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Synthetic Aminosugar Derivatives as Potential Antimicrobials; N-Substituted Derivatives of 6-Amino-6-Deoxy-D-glucose Ethylene Dithioacetal

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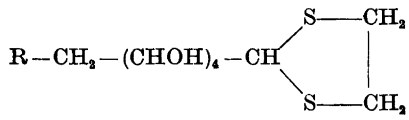
A series of N-substituted derivatives of 6-amino-6-deoxy-D-glucose ethylene dithioacetal has been prepared and tested as antimicrobials.

The occurrence of aminosugars is a general characteristic of the structures of several antibiotics [1,2]. Recent developments in the chemistry of antibiotics, therefore, have stimulated the interest in synthetic aminosugar derivatives as potential antimicrobials.

In some of the antibiotics the aminogroup is found in position 3 of the sugar moiety in question. Thus, 3-amino-3-deoxy-D-ribose is the carbohydrate component of puromycin, and rare 3-dimethylamino-hexoses (desosamine, mycaminose) occur in the so-called macrolide antibiotics. Streptomycin is a well-known example of an antibiotic containing a 2-aminosugar (2-methylamino-2-deoxy-L-glucose) and an ω-aminosugar (6-amino-6-deoxy-D-glucose) occurs in the recently described antibiotic kanamycin [3].

Among these aminosugars constituting a part of the specific structure of antibiotics, 6-amino-6-deoxy-D-glucose is of interest because of the possibility of replacing its terminal primary aminogroup by a variety of amine components according to known synthetic methods [4]. The 6-amino-6-deoxy-D-glucose derivatives described in the present work have a tertiary aminogroup linked to carbon 6 and their reducing function blocked in a cyclic dithioacetal group, these features creating a structure which may be expected not to fit into the pattern of the general metabolism of D-glucose. In order to obtain some information about the possible antimicrobial effect of compounds possessing this structure only a short series of derivatives has been prepared, Table 1. One reason for choosing the ethylene dithioacetal group to block the reducing function was that derivatives of 1,3-dithiolane apparently have received some attention as fungicides [5].

Table 1. Analysis of the synthesized N-substituted derivatives of 6-amino-6-deoxy-D-glucose ethylene dithioacetal.



R	m.p. °C	$[\alpha]_D^{23}$ in chloroform	Elementary analysis				
				% C	% H	% N	% S
A. Piperidino-	104–105	−24.1°	found	48.71	7.73	4.40	19.64
		(*c*=2)	calc.	48.27	7.79	4.33	19.83
B. Morpholino-	120–121	− 6.9°	found	44.52	7.13	4.38	20.04
		(*c*=2)	calc.	44.29	7.12	4.31	19.71
C. Pyrrolidino-	113–114	−18.2°	found	46.64	7.51	4.62	20.72
		(*c*=2)	calc.	46.58	7.49	4.53	20.73
D. Dibenzylamino-	137–138	+4.9°	found	60.70	6.68	3.23	14.54
		(*c*=3.8)	calc.	60.68	6.71	3.22	14.72

The compounds listed in Table 1 were tested by the "agar cup" method against a selection of pathogenic bacterium strains (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus faecalis*) growing on 10 % peptone-free horse-blood agar, and found to be without antibacterial activity. The same substances were also tested against a selection of pathogenic fungus strains (*Candida albicans, Cryptococcus neoformans, Aspergillus flavus, Aspergillus niger, Trichophyton mentagrophytes*) using the same experimental technique (10 % peptone-free horse-blood agar containing 1 % of D-glucose). The results were in most cases completely negative as to antifungal activity. But two of the compounds of Table 1, A and C, were able at a high concentration (1 %) to inhibit the growth of the highly resistant fungus strains *Cryptococcus neoformans* and *Aspergillus niger*. Since the parent nitrogen-free sugar derivative, D-glucose ethylene dithioacetal [6], was inactive towards all the microorganisms tested, the observed activity of substances A and C (Table 1) should encourage further research on aminosugar derivatives of a structure corresponding to the pattern outlined in the present work.

The synthesis of each of the compounds listed in Table 1 was achieved by classical reactions using 5,6-anhydro-1,2-*iso*-propylidene-D-glucofuranose as starting material: after opening of the epoxide ring by various amines, the *iso*propylidene residue was removed under mild conditions and the resulting aminosugar (without isolation) condensed with ethane-1,2-dithiol. The starting material was prepared from 1,2-*iso*propylidene-D-glucofuranose obtainable either from 1,2-5,6-di-*iso*propylidene-D-glucofuranose or direct from D-glucose. The modification of the direct method [7] described in the experimental section gave in repeated experiments a satisfactory yield of pure 1,2-*iso*propylidene-D-glucofuranose.

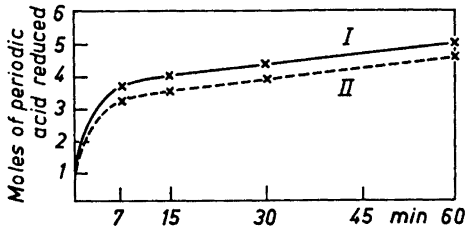


Fig. 1. Periodate uptake in acid solution of D-glucose ethylene dithioacetal, curve I, and of the corresponding 6-amino-6-deoxy-derivatives of Table 1, curve II. Curve II represents the arithmetic mean of the titrations of all four compounds of Table 1, the average deviation of a single measurement at each time interval being 1 % or less. Experimental conditions: 10^{-5} mole of each substance (1 ml) + 10^{-4} mole of sodium metaperiodate (10 ml) + 1 N sulphuric acid (5 ml). Periodate uptake by iodometry.

The opening of the epoxide ring of 5,6-anhydro-1,2-*iso*propylidene-D-glucofuranose by the various amines is, according to the theory [8], expected to proceed as a nucleophilic attack exclusively on carbon 6 and without inversion on carbon 5. Oxidation of the final products (of Table 1) with periodate gave results which tend to confirm that the aminogroup — as predicted by the general reaction mechanism — is located in position 6 of the sugar derivatives prepared.

In strongly acid solution α-amino-alcohols are oxidized by periodate at a considerably slower rate than the corresponding α-glycols [9]. A comparison between the oxidation in acid solution of D-glucose ethylene dithioacetal and the corresponding 6-aminoderivatives (of Table 1) gave results as shown in Fig. 1. The aminosugar derivatives consume about half a molecule of periodate less than the corresponding unsubstituted D-glucose mercaptal and, in contrast to the latter, they do not liberate formaldehyde at the end of one hour's oxidation (colorimetry with chromotropic acid [10]). These results are consistent with the expected effect of the replacement of the hydroxyl group on carbon 6 by an aminogroup, which is to render one carbon to carbon bond (C_6—C_5) more resistant towards periodate in acid solution. The periodate uptake (Fig. 1) is however, always higher than the quantity required to split the α-glycol groups of the compounds studied. A similar over-oxidation of non-cyclic sugar mercaptals has been observed previously [11].

EXPERIMENTAL

All melting points are micro melting points (Kofler's hot-stage microscope).

1. *1,2-Isopropylidene-D-glucofuranose.* D-Glucose (50 g) is shaken for 3—4 h with acetone (1 litre) containing concentrated sulphuric acid (40 ml). After filtration the reaction mixture is neutralized with sodium carbonate monohydrate (100 g) dissolved in water (200 ml). Solid material is removed by filtration and the filtrate concentrated at reduced pressure to 150—200 ml. The solution is heated to 60° and acidified by concentrated hydrochloric acid (about 0.5 ml); the colour of the solution changes from yellowish brown to brown upon acidification. One minute after the acid has been added the solution is cooled and extracted with chloroform (3 × 25 ml), which removes remaining diacetone-glucose and impurities. The colourless aqueous layer is neutralized to pH about 8

by means of a little sodium carbonate solution and then concentrated to dryness at a temperature below 40°. The residue is dried (15 mm Hg, P_2O_5) and then extracted with methanol (30°—40°) which on cooling gives crude, crystalline 1,2-*iso*propylidene-D-glucofuranose, m.p. 150°—160°, yield 50 %. Purification to m.p. 160—161°[7] by recrystallizations from light petroleum (b.p. 60°—80°).

2. *5,6-Anhydro-1,2-iso*propylidene-D-*glucofuranose.* 1,2-*Iso*propylidene-D-glucofuranose was tosylated[12,13] and the 6-tosyl-derivative (m.p. 106°) converted[14,15] into 5,6-anhydro-1,2-*iso*propylidene-D-glucofuranose (m.p. 133°—134°).

3. *Opening of the epoxide ring with amines.* 5,6-Anhydro-1,2-*iso*propylidene-D-glucofuranose (2×10^{-2} mole) dissolved in anhydrous methanol (75 ml) is poured into a solution of 4×10^{-2} mole of the amine in the same solvent (30 ml). Allow to react for half an hour and complete the reaction by smooth reflux for 2 h. The solvent and the majority of the unreacted amine are removed at reduced pressure below 50°. The residual syrups crystallize when kept in a vacuum over P_2O_5. The products were purified by recrystallization from light petroleum (b.p. 60°—80°). Yields 60 to 80 %. Melting points, optical rotations, and the results of potentiometric titration of the various N-substituted 6-amino-6-deoxy-1,2-*iso*propylidene-D-glucofuranoses are enumerated below; the parent amine in brackets: [1: (piperidine) 109—110°; $[\alpha]_D^{20} = +2.8°$ (chloroform, $c = 5$); 98,8 %. [2: (morpholine) 99.5°; $[\alpha]_D^{20} = +7.1°$ (water, $c = 5$); 99.4 %. [3: (pyrrolidine) 92—93°; $[\alpha]_D^{20} = +10.3°$ (water, $c = 5$); 98,0 %. [4. (dibenzylamine) 114—115°; $[\alpha]_D^{20} = +35.6°$ (chloroform, $c = 3.5$); 99.1 %.

The potentiometric titrations were carried out in aqueous solution except for the dibenzylamine derivative which was titrated with perchloric acid in a solution of acetic acid and dioxane.

Two of the four compounds listed above (Nos. 1 and 4) have been described previously by Ohle *et al.*[4]. The melting point (133°) and the optical rotation, $[\alpha]_D^{21} = +1.5°$ (chloroform, $c = 4.41$), attributed to 6-dibenzylamino-6-deoxy-1,2-*iso*propylidene-D-glucofuranose by these authors differ widely from the data recorded in the present work. The substance in question (No. 4) was therefore submitted to a degradation by periodate which gave results in complete agreement with the formula of 6-dibenzylamino-6-deoxy-1,2-*iso*propylidene-D-glucofuranose: In alcaline solution (pH 8) it reduced one molecule of periodate and gave rise to dibenzylamine (detected by paper chromatography) and a reducing compound which on chromatograms (solvent system: acetone, *n*-propanol, water 45:45:10 v/v)) could not be distinguished from 5-aldo-1,2-*iso*propylidene-D-xylofuranose[16] obtained by oxidation of 1,2-*iso*propylidene-D-glucofuranose by periodate (spots, $R_F^{20} = 0.83$, revealed by silver nitrate and ethanolic sodium hydroxide[17]). Further, after removal of the *iso*propylidene residue by mild hydrolysis, our dibenzylamine derivative reduced in acid solution one molecule of periodate less than D-glucose, as expected for an ω-amino-glucose.

4. *Conversion of the N-substituted derivatives of 6-amino-6-deoxy-1,2-iso*propylidene-D-*glucofuranose to the corresponding derivatives of 6-amino-6-deoxy-*D-*glucose ethylene dithioacetal.* Removal of the *iso*propylidene residue is achieved by hydrolysis at room temperature (controlled by paper chromatography): 2×10^{-2} mole of each of the products described above (3) are dissolved in 1 N hydrochloric acid (150 ml) and kept for three days. The hydrolysate is concentrated at reduced pressure (bath temperature below 30°) to 10—15 ml and then completely saturated with HCl gas under cooling in crushed ice. Ethane-1,2-dithiol (equimolecular quantity + 10 % excess) is then added and the mixture is shaken under continuous cooling until it becomes homogeneous. After standing for 2 h in the ice bath the faintly yellow solution is poured into water (100 ml) and left over night. The mixture is made weakly alkaline with 2 N sodium hydroxide and extracted with chloroform (4×200 ml). The combined chloroform extracts are dried (sodium sulphate) and concentrated to dryness at 20°. The partly crystalline residue is (after drying in vacuum over silica gel) recrystallized from benzene. The products are further purified by recrystallization from acetone-ether mixtures. Substance remaining in the mother liquids may be recovered by precipitation with light petroleum (b.p. 60°—80°). Yields are in the range of 50 % of the theoretical. Analysis and properties of the products are summarized in Table 1.

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