151. *Synthesis of New Sugar Derivatives of Potential Antitumour Activity. Part I.* Ethyleneimino- and 2-Chloroethylamino-derivatives.*

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

When 5 : 6-anhydro-1 : 2-*O-iso*propylidene-D-glucofuranose (I) and 1 : 2-5 : 6-dianhydro-3 : 4-*O-iso*propylidene-D-mannitol (III) are treated with ethyleneimine, the corresponding ethyleneimino-derivatives (II) and (IV) are formed with cleavage of the epoxide ring. When 1 : 6-dideoxy-1 : 6-diethyleneimino-3 : 4-*O-iso*propylidene-D-mannitol (IV) is treated with hydrochloric acid, the ethyleneimino-ring splits, yielding the 1 : 6-bis-2-chloroethylamino-1 : 6-dideoxy-D-mannitol dihydrochloride (V), the structure of which is confirmed by an alternative synthesis. For biological test, the hydroxyl-free analogue (IX) and the 2-chloroethylamides of gluconic, glucosaccharic, and mannosaccharic acid have been prepared. Of these compounds, the amine (V) showed strong cytoactive and tumour-inhibiting activity.

Although compounds of very different type occur among the numerous known cytoactive substances, the only cytoactive sugar derivatives described are natural glucosides.[1] Synthetic cytoactive substances described in the literature, except for some amino-acid

* Preliminary communication, *Naturwiss.*, 1955, **42**, 582.

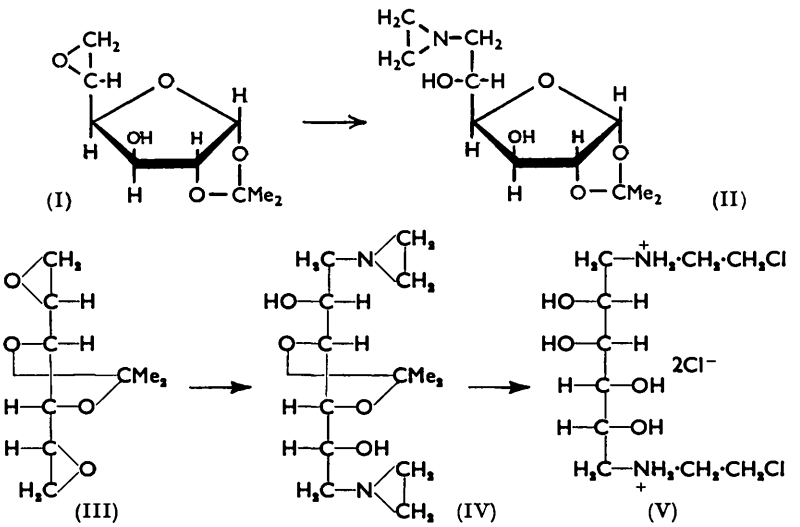
[1] Stoll, Renz, and von Wartburg, *Helv. Chim. Acta*, 1954, **37**, 1747.

derivatives,[2] are derivatives of compounds which do not occur in the body. However, it is known that compounds essential from a biological point of view, *e.g.*, natural amino-acids or sugars, readily pass through the cell membrane, and the present aim has been to study derivatives of these compounds. It was expected to find among them substances of stronger activity and greater selectivity than were known so far. Attention was first turned to the sugar group and we prepared ethyleneimino- and 1-di-(2-chloroethyl)amino-derivatives of sugars and similar compounds. The ethyleneimino-derivatives of sugars seemed also of interest from the chemical point of view because the three-membered heterocyclic ring is capable of manifold conversions not studied as yet in the sugar group.

For the preparation of ethyleneimino-derivatives of this type the epoxides of sugars and sugar alcohols seemed to be the most suitable starting materials, since generally on reaction with ammonia or organic bases, the anhydro-ring is opened and amino-compounds are formed. Similar behaviour with ethyleneimine was expected. First, the reaction of 5 : 6-anhydro-1 : 2-*O*-*iso*propylidene-D-glucofuranose [3] and of 1 : 2-5 : 6-dianhydro-3 : 4-*O*-*iso*propylidene-D-mannitol [4] (III) with ethyleneimine was studied.

Experiment confirmed that ethyleneimine reacts with the epoxides mentioned in the same way as other bases do. Addition starts at room temperature, is exothermic, and affords the ethyleneimino-derivatives (II) and (IV). 6-Ethyleneimino-1 : 2-*O*-*iso*propylidene-D-glucofuranose (II) is crystalline and stable for years. The *iso*propylidene group is not removed by the usual acid hydrolysis without the simultaneous cleavage of the ethyleneimino-ring. The constitution (II), *i.e.*, the 6-position of the ethyleneimino-group, is very probable by analogy, since in opening of the 5 : 6-anhydro-ring the basic group generally becomes attached [5] to position 6, and no example is known of an addition to position 5.

1 : 6-Dideoxy-1 : 6-diethyleneimino-D-mannitol (IV) was, however, unstable: it is converted within a few days into a glass of high molecular weight and insoluble in water. Concentrated hydrochloric acid converts it into crystalline 1 : 6-dideoxy-1 : 6-



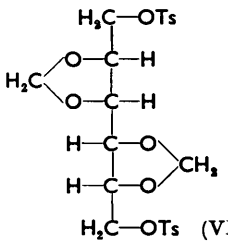
di-(2-chloroethylamino)-D-mannitol dihydrochloride (V); the free base is only slightly soluble in water and other solvents and also crystallises readily.

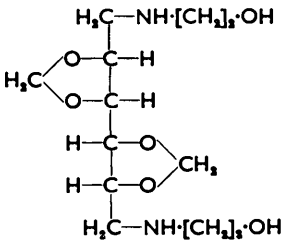
Although the 1 : 6-positions of the imino-groups are, by analogy, very probable, it seemed

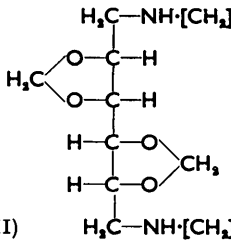
[2] Bergel, *J.*, 1954, 2409; Ross, *J.*, 1949, 183; Ross, Warwick, and Roberts, *J.*, 1955, 3110.
[3] Ohle and Vargha, *Ber.*, 1929, **62**, 2435.
[4] Wiggins, *J.*, 1946, 384.
[5] Peat, *Adv. Carbohydrate Chem.*, 1946, **2**, 68.

necessary to confirm the structures (IV) and (V) by an unambiguous synthesis, because the yield from the diepoxide (III) was only 50%, indicating the possibility of a simultaneous formation of isomers. 2 : 3-4 : 5-Di-*O*-methylene-D-mannitol 1 : 6-ditoluene-*p*-sulphonate [6] (VI) was converted by ethanolamine into 1 : 6-dideoxy-1 : 6-di-(2-hydroxyethylamino)-2 : 3-4 : 5-di-*O*-methylene-D-mannitol (VII), which withthionyl chloride gave the di-(2-chloroethylamino)-derivative (VIII). The product obtained from this after removal of the methylene groups was proved (m. p., mixed m. p., analysis, and optical rotation) to be identical with that (V) prepared from the dianhydro*iso*propylidenemannitol (III). The diamine (V) was obtained similarly from di-*O*-dibenzylidene-D-mannitol 1 : 6-ditoluene-*p*-sulphonate, described by Hudson and co-workers.[7]

To decide whether the presence of hydroxyl groups plays a rôle in the antitumour


(VI)


(VII)


(VIII)

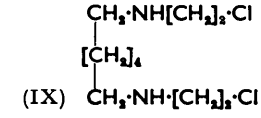
Ts = *p*-$C_6H_4Me{\cdot}SO_2$

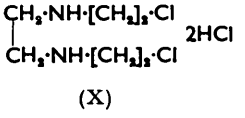
activity, the hydroxyl-free analogue of (V), namely, 1 : 6-di-(2-chloroethylamino)-*n*-hexane dihydrochloride (IX), and a lower homologue, 1 : 2-di-(2-chloroethylamino)ethane dihydrochloride (X), were prepared from 1 : 6-dichloro-*n*-hexane [8] and 1 : 2-dichloroethane, respectively, as above.

Further, the 2-chloroethylamides of D-gluconic, D-glucosaccharic, and of D-mannosaccharic acid were prepared.

All these compounds, except (IV) which is unstable, were tested by the Hungarian Oncological Institute for antitumour action and therapeutic value. The results of these tests, published in detail elsewhere,[9] may be summarised as follows.

The hexane and ethane derivatives (IX, X) and the amides showed no inhibiting action on Guerin rat carcinoma, N-1 rat sarcoma, Crocker mouse sarcoma, or Ehrlich ascites tumour in doses up to 50 mg./kg., whereas the glucose derivative (II) was slightly active. 75% inhibition of growth was observed in the mentioned tumours when 1 : 6-di-(2-chloroethylamino)-1 : 6-dideoxy-D-mannitol dihydrochloride (" BCM ") (V) was applied in daily doses of 10—20 mg./kg. LD50 was found to be 60—80 mg. with mice or rats. The substance showed inhibiting effect also in the leukemia of mice. On the basis of clinical investigations it was suitable mainly for therapy of malignant hematological diseases such as lymphogranulomatosis (Hodgkin's disease), chronic lymphoid and myeloid leukemia, and lymphosarcoma. Good results were obtained also in cases resistant to *X*-ray irradiation and to " nitrogen mustard."


(IX)


(X)

This substance, a crystalline compound stable in aqueous solution, represents a new type of biological alkylating agent of antitumour activity, not only since it may be considered as a sugar derivative, but also because in contrast to " nitrogen mustard " and

[6] Haworth, Jones, Stacey, and Wiggins, *J.*, 1944, 61.
[7] Haskins, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1943, **65**, 1419.
[8] Gensler and Thomas, *J. Amer. Chem. Soc.*, 1951, **73**, 4601.
[9] Kellner, Németh, and Sellei, *Naturwiss.*, 1955, **42**, 482; Sellei, Eckhardt, Hartai, and Dumbovich, *Lancet*, 1956, 785; Kellner and Németh, *Z. Krebsforsch.*, 1956, **61**, 165.

its derivatives, it contains two secondary nitrogen atoms. Although 2-chloroethyl derivatives with one secondary nitrogen have been described,[10] these had no cytoactivity. Since the hydroxyl-free analogue (IX) proved inactive, the presence of hydroxyl groups seems indispensable for cytoactivity in this type of compound.

Experimental

6-Dideoxy-6-ethyleneimino-1 : 2-O-isopropylidene-D-glucofuranose (II).—To a suspension in dry ether (25 ml.) of 5 : 6-anhydro-1 : 2-*O-iso*propylidene-D-glucofuranose[3] (I) (10 g.), ethyleneimine (15 ml.) was added. After 4 days at room temperature the solvent was removed. The syrupy residue solidified slowly and was recrystallised from hot benzene. The *imine* (8 g.) was readily soluble in water, ethanol, or chloroform, but insoluble in light petroleum) and had m. p. 131—132°, $[\alpha]_D^{20}$ +17·1° (*c* 2·916 in $CHCl_3$), −8·0° (*c* 2·534 in H_2O) (Found: C, 54·9; H, 7·85; N, 5·6. $C_{11}H_{19}O_5N$ requires C, 55·1; H, 7·8; N, 5·7%).

1 : 6-Dideoxy-1 : 6-diethyleneimino-3 : 4-O-isopropylidene-D-mannitol (IV).—1 : 2-5 : 6-Dianhydro-3 : 4-*O-iso*propylidene-D-mannitol[4] (III) (20 g.), mixed with ethyleneimine (30 ml.), gradually became warm. The temperature was kept below 50°. After being kept overnight, the ethyleneimine was distilled under reduced pressure and the syrupy residue evaporated twice with methanol under reduced pressure to remove traces of ethyleneimine. The thick syrup was purified by dissolution in absolute ether, filtration, and evaporation under reduced pressure. Attempts to obtain the substance crystalline failed. It is readily soluble in water, benzene, and ethanol, and has $[\alpha]_D^{20}$ +51·6° (*c* 1·835 in $CHCl_3$).

1 : 6-Di-(2-chloroethylamino)-1 : 6-dideoxy-D-mannitol Dihydrochloride (V).—The crude derivative (IV) (20 g.) was added in methanol (20 ml.) slowly with stirring at 0° to concentrated hydrochloric acid (80 ml.). Slow crystallisation took place. Next day the mixture was kept at 0° for some hours, the precipitated *salt* was filtered off, washed with cold concentrated hydrochloric acid and with 80% ethanol, dried under reduced pressure (KOH), and recrystallised from 75—80% ethanol (yield 20 g.). The salt was soluble in water, slightly so in ethanol, but insoluble in other organic solvents, and had m. p. 239—241° (decomp.), $[\alpha]_D^{20}$ +18·46° (*c* 1·812 in H_2O) (Found: C, 31·6; H, 6·5; N, 7·65; Cl, 37·5; Cl^-, 18·5. $C_{10}H_{24}O_4N_2Cl_4$ requires C, 31·7; H, 6·4; N, 7·4; Cl, 37·5; Cl^-, 18·75%). The aqueous solution is stable for weeks without a considerable rise of the chloride ions.

To a solution of the dihydrochloride (0·945 g.) in water (3 ml.), 2·0N-sodium hydroxide (2·5 ml.) was added with cooling by ice. On rubbing, a white crystalline precipitate appeared immediately. The *base* (0·6 g.) was slightly soluble in water, ethanol, and pyridine, and shrinks above 250°, decomposing at 278° (Found: C, 39·2; H, 7·1; N, 9·3; Cl, 23·2. $C_{10}H_{22}O_4N_2Cl_2$ requires C, 39·35; H, 7·3; N, 9·2; Cl, 23·2%). Aqueous-ethanolic hydrochloric acid reconverts it into the dihydrochloride, m. p. 239—241°.

1 : 6-Dideoxy-1 : 6-di-(2-hydroxyethylamino)-2 : 3-4 : 5-di-O-methylene-D-mannitol (VII).—2 : 3 : 4 : 5-Di-*O*-methylene-D-mannitol 1 : 6-ditoluene-*p*-sulphonate[6] (VI) (4 g.) and ethanolamine (10 g.) were heated at 150—160° for 8 hr. After cooling, the mixture was warmed with a solution of barium hydroxide (5 g. of hydrate) in water (40 ml.) for 30 min. at 90—95°, water and excess of ethanolamine were removed at 1—3 mm., and the residue was extracted with propan-2-ol (4 × 50 ml.). Attempts to crystallise the brown syrup (2 g.) obtained on evaporation of the propan-2-ol solution were unsuccessful. Treating the syrup in absolute ethanol with oxalic acid gave a *di(hydrogen oxalate)*, m. p. 190° (decomp.) (from aqueous ethanol), m. p. 190°, $[\alpha]_D^{20}$ +32·6° (*c* 0·411 in H_2O) (Found: C, 40·8; H, 5·9; N, 5·8. $C_{16}H_{28}O_{14}N_2$ requires C, 40·7; H, 5·9; N, 5·9%).

1 : 6-Di-(2-chloroethylamino)-1 : 6-dideoxy-D-mannitol Dihydrochloride (V) *from* (VII).—Crude 1 : 6-dideoxy-1 : 6-di-(2-hydroxyethylamino)-2 : 3-4 : 5-di-*O*-methylene-D-mannitol (VII) (1·8 g.) was evaporated to dryness with N-hydrochloric acid (16 ml.) under reduced pressure, and the residue treated with thionyl chloride (20 ml.) to yield a clear liquid (30 min. at 65°). After removal of the thionyl chloride under reduced pressure, the residual dark amorphous product was boiled with 10% hydrochloric acid for 16 hr. to remove the methylene groups, then treated with carbon, and evaporated under reduced pressure. The solid residue, recrystallised (carbon) from 70% ethanol, gave the *salt* (0·3 g.), m. p. and mixed m. p. 240—242°, $[\alpha]_D^{20}$ +18·6° (*c* 1·80 in H_2O) (Found: C, 31·6; H, 6·4; N, 7·5; Cl, 37·45; Cl^-, 18·5%).

[10] Wilson and Tishler, *J. Amer. Chem. Soc.*, 1951, **73**, 3635.

1 : 6-*Dideoxy*-1 : 6-*di*-(2-*hydroxyethylamino*)-*di*-O-*benzylidene*-D-*mannitol*.—Di-*O*-benzylidene-D-mannitol 1 : 6-ditoluene-*p*-sulphonate [7] (5 g.) was heated with ethanolamine (30 ml.) for 8 hr. at 150—160°, excess of ethanolamine removed at 1—2 mm., and the residue heated with barium hydroxide (5 g. of hydrate) in water (100 g.) on the water-bath for 30 min. and evaporated to dryness. The residue was extracted with propan-2-ol (3 × 100 ml.), and the solvent removed, giving 3·3 g. of a syrup, which in absolute ethanol gave a *di*(*hydrogen oxalate*), decomp. 212—214° (from aqueous propan-2-ol), $[\alpha]_D^{20}$ +49·3° (*c* 0·772 in H_2O) (Found : C, 53·5; H, 5·9; N, 4·2. $C_{28}H_{36}O_{14}N_2$ requires C, 53·8; H, 5·8; N, 4·5%).

The crude product (3 g.) was boiled for 15 min. with thionyl chloride (30 ml.), then the thionyl chloride was removed under reduced pressure, the residue boiled with 5% hydrochloric acid (50 ml.) for 10 min., treated with carbon, and evaporated under reduced pressure. The solid residue of the salt (V) (0·7 g.), recrystallised from 70% ethanol, had m. p. and mixed m. p. 239—241° (decomp.), $[\alpha]_D^{20}$ +18·4° (*c* 1·82 in H_2O) (Found : N, 7·2; Cl, 37·4; Cl^-, 18·55%).

1 : 6-*Di*-(2-*hydroxyethylamino*)*hexane*.—1 : 6-Dichlorohexane [8] (40 g.) was added dropwise to ethanolamine (100 ml.) at 120—130° with stirring for 20 min. The mixture was kept at 150—160° for 6 hr., cooled, treated with sodium hydroxide (25 g.) in methanol (500 ml.), and kept for several hours at 0°. The precipitated sodium chloride was filtered off, the methanol removed by distillation, and the residue fractionated : ethanolamine was followed at 185—195°/8 mm. by the *diamine* (17 g.), m. p. (from ethanol) 78—80° (Found : C, 58·5; H, 12·05; N, 13·3. $C_{10}H_{24}O_2N_2$ requires C, 58·8; H, 11·8; N, 13·7%).

1 : 6-*Di*-(2-*chloroethylamino*)*hexane*.—1 : 6-Di-(2-hydroxyethylamino)hexane (10 g.) was boiled for 100 min. with thionyl chloride (100 ml.), the latter was removed under reduced pressure, the residue rubbed with propan-2-ol, and the dark powder filtered off, washed with propan-2-ol, and extracted with hot propan-2-ol (2 l.) (carbon). After 20 hr. at 0°, the precipitated *dihydrochloride* (IX) was filtered off. The crude product was extracted twice more with the mother-liquor from this salt. The product (total yield 4—5 g.) decomposed at 250—253° (Found : N, 8·85; Cl, 45·3; Cl^-, 22·8. $C_{10}H_{24}N_2Cl_4$ requires N, 8·9; Cl, 45·2; Cl^-, 22·6%).

1 : 2-*Di*-(2-*chloroethylamino*)*ethane Dihydrochloride* (X).—1 : 2-Di-(2-hydroxyethyl)ethane [11] (6 g.) was stirred with thionyl chloride (50 ml.) at 15° for 20 min., then for an hour at 80—85°. Working up as in the preceding case gave the *salt* (total yield, 4—5 g.), decomp. at 210—212° (Found : N, 10·7; Cl, 55·1; Cl^-, 27·9. $C_6H_{16}N_2Cl_4$ requires N, 10·85; Cl, 55·0; Cl^-, 27·5%).

N-2-*Chloroethyl*-D-*gluconamide*.—To a suspension of 2-chloroethylamine hydrochloride (18·47 g.) and D-gluconolactone (24·82 g.) in methanol (600 ml.), sodium methoxide (3·4 g. of sodium in 60 ml. of methanol) was added with stirring. After being kept overnight, the *amide* was filtered off, washed with water and methanol, and recrystallised from methanol. It (20 g.) had m. p. 144—145°, $[\alpha]_D^{20}$ +28·18° (*c* 1·856 in H_2O) (Found : C, 37·4; H, 6·4; N, 5·3; Cl, 13·7. $C_8H_{16}O_6NCl$ requires C, 37·3; H, 6·3; N, 5·4; Cl, 13·8%).

NN'-*Di*-(2-*chloroethyl*)-D-*saccharodiamide*.—To a solution of the calcium chloride compound [12] of diethyl D-saccharate (3·54 g.) in methanol (63 ml.), were added 2-chloroethylamine hydrochloride (5·1 g.), then slowly (ice-cooling) 2·0N-methanolic sodium methoxide (20·2 ml.). After filtration and storage at room temperature for 4 hr., the solvent was removed under reduced pressure, the residue treated with water (10 ml.), and the precipitate (2·26 g.) washed with water. Recrystallisation from methanol gave the *amide*, m. p. 173—174° (decomp.), slightly soluble in water, $[\alpha]_D^{20}$ +22·15° (*c* 0·50 in MeOH) (Found : C, 35·8; H, 5·6; N, 8·4; Cl, 21·3. $C_{10}H_{18}O_6N_2Cl_2$ requires C, 36·0; H, 5·4; N, 8·4; Cl, 21·3%).

NN'-*Di*-(2-*chloroethyl*)-D-*mannosaccharodiamide*.—To 2-chloroethylamine hydrochloride (3·48 g.) and D-mannosaccharodilactone (1·6 g.) in water (5 ml.), 5·0N-sodium hydroxide (5·45 ml.) was added at 0° in portions. The dilactone dissolved, then the sparingly soluble *diamide* crystallised. When washed with water and recrystallised from methanol, it (1·6 g.) had m. p. 179—180° (decomp.), $[\alpha]_D^{20}$ −26·38° (*c* 0·50 in MeOH) (Found : C, 36·3; H, 5·7; N, 8·4; Cl, 21·0. $C_{10}H_{18}O_6N_2Cl_2$ requires C, 36·0; H, 5·4; N, 8·4; Cl, 21·3%).

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.]

[11] D.R.P. 635,903.
[12] Heintz, *Ann. Physik*, 1858, **105**, 231.