corrected to m. p. 75—77°,  $[\alpha]_D + 1^\circ$ .

*2-Acetamido-3:4:5:6-tetra-O-acetyl-2-deoxy-D-glucose (Penta-acetyl-aldehydo-D-glucosamine)*.—To penta-acetyl-D-glucosamine diethyl mercaptal (2.9 g.) in 30% aqueous acetone (60 ml.), cadmium carbonate (20 g.) and mercuric chloride (5 g.) in acetone (30 ml.) were added. After 24 hr. the mixture was heated at 50° for 15 min. and finally at the b. p. for 15 min. The filtrate was freed from traces of a mercury complex by chromatography on powdered cellulose with *n*-butanol-ethanol-water (4:1:5) as eluant (Hough, Jones, and Wadman, *J.*, 1949, 2511). The product was detected in the effluent by its colour with aniline hydrogen phthalate. The fractions containing aldehydo-*penta-acetylglucosamine* were concentrated to a syrup; crystallised from isopropyl ether this (0.2 g.) had m. p. 156—157° (decomp.),  $[\alpha]_D^{25} + 36.7^\circ$  (*c.* 0.1 in  $CHCl_3$ ),  $R_F$  0.92 (Found: C, 49.1; H, 5.75; N, 3.3.  $C_{16}H_{23}O_{10}N$  requires C, 49.3; H, 5.9; N, 3.6%).

*2-Amino-2-deoxy-D-galactose Diethyl Mercaptal Hydrochloride (D-Galactosamine Diethyl Mercaptal Hydrochloride)*.—*D*-Galactosamine (0.5 g.) was treated with ethanethiol (2.5 ml.) and fuming hydrochloric acid (5 ml.) under the conditions described for glucosamine diethyl-mercaptal. The product ( $n_D^{25}$  1.5302) failed to crystallise.

*2-Acetamido-3:4:5:6-tetra-O-acetyl-2-deoxy-D-galactose Diethyl Mercaptal*.—The preceding hydrochloride (0.5 g.) with acetic anhydride (2 ml.) in dry pyridine (4 ml.) gave the *amide*, m. p. 184° (from ethyl acetate),  $[\alpha]_D^{20} - 22^\circ$  (*c.* 0.36 in  $CHCl_3$ ),  $R_F$  0.95 (Found: C, 49.0; H, 6.6.  $C_{20}H_{33}O_9NS_2$  requires C, 49.5; H, 6.7%).

*2-Benzoyloxycarbonylamino-2-deoxy-D-galactose Diethyl Mercaptal*.—The mercaptal hydrochloride was treated with benzyl chloroformate as in the preceding case. The product had m. p. 59—61°,  $[\alpha]_D^{25} + 33^\circ$  (*c.* 0.3 in  $CHCl_3$ ),  $R_F$  0.88 (Found: C, 51.15; H, 6.8.  $C_{18}H_{29}O_6NS_2$  requires C, 51.4; H, 6.9%).

*Colour Reactions*.—(i) *Molisch test*. The modification described by Devor (*J. Amer. Chem. Soc.*, 1950, 72, 2008) was employed. No positive reaction (increased absorption at 570 m $\mu$ )

was given by pyranose forms of D-glucosamine (free sugar, glucosaminides) or by any of the acyclic derivatives in the concentration range 0.1—1.0 mg./ml. By contrast 3-amino-3-deoxyaltrose hydrochloride and 2-chloro-2-deoxyaltrose (kindly presented by Dr. L. F. Wiggins) gave strong colours under similar conditions.

(ii) *Elson-Morgan reaction* (*Biochem. J.*, 1933, **27**, 1824; 1934, **28**, 988). The diethyl mercaptals failed to give the characteristic colour reaction given by D-glucosamine hydrochloride and *N*-acetyl- and *N*-benzyloxycarbonyl-glucosamine. *aldehydo*-Penta-acetyl-D-glucosamine and the isomeric 1 : 3 : 4 : 6-tetra-*O*-acetyl-*N*-acetylglucosamine gave strong colours. 3-Amino-3-deoxyaltrose and 6-amino-6-deoxyglucose were without reaction. These findings appear to be in accordance with the findings of Morgan (*Chem. and Ind.*, 1938, **57**, 1191) and Morgan and King (*Biochem. J.*, 1943, **37**, 640).

(iii) *Dische-Borenfreund reaction* (*J. Biol. Chem.*, 1950, **184**, 517). D-Glucosamine diethyl mercaptal gave a pink colour in contrast to the orange colour from glucosamine and glucosaminide hydrochlorides, in concentrations of 0.1—1.0 mg./ml.

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DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF OXFORD.

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