677. *Reactions at Position 1 of Carbohydrates. Part I. The Polarographic Reduction of Carbohydrates.*

By W. G. Overend, A. R. Peacocke, and J. B. Smith.

An investigation has been made of the polarography of a variety of sugars and their derivatives in a buffer solution based on the tetramethylammonium ion. Reasonable polarographic waves were obtained with limiting currents proportional to sugar concentration over a wide range. For unsubstituted sugars the limiting current was independent of the mercury-reservoir height and so was determined by the rate of transformation of the αβ-equilibrium mixture of the sugar from ring to reducible form at the mercury surface, and not by the rate of diffusion of the sugar to the mercury drop. With some methylated pentose derivatives diffusion became the rate-controlling step.

In 1932, Heyrovsky and Smoler [1] reported that glucose and several other simple sugars were not reduced at the dropping-mercury electrode, a conclusion which was revised when Cantor and Peniston [2] obtained polarographic waves for various aldoses. Relatively high concentrations (*ca.* 0·1M) were required and the limiting currents observed were low. This was explained by suggesting that the *aldehydo*-form of the sugar was in equilibrium with a much larger amount of the ring form and that the *aldehydo*-form alone was reducible so that only low limiting currents, controlled by the rate of diffusion of this form to the drop surface, could be expected. This interpretation was disproved by Wiesner [3] who obtained polarographic waves for several aldoses and demonstrated that the heights of such waves were independent of the head of mercury above the drop. Wiesner accordingly concluded that the limiting current was determined, not by the rate of diffusion of the *aldehydo*-form to the mercury drop, but by the rate of transformation of the αβ-equilibrium mixture from the ring to the open-chain reducible form at the surface of the mercury drop. Further, he showed that the equilibrium concentration of the *aldehydo*-form was negligible. In view of the proposal that the limiting current was determined by the rate of transformation of the ring-form to the open-chain form of the sugar, it was decided to investigate the effect, on this rate, of factors which are likely to alter the stability of the ring-form relative to that of the open-chain structure.

The polarography of a variety of sugars and their derivatives was investigated, the buffer solution being based on the tetramethylammonium ion. This solution consisted of a mixture of tetramethylammonium phosphate and phosphoric acid at pH 8·0 and gave a constant concentration of 0·002M-tetramethylammonium ion in the polarographic test solution. The advantage of using this buffer lies in the high decomposition potential of the tetramethylammonium ion (about —2·5 v). The half-wave potentials of 2-deoxy-sugars were found to be in the region —2·1 to —2·2 v, and only in this buffer solution did these sugars give discrete waves amenable to measurement. It was essential to remove all traces of alkali-metal ions from the sugars, since the reduction waves of these ions otherwise overlapped and obscured those of the sugars themselves.

A preliminary account of some of this work was published recently.[4]

Results

The sugars were found to give reproducible polarographic waves in the tetramethylammonium phosphate buffer solution, and representative curves are shown in Figs. 1, 2, and 3. For all except two of the sugars studied, the limiting current generated by the sugar was independent

---

[1] Heyrovsky and Smoler, *Coll. Czech. Chem. Comm.*, 1932, **4**, 521.
[2] Cantor and Peniston, *J. Amer. Chem. Soc.*, 1940, **62**, 2113.
[3] Wiesner, *Coll. Czech. Chem. Comm.*, 1947, **12**, 64.
[4] Overend, Peacocke, and Smith, *Chem. and Ind.*, 1957, 113, 1383.

of the mercury-reservoir height. This result, first observed by Wiesner,[3] shows that the rate of polarographic reduction is not determined by the rate of diffusion to the mercury drop, but by the rate of transformation of the αβ-equilibrium mixture from ring to reducible form at the mercury surface. The two exceptional compounds, 2,3,4-tri-*O*-methyl-L-arabopyranose and 2,3,5-tri-*O*-methyl-D-ribofuranose generated limiting currents which were accurately proportional to the square root of the head of mercury. This result, which is illustrated in Table 1, shows that for these two compounds the rate of transformation from ring to reducible form is sufficiently high to render diffusion to the mercury drop surface the slowest, rate-controlling step.

FIGS. 1—3. *Polarographic waves in* 0·002M-*tetramethylammonium phosphate buffer* (pH 8·0) *at* 25° *at various heights of the mercury reservoir* (*see below*). *The scale of applied voltage on the abscissa is moved to the right by* 0·4 v *for each successive curve, reading from left to right.*

FIG. 1. FIG. 3.

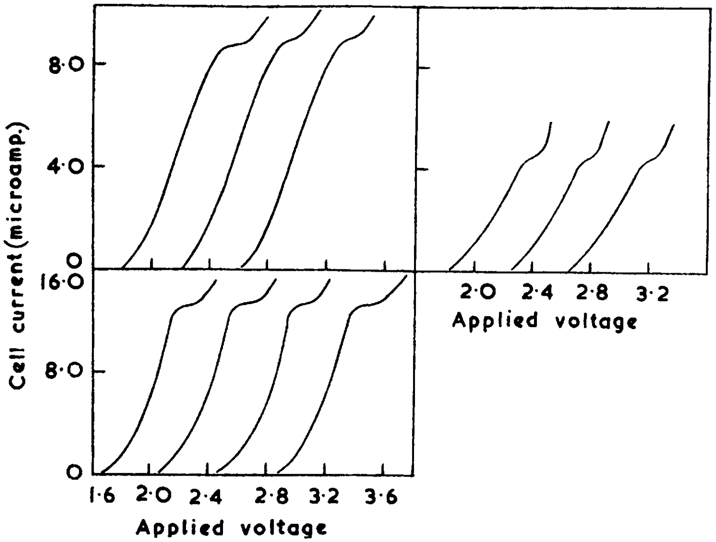


FIG. 2.

FIG. 1. D-Ribose (0·008M). Reservoir heights (from left to right): 45·5, 38·2, 30·8 cm.
FIG. 2. 2-Deoxy-D-galactose (0·008M). Reservoir heights (from left to right): 51·6, 48·0, 45·2, 41·4 cm.
FIG. 3. 2-Deoxy-D-ribose (0·008M). Reservoir heights (from left to right): 44·2, 41·2, 34·9 cm.

TABLE 1. *Relation between limiting current and mercury head at* 25°.

2,3,4-*Tri-O-methyl-*L-*arabopyranose* (0·002M; pH 8·0; 25°)

Mercury head (H) (mm.)	534	498	458	421	382	351
Limiting current (i) (microamp.)	6·3	6·0	5·8	5·5	5·2	5·0
$H^{\frac{1}{2}}/i$	3·7	3·7	3·7	3·7	3·7	3·7

2,3,5-*Tri-O-methyl-*D-*ribofuranose* (0·043M; pH 8·0; 25°)

Mercury head (H) (mm.)	430	389	338	295	248
Limiting current (i) (microamp.)	14·8	14·0	13·0	12·1	11·1
$H^{\frac{1}{2}}/i$	1·4	1·4	1·4	1·4	1·4

In order to provide a standard scale of reference, all half-wave potentials were measured relatively to the saturated calomel electrode. The results appear in Table 2. The half-wave potentials of the 2-deoxy-sugars lie in the range −2·1 to −2·18 v, whereas those of the normal sugars vary from −1·87 to −2·12 v. It is particularly noticeable that 2,3,4-tri-*O*-methyl-L-arabopyranose and 2,3,5-tri-*O*-methyl-D-ribofuranose, which both generate diffusion-controlled waves, exhibit more positive half-wave potentials (−1·36 and −1·57 v, respectively) than all of the other sugars studied. The half-wave potentials were independent of sugar concentation and became more negative when the pH of the solution was increased.

Effect of Sugar Concentration on Limiting Current.—Ideally, the limiting current should be directly proportional to the sugar concentration, and this relation has been found to apply over limited ranges of concentration for all compounds examined. At high sugar concentrations deviations from proportionality occur as exemplified by curve C in Fig. 4 for D-ribose, and have been observed also with D-galactose, 2-deoxy-D-galactose, and 3,5,6-tri-*O*-methyl-D-glucofuranose. This decrease in the ratio of limiting current to concentration which occurs at high concentrations (*ca.* 0·1M) may be the result either of a change from a first-order to a more complex reaction or to an increase in viscosity, causing a decrease in the diffusion coefficient to the point where it determines the rate of polarographic reduction. A decrease in the value of the diffusion coefficient would cause a corresponding decrease in the limiting current, in accordance with Ilkovic's equation[5] for diffusion-controlled polarographic reduction. Friedman and Carpenter[6] have shown that the diffusion coefficient of D-glucose at 25° is a linear function of the square root of the sugar concentration, so that deviations from strict proportionality between limiting current and concentration are possible over wide ranges of concentration.

Determination of Rate Constants.—A quantity A, the limiting current generated per unit concentration, was obtained from the linear section of the current–concentration graph for each sugar, and these A values are shown in Table 2. There are two treatments which relate the ratio A to the rate constants for the transformation of the αβ-equilibrium mixture from ring to reducible form:

(i) Delahay[7] considered the reduction of the αβ equilibrium mixture, and assumed the reaction scheme $\alpha\beta \xrightleftharpoons{k_f^{\alpha\beta}} \gamma$, where γ represents the reducible intermediate, presumably the *aldehydo*-form. The overall equilibrium constant K for the reaction is equal to $[\gamma]/[\alpha\beta]$. The limiting kinetic current, i (microamp.), is then shown to be given by the equations:

$$i = 0{\cdot}6Pn(m\tau)^{2/3}ck_s^{\alpha\beta} \qquad (1)$$

and

$$i = 0{\cdot}6Pn(m\tau)^{2/3}c(DKk_f^{\alpha\beta})^{1/2} \qquad (2)\ (A = i/c)$$

In these equations, P is a constant equal to 1255 at 25°, n is the number of electrons required for the reduction of one sugar molecule (a value $n = 2$ is consistent with the reduction mechanism proposed), m is the mercury flow rate in mg. sec.$^{-1}$, τ is the mercury drop time in seconds, c is the sugar concentration in millimoles litre^{-1}, and D is the sugar diffusion coefficient in cm.^{2} sec.$^{-1}$: $k_s^{\alpha\beta}$ and $k_f^{\alpha\beta}$ are, respectively, the heterogeneous and the homogeneous rate constants for the transformation from cyclic to reducible form, and K is the overall equilibrium constant of the transformation as already defined. The value of $k_s^{\alpha\beta}$ is expressed in cm. sec.$^{-1}$ and that of $k_f^{\alpha\beta}$ in sec.$^{-1}$, and Delahay related these constants by assuming that the transformation reaction occurs essentially in a " monolayer " at the surface of the mercury drop. Then $k_f^{\alpha\beta} = k_s^{\alpha\beta}/\delta$ where δ is the thickness (in cm.) of a " monolayer " of the sugar ring form, and this thickness was arbitrarily assumed[7] to equal the mean distance between two sugar molecules in solution. Equations (1) and (2) may be compared, showing that

$$K = (k_s^{\alpha\beta})^2/Dk_f^{\alpha\beta}, \qquad (3)$$

so that if D, the sugar diffusion coefficient is known, the rate and equilibrium constants may be calculated from experimentally determined quantities. The values of $k_s^{\alpha\beta}$, $k_f^{\alpha\beta}$, and K for the sugars studied are shown in Table 2 and have a reproducibility of 5—7%.

(ii) In a polarographic study on the mutarotation of D-glucose, Los, Simpson, and Wiesner[8] adopted the reaction scheme $\alpha \xrightleftharpoons[k_b^{\alpha}]{k_f^{\alpha}} \gamma \xrightleftharpoons[k_f^{\beta}]{k_b^{\beta}} \beta$, where γ again represents the reducible intermediate. (This formulation involves the rate constants for the equilibrium $\alpha \rightleftharpoons \beta$, so these need not be further specified.) From the equations derived by these workers, it can be shown that the limiting current i ($= Ac$) is given by the relation:

$$i = Gn(m\tau)^{2/3}D^{1/2}\frac{(k_f^{\alpha}c_{\alpha} + k_f^{\beta}c_{\beta})}{(k_b^{\alpha} + k_b^{\beta})^{1/2}}, \qquad (4)$$

---

[5] Ilkovic, *Coll. Czech. Chem. Comm.*, 1934, **6**, 498.

[6] Friedman and Carpenter, *J. Amer. Chem. Soc.*, 1939, **61**, 1745.

[7] Delahay, *J. Amer. Chem. Soc.*, 1951, **73**, 4944; 1952, **74**, 3506.

[8] Los, Simpson, and Wiesner, *J. Amer. Chem. Soc.*, 1956, **78**, 1564.

in which G is a constant equal to 493 at 25°, c_α and c_β are respectively the concentrations in millimoles l.$^{-1}$ of the α- and the β-anomer at mutarotation equilibrium, the rate constants are expressed in sec.$^{-1}$, and other factors have their previous significance. Thus, the experimental quantity $A = i/c = i/(c_\alpha + c_\beta)$ depends on the ratio of quantities involving the first powers of k_f^α and k_f^β to $(k_b^\alpha + k_b^\beta)^{1/2}$, and this fuller analysis shows the limitations of Delahay's treatment which ignores differences between the rates of the back reactions $\gamma \longrightarrow \alpha$ and $\gamma \longrightarrow \beta$. However, if $k_f^\alpha = k_f^\beta = k_f^{\alpha\beta}$, and $k_b^\alpha = k_b^\beta = k_b^{\alpha\beta}$, equation (4) reduces to

$$i = Gn(m\tau)^{2/3}D^{1/2}c\frac{\sqrt{2}\,.\,k_f^{\alpha\beta}}{(k_b^{\alpha\beta})^{1/2}} \qquad (5)$$

or

$$i = Gn(m\tau)^{2/3}D^{1/2}cB, \qquad (5a)$$

where B is the ratio $\sqrt{2}\,.\,k_f^{\alpha\beta}/(k_b^{\alpha\beta})^{\frac{1}{2}}$. The value of B will increase with increase in the rate of the forward reaction ($\alpha\beta \longrightarrow \gamma$) and with decrease in that of the back-reaction ($\gamma \longrightarrow \alpha\beta$). Thus the ratio B provides a measure of the ease of transformation from cyclic to reducible form which is appropriately weighted to allow for the rate of the back-reaction, and accordingly represents a quantity more valuable and informative for carbohydrate reactivity studies than does the supposed $k_f^{\alpha\beta}$ derived from Delahay's analysis. In Table 2 the sugars studied are arranged in order of B values. The sugar diffusion coefficient (D) was assumed throughout to be 6·7 × 10^{-6} cm.^{2} sec.$^{-1}$, the value obtained by Friedman and Carpenter[6] for 0·1M-D-glucose at 25°.

TABLE 2. *General properties of the polarographic waves of sugars at* pH 8·0 *and* 25° *in* 0·002M-*tetramethylammonium phosphate buffer.*

Sugar or sugar derivative	Mercury head dependence	$E_{1/2}$ (S.C.E.) (v)	A (microamp. l. mmole^{-1})	$10^5k_s^{\alpha\beta}$ (cm. sec.$^{-1}$)	$k_f^{\alpha\beta}$ (sec.$^{-1}$)	10^8K	B (sec.$^{-\frac{1}{2}}$)
2,3,4-Tri-*O*-methyl-L-arabopyranose	$\propto H^{\frac{1}{2}}$	−1·36	2·5	>1300			
2,3,5-Tri-*O*-methyl-D-ribofuranose	$\propto H^{\frac{1}{2}}$	−1·57	0·4	>1300			
2-Deoxy-L-ribose	$\propto H^{\circ}$	−2·10	1·9	63	890	6600	0·37
2-Deoxy-D-ribose	,,	−2·10	1·9	62	880	6500	0·37
D-Ribose	,,	−2·03	1·2	36	600	3200	0·22
2-Deoxy-D-allose	,,	−2·16	0·61	23	530	1500	0·14
2-Deoxy-D-galactose	,,	−2·13	0·68	25	260	3500	0·14
3,5,6-Tri-*O*-methyl-D-glucofuranose	,,	−1·91	0·28	8·5	87	1200	0·050
2-Deoxy-D-glucose	,,	−2·18	0·20	7·6	180	500	0·045
3-Deoxy-D-allose	,,	−2·03	0·14	5·0	130	300	0·029
L-Arabinose	,,	−2·12	0·14	4·7	80	410	0·028
2,3,4-Tri-*O*-methyl-D-ribopyranose	,,	−2·21	0·10	3·4	98	180	0·020
D-Lyxose	,,	−1·93	0·088	2·2	80	89	0·013
D-Xylose	,,	−2·05	0·071	2·1	35	180	0·012
D-Galactose	,,	−1·97	0·040	1·3	38	68	0·0078
2-*O*-Methyl-L-arabinose	,,	−1·71	0·036	0·96	37	37	0·0057
D-Mannose	,,	−1·91	0·033	0·90	33	37	0·0051
D-Glucose	,,	−1·87	0·01	0·27	13	9·0	0·0017
3-*O*-Methyl-D-glucose	,,	−1·73	0·004	0·10	2·5	6·6	0·00063
2,3,4,6-Tetra-*O*-methyl-D-glucopyranose			⩽0·1	⩽1			⩽0·1

From a comparison of equations (1) and (5a), the ratio B may be related to the rate constant $k_s^{\alpha\beta}$ of Delahay's treatment. Thus, $B = 0{\cdot}6Pk_s^{\alpha\beta}/GD^{\frac{1}{2}}$, and at 25° when $P = 1255$ and $G = 493$, $B = 1{\cdot}53k_s^{\alpha\beta}/D^{\frac{1}{2}} = 590k_s^{\alpha\beta}$ when the above value for D is used: hence B and k_s are directly proportional.

These equations show that a limiting current which is controlled by a reaction rate is dependent on the product $(m\tau)^{2/3}$, which is proportional to the surface area of the mercury drop. Since the area of the mercury drop is independent of the mercury-reservoir height, the magnitudes of limiting currents controlled by reaction rates show a similar independence. When the limiting current depends on a diffusion rate, the instantaneous current generated depends not only on the area of the drop, but also on a function of the mercury drop time. This arises because the amount of material diffusing to the mercury surface depends on these same two factors and the limiting current is proportional to $m^{2/3}\tau^{1/6}$, as in the Ilkovic equation.

When the rates of diffusion and reaction are joint controlling factors, the limiting current depends on a power of τ between 2/3 and 1/6. Thus, the magnitude of a limiting current which is controlled to any extent by diffusion should not be independent of the mercury reservoir height.

In the cases of 2,3,4-tri-*O*-methyl-L-arabopyranose and 2,3,5-tri-*O*-methyl-D-ribofuranose, the polarographic reduction was diffusion-controlled, so that the rate constants for the transformation from cyclic to reducible form could not be calculated on the basis of the above two treatments. Nevertheless, lower limiting values of $k_s^{\alpha\beta}$ were available since Delahay showed that for a diffusion-controlled reduction of this type, the product $k_s^{\alpha\beta}D^{-1/2}$ must exceed five. This result does not depend on the assumptions involving the reaction layer thickness " δ," and lower limiting values of $k_s^{\alpha\beta}$ for these two methylated sugars are shown in Table 2.

Effect of Temperature Variation.—(a) 2,3,4-*Tri*-O-*methyl*-L-*arabopyranose and* 2,3,5-*tri*-O-*methyl*-D-*ribofuranose*. Even in the region of 0°, the limiting currents generated by these compounds remained accurately proportional to the square root of the head of mercury (see Table 3). Evidently the rate of transformation to the reducible form is much faster for these compounds than for the other sugars studied. The approximate temperature coefficients of limiting current in the region of 25° were +2·3% per degree for 2,3,4-tri-*O*-methyl-L-arabopyranose, and +1·7% per degree for 2,3,5-tri-*O*-methyl-D-ribofuranose. These figures are typical of a diffusion-controlled reduction (the temperature coefficients of diffusion current for many common ions range from +1·3 to +2·3% per degree [9]).

TABLE 3. *Relation between limiting current and mercury head at low temperatures.*

(*a*) 2,3,4-Tri-*O*-methyl-L-arabopyranose (0·005M; pH 8·0; 0·1°)

Mercury head (*H*) (mm.)	480	420	390	360	320
Limiting current (*i*) (microamp.)	9·0	8·4	8·0	7·7	7·3
$H^{\frac{1}{2}}/i$	2·4	2·4	2·5	2·5	2·4

(*b*) 2,3,5-Tri-*O*-methyl-D-ribofuranose (0·043M; pH 8·0; 1·1°)

Mercury head (*H*) (mm.)	449	419	379	339	299
Limiting current (*i*) (microamp.)	9·9	9·6	9·1	8·8	8·1
$H^{\frac{1}{2}}/i$	0·68	0·67	0·68	0·67	0·68

(b) D-*Ribose and* 2-*deoxy*-D-*ribose*. The variation with temperature of the values of *A* and *B* was studied for these two sugars. *A* and *B* are related by equation (5*a*) in which the constant *G* is equal to 493 at 25°, and at other temperatures this value must be corrected (it has a very small temperature coefficient [10] of +0·00012 deg.$^{-1}$).

By using equation (5*a*) with the appropriately corrected *G* value, the ratios *A* and *B* were determined for D-ribose and 2-deoxy-D-ribose at various temperatures between 0° and 60°, and log *B* was plotted against the reciprocal of the absolute temperature (Fig. 5). Since *B* is directly proportional to the " heterogeneous " rate constant of the transformation from cyclic to reducible form (Delahay's $k_s^{\alpha\beta}$), the gradient of the plot in Fig. 5 may be regarded as a measure of the activation energy involved in electroreduction. Above about 30°, these plots deviate markedly from linearity, and this may arise for two reasons. First, the rate of transformation from ring to reducible form may have increased to such an extent that diffusion is becoming a rate-controlling factor: the temperature coefficients of *A* (and hence of *B*) for D-ribose and 2-deoxy-D-ribose would then decrease from a value of +10% per degree shown by both sugars in the region of 20°, and approach the value of +2% per degree, which is characteristic of the two tri-*O*-methyl sugars generating diffusion-controlled limiting currents. Secondly, at higher temperatures the reduction may proceed by an alternative mechanism of lower overall activation energy.

The activation energies involved in the polarographic reduction of D-ribose and 2-deoxy-D-ribose were calculated from the gradients of the linear sections of Fig. 5 as 25 and 23 kcal./mole, respectively. These activation energies are greater than the value of

---

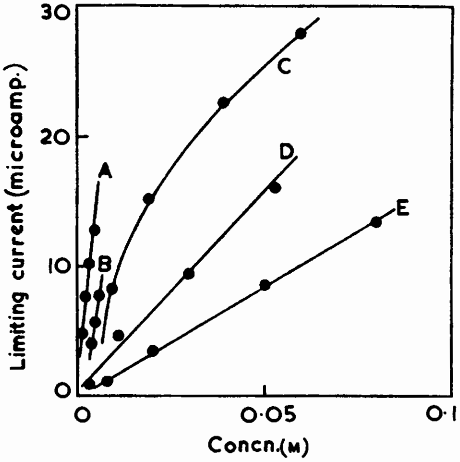
[9] Nejedly, *Coll. Czech. Chem. Comm.*, 1929, **1**, 319.

[10] Cf. Kolthoff and Lingane, " Polarography," Interscience Publ. Inc., New York, 1952, 2nd edn., Vol. I, Chap. 4.

16·5 kcal./mole obtained by Delahay and Strassner[11] for the reduction of L-arabinose, D-xylose, D-galactose, D-mannose, and D-glucose at pH 7·75. However, the results obtained by these workers are not readily interpreted since they used highly concentrated (0·5M) sugar solutions; they would exhibit too low a ratio of limiting current to concentration (*A* value) for under these conditions the current–concentration curves are not linear (Fig. 4).

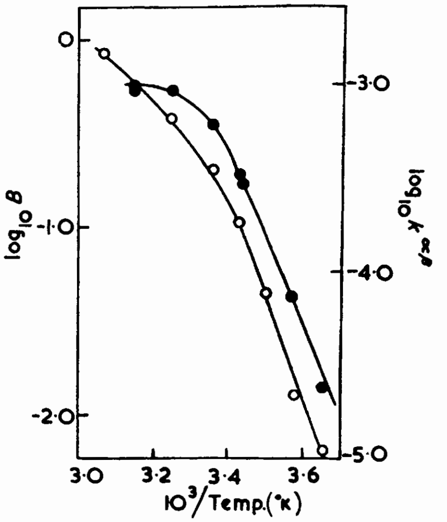
Effect of pH *Variation.*—(a) 2,3,4-*Tri*-O-*methyl*-L-*arabopyranose and* 2,3,5-*tri*-O-*methyl*-D-*ribofuranose*. Variation of pH over a wide range had no significant effect on the *A* value for either of these compounds (Table 4). This accords with the view that these compounds generate limiting currents which are determined by the rate of diffusion to the mercury drop surface since this rate does not depend on pH.

FIG. 4. *The effect of sugar concentration on the limiting polarographic current in* 0·002M-*tetramethylammonium phosphate buffer* (pH 8·0) *at* 25°.



A, 2,3,4-Tri-*O*-methyl-L-arabinose; *B*, 2-deoxy-D-ribose; C, D-ribose; D, 2,3,5-tri-*O*-methyl-D-ribose; E, 2-deoxy-D-glucose.

FIG. 5. *Dependence of the quantities B and* $k^{\alpha\beta}$ *on temperature at* pH 8·0.



○ D-Ribose; ● 2-deoxy-D-ribose.

TABLE 4. *Variation of A values with* pH *at* 25°.

(*a*) 2,3,4-Tri-*O*-methyl-L-arabopyranose

pH	3·30	4·98	6·25	7·48
A (microamp. mmole l.$^{-1}$)	2·11	2·14	2·19	2·14

(*b*) 2,3,5-Tri-*O*-methyl-D-ribofuranose

pH	3·18	3·98	5·79	7·24
A (microamp. mmole l.$^{-1}$)	0·23	0·22	0·22	0·23

(b) D-*Ribose and* 2-*deoxy*-L-*ribose*. Variation in pH was found to have a marked effect on the values of *A* and *B* for these sugars (Table 5). The results obtained for D-ribose indicate that the transformation of the αβ-equilibrium mixture from ring to reducible form is catalysed by hydrogen and hydroxyl ions with a minimum velocity at pH 3·5—6. The values of the catalytic constants for the reduction of D-ribose were: $k_{\mathrm{H}^+} = 9{\cdot}9 \times 10^3$ l. mole^{-1} sec.$^{-1}$, $k_{\mathrm{OH}^-} = 1{\cdot}6 \times 10^6$ l. mole^{-1} sec.$^{-1}$ and $k_{\mathrm{OH}^-}/k_{\mathrm{H}^+} = 162$ at 25°. (The constants k_{H^+} and k_{OH^-}, when multiplied by the concentration of hydrogen or hydroxyl ion respectively, represent the contribution of these catalytic effects to the rate constants of the forward reaction.) These results are in accordance with the work of Delahay and Strassner who showed that increase in pH increased the limiting currents of several simple sugars. These workers did not study the pH effect below pH 6·5, and so did not observe hydrogen-ion catalysis, although Cantor and

---

[11] Delahay and Strassner, *J. Amer. Chem. Soc.*, 1952, **74**, 893.

Peniston reported hydrogen-ion catalysis in the reduction of D-ribose and L-allose and suggested that the effect might be general.

TABLE 5. *Variation of A and B values with* pH *at* 25°.

(*a*) D-Ribose

pH	*A* (microamp. mmole l.$^{-1}$)	*B* (sec.$^{-1/2}$)	$10^4 . k_s\alpha\beta$ (cm. sec.$^{-1}$)
1·76	0·50	0·067	1·13
2·66	0·51	0·039	0·66
3·51	0·097	0·0077	0·13
4·20	0·11	0·017	0·285
6·00	0·14	0·023	0·385
7·22	0·56	0·089	1·51
8·72	0·93	0·15	2·47
9·90	1·2	0·19	3·16

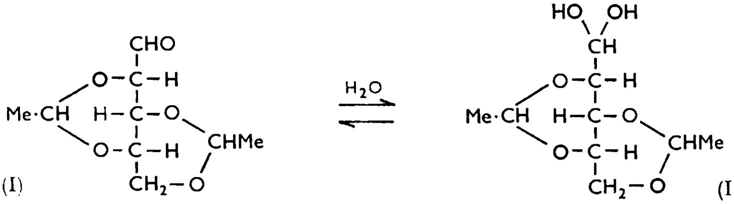
(*b*) 2-Deoxy-L-ribose

pH	*A* (microamp. mmole l.$^{-1}$)	*B* (sec.$^{-1/2}$)	$10^4 . k_s\alpha\beta$ (cm. sec.$^{-1}$)
4·42	$\leqslant 1$	—	—
5·92	0·18	0·030	0·51
8·34	0·15	0·24	4·12
10·48	0·17	0·28	4·71

Polarography of 2,4:3,5-*Di*-O-*ethylidene*-aldehydo-L-*xylose*.—With this compound in the usual 0·002M-tetramethylammonium phosphate–phosphoric acid mixture fluctuating non-reproducible waves were obtained and this was attributed to failure to maintain steady pH conditions in the region of the mercury drop surface. Accordingly further work was conducted with 0·01M-sodium borate buffer (pH 9·18) which has a much higher buffering capacity than the 0·002M-tetramethylammonium solution. In the borate medium the *aldehydo*-sugar generated clear reproducible waves (half-wave potential = −1·42 v) with limiting currents proportional to sugar concentration over a wide range. The limiting current was independent of the mercury head, indicating that the reduction rate was controlled by the rate of transformation of the *aldehydo*-sugar from a non-reducible to a reducible form at the mercury drop surface.

This behaviour may be compared with the polarography of simple aldehydes. Veseley and Brdicka [12] have shown that formaldehyde gives kinetic limiting currents determined by the acid–base-catalysed dehydration rate of the hydrated methylene glycol structure: $H_2C(OH)_2 \rightleftharpoons H_2C{=}O + H_2O$. Acetaldehyde [13] and propionaldehyde [14] give reduction waves which are less dependent on temperature and pH since these aldehydes are less hydrated than formaldehyde. Their limiting currents are controlled partly by diffusion and partly by the rate of dehydration of the hydrated structure. The dehydration of acetaldehyde hydrate has been studied extensively [15] and has been shown to undergo general acid–base catalysis.[16]

In common with other *aldehydo*-sugars,[17] 2,4:3,5-di-*O*-ethylidene-*aldehydo*-L-xylose (I) has been shown [18] to react instantaneously with water to give the hydrated form (II). It seems


(I) (II)

probable, therefore, that the rate of polarographic reduction of the *aldehydo*-sugar is controlled exclusively by the dehydration rate of the non-reducible form (as II). Acid–base catalysis of the reduction may then be interpreted in terms of a mechanism of the type proposed by Bell and Higginson [16] for acetaldehyde hydrate.

---

[12] Veseley and Brdicka, *Coll. Czech. Chem. Comm.*, 1947, **12**, 313.

[13] Bieber and Trumpier, *Helv. Chim. Acta*, 1947, **30**, 2000.

[14] Bieber and Trumpier, *Helv. Chim. Acta*, 1948, **31**, 5.

[15] Herold and Wolf, *Z. phys. Chem.*, 1931, *B*, **12**, 165; Herbert and Lander, *Trans. Faraday Soc.*, 1938, **34**, 432, 1219.

[16] Bell and Higginson, *Proc. Roy. Soc.*, 1949, *A*, **197**, 141; Bell and Darwent, *Trans. Faraday Soc.*, 1950, **46**, 34.

[17] Levene and Meyer, *J. Biol. Chem.*, 1927, **74**, 695; Wolfrom, *J. Amer. Chem. Soc.*, 1931, **53**, 2275; Hann, Ness, and Hudson, *ibid.*, 1944, **66**, 665; Stacey and Turton, *J.*, 1946, 661.

[18] Corbett, Ph.D. Thesis, Birmingham University, 1951.

Thus, the reduction may be assumed to proceed by the reaction scheme: hydrated form (non-reducible) $\rightleftharpoons$ *aldehydo*-form (reducible), and may be treated by the analysis of Delahay or by that of Los, Simpson, and Wiesner if the presence of anomeric α- and β-forms is disregarded. The limiting current of the *aldehydo*-sugar showed temperature- and pH-dependence typical of kinetically controlled currents. Increase of temperature increased A, B, and $k_s^{\alpha\beta}$ and the plot of log B against the reciprocal of the absolute temperature was linear up to about 25°: the temperature coefficient of A and of B was $+14\%$ per degree in the region of 20°. Above 25° the plot had a downward deviation from linearity which may have been caused either by the emergence of diffusion as a rate-controlling factor or by the adoption of a new reduction mechanism having a lower overall activation energy. The activation energy involved in reduction was calculated to be 42 kcal./mole from the gradient of the linear section of the plot of log B against $1/T$.

Values of A, B, and $k_s^{\alpha\beta}$ were determined for 2,4:3,5-di-*O*-ethylidene-*aldehydo*-L-xylose in

TABLE 6. *Polarographic reduction of* 2,4:3,5-*di*-O-*ethylidene*-aldehydo-L-*xylose at various* pH *values at* 25°.

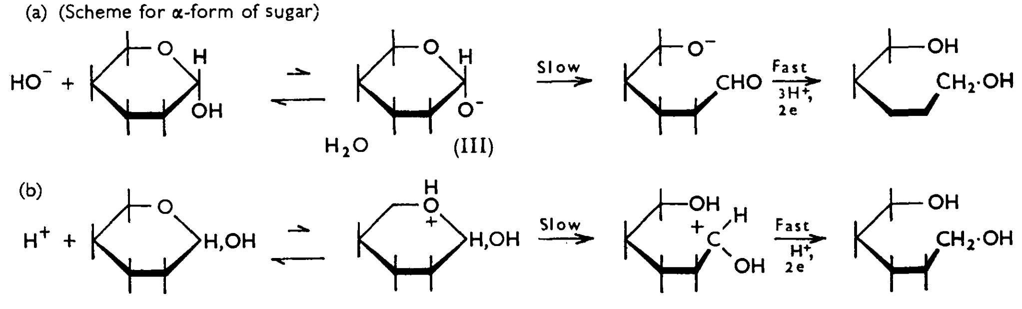
pH	A (microamp. millimole l.$^{-1}$)	B (sec.$^{-1/2}$)	$10^{-5}k_s^{\alpha\beta}$ (cm. sec.$^{-1}$)	Buffer
2·45	0·14	0·017	2·8	KCl–HCl
2·77	0·14	0·017	2·8	K H phthalate–HCl
3·02	0·14	0·017	2·8	
3·61	0·13	0·016	2·7	
4·01	0·10	0·013	2·2	K H phthalate
6·08	0·12	0·015	2·6	Phosphate mixtures
6·62	0·12	0·015	2·5	
7·06	0·17	0·022	3·7	
7·90	0·18	0·023	3·9	Na borate–HCl
9·18	0·83	0·17	28·0	Na borate

buffer solutions of varying pH, and the polarographic reduction was found to be catalysed by hydrogen and hydroxyl ions (Table 6).

In the pH range 2—6, the velocity of the dehydration reaction is almost independent of pH. The values of the catalytic constants for the dehydration were: $k_{\mathrm{H}^+} = 5{\cdot}4 \times 10^2$ l. mole^{-1} sec.$^{-1}$, $k_{\mathrm{OH}^-} = 3{\cdot}9 \times 10^7$ l. mole^{-1} sec.$^{-1}$, and $k_{\mathrm{OH}^-}/k_{\mathrm{H}^+} = 7{\cdot}2 \times 10^4$ at 25°.

DISCUSSION

Information on the relative stability of the 1-hydrogen atom and 1-hydroxyl group in D-glucose, together with the fact that the product of polarographic reduction of a sugar has been shown to be a polyalcohol, leads to the following proposals for the reduction mechanism.



Two electrons are required for the reduction of one sugar molecule, as for the reduction of a simple aldehyde to the corresponding alcohol. This assumption is supported by the work of Sugino and Hayashi[19] who showed that the polarographic reduction of D-glucose yielded D-glucitol. The reduction, under alkaline conditions, is considered to proceed by scheme (a) since Fredenhagen and Bonhoeffer[20] demonstrated that the 1-H bond of

---

[19] Sugino and Hayashi, *J. Chem. Soc., Japan*, 1944, **65**, 458; *Chem. Abs.*, 1947, **41**, 3758*b*.

[20] Fredenhagen and Bonhoeffer, *Z. phys. Chem.*, 1938, **181**, 392.

D-glucose is not broken during alkali-catalysed mutarotations. The intermediate (III) is present in only very small proportion at pH 8 since the pK_a of the 1-hydroxyl group of glucose [21] is of the order of 13. However, the intermediate (III) is removed continuously as reduction proceeds and the reaction shown as scheme (a) is driven to the right-hand side. This mechanism is similar to that proposed by Delahay and Strassner. Both schemes (a) and (b) involve a change from the cyclic to the open-chain form of the sugar and so the rate overall would probably be assisted by any factor which decreases the stability of the ring-form relatively to that of the open-chain or which, in the case of hydroxyl-ion catalysis, facilitates access to the 1-position.

The hexoses generally exhibit lower B values than the pentoses. The B value for ribose appears to be abnormally high but there are other indications, *e.g.*, mutarotation, that ribose shows anomalous behaviour, possibly connected with the higher incidence of furanose forms.

Methylation at the 2-position and, to a smaller extent, at the 3-position, has a marked effect on the B value since 2-*O*-methyl-L-arabinose (0·0057 sec.$^{-1/2}$) and 3-*O*-methyl-D-glucose (0·00063 sec.$^{-1/2}$) both show B values which are considerably lower than those of the parent sugars, L-arabinose (0·028 sec.$^{-1/2}$) and D-glucose (0·0017 sec.$^{-1/2}$). Further, 2,3,4,6-tetra-*O*-methyl-D-glucopyranose appears to be completely non-reducible in concentrations up to 0·1M, the maximum which it was practicable to use with this compound. Similarly, 2,3,4-tri-*O*-methyl-D-ribopyranose shows a lower B value than does D-ribose. Since the rate-determining step is more likely to be that of ring opening in schemes (a) and (b) and since, in acid-catalysis, attack by a hydrogen ion is not usually sterically hindered, such steric effects are more likely to be operative in altering ring stability than in changing the susceptibility to attack by hydrogen or hydroxyl ions, in the first stage of (a) and (b). Moreover, the relatively greater stability of the pyranose ring form, in which these two compounds are constrained, must be of significance.

In contrast, 2-deoxy-D-glucose (0·045 sec.$^{-1/2}$) and, to a smaller extent, 3-deoxy-D-glucose (= 3-deoxy-D-allose; 0·029 sec.$^{-1/2}$) show higher B values than the parent sugar.

2,3,4-Tri-*O*-methyl-L-arabopyranose and 2,3,5-tri-*O*-methyl-D-ribofuranose generated limiting currents which were controlled by the rate of diffusion of the ring form to the mercury drop surface. This indicated that a fast reaction occurred before the electro-reduction stage. When set up in Courtauld-type models [22] these two compounds proved to have very hindered ring structures, so that the ring form is destabilised relatively to the open-chain *aldehydo*-form. Thus, in aqueous solution there are two possibilities: either there is a larger proportion of the *aldehydo*-form of the sugar or there is a high rate of transformation from ring to reducible form. But 2,4:3,5-di-*O*-ethylidene-*aldehydo*-L-xylose, a sugar derivative fixed in the open-chain form (I), has been shown above to generate kinetic currents determined by the dehydration rate of the hydrated form (II). Thus, since 2,3,4-tri-*O*-methyl-L-arabopyranose and 2,3,5-tri-*O*-methyl-D-ribofuranose yield diffusion-controlled currents, they must exist in aqueous solution predominantly as the ring form, but they show a very high rate of transformation to the reducible form. If they existed to an appreciable extent as *aldehydo*-sugars they would be in the hydrated form in aqueous solution and would exhibit kinetic limiting currents.

This work is being continued in an endeavour to obtain further evidence about the steric factors which affect the equilibrium between open-chain and cyclic forms of sugars.

EXPERIMENTAL

Materials.—To obtain clear reproducible polarographic reduction waves it was essential to purify carefully all carbohydrate derivatives. It was necessary to remove alkali-metal impurities which tended to cause coalescence of the buffer and sugar waves. Solid sugars were

---

[21] Los and Simpson, *Rec. Trav. chim.*, 1954, **73**, 941; 1957, **75**, 267.

[22] Hartley and Robinson, *Trans. Faraday Soc.*, 1952, **48**, 847.

purified from solvents containing low proportions of water. Syrupy products were distilled under diminished pressure. The following sugars were purified by slow recrystallisation twice from 80% aqueous ethanol and were dried *in vacuo* over phosphoric oxide: α-D-glucose, m. p. 146°, $[\alpha]_D^{20}$ +52·5° (*c* 4 in water); α-D-galactose, m. p. 167—168°, $[\alpha]_D^{20}$ +80·4° (*c* 4 in water); α-D-mannose, m. p. 132°, $[\alpha]_D^{20}$ +14·1° (*c* 4 in water); β-L-arabinose, m. p. 158°, $[\alpha]_D^{20}$ +104° (*c* 4 in water); β-D-lyxose, m. p. 118—119°, $[\alpha]_D^{20}$ −14° (*c* 4 in water); α-D-ribose, m. p. 90°; α-D-xylose, m. p. 146—147°, $[\alpha]_D^{20}$ −18·8° (*c* 4 in water).

Deoxy-sugars were purified to the constants shown: 2-deoxy-D-allose was prepared from methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-alloside [23] by Prins's method.[24] The product was recrystallised from ether to afford the α-form of the sugar, m. p. 126—128°, $[\alpha]_D^{20}$ +150° (*c* 0·6 in $CHCl_3$) (Found: C, 43·8; H, 7·2. Calc. for $C_6H_{12}O_5$: C, 44·0; H, 7·3%); 2-deoxy-β-D galactose, m. p. 126—128°; 2-deoxy-α-D-glucose (crystallised from methanol–acetone), m. p. 146°; 2-deoxy-β-D-ribose, m. p. 77°; 2-deoxy-β-L-ribose, m. p. 74—76°; 3-deoxy-D-allose, colourless syrup, n_D^{18} 1·4735, $[\alpha]_D^{20}$ +8° (*c* 0·25 in water).

Methylated derivatives: 3-*O*-methyl-D-glucose was prepared by the method of Glen, Myers, and Grant,[25] and was crystallised twice from methanol as white plates, m. p. 167—168°; 3,5,6-tri-*O*-methyl-D-glucofuranose was synthesised by the method of Irvine and Scott [26] as modified by Coles *et al.*[27] and Freudenberg and Vajda.[28] Fractional distillation gave a mobile syrup, b. p. 115—120° (bath-temp.)/0·01 mm., n_D^{15} 1·4549, $[\alpha]_D^{20}$ +39° (*c* 1·1 in methanol) (Found: C, 49·0; H, 8·0; OMe, 41·8. Calc. for $C_9H_{18}O_6$: C, 48·9; H, 8·15; OMe, 42·0%); 2,3,4,6-tetra-*O*-methyl-D-glucose, needles, m. p. 84°, $[\alpha]_D^{20}$ +84·5° (*c* 1 in water); 2-*O*-methyl-L-arabinose, syrup, $[\alpha]_D^{20}$ +61° (*c* 0·18 in water); 2,3,4-tri-*O*-methyl-L-arabinose, prepared by the method of Purdie and Rose [29] except that the methylation was carried out with the Haworth reagent, was a mobile syrup, b. p. 98—99° (bath-temp.)/0·02 mm., n_D^{18} 1·4823, $[\alpha]_D^{20}$ +43° (*c* 0·42 in water) (Found: C, 50·2; H, 8·4; OMe, 48·0. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·34; OMe, 48·4%); 2,3,4-tri-*O*-methyl-D-ribose, m. p. 83°; 2,3,5-tri-*O*-methyl-D-ribose,[30] b. p. 75° (bath-temp.)/0·05 mm., n_D^{18} 1·4540, $[\alpha]_D^{20}$ +40° (*c* 1·1 in methanol) (Found: C, 50·1; H, 8·3; OMe, 48·6. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·34; OMe, 48·4%).

2,4:3,5-Di-*O*-ethylidene-*aldehydo*-L-xylose was prepared by the method of Bladon and Owen [31] as modified by Corbett.[18] After being recrystallised twice from dry redistilled dioxan it was obtained as needles, m. p. 160—161°, $[\alpha]_D^{20}$ −12·5° (*c* 2 in water).

Buffer solution: the buffer was tetramethylammonium phosphate solution containing phosphoric acid to adjust the pH to any desired value. Tetramethylammonium iodide (3·015 g.), recrystallised twice from water, in warm distilled water (400 ml.) was mixed with an excess of powdered silver phosphate in a 500 ml. bottle covered with black paper to prevent photodecomposition. (If allowed to proceed unhindered, this decomposition produced much colloidal matter in the buffer and gave large irregularities in the polarographic wave.) The mixture was shaken mechanically overnight and then filtered through a fine Whatman paper to remove silver iodide and the excess of silver phosphate, leaving a clear solution of tetramethylammonium phosphate. 1% Phosphoric acid ("AnalaR") was added to the filtrate to adjust the pH to 8·0 and the solution was diluted to 1 l., giving 0·1M-tetramethylammonium ion. The buffer was stored in a Polythene bottle, and its pH was stable over long periods. The final concentration of tetramethylammonium ion in the solution used in the polarographic cell was 0·002M and this buffer was used throughout unless otherwise stated. Values of pH other than 8 were obtained by variation of the phosphoric acid content.

Methods.—The polarograph was based on the "Voltamoscope" of the Cambridge Instrument Company. The cell was conventional with two side-arms, one for making electrical contact to the pool of mercury at the bottom of the cell, and the other for the passage of hydrogen. The capacity of the cell was 5 ml. Half-wave potentials were measured relative to a saturated calomel electrode which was connected through a saturated potassium chloride

---

[23] Jeanloz, Prins. and Reichstein, *Experientia*, 1945, **1**, 336; *Helv. Chim. Acta*, 1946, **29**, 371.

[24] Prins, *J. Amer. Chem. Soc.*, 1948, **70**, 3955.

[25] Glen, Myers, and Grant, *J.*, 1951, 2568.

[26] Irvine and Scott, *J.*, 1913, **103**, 564.

[27] Coles, Goodhue, and Hixon, *J. Amer. Chem. Soc.*, 1929, **51**, 519.

[28] Freudenberg and Vajda, *J. Amer. Chem. Soc.*, 1937, **59**, 1955.

[29] Purdie and Rose, *J.*, 1906, **89**, 1204.

[30] Barker, *J.*, 1948, 2035.

[31] Bladon and Owen, *J.*, 1950, 591.

bridge to a side-arm of the polarographic cell. Values of pH were measured by using a bench pH meter in conjunction with a glass electrode. A vigorous stream of pure hydrogen was bubbled through the test solution to remove dissolved oxygen. (Hydrogen was preferred since the smallest traces of oxygen could then be readily removed.)

The experiments were carried out in a water-thermostat at 25° ± 0·05° except where otherwise stated. The galvanometer of the polarograph was calibrated so that limiting currents could be measured directly in microampères. The mercury drop period (τ) was measured with a stop watch, and the mercury flow rate (m) was determined by weighing the mercury dropping from the capillary during a known time interval whilst a voltage corresponding to the "plateau" of the polarographic wave in question was applied between the electrodes. Two capillaries were used: in distilled water at 25° with 480 mm. mercury head and an applied voltage of 2·0 v, the first gave $\tau = 2{\cdot}21$ sec. and $m = 2{\cdot}01$ mg. sec.$^{-1}$; the second gave $\tau = 2{\cdot}87$ sec. and $m = 1{\cdot}429$ mg. sec.$^{-1}$. For each of the sugar solutions studied a residual current–voltage curve was recorded. The difference between this curve and the wave generated by the sugar solution represented the current–voltage curve produced solely by the reduction of the sugar.

The authors thank Professor M. Stacey, F.R.S., for his interest and encouragement and Professor D. G. Catcheside, F.R.S., for hospitality in his laboratory. They are indebted to Dr. P. Zuman (Prague) and Dr. J. E. B. Randles (Birmingham) for helpful criticism. The loan of apparatus by Imperial Chemical Industries Limited, and the receipt by one of them (J. B. S.) of a grant from the Department of Scientific and Industrial Research, are gratefully acknowledged.

CHEMISTRY DEPARTMENT, BIRKBECK COLLEGE, LONDON, W.C.1.
CHEMISTRY DEPARTMENT, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM, 15.

[*Received, March* 15*th*, 1960.]