[CONTRIBUTION FROM DIVISION OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Reduction of Sugars with Sodium Borohydride[1]

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The reduction of sugars to the corresponding alcohols is shown to be smoothly effected by sodium borohydride in aqueous solution.

Sodium borohydride and lithium aluminum hydride were shown by Brown and his associates to be extremely effective reducing agents in organic chemistry.[2a,b] Although sodium borohydride is much less reactive than the lithium compound it has the advantage as far as carbohydrates are concerned of being effective in aqueous solution.

In the field of carbohydrate chemistry it has already been shown that lithium aluminum hydride reduces the esters of methylated polyuronides, methylated plant gums and methylated uronic and aldobionic acids as well as certain sugar derivatives containing a potential aldehydic or a lactone group to the corresponding alcohols.[3,4] The glycosyl halides behave similarly when reduced with lithium aluminum hydride.[5]

The work reported herein has demonstrated that sodium borohydride is an excellent reagent for converting carbohydrates with a reducing group into the corresponding alcohols. The method is very simple and thus appears to offer certain advantages over the classical sodium amalgam method[6] and the more recent by electrolytic[7] and catalytic[8] hydrogenation procedures. Chaikin and Brown first carried out the reduction of glucose and noted[2] as we did in this work that products, possibly boric acid complexes, were formed which interfered with the crystallization of the sugar alcohols. The difficulty is shown herein to be a minor one for it was overcome by converting the reduction products into the fully acetylated derivatives. The latter usually crystallized with ease and from them the parent alcohols could be regenerated in the usual way.[9,10] The difficulty arising from the borate complex formation was also overcome by the use of ion exchange resins and also by treatment of the crude alcohols with methanolic hydrogen chloride.

Judged either by loss of reducing power or by change in rotation the rate of reduction of free sugars by sodium borohydride was fairly rapid and dependent upon the amount of sodium borohydride used.

Since this work was completed it has been reported that sodium borohydride will reduce aldonic lactones to sugar alcohols (glycitols) and ester glycuronides to the corresponding glycosides.[11]

Sodium borohydride was also found to convert amylose to a non-reducing polysaccharide ("amylitol"). This observation appears to offer the possibility of ascertaining what effect, if any, the reducing group of amylose has upon its chemical and physical properties and also upon its behavior with enzymes. Moreover, the quantitative determination of the terminal sugar alcohol would appear to provide a chemical method for determining the chain length and the molecular weight of the "amylitol" and therefore of the parent amylose. From the ratio of the terminal glucose residues to the terminal sorbitol residue it should also be possible to ascertain whether there is any branching in the various amyloses.

The reducing carbohydrate laminarin (extracted from *Laminaria cloustoni*), which consists of twenty anhydro-D-glucose units joined by 1:3 glycosidic bonds and possesses a free reducing group,[12] was also transformed into a non-reducing polysaccharide by means of sodium borohydride.

Further details of these investigations into amylose, laminarin and other reducing polysaccharides and oligosaccharides will be reported later.

Experimental

General Procedure for the Reduction of Sugars.—To a solution of the sugar (1.0 g.) in water (20 ml.) was added a solution of sodium borohydride (0.1–0.15 g.) in water (10 ml.). The reaction mixture which became faintly alkaline to litmus paper was kept at room temperature (20–25°) either until a drop of it after acidification with acetic acid to destroy the excess of sodium borohydride no longer reduced Fehling solution or until the optical rotation became constant.

The reaction usually took one to two hours. Thus, when a freshly prepared solution of D-galactose was treated as above it showed $[\alpha]^{25}D$ +40° (after four minutes); +28° (six minutes); +21° (eight minutes); +17° (ten minutes); +12° (fifteen minutes); +8° (twenty minutes); +6° (thirty minutes); +4° (forty-five minutes); +3° (eighty minutes) constant value. After acidification the solution was non-reducing to boiling Fehling solution. The slight positive final rotation may be due either to asymmetric reduction[13] or to an impurity in the D-galactose.

The rate of reduction depended upon the amount of sodium borohydride added. For instance when D-xylose was treated with an equal weight of sodium borohydride reduction was complete within five minutes. Similarly D-galactose, treated with half its weight of sodium borohydride, underwent reduction in fifteen minutes.

When reduction was complete the reaction mixture was acidified with acetic acid to destroy the excess of the sodium borohydride and evaporated to dryness *in vacuo*. Attempts to crystallize the alcohols directly at this stage invariably failed; *cf.* ref. 2. The dry residue containing the sugar alcohol was shaken with acetic anhydride (15 ml.) containing sulfuric acid (1 ml.) until most of the solid had

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TABLE I

REDUCTION OF SUGARS WITH SODIUM BOROHYDRIDE

Sugar	Acetylated product	M.p., °C.	$[\alpha]^{25}D$	Yield, %
D-Glucose	Sorbitol hexaacetate	99.5	+50 (C_6H_6)	78
D-Mannose	D-Mannitol hexaacetate	126	+18.5 (C_5H_5N)	92
D-Galactose	Dulcitol hexaacetate	171	Inactive	87
L-Arabinose	L-Arabitol pentaacetate[a]	76	+38 ($CHCl_3$)	87.5
D-Xylose	Xylitol pentaacetate[b]	61–62	Inactive	80
D-Fructose	A mixture of sorbitol and D-mannitol hexaacetates	91–92	+11.5 (C_6H_6)	75
Maltose	Maltitol nonaacetate[c]	87	+85 ($CHCl_3$)	70

[a] *Anal.* Calcd. for $C_{15}H_{22}O_{10}$: C, 49.7; H, 6.1. Found: C, 49.6, H, 6.1. Deacetylation in acetone with a slight excess of alcoholic potassium hydroxide gave arabitol m.p. and mixed m.p. 103° (from ethanol). [b] *Anal.* Calcd. for $C_{15}H_{22}O_{10}$: C, 49.7; H, 6.1. Found: C, 50.1; H, 6.2. [c] Acetylation carried out with acetic anhydride and sodium acetate at 100°.[15,16] *Anal.* Calcd. for $C_{33}H_{42}O_{20}$: C, 49.8; H, 5.9. Found: C, 49.8; H, 6.0.

dissolved and then warmed for ten minutes at 50–60°. The reaction mixture was allowed to cool and poured with stirring into ice-water (50 ml.). The acetate of the alcohol, which usually crystallized with ease, was filtered, washed with water, dried and recrystallized in the usual way.

In some cases acetylation was effected by suspending the dry residue containing the sugar alcohol (from 1 g. of sugar) in a mixture of acetic anhydride (10 ml.) and acetic acid (10 ml.) and adding perchloric acid (0.2 ml.). After the heat of the reaction had subsided the mixture was heated for one hour at 50–60°.[14] The solution was allowed to cool, poured into water, and the acetate isolated as above.

The results with a number of sugars are given in Table I.

It is of interest to note that reduction of D-fructose and acetylation of the product gave a compound m.p. 92° the rotation ($[\alpha]^{25}D$ +11.5° in benzene) of which indicated that it was mixture of about equal parts of D-mannitol hexaacetate and sorbitol hexaacetate.

In an experiment with D-galactose it was shown that if an aqueous solution of the crude reduction product was passed successively through a cation (IR-100 Rohm and Haas) and an anion exchange resin (IRA-400, Rohn and Haas) removal of inorganic impurity was effected and upon evaporation the dulcitol crystallized directly (yield 40%). The product which showed m.p. and mixed m.p. 188–189° (after recrystallization from aqueous ethanol) was optically inactive.

In the case of D-mannose the crude dried reduction product was boiled for one hour with 6% methanolic hydrogen chloride. After filtering the sodium chloride and cooling the solution, D-mannitol readily crystallized (yield 90%) m.p. and mixed m.p. 163°. An aqueous solution of the product showed a slight negative rotation.

Lactitol and cellobiitol were prepared in the same manner but they failed to crystallize in a reasonable time either in the free state or as their acetates. Lactitol[17] and cellobiitol[18] were non-reducing. Upon hydrolysis the former was shown by chromatographic analysis, using phenol–water as the irrigating solvent, to give galactose (R_f, 0.44) and sorbitol (R_f, 0.48) while the latter yielded glucose (R_f, 0.4) and sorbitol (R_f, 0.48).

Reduction of Amylose with Sodium Borohydride (a).—To a solution of corn amylose (0.5 g.) in 0.2 *N* sodium hydroxide (30 ml.), sodium borohydride (0.118 g.) was added. After keeping for one day at room temperature the solution was acidified with acetic acid and the polysaccharide precipitated by adding methanol (two volumes). The product was purified by dissolving it in dilute sodium hydroxide and adding a slight excess of acetic acid followed by methanol to effect precipitation. The reduced polysaccharide ("Amylitol") was washed with methanol, ethanol, petroeum ether and dried *in vacuo*. The product was sparingly soluble in water, soluble in dilute sodium hydroxide and in hot water containing a little 1-butanol. Like the original amylose it crystallized from water saturated with 1-butanol and it gave the characteristic blue color with iodine. It was, however, non-reducing (tested by the ferricyanide method).[19] A solution of the material in water containing 1-butanol (2%) showed $[\alpha]^{25}D$ +177° (*c*, 0.5).

(b).—Amylose (0.2 g.) moistened with 1-butanol (0.1 ml.) was dissolved in boiling water (15 ml.), the solution was cooled and treated with sodium borohydride (0.06 g.). The product isolated as above was found to be non-reducing.

In another experiment a solution of the amylose (0.2 g.) in water (15 ml.) containing butanol prepared as in (b), was mixed with a solution made by dissolving sodium borohydride (0.06 g.) in water (5 ml.) and adding a slight excess of acetic acid. The polysaccharide isolated as described above proved to behave like the original amylose and possessed almost the same reducing power.

Reduction of Laminarin.—A solution of laminarin (0.2 g.) (extracted from *Laminaria cloustoni*) in water (10 ml.) was treated with sodium borohydride (0.07 g.) during 2 days at room temperature. Acidification with acetic acid followed by the addition of ethanol (40 ml.) gave a flocculent precipitate which was purified by three precipitations from aqueous solution with ethanol. The amorphous white product ("laminaritol") showed only slight reducing activity by the ferricyanide method.[18]

Acknowledgment.—The authors thank the Corn Industries Research Foundation for their generous support. They also wish to thank Mr. C. E. Rist of the Northern Regional Research Laboratory, Peoria, Illinois, for the sample of corn amylose and Dr. E. G. V. Percival of the University of Edinburgh, Scotland, for the laminarin. One of us (M. A.-A.) thanks the Fouad I University, Cairo, Egypt, for a scholarship.

ST. PAUL, MINNESOTA RECEIVED APRIL 12, 1951

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