870. *Quantitative Aspects of Reductions of Carbohydrates by Potassium Borohydride.*

By P. D. Bragg and L. Hough.

Estimations of the uptake of hydrogen from borohydride in borate buffer, by various monosaccharides and their derivatives, have shown that 3-*O*-substituted aldoses and 4-*O*-substituted hexuloses react slowly because of steric hindrance. Smaller but significant differences in reaction rates have been attributed to the formation of borate complexes.

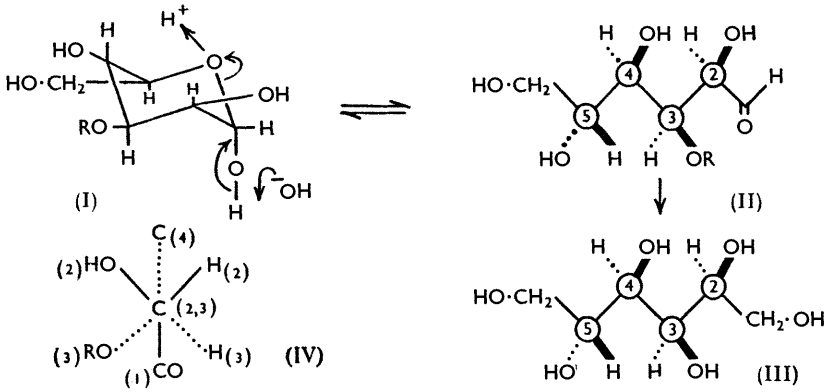
Reduction by borohydride has been utilised for quantitative semimicro-estimation of monosaccharides and reducing oligosaccharides by determining the consumption of hydrogen,[1-3] and also of oligosaccharides by colorimetric estimation of the carbohydrate before and after reduction.[4] We have studied the rate of reduction of various reducing carbohydrates, using Lindberg and Theander's procedure,[1,2] but with an improved apparatus. The reductions were in aqueous borate buffer at pH 10·3, which has been found optimum for this reaction.[2,5]

In the presence of excess of borohydride, the conversion of aldoses into glycitols [6] is generally accepted as being complete within a few hours. However, determinations of the rates of reduction of various mono- and di-saccharides have revealed that, under the

[1] Lindberg and Missiorny, *Svensk. Papperstidning*, 1952, **55**, 13.
[2] Lindberg and Theander, *ibid.*, 1954, **57**, 83.
[3] Skell and Crist, *Nature*, 1954, **173**, 401.
[4] Peat, Whelan, and Roberts, *J.*, 1956, 2258.
[5] Head, *Shirley Institute Memoirs*, 1955, **28**; *J. Text. Inst.*, 1955, **46**, T 400.
[6] Abdel-Akher, Hamilton, and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 4691.

conditions described herein, whilst the majority (Table 1; group A) were reduced stoicheiometrically within 1—2 hr., there were exceptions (Table 1; group B) which required at least 6 hr. for complete reduction. Of the aldoses studied, only those with a 3-*O*-substituent fell into group B (see Table 1), namely, 3-*O*-methyl-D-glucose (I; R = OMe), laminaribiose (I; R = β-D-glucopyranosyl), 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose. These results were confirmed by following the changes in the amounts of formaldehyde produced on periodate oxidation of mixtures of aldoses and borohydride.[7]

The reduction of an aldose (*e.g.*, I) to a glycitol (*e.g.*, III) will be preceded by ring-opening of the cyclic modification (I) of the aldose to the *aldehydo*-form in the acyclic staggered zig-zag conformation (II).[8,9] These results are consistent with the view that a bulky substituent at $C_{(3)}$ of this conformation (II) causes the approach of a borohydride ion to the aldehyde group to be sterically hindered (see the projection along the $C_{(2)}$–$C_{(3)}$



bond; IV). Thus 3-*O*-methyl-D-glucose (I; R = OMe) reacted more rapidly with borohydride than laminaribiose (I; R = β-D-glucopyranosyl) with its more bulky substituent at $C_{(3)}$. Under the same conditions laminarin, a polysaccharide containing β-1 : 3-D-glucopyranosyl units, did not consume any measurable amount of borohydride.

Nevertheless, in group A there were small but distinct differences in the rates of reaction which must be attributed to other stereochemical factors involving the conformations of the cyclic modifications of the aldoses and the stability of derived borate complexes.[10-12] The formation of borate complexes which involve substitution at either $C_{(1)}$ or $C_{(3)}$ of the open-chain forms should also reduce the rate of reduction, but in view of the following results is not considered to be of importance. Acetaldehyde, D-glyceraldehyde, 4 : 6-*O*-ethylidene-D-glucose, and 2-deoxy-D-glucose were more rapidly reduced (<15 min.) than D-xylose (30 min.) and D-glucose (1 hr.). These observations are in accord with previous results[10,11,13,14] on borate-complex formation with various derivatives of D-glucose and suggest that D-xylose and D-glucose form fairly stable borate complexes in their furanose forms at $C_{(1)}$ and $C_{(2)}$. Böeseken[13] has shown that the strongest boric acid complex formation occurs with true *cis*-glycols which are found in the furanose forms of monosaccharides but not in the pyranosides in their chair conformations. In the case of D-glucose, all its free hydroxyls appear to be capable of borate-complex formation to a certain extent,[11] which probably accounts for its slower reaction than that of D-xylose.

[7] Hough, Perry, and Woods, *Chem. and Ind.*, 1957, 1100.
[8] Barker, Bourne, and Whiffen, *J.*, 1952, 3865.
[9] Barton and Cookson, *Quart. Rev.*, 1956, **10**, 44.
[10] Foster, *J.*, 1953, 982.
[11] Foster and Stacey, *J.*, 1955, 1778.
[12] Foster, *J.*, 1957, 1395.
[13] Böeseken, *Adv. Carbohydrate Chem.*, 1949, **4**, 189; *Rec. Trav. chim.*, 1921, **40**, 553.

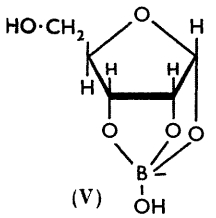
Cellobiose (4-*O*-β-D-glucopyranosyl-D-glucose), which cannot pass into the furanose form, was reduced more rapidly than melibiose (6-*O*-β-D-galactopyranosyl-D-glucose). The slower reaction of glucose than galactose and mannose can be correlated with the greater conformational stability of the 1 : 2-borate complex of glucofuranose which has fewer *endo*-groups than those derived from the latter hexoses.

Of the aldopentoses D-ribose behaved atypically, since L-arabinose, D-lyxose, and D-xylose were reduced at an appreciably faster rate. Since D-ribose exists in aqueous solution to an appreciable extent (10—30%) in the *aldehydo*-form whereas other aldopentoses do not (<1%),[15,16] it follows that in borate solution a relatively stable complex of D-ribose was formed. These results, the ready formation of 2 : 3-*O*-cyclic acetals of D-ribofuranose,[17] and the greater mobility during ionophoresis of D-ribofuranoside 5-phosphate than of the 2- and 3-phosphates,[18] suggested that this pentose may have reacted in the furanose form with borate at the true *cis*-hydroxyls at $C_{(1)}$, $C_{(2)}$, and $C_{(3)}$ to give a tridentate structure (V).[19] Angyal and McHugh [19] have found that certain cyclitols with borate form tridentate structures which are relatively strong acids containing a 1 : 1 ratio of borate to cyclitol. Using their procedure, we found ribose to depress the pH of a borate solution to a greater extent than other pentoses (Table 3), but calculation of the equilibrium constants for the formation of a tridentate complex did not give constant K values over a wide range of concentrations of pentose (Table 4). However, D-ribose, D-xylose, and L-arabinose followed approximately the equation $K = [C^-]/[B^-][P]^x$, where $x = 1{\cdot}84$, 1·84, and 1·90, respectively, indicating the presence of both 1 : 1 and 2 : 1 complexes [20] (Table 4) ([C^-], [B^-], and [P] are the concentrations of complex anion, borate ion, and pentose, respectively). Complex formation was more extensive with ribose ($K = 5{\cdot}14 \times 10^4$) than xylose ($K = ca.\ 4{\cdot}2 \times 10^3$) and arabinose ($K = 1{\cdot}73 \times 10^3$) which would suggest that the hydrolysis of the borate complex is a rate-determining step in the reduction by borohydride. We conclude that ribose reacts in the furanose form at $C_{(1)}$ and $C_{(2)}$ with borate, as also suggested by the behaviour of various methyl ethers of ribose during ionophoresis,[21] and that there is little, if any, tridentate formation. Measurement of the optical rotation of D-ribose in borate gave a constant value of +8·5° as compared with −19·8° found in aqueous solution, thus confirming that complexes had been formed. D-Glucose, D-mannose, D-galactose, and L-arabinose also showed changes (Table 2), but interpretation is complicated by the various types of borate complexes present in the equilibrium mixtures.[22]

That ketoses were reduced more slowly than aldoses, was shown by comparing dihydroxyacetone with D-glyceraldehyde, and D-fructose with D-glucose and D-mannose. This difference is related to the increased steric hindrance of the carbonyl group in ketoses by neighbouring substituents, a conclusion supported by the slow consumption of borohydride by turanose (3-*O*-α-D-glucopyranosyl-D-fructose) compared with D-fructose. As noted by Peat, Whelan, and Roberts,[4] maltulose (4-*O*-α-D-glucopyranosyl-D-fructose) was resistant to reduction during 9 hr. The behaviour of maltulose thus paralleled that of 3-*O*-substituted aldoses and may be accounted for by the intermediary formation of the staggered zig-zag conformation (VI) in which the bulky substituent at $C_{(4)}$ interferes with the reduction at $C_{(2)}$. Amino- and acetamido-groups adjacent to the aldose carbonyl group had little influence on the rates of reduction as revealed by examining 2-amino- and 2-acetamido-2-deoxy-D-glucose; from the latter 2-acetamido-2-deoxy-D-glucitol was prepared *via* the penta-acetate.

[14] Lock and Richards, *J.*, 1955, 3024.
[15] Cantor and Peniston, *J. Amer. Chem. Soc.*, 1940, **62**, 2113.
[16] Overend, Peacocke, and Smith, *Chem. and Ind.*, 1957, 113.
[17] Jeanloz and Fletcher, *Adv. Carbohydrate Chem.*, 1951, **6**, 168.
[18] Burke and Foster, *Chem. and Ind.*, 1955, 94.
[19] Angyal and McHugh, *J.*, 1957, 1423.
[20] Antikainen, *Acta Chem. Scand.*, 1955, **9**, 1008.
[21] Brown, Magrath, and Todd, *J.*, 1954, 1442.
[22] Isbell, Brewster, Holt, and Frush, *J. Res. Nat. Bur. Stand.*, 1948, **40**, 120.

The reduction of lactones to glycitols was incomplete (see Table 1; group C) owing to competing hydrolysis of the lactone to form non-reducible sodium salts.[23,24] Thus, L-gulono-1 → 4-lactone and D-glucofuranurono-6 → 3-lactone consumed *ca.* 0·6 mol. and *ca.* 2·0 mol. of hydrogen (constant values) respectively as compared with 2 mol. and 3 mol. required in theory. These results could also be interpreted by partial reduction



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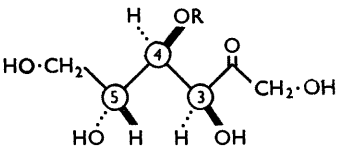


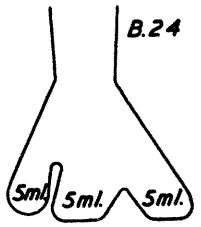
(VI)

of the lactones to intermediary aldoses,[23,25] but reducing substances were not found. High yields of glycitols from γ-lactones have been obtained in reductions in either acid medium or anhydrous methanol,[24] thus obviating hydrolysis.

Re-examination [4] of the borohydride reduction of lactose has shown that lactitol was formed in high yield and that the reductive fission previously indicated [26] was anomalous owing to the conditions [27] used for isolation of the product. In the previous investigation, sodium ions were removed from the mixture by ion exchange on Amberlite IR-120(H^+) resin, giving unexpectedly a strongly acidic solution, distillation of which with methanol caused methanolysis of the lactitol and formation of sorbitol and methyl glucosides.

Experimental

Method.—The reducing substance (*ca.* 200—300 mg.; accurately weighed) was dissolved in 0·1M-boric acid solution (25·0 ml.) and an aliquot portion (3·0 ml.) transferred into one of the two lower compartments of the reaction flask (50 ml.) (see Inset), which was designed for easy filling and mixing. Into the other compartment a 0·13M-solution (3·0 ml.) of potassium borohydride in 0·1N-sodium hydroxide was introduced. The side-arm of the flask was carefully charged with 10N-sulphuric acid (*ca.* 2 ml.). The vessel was then connected through a ground-glass joint (B.24) to a gas-burette (50 ml.) and the volume recorded before the boric acid and borohydride solutions were thoroughly mixed by tilting and gentle rotation of the apparatus. To minimise loss of hydrogen, the gas pressure in the apparatus was reduced to atmospheric pressure from time to time by altering the height of the water reservoir. If the evolution of hydrogen was slow, the reaction could be allowed to continue for 24 hr. without appreciable loss of hydrogen, but generally reduction times of a few hours were employed. After a suitable period, the reaction was stopped by tipping the acid from the side-arm into the solution. The total volume of hydrogen evolved was measured in the gas-burette after vigorous shaking had freed the solution of hydrogen. A blank containing no reducing substance was run concurrently with every determination. By difference, the amount of hydrogen consumed by the substance was obtained. All reactions were carried out at 22°. The accuracy of the determination was ±5%.



Effect of Borate on the Optical Rotation of Monosaccharides.—The optical rotations of the following monosaccharides were measured in water and in 0·1M-boric acid–0·1N-sodium hydroxide solution, a 1 dm. tube being used, until a constant value was obtained.

Determination of the Equilibrium Constants for the Pentose–Borate Reactions.—The determination was carried out in 0·0025M-sodium tetraborate by Angyal and McHugh's method.[19]

[23] Wolfrom and Wood, *J. Amer. Chem. Soc.*, 1951, **73**, 2933.
[24] Frush and Isbell, *ibid.*, 1956, **78**, 2844.
[25] Macdonald and Fischer, *ibid.*, p. 5025.
[26] Hough, Jones, and Richards, *Chem. and Ind.*, 1953, 1064.

TABLE 1. *Hydrogen uptake from borohydride (moles of H_2 per mole of substance).*

Substance	Wt. of sample (mg.)	Time of reduction: 5 min.	10 min.	15 min.	30 min.	60 min.	3 hr.	4 hr.	5 hr.	24 hr.
Group A										
Acetaldehyde	31·6	1·03	1·07			1·06 [e]				
DL-Glyceraldehyde	26·4	0·93	0·95	1·00	1·00					
D-Xylose	44·0	0·66	0·75	0·79	1·03	1·03				
D-Ribose	45·2			0·38	0·56	0·89		1·06		
L-Arabinose	39·5	0·74		0·94	1·05 [a]					
D-Lyxose	48·4	0·67	0·98	0·96	1·05					
D-Glucose	52·3			0·67	0·85 [b]	1·02	1·02			0·98
D-Mannose	51·7	0·38	0·68	0·81	0·98					
D-Galactose	59·0	0·46		0·79	0·96					
L-Rhamnose monohydrate	32·9					1·00	1·04			
2-Deoxy-D-glucose	51·9	0·88	1·00	1·00	1·01	1·04		1·03		
4 : 6-*O*-Ethylidene-D-glucose	21·9	0·98	1·04	1·04	1·04	1·05		1·04		
Cellobiose	60·2	0·47	0·71	0·85	1·02	0·96				1·01
Lactose monohydrate	47·7					0·98		0·98		1·06
Melibiose	61·5			0·59	0·73	0·98		0·94		
D-*glycero*-D-*galacto*-Heptose	24·7		0·78	0·88 (20 min.)	0·99	0·96				
2-Amino-2-deoxy-D-glucose	33·1					1·05	1·07			
2-Acetamido-2-deoxy-D-glucose	30·7					1·00		1·04		
Methyl α-D-glucoside	30·0									0·0
Dihydroxyacetone	25·1			0·84	0·90	1·00				
D-Fructose	54·4			0·54	0·78	0·95		1·02		1·02
Group B										
3-*O*-Methyl-D-glucose	18·5					0·28	0·38			0·94 [d]
										1·02
2 : 3 : 4 : 6-Tetra-*O*-methyl-D-glucose	28·4					0·27				
2 : 3 : 4 : 6-Tetra-*O*-methyl-D-galactose	15·3					0·53	0·69			0·97 [e]
Laminaribiose	8·3					0·5 (± 0·15)				
	18·0								0·55	0·6 [f]
Laminarin (insoluble)	285·0								0·0	0·0 [g]
Turanose	22·9					0·44		0·73		1·03
Maltulose monohydrate	22·1					0·0		0·0		0·0 [a]
Group C										
Sodium D-glucuronate monohydrate	62·1	0·58		0·93	0·98	0·93		1·07		1·06
D-Glucofuranurono-6→3-lactone	35·3				1·50	1·70 [i]		1·96		2·0
1 : 2-*iso*Propylidene-D-glucofuranurono-6→3-lactone	13·6					0·4	0·3			0·4 [j]
Sodium D-gluconate	22·3					0·0		0·0		0·0
D-Glucono-1→5-lactone	13·6					0·5	0·71			0·91
L-Gulono-1→4-lactone	15·1					0·66			0·61	0·62 [j]
	18·3					0·60		0·62		
D-Mannono-1→4-lactone	12·3					0·71				1·01 [k]
L-Galactono-1→4-lactone	16·5						1·07			
Lactobiono-1→5-lactone	12·4					0·0				0·0 [j]

[a] 1·03 at 45 min. [b] 0·95 at 45 min. [c] 1·06 at 2 hr. [d] At 12 hr. [e] At 10 hr. [f] At 12 hr. [g] At 20 hr. [h] At 9 hr. [i] 1·9 at 2 hr. [j] At 8 hr. [k] At 6 hr.

TABLE 2.

	$[\alpha]_D^{24}$ in borate: Concn. (%)	Time (min.) 1	2	3	4	5	10	15	30	$[\alpha]_D^{24}$ in water: $[\alpha]_D$			Concn. (%)
β-L-Arabinose	5·97	+139°	129°	120°	113°	108°	96°	90°	88·7°	191°	→	91·9°	6·02
α-D-Xylose	4·91	+38·9	30·8	25·0	21·6	20·1	18·5	18·5	18·5	93·6	→	17·0	5·11
D-Ribose	4·51	—	+8·4	10·0	—	9·1	—	8·5	8·5	−18·4	→	−19·8	5·15
α-D-Glucose	5·16	+54·0	51·2	48·6	47·7	46·6	46·3	46·1	45·9	112	→	49·1	5·03
β-D-Mannose	5·79	+ 5·7	9·0	9·9	9·9	9·9	10·0	9·9	—	−17·0	→	13·7	5·10
α-D-Galactose	2·35	+67·7	61·7	56·7	56·2	55·8	55·3	—	55·3	151	→	76·0	2·35

Reduction of 2-Acetamido-2-deoxy-α-D-glucose.—A mixture of 2-acetamido-2-deoxy-D-glucose (0·55 g.) in 0·1M-boric acid solution (50 ml.) and 0·13M-potassium borohydride in 0·1N-sodium hydroxide solution (50 ml.) was kept for 2¼ hr. at room temperature. Excess of borohydride was destroyed by a slight excess of glacial acetic acid, and the resulting solution concentrated to a dry white solid under reduced pressure. The solid was dissolved in a mixture of

TABLE 3.

pH *at* 19°

Concn.*	Ribose	Arabinose	Lyxose	Xylose	Concn.*	Ribose	Arabinose	Lyxose	Xylose
0·5	8·71	9·19	9·08	9·07	3	7·46	8·57	8·40	8·43
1	8·39	—	8·87	8·87	4	7·19	—	8·25	8·28
2	7·95	8·78	8·60	8·62	5	7·01	8·27	8·10	8·16

* Moles of pentose per mole of boric acid.

TABLE 4.

Pentose	Concn.*	pH at 17°	$K = [C^-]/[B^-][P]$	$K = [C^-]/[B^-][P]^x$	x
Ribose	8	6·63	$5·21 \times 10^3$	$4·39 \times 10^4$	1·84
	12	6·23	$8·52 \times 10^3$	$5·13 \times 10^4$	
	16	5·98	$1·13 \times 10^4$	$5·29 \times 10^4$	
	20	5·82	$1·29 \times 10^4$	$5·01 \times 10^4$	
Arabinose	8	8·09	$1·67 \times 10^2$	$1·73 \times 10^3$	1·90
	12	7·75	$2·48 \times 10^2$	$1·77 \times 10^3$	
	16	7·51	$3·25 \times 10^2$	$1·75 \times 10^3$	
	20	7·35	$3·77 \times 10^2$	$1·65 \times 10^3$	
Xylose	8	7·89	$2·72 \times 10^2$	$2·35 \times 10^3$	1·84
	12	7·35	$6·4 \times 10^2$	$3·96 \times 10^3$	
	16	7·08	$8·87 \times 10^2$	$4·24 \times 10^3$	
	20	6·86	$1·18 \times 10^3$	$4·68 \times 10^3$	

* Moles of pentose per mole of boric acid.

formamide (15 ml.) and dry pyridine (15 ml.), and redistilled acetic anhydride (15 ml.) was added. After 16 hr. the mixture was poured into ice-water (200 ml.), and the aqueous solution extracted with chloroform (2 × 200 ml.). The chloroform extract was shaken successively with 3N-hydrochloric acid (200 ml.), saturated aqueous sodium hydrogen carbonate (200 ml.), and water (400 ml.), and finally dried (Na_2SO_4). Concentration of the chloroform extract gave a syrup (0·31 g.) which on de-*O*-acetylation with ammonia (*d* 0·88; 6 ml.) in methanol (15 ml.) gave a crystalline product. After recrystallisation from ethanol, 2-acetamido-2-deoxy-D-glucitol was obtained as fine needles, m. p. 160°, $[\alpha]_D^{24}$ −9° (*c* 1·71 in water) (Found: C, 43·3; H, 7·8; N, 5·7. Calc. for $C_8H_{17}O_6N$: C, 43·1; H, 7·6; N, 6·3%). Karrer and Meyer [28] record m. p. 153°, $[\alpha]_D$ −11°.

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[27] Zill, Khym, and Cheniae, *J. Amer. Chem. Soc.*, 1953, **75**, 1339.
[28] Karrer and Meyer, *Helv. Chim. Acta*, 1937, **20**, 407.