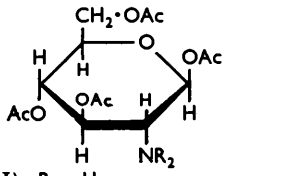
152. *Synthesis of New Sugar Derivatives of Potential Antitumour Activity. Part II.* Di-2-(chloroethyl)-D-glucosamine Hydrochloride.*

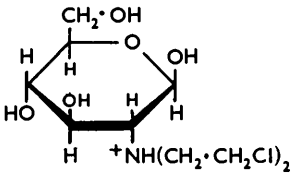
By L. Vargha, Ö. Fehér, and S. Lendvai.

For study of its possible tumour-inhibiting action *N*-di-2-(chloroethyl)-D-glucosamine hydrochloride has been synthesized from tetra-*O*-acetyl-D-glucosamine.

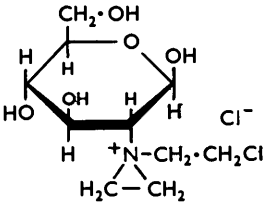
1 : 6-Di-(2-chloroethylamino)-1 : 6-deoxy-D-mannitol dihydrochloride * showed in animal tests and in clinical experience significant antitumour action and cytoactivity. Accordingly, it seemed desirable to prepare sugar derivatives carrying the di-(2-chloroethyl)amino-group characteristic of "nitrogen mustards." Natural D-glucosamine was chosen as starting material. According to Quastel,[1] D-glucosamine itself possesses tumour-inhibiting activity, but this has not been confirmed by Lettré [2] or by Németh and Sellei.[3] In order to obtain the *NN*-di-(2-chloroethyl) derivative (IV), tetra-*O*-acetyl-D-glucosamine [4] (I) was treated with ethylene oxide to give the *NN*-di-(2-hydroxyethyl) derivative (II), which was converted by thionyl chloride into the *NN*-di-(2-chloroethyl) derivative



(I) R = H
(II) R = $CH_2 \cdot CH_2 \cdot OH$
(III) R = $CH_2 \cdot CH_2Cl$
(VI) R = $CH_2 \cdot CH_2 \cdot O \cdot SO_2 \cdot Me$



(IV)



(V)

(III). Both compounds were isolated in homogeneous crystalline form. The usual methods for removing acetyl groups requiring an alkaline medium were not applicable owing to the sensitivity of the carbon–chlorine bonds. However, hydrolysis by hydrochloric acid gave fair yields. The structure of *NN*-di-(2-chloroethyl)-D-glucosamine hydrochloride (IV) obtained in this way is confirmed by analysis and by reconversion into the tetra-acetate (III). Although the derivative (IV) did not mutarotate in water, its methanolic solution showed rising mutarotation, indicative of the β-configuration. When mutarotation stopped, the product was recovered unchanged, confirming that the mutarotation is due to anomerisation.

The compound (IV), on treatment with phenylhydrazine, loses the tertiary amine group, yielding D-glucosazone, just as glucosamine does.

Although the aqueous solution of 1 : 6-di-(2-chloroethylamino)-1 : 6-deoxy-D-mannitol dihydrochloride was stable for months, that of the glucosamine derivative (IV) altered in several days, in that about half of the chlorine was converted into chloride ions, presumably with formation of the iminium cation (V) : Hanby and his co-workers demonstrated [5] the conversion of tertiary 2-chloroethylamine derivatives into ethyleneiminium cations in aqueous solution.

Attempts were made to prepare also the methane- and toluene-*p*-sulphonyloxy-derivatives analogous to (IV) in the hope of finding them also cytoactive. The compound (II)

* Part I, preceding paper.

[1] Quastel, *Nature*, 1953, **171**, 252.
[2] Lettré, *Naturwiss.*, 1953, **40**, 513.
[3] Personal communication.
[4] Bergmann and Zervas, *Ber.*, 1931, **64**, 975.
[5] Hanby, Hartley, Powell, and Rydon, *J.*, 1947, 519.

with methanesulphonyl chloride gave the expected diester derivative (VI), but with toluene-*p*-sulphonyl chloride the hydroxyl groups were replaced by chlorine.[6] Efforts to remove the acetyl groups from the dimethanesulphonate (VI) were unsuccessful.

According to the tests carried out by Kellner and Németh,[7] *NN*-di-(2-chloroethyl)-D-glucosamine hydrochloride is almost as toxic as " nitrogen mustard," its lethal dosage being 5 mg./kg. for rats, 8—10 mg./kg. for mice, the symptoms being similar to those of " nitrogen mustard." Daily doses of 2 mg. applied for a week showed 50% inhibition of growth of Guerin tumour. However, the experimental animals died in ten days.

Experimental

Tetra-O-acetyl-N-di-(2-hydroxyethyl)-D-glucosamine (II).—To an aqueous solution (529 g.) containing ethylene oxide (188 g.), tetra-*O*-acetyl-D-glucosamine (I) (39·3 g.) was added at 10—15°. The mixture was kept for 9—12 days at 10—15°, then evaporated to about 100 ml. under reduced pressure, and the precipitate was filtered off (23—26 g.). The *product* had m. p. 131·5—132·5°, $[\alpha]_D^{20}$ +38·6° (*c* 1·0 in $CHCl_3$), after recrystallization from ethanol (Found: C, 49·8; H, 6·8; N, 3·2. $C_{18}H_{29}O_{11}N$ requires C, 49·7; H, 6·7; N, 3·2%).

Tetra-O-acetyl-N-di-(2-chloroethyl)-D-glucosamine (III).—(*a*) To a solution of the foregoing (II) (10 g.) in chloroform (80 ml.) and pyridine (7·5 ml.) at 0°, thionyl chloride (10 ml.) was added; the mixture was boiled for 5 hr. and the solvent distilled off under reduced pressure. The residue was mixed with ice-water, the water discarded, and this procedure repeated several times, gives a crystalline *amine* (10·23 g.) which, recrystallized from ethanol, had m. p. 103—104°, $[\alpha]_D^{20}$ +39·1° (*c* 1·0 in $CHCl_3$) (Found: C, 46·0; H, 5·8; N, 3·0; Cl, 15·1. $C_{18}H_{27}O_9NCl_2$ requires C, 45·8; H, 5·8; N, 3·0; Cl, 15·0%).

(*b*) To tetra-*O*-acetyl-*N*-di-(2-hydroxyethyl)-D-glucosamine (0·22 g.) in pyridine (1·1 ml.), toluene-*p*-sulphonyl chloride (0·24 g.) was added and the mixture was kept for 2 hr. at 20—25°, then heated on the water-bath for 30 min. Pouring the mixture on ice, filtering off the precipitate (0·1 g.), and recrystallizing it from ethanol, gave a product m. p. 102—104°, identical (analysis; mixed m. p.) with that obtained as in (*a*).

Tetra-O-acetyl-N-di-(2-methanesulphonyloxyethyl)-D-glucosamine (VI).—To tetra-*O*-acetyl-*N*-di-(2-hydroxyethyl)-D-glucosamine (1 g.) in pyridine (2·5 ml.), methanesulphonyl chloride (0·89 g.) was added at 0°. The mixture was kept for 10 min. at 0° and for 45 min. at 20—25°, then poured on ice; the precipitated *ester* was filtered off, mixed with methanol (2·4 ml.), again filtered, and dried (yield 1·06 g.); it had m. p. 125—127° (decomp.), $[\alpha]_D^{20}$ +18·8° (*c* 1·0 in $CHCl_3$) (Found: N, 2·3; S, 10·8. $C_{20}H_{33}O_{15}NS_2$ requires N, 2·4; S, 10·8%).

N-Di-(2-chloroethyl)-D-glucosamine Hydrochloride (IV).—Tetra-*O*-acetyl-*N*-di-(2-chloroethyl)-D-glucosamine (10 g.) in concentrated hydrochloric acid (95 ml.) was kept for 7—8 hr. at 50°, then evaporated under reduced pressure, and the residue mixed with acetone. The precipitated crystals (6·9 g.) were filtered off, dissolved in acetone (41 ml.) containing 20% of water, clarified (carbon), and diluted with small portions of acetone (total 83 ml.). The resulting white needles were collected (4·27 g.). From the mother-liquor, a further 1·33 g. of the substance were recovered. The substance contains 3 mols. of water. At 60°/0·5 mm. it loses 12·6% in weight. The *hydrate* has m. p. 75·5—76·5°, $[\alpha]_D^{20}$ +25·4° (*c* 1·14 in MeOH) after 10 min., +42·3° after 8 hr. (Found: C, 30·6; H, 6·6; N, 3·45; Cl, 27·1; Cl^-, 8·8; H_2O, 12·6. $C_{10}H_{20}O_5NCl_3,3H_2O$ requires C, 30·4; H, 6·6; N, 3·55; Cl, 26·95; Cl^-, 9·0; H_2O ,13·7%).

The needles are converted in acetone containing 10% of water, in several days, into thick prisms without change in analytical values, m. p., or rotation. These prisms, however, at 60°/0·5 mm. lose only 2·2% of water. In aqueous solution the chloride ion content rose from 8·8% to 17·7% in 7 days.

A solution of the salt (IV) (0·2 g. of hydrate) in pyridine (1 ml.) containing acetic anhydride (0·6 ml.) was kept at 20—25° for 24 hr., then poured on ice. On recrystallization from absolute ethanol, the acetate had m. p. 102—104°, and on the basis of analysis, m. p., mixed m. p., and rotation, was indentical with the tetra-*O*-acetyl-*N*-di-(2-chloroethyl)-D-glucosamine prepared as above.

[6] Cf. Tipson, *Adv. Carbohydrate Chem.*, 1953, **8**, 121.
[7] Kellner and Németh, personal communication.

A solution of the salt (IV) (0·5 g. of hydrate), phenylhydrazine hydrochloride (1 g.), and sodium acetate (1·5 g.) in water (10 ml.) was heated for 20 min. on the water-bath; the precipitate, recrystallized from ethanol, had m. p. 202—205° (decomp.) alone or mixed with glucosazone. The osotriazole prepared from the product had m. p. and mixed m. p. identical with that of phenyl-D-glucosotriazole (195—196°) obtained from authentic glucosazone.

Thanks are offered to B. Dumbovich, M.D., for his interest.

RESEARCH INSTITUTE OF THE PHARMACEUTICAL INDUSTRY,
BUDAPEST, VII, ROTTENBILLER U. 26, HUNGARY.

[*Received, July 30th*, 1956.]