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Note

Hydrolysis of glycosides under reducing conditions

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Total and graded hydrolysis with aqueous acid are standard procedures in structural studies of oligo- and poly-saccharides. Such sugars as 3,6-anhydro-galactose, 3-deoxyketoaldonic acids, and, to a lesser extent, 3,6-dideoxyhexoses and ketoses are degraded under the normal conditions of hydrolysis; hence, methanolysis[1] and mercaptolysis[2] have been used to obtain more stable derivatives. Hydrolysis with acid under conditions where the sugars released are reduced rapidly to the alditols would be an attractive alternative. However, the most common reducing agents used in aqueous solution, namely, sodium borohydride and sodium cyanoborohydride, are decomposed at low pH. We now report on the use of sodium dicyanoborohydride[3,4], and the commercially available 4-methylmorpholine–borane[5].

Sodium dicyanoborohydride. — It has been reported[4] that sodium dicyanoborohydride[3] cannot be used for the reduction of carbonyl compounds. Preliminary experiments, however, demonstrated that benzophenone and 4-hydroxy-3-methoxybenzaldehyde were reduced to diphenylmethane and 4-hydroxy-3-methoxybenzyl alcohol, respectively, when treated with dicyanoborohydride and trifluoroacetic acid in boiling tetrahydrofuran. Hydrogen dicyanoborohydride was obtained as a stable white powder after passage of an aqueous solution of the sodium salt over a cation-exchange resin. When solutions of aldopentoses and aldohexoses in 2M trifluoroacetic acid containing 5% of sodium dicyanoborohydride were kept at room temperature, reduction was insignificant, but extensive reduction occurred for 40 h at 100° (Table I). In addition to the alditols, 1,4- and 1,5-anhydroalditols were also formed, as shown by g.l.c.–m.s. of the acetylated products of reduction. These anhydrides may be formed either by reduction of the sugars or by dehydration of the alditols originally formed[6,7]; when the alditols were treated under similar conditions, the results in Table I showed that part of the anhydroalditols, and practically

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all of the 1,5-anhydroalditols, are formed by reduction of the sugars. Treatment of the methyl pyranosides (α except for arabinose) gave essentially the same results as those obtained with the free sugars. There were no increases in the yields of 1,5-anhydroalditols, demonstrating that it is mainly, or exclusively, the free sugar and not the glycoside that is reduced.

For solution of D-fructose in 0.2M trifluoroacetic acid at 100°, mainly anhydrohexitols together with some D-glucitol and D-mannitol were formed. In 2M trifluoroacetic acid, however, only 2,5-anhydro-D-mannitol (36%), 2,5-anhydro-D-glucitol (28%), and 1,5(2,6)-anhydro-D-mannitol (33%) were obtained (identified by g.l.c. and g.l.c.–m.s. of the acetylated compounds). The amount of 2,5-anhydro-D-mannitol was not increased on similar treatment of phenyl D-fructopyranoside and sucrose, again indicating that hydrolysis precedes reduction.

A solution of 2-deoxy-D-*arabino*-hexose (2-deoxy-D-glucose) in 0.15M trifluoroacetic acid gave, after 2 h at room temperature, a mixture of 1,4- and 1,5-anhydro-2-deoxy-D-*arabino*-hexitol and 2-deoxy-D-*arabino*-hexitol. The same compounds were formed in the ratios 45:39:16 when the reaction was performed for 1 h at 100°. No other products and no discoloration of the solution were observed, and treatment of methyl 2-deoxy-β-D-*arabino*-hexoside gave the same result.

Treatment of methyl 3,6-dideoxy-α-D-*xylo*-hexopyranoside (abequoside) with sodium dicyanoborohydride in 0.5M trifluoroacetic acid for 1 h at 100° yielded a 2:3 mixture of the alditol and 1,4-anhydro-3,6-dideoxy-D-*xylo*-hexitol.

These results demonstrate that glycosides can be hydrolysed and the resulting sugars reduced on treatment with sodium dicyanoborohydride in aqueous acid. Acid-labile sugars, such as D-fructose, 3,6-dideoxy-D-*xylo*-hexose, and 2-deoxy-D-*arabino*-hexose, are not degraded under these conditions, A disadvantage, how-

TABLE I

ALDITOLS AND ANHYDROALDITOLS OBTAINED ON TREATMENT OF ALDOSES AND ALDITOLS WITH SODIUM DICYANOBOROHYDRIDE IN 2M TRIFLUOROACETIC ACID (FOR 40 h AT 100°)

Substance	*Alditol (%)*	*1,4-Anhydroalditol (%)*	*1,5-Anhydroalditol (%)*
L-Arabinose	71	17[a]	11
L-Arabinitol	93	7[a]	
D-Xylose	60	34	6
Xylitol	78	22	
D-Glucose	76	24[a]	
D-Glucitol	72	28[a]	
D-Galactose	65	25	11
Galactitol	75	25	
D-Mannose	82	11	7
D-Mannitol	100		
L-Fucose	42	15	43
L-Rhamnose	68	7	25

[a]Mixture of two isomers.

ever, is that not only the alditols but also one or several anhydroalditols are formed, both by reduction of the sugar and by dehydration of the alditol. The practical value of the method is therefore limited.

Sodium cyanoborohydride and a strong acid in an organic solvent have been used for the reductive cleavage of benzylidene acetals to benzyl ethers. Sodium dicyanoborohydride can be used also. Thus, methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside yielded methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (75%), and 1,4,5,6-tetra-*O*-benzyl-2,3-*O*-benzylidene-*myo*-inositol (*exo,endo* ~1:1) yielded a mixture (77%) of 1,2,4,5,6- and 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol. The same results[8,9] were obtained with sodium cyanoborohydride and the use of sodium dicyanoborohydride thus offers no advantages.

4-Methylmorpholine–borane. — This reagent[5] is not as stable in aqueous acid as sodium dicyanoborohydride and was applied only to sugars that are degraded easily on treatment with acid and also give acid-sensitive glycosides.

When a solution of sucrose in 0.5M aqueous trifluoroacetic acid containing 4-methylmorpholine–borane was kept for 15 h at room temperature, hydrolysis was complete; all of the fructose, but only part of the glucose, was reduced and anhydroalditols were not formed. When 3M acid was used, conversion into the alditols was complete, but reduction was incomplete in 6M acid, reflecting decomposition of the 4-methylmorpholine–borane. On treatment of methyl 2-deoxy-β-D-*arabino*-hexopyranoside with the borane in 0.5M trifluoroacetic acid for 60 min at room temperature, hydrolysis and reduction were complete and 2-deoxy-D-*arabino*-hexitol was the sole product. The reaction was so fast that, even at 60° or 100°, no degradation was observed.

Glycosides of 3-deoxy-D-*manno*-octulosonic acid (KDO) are cleaved readily with acid and the sugar is degraded. On treatment with 4-methylmorpholine–borane in aqueous 10% acetic acid for 1 h at 100°, KDO was completely reduced to a ~1:1 mixture of 3-deoxy-D-*glycero*-D-*galacto*- and -D-*glycero*-D-*talo*-octono-1,4-lactone, as shown by g.l.c.–m.s. of the derived acetates. The same results were obtained when the reduction was performed in 0.5 or 1.5M trifluoroacetic acid for 30 min at room temperature.

On treatment of agarose with 0.5M trifluoroacetic acid containing 4-methylmorpholine–borane, for 15 h at 60° or 100°, hydrolysis was complete and all of the 3,6-anhydro-L-galactose was reduced to its alditol, but virtually no D-galactose was reduced. It is evident that the 3,6-anhydro-L-galactose residues are released rapidly and reduced, but that the reducing agent is destroyed before significant amounts of D-galactose residues are formed.

Aldonolactones can be reduced on treatment with aqueous sodium borohydride, but treatment of the lactones obtained on reduction of KDO and of D-glucono-1,5-lactone with 4-methylmorpholine–borane in aqueous acid effected no reduction.

Sugars that are easily degraded in aqueous acid generally give glycosides which are hydrolysed much faster than glycosides of more stable sugars. When

such glycosides are hydrolysed in the presence of 4-methylmorpholine–borane, the reduction of the sugar to the alditol(s) is so fast that virtually no degradation occurs. This procedure may be of value in studies of oligo- and poly-saccharides containing such acid-labile sugars.

EXPERIMENTAL

General methods. — G.l.c. was performed on a Hewlett–Packard 5830A instrument, fitted with a flame-ionisation detector and an SE-54 fused-silica capillary column. G.l.c.–m.s. was performed on a Hewlett–Packard 5970 instrument, using the same phase. The interpretations of mass spectra were unambiguous and will not be discussed. When stereoisomers were found, they were identified from their retention times, compared with authentic substances.

Reductions. — The sugar (0.1 mmol) and reducing agent (1 mmol) in a capped serum bottle were dissolved in aqueous trifluoroacetic acid (2 mL). The molarity of the acid, reaction temperature, and time are given in the Discussion. The solution was concentrated to dryness and the residue was acetylated by treatment with trifluoroacetic acid (0.2 mL) and acetic anhydride (0.2 mL) at 100° for 10 min. Water (1 mL) was added, the solution was concentrated to dryness, and the residue was partitioned between dichloromethane (1 mL) and water (1 mL). The dichloromethane solution was concentrated and the product characterised by g.l.c. and g.l.c.–m.s.

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