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Note

A re-investigation of the borohydride reduction of carbohydrates*

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In the estimation of the d.p. of oligo- and poly-saccharides, the ratio of reducing sugars and alditols in hydrolysates of borohydride-reduced polysaccharides can be determined by g.l.c. of the derived trimethylsilyl ethers[1] or alditol acetates[2]. Alternatively, a comparison can be made of the reducing power of hydrolysates of an oligosaccharide with that of a borohydride-reduced oligosaccharide[3]. Enzymic methods, specific for the measurement of alditols and aldoses, have also been developed[4–6]. In a microchemical method[2] for the determination of the d.p. of neutral oligo- and poly-saccharides, trace amounts of alditols were isolated on basic ion-exchange resins, from mixtures containing large excesses of monosaccharides, prior to the g.l.c. When this procedure was applied to dextrans, D-glucitol and D-mannitol were detected after borohydride reduction and acid hydrolysis of the polysaccharide. Since D-mannose does not occur as the reducing end group in dextrans, the D-mannitol appeared to be derived by rearrangement of reducing terminal D-glucose residues.

The role of borohydride reduction of carbohydrates has been re-examined, using two enzymic methods for the determination of the alditols. In the study by Yamaguchi *et al.*[2], 2M sodium borohydride at 40° was employed for the reduction of dextrans of molecular weights 8.6×10^3, 3.4×10^4, and 1.06×10^5. The D-mannitol detected was 2.7, 4.2, and 4.7%, respectively, of the total alditols. The most probable reason for the production of both D-glucitol and D-mannitol from a D-glucose end group of the polysaccharide was the combination of the pH of the reduction stage and temperature.

A series of D-*manno*-oligosaccharides was subjected to end-group analysis after borohydride reduction. Two enzymic methods were used to determine the liberated alditols, one capable of measuring D-glucitol in the presence of D-mannitol, and the other D-mannitol in the presence of D-glucitol. Each of the oligosaccharides examined, with the exception of α-Man-(1→3)-α-Man-(1→2)-Man, produced some D-glucitol on borohydride reduction (Table I). This result is to be expected by ring opening of the reducing-end D-mannose residues being followed by Lobry de Bruyn–Alberda van

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TABLE I

Production of D-mannitol and D-glucitol on borohydride reduction of D-*manno*-oligosaccharides at pH 12.1 and 18°

Oligosaccharide	D-*Mannitol* (*mol.%*)	*D.p.*[a]	D-*Glucitol* (*mol.%*)	*D.p.*[b]
α-Man-(1→6)-Man	98.7	1.95	1.3	1.98
β-Man (1→4)-Man	98.5	1.92	1.5	1.95
α-Man-(1→6)-α-Man-(1→6)-Man	95.2	2.75	4.8	2.90
β-Man-(1→4)-β-Man-(1→4)-Man	95.9	2.91	4.1	3.03
α-Man-(1→3)-α-Man-(1→2)-Man	100.0	3.00	0	3.00

[a] Based on D-mannitol. [b] Based on D-mannitol plus D-glucitol.

Ekenstein transformation. This transformation does not appear to take place where the reducing end group of an oligosaccharide is 2-substituted. Whereas the amounts of D-glucitol produced in the reduction of D-*manno*-oligosaccharides appeared to increase with increasing d.p., this did not cause major inaccuracies in the measurement of the d.p. Reduction of D-mannose with sodium borohydride gave only D-mannitol.

The influence of temperature and pH on the borohydride reduction of maltotriose was studied (Table II). When the reduction was carried out at 50° and pH 12.1, the amount of D-mannitol produced was approximately twice that produced at the same pH and at 4°. At pH 9.1, the same levels of D-mannitol were detected at all of the temperatures studied. When the D-*manno*-oligosaccharides used in the earlier study were reduced at pH 9.1 and 18°, the ratio of D-mannitol to D-glucitol was ~99:1 (data not included).

Thus, the borohydride reduction of D-*manno*- and D-*gluco*-oligosaccharides can yield epimeric alditols, the proportions of which are related to both the temperature and the pH of the reaction.

TABLE II

Influence of temperature and pH on the composition of the alditol mixture obtained on borohydride reduction of maltotriose

Temp. (°)	*pH 12.1*		*pH 9.1*	
	D-*Glucitol* (*%*)	D-*Mannitol* (*%*)	D-*Glucitol* (*%*)	D-*Mannitol* (*%*)
50	96.50	3.50	98.93	1.07
40	97.22	2.78	98.93	1.07
18	98.02	1.98	98.95	1.05
4	98.18	1.82	98.95	1.05

EXPERIMENTAL

D-Mannitol dehydrogenase (EC 1.1.1.138) was isolated and purified from *Agaricus bisporus* as reported[7], glucitol dehydrogenase (EC 1.1.1.14) was obtained from Boehringer, and α-D-*manno*-oligosaccharides were a gift from Professor C. E. Ballou.

Reduction and hydrolysis of oligosaccharides. — To solutions of samples (1–2 mg) in water (1 mL) was added aq. sodium borohydride (0.1 mL, 10 mg/mL). Reduction was allowed to proceed for 2 h at 18°. The reduced oligosaccharides were hydrolysed in M H_2SO_4) for 1 h at 100°, the hydrolysate was cooled to room temperature, and the pH was adjusted to 8.5 (phenolphthalein). A portion of this solution was used for the estimation of the D-glucitol and D-mannitol formed. Another portion was treated with more aq. sodium borohyride (0.1 mL, 10 mg/mL). Reduction of the aldoses to the corresponding alditols was allowed to proceed for 2 h, then excess of borohydride was destroyed by the addition of 2M acetic acid (0.1 mL), and the pH was again adjusted to 8.5. D.p. values were calculated from the ratio of D-mannitol derived from the total hydrolysis of the oligosaccharide to that derived from the reducing end group.

Analytical methods. — D-Mannitol was measured as previously reported[7]. D-Glucitol was determined using the glucitol dehydrogenase assay[6].

REFERENCES

1 G. G. S. Dutton, P. E. Reid, J. J. M. Rowe, and K. L. Rowe, *J. Chromatogr.*, 47 (1970) 195–198.
2 H. Yamaguchi, S. Inamura, and K. Makino, *J. Biochem. (Tokyo)*, 79 (1976) 299–303.
3 S. Peat, W. J. Whelan, and J. G. Roberts, *J. Chem. Soc.*, (1956) 258–260.
4 D. J. Manners, A. J. Masson, and R. J. Sturgeon, *Carbohydr. Res.*, 17 (1971) 109–114.
5 R. J. Sturgeon, *Carbohydr. Res.*, 30 (1973) 175–178.
6 R. J. Sturgeon, *Methods Carbohydr. Chem.*, 8 (1980) 77–80.
7 S. Berezenko and R. J. Sturgeon, *Carbohydr. Res.*, 216 (1991) 505–509.