Anal. Calcd. for $C_{27}H_{16}O_4NK$: K, 8.56. Found: K, 8.73.

When recrystallized from *n*-butyl alcohol we obtained flaky red crystals which contained alcohol of crystallization. Had this been the case with the mono-benzoyl compound we might have been successful in isolating it in an active state.

Anal. Calcd. for $C_{27}H_{16}O_4NK \cdot C_4H_9OH$: K, 7.35. Found: K, 7.06, 7.15.

To a boiling filtered solution of 5 g. of potassium 9-*aci*-nitro-2,7-dibenzoylfluorene in 400 cc. of ethyl alcohol was added 4.74 g. of brucine hydrochloride in 100 cc. of alcohol. When mixed the temperature was 80°. On cooling to 50°, crystals began to separate when the sides of the container were scratched. The solution had been red but turned yellow when crystallization took place.

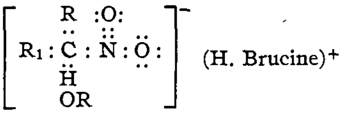
The solid was filtered and triturated with water to remove potassium chloride, washed with alcohol and dried in air. The yield was 5 g. or 51%.

A sample of 0.2204 g. in 25 cc. of chloroform in a 2-dm. tube gave α_D +1.18°, $[\alpha]^{24}_D$ +66.92°. In pyridine there was no mutarotation over a period of two hours; the specific rotation then slowly changed from +78.07 to +72.56° in the course of six hours.

Summary

We have presented evidence to show that the resolution of the 9-*aci*-nitro-2-benzoylfluorene ion into its antipodes has been accomplished with brucine.

The brucine salt contains alcohol of crystallization which we think accounts for its activity by stabilizing the "lone pair" of electrons as follows. This structure is similar to that sug-



$$\left[\begin{array}{c} R\ \ :O: \\ R_1:\ddot{C}:\ddot{N}:\ddot{O}: \\ \ddot{H} \\ OR \end{array}\right]^- \text{(H. Brucine)}^+$$

gested by Young and Shriner[1] for the coördination compound of the sodium salt of 2-nitroöctane and alcohol.

Neither the free 9-*aci*-nitro-2-benzoylfluorene nor its potassium salt crystallized with alcohol of coördination and neither could be obtained in an active form, although in solution both showed a transitory activity.

The symmetrical compound, 9-*aci*-nitro-2,7-dibenzoylfluorene, has been prepared and found to differ radically in optical properties from the unsymmetrical 9-*aci*-nitro-2-benzoylfluorene.

If an *aci*-nitro compound or its salt can be obtained that separates with a coördinating molecule, we predict that the application of the technique here described will result in its isolation in an optically active form.

CINCINNATI, OHIO RECEIVED JUNE 13, 1940

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE CORN PRODUCTS REFINING COMPANY]

The Reduction of Aldoses at the Dropping Mercury Cathode: Estimation of the *aldehydo* Structure in Aqueous Solutions[1]

BY SIDNEY M. CANTOR AND QUINTIN P. PENISTON

Investigations on aqueous solutions of the reducing sugars at equilibrium rotation have demonstrated that a condition of dynamic equilibrium between several components is involved and that, depending on conditions and reagents, the equilibrium may be shifted to form derivatives of several parent structures. While it has been possible to estimate from various data the relative amounts of alpha and beta pyranose forms for certain of the aldoses at equilibrium, it has hitherto not been possible to measure the concentration of or indeed adequately to demonstrate the presence of the so-called "free aldehyde" or *aldehydo* structure. Such a non-cyclic tautomer must necessarily be postulated as the intermediate through which mutarotation, ring shift and other structural transformations occur.

Indications that more than two isomers are concerned in the mutarotation of the reducing sugars are contained in the extensive researches of the Lowry, Hudson and Isbell[2] groups. While the first order mechanism adequately represents the data obtained in some cases, in other cases this interpretation does not hold and the mutarotation can only be described as anomalous.

Several attempts have been made to establish the *aldehydo* structure. Thus, ultraviolet studies on aldose solutions originally conducted by Niederhoff[3]

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(1) Presented at the Cincinnati meeting of the American Chemical Society, April 10, 1940.

(2) (a) Lowry, *J. Chem. Soc.*, 1913 (1903); Lowry and Richards, *ibid.*, 1385 (1925); Smith and Lowry, *ibid.*, 666 (1928); (b) Hudson, *Sci. Pap. Bur. Standards* No. 533 (1926); (c) Isbell and Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

(3) Niederhoff, *Z. physiol. Chem.*, **165**, 130 (1927).

showed a band at 2800 Å. which was believed to be indicative of the carbonyl group. Later work by several investigators,[4] particularly with regard to glucose, has shown that this band was due to an impurity and has led to the conclusion that the data published by Niederhoff are unreliable and that the *aldehydo* glucose concentration is below the limit of spectroscopic investigation, namely, 0.3% of the total sugar. This conclusion is in accord with the present investigation. Lippich[5] estimated the amount of *aldehydo* form in several aldoses by determining the extent of "instantaneous" combination with hydrocyanic acid in alkaline solutions. Although a number of fundamental objections may be raised to the interpretation of Lippich's data as a measure of an equilibrium concentration, it will be seen that values for *aldehydo* concentration may be calculated from these data which agree in order of magnitude with the present results.

The extensive researches of the Heyrovsky school on the application of the polarographic method to the electro-reduction of numerous aldehydes and ketones suggested that this procedure should be applicable to the study of the *aldehydo* form in sugar solution systems. Heyrovsky and Smoler[6] studied several simple sugars with the polarograph and while they were able to demonstrate that ketoses were reducible and determinable with the dropping mercury cathode, they concluded that aldoses showed no similar reduction "wave." The ability of ketoses to undergo reduction was attributed to an "easily electro-reducible" form, probably meaning the *keto* structure. Reduction waves were observed with ketose concentrations as low as 1.7×10^{-3} molar. Aldoses were studied only at low concentrations and the slight waves obtained were attributed to impurities of a ketose nature.[7] In the present investigation it is shown that aldoses are reducible at the dropping mercury cathode, although the concentration of reducible form is much less than for ketose sugars, and that the determination of the reducible form is of value in a study of the equilibrated solutions as affected by various conditions of *p*H, concentration, and temperature. It is also shown that the amount of the reducible form can be correlated with configuration in the sugar series and with other manifestations of physical behavior such as the rate of mutarotation.

Results and Discussion

Initial indications that aldoses are reducible at the dropping mercury cathode were obtained in the examination of glucose solutions at various concentrations. Waves were obtained which varied in height with the sugar concentration. It was established that the reduction waves were not due to impurities by examination of sugar samples of varying degree of purity. Repeated recrystallization was without effect on the waves obtained. That the observed reducible substance was not due to ketose formed by Lobry de Bruyn transformation was demonstrated by repeating one experiment at *p*H 8 after forty-eight hours of standing at room temperature. The wave height remained unchanged. It was apparent that the reduction was due to some form other than the hemiacetal, either the *aldehydo* structure or its equivalent hydrate.

Preliminary experiments also showed that the amount of reducible substance varied widely with *p*H, particularly in the alkaline region. Determinations in solutions buffered at acidic *pH* values showed waves of complex character apparently complicated by hydrogen discharge. In unbuffered solutions a wave was obtained which also varied with sugar concentration. However, it was possible to show, by introducing a few drops of phenolphthalein into the solution under observation, that the region around the mercury cathode was definitely alkaline. It is thus indicated that in unbuffered solutions the *p*H of the reduction interface is determined by the electrode and that reliable results can only be obtained in adequately buffered solutions.

Effect of Variation in *p*H and Concentration on the Glucose Reduction Wave.—A series of polarographic reduction waves obtained in 0.50 molar glucose solution at several *p*H values is shown in Fig. 1. Thallous chloride was used at various minute concentrations to provide a standard reference point for estimation of half-wave potentials. Since the half-wave potential for thallous ion is −0.48 volt in either acid, neutral, or alkaline solution (referred to the normal calomel electrode) and the constant increment

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[4] (a) Henri and Schou, *Z. Physiol. Chem.*, **174**, 295 (1928); (b) Goos, Schlubach and Schröter, *ibid.*, **186**, 148 (1930); (c) Fischer, Hauss and Täufel, *Biochem. Z.*, **227**, 156 (1930).

[5] Lippich, *ibid.*, **248**, 280 (1932).

[6] Heyrovsky and Smoler, *Collection Czechoslov. Chem. Commun.*, **4**, 521 (1932).

[7] It is of interest to observe that lyxose, the sugar which these investigators found to have the most of such "ketose impurity," is shown in this investigation to have a relatively high free aldehyde concentration compared to the hexose sugars.

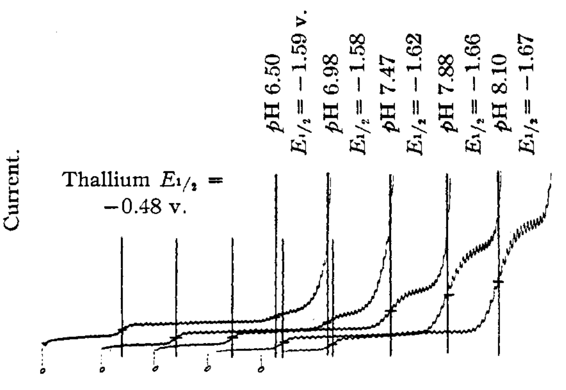

Fig. 1.—Glucose reduction "waves" for 0.50 M glucose, pH varying: 2×10^{-2} M buffer (KOH–KH_2PO_4); 1×10^{-1} M LiCl; 2×10^{-4} M TlCl; $S = {}^1/_{30}$.

for the polarograph was determined as 0.1815 volt per centimeter, half-wave potentials are thus obtained directly without corrections for variation in anode potential.

Apparent is the progressive increase in wave height and the shift in half-wave potential to more negative values with increasing pH. In Fig. 2 is presented a summary of the experimental results for glucose at 25°. The curves represent the variation in observed wave height with pH at several concentrations. It may be seen that at the lower concentrations the wave height is a linear function of pH. At half molar concentration and above, deviation from the linear form becomes apparent and the amount of reducible form is augmented by the increased sugar concentration. The relation is apparently complex in character since at higher pH values proportionality does not hold between the amount of reducible form and the concentration.

The Relation of "Wave" Height to Amount of Reducible Form.—Accurate interpretation of the heights of current-voltage "waves" in terms of the amount of reducible form existing in solution presents some difficulty. The limiting current forming the top of the observed wave is composed of two factors: a migration current, carried by all ions in solution, and a diffusion current, determined by the rate of diffusion of the reducible substance into the depleted region surrounding the mercury drop. When the "indifferent electrolyte" concentration is high with respect to that of the reducible substance, the migration current is independent of the concentration of the reducible substance and the wave height is then proportional to the diffusion current.

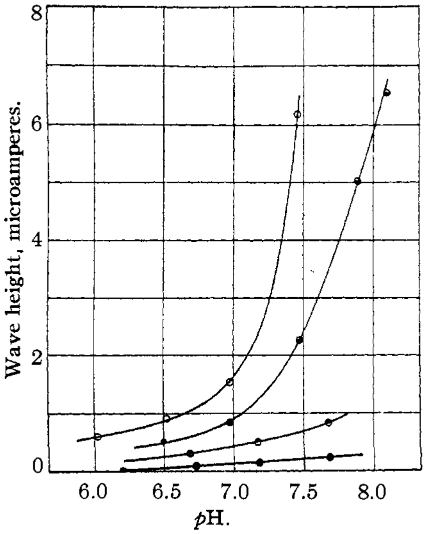

Fig. 2.—D-Glucose, variation of reducible form with pH and concentration: ○, 1.00 M; ◒, 0.50 M; ◓, 0.25 M; ●, 0.10 M.

If the properties of the capillary electrode remain constant, the proportionality of current with the concentration of reducible substance depends according to Ilkovic[8] upon the square root of the diffusion coefficient. For ions of the same molecular weight and valence and similar molar volumes the value of the diffusion coefficient should not vary greatly and would be primarily dependent on the viscosity of the solvent. It thus seemed reasonable to assume that the square root of the diffusion coefficient would be fairly constant for the sugars and concentrations investigated and that the concentration of the reducible form could be considered approximately proportional to the height of the observed wave.

This assumption is well supported by the data of Winkel and Proske[9] with regard to the diffusion currents of a series of structurally divergent aldehydes and ketones. The diffusion currents for such dissimilar compounds as chloroacetone and vanillin differed by only 0.3 microampere, about 6%.

In order to establish a reference standard for the proportionality of concentration and wave height, it was necessary to determine the wave heights for known concentrations of a known monomeric aldehyde. 5-Hydroxymethylfurfural was chosen as the standard since from its similarity in molecular size and structure to the hexose sugars it could be assumed that its diffusion co-

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(8) Ilkovic, *Collection Czechoslov. Chem. Commun.*, **6**, 498–513 (1934).

(9) Winkel and Proske, *Ber.*, **69**, 693, 1917 (1936).

efficient was similar to that of the sugars investigated.

Waves obtained with 5-hydroxymethylfurfural at various concentrations in solutions buffered at *p*H 7.60 are shown in Fig. 3. The wave heights are not directly comparable since they were obtained at different sensitivities. It can be seen, however, that they increase with increasing concentration. The lower half-wave potential as compared to the glucose waves is also apparent.

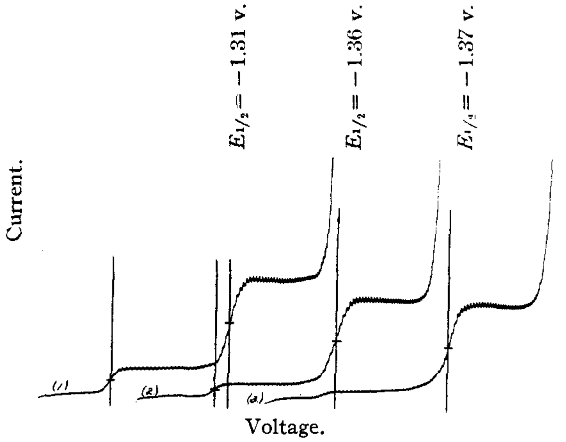


Fig. 3.—Determination of 5-hydroxymethylfurfural: 2×10^{-2} *M* buffer ($KOH-KH_2PO_4$); 1×10^{-1} LiCl; 2×10^{-4} TlCl; *p*H 7.60.

(1) at $S = 1/20$ $C = 6.7 \times 10^{-4}$ *M*
(2) at $S = 1/40$ $C = 13.1 \times 10^{-4}$ *M*
(3) at $S = 1/70$ $C = 26.3 \times 10^{-4}$ *M*

Standardization data obtained for 5-hydroxymethylfurfural are plotted in Fig. 4. It may be seen that below 2×10^{-3} molar the wave height is substantially linear with concentration and almost independent of *p*H. Almost all of the aldose waves measured fell within this range. It should be noted that variations in curves with both *p*H and concentration are in the opposite direction to those obtained for glucose. The lower values at *p*H 7.60 can be interpreted as a measure of the tendency of 5-hydroxymethylfurfural to undergo polymerization in alkaline solution. The slightly lower values obtained after seventeen hours of standing at *p*H 7.60 indicate that the rate of decomposition in neutral solution is slow.

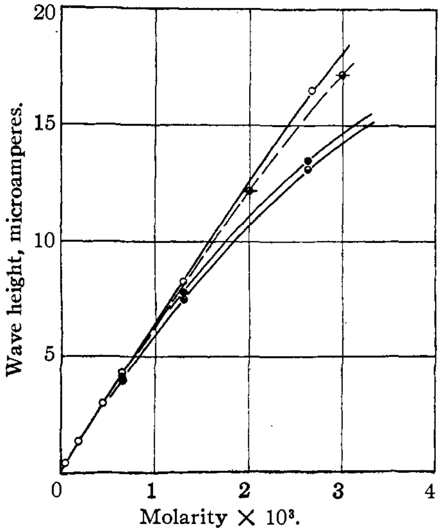


Fig. 4.—Determination of 5-hydroxymethylfurfural: ○, *p*H 6.70; ●, *p*H 7.60; ⊖, *p*H 7.60 after 17 hours; -⊖-, *p*H 7.00—interpolated curve.

The Relation of Reducible Form to Total Glucose Concentration.—In Fig. 5 are shown the glucose data represented as molarity of reducible form *vs.* total sugar molarity for three *p*H values. At *p*H 6.5 and 7.0 the amount of reducible form is found to be a linear function of the sugar concentration but at higher *p*H values intermolecular forces act to decrease the stability of the pyranose ring and increase the amount of *aldehydo* form. Similar behavior was encountered with all the sugars investigated.

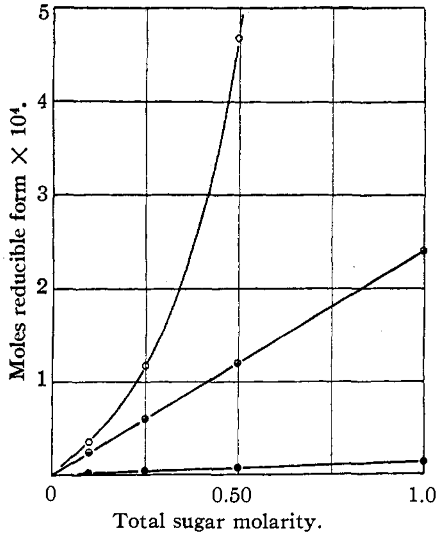


Fig. 5.—Relation of reducible form to total sugar concentration: *D*-glucose, ○, *p*H 7.60; ⊖, *p*H 7.00; ●, *p*H 6.50.

The Variation of Reducible Form of Glucose with Temperature.—The equilibrium constant for the reaction pyranose form $\rightleftarrows$ *aldehydo* form is plotted on a logarithmic scale against the reciprocal of the absolute temperature in Fig. 6. The curves represent the equations given and the points are those experimentally determined for 0.25 and 0.50 *M* glucose at *p*H 6.95. Complica-

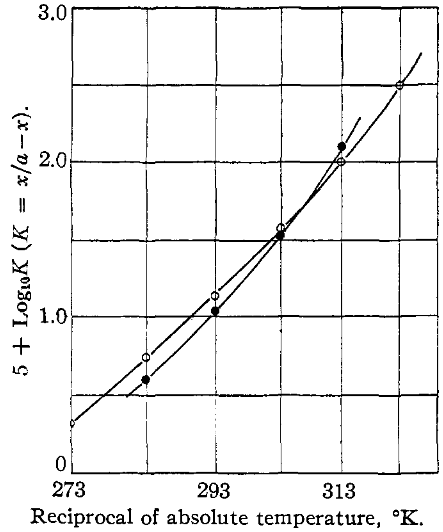


Fig. 6.—Variation of reducible form with temperature, pH 6.95: ○, 0.25 M glucose, $\log_{10}K = (3882/T) + 0.08622T - 42.383$; ●, 0.50 M glucose, $\log_{10}K = (11042/T) + 0.1748T - 92.865$.

tion of the equilibrium at higher concentration is shown by the greater deviation from the linear of the 0.5 M curve. It should be pointed out that a truly linear relationship between $\log_{10} K$ and $1/T$ could hardly be expected in these data for several reasons: (1) The equilibrium equation pyranose form $\rightleftarrows$ *aldehydo* form is undoubtedly an over-simplification of the real situation. This is also established by mutarotation measurements.[2] (2) In the standardization of the amount of reducible form, variations in the diffusion coefficient have been neglected. (3) The van't Hoff relation for the variation in the equilibrium constant with temperature could not be expected to yield a simple integral for the equilibrium involved in sugar solutions since there is no reason to assume that ΔH is independent of temperature.

Although the relation between amount of reducible form and temperature is found to be not a simple one, it is believed that the complexities of the glucose equilibrium receive further emphasis from the data obtained.

The fact that analyses which closely agree with the determined curves may be obtained on the same solution, varying the temperature in either direction, is substantial proof that a true and very mobile equilibrium is involved and that an impurity is not the cause of the observed waves. This is further substantiated by the very large variation in amount of the reducible form with temperature as opposed to the slight variation noted for the thallium wave. It is of interest to note that the rate of mutarotation for glucose is also strongly dependent on temperature. As determined by Isbell and Pigman,[2] the value of $K_1 + K_2$ increases 8.5 fold between 0 and 20°.

The Variation in Reducible Form with pH and Concentration for Other Aldoses.—A summary of the data obtained for mannose solutions is given in Fig. 7. While 0.25 molar glucose solutions showed a linear relationship between pH and amount of reducible form, it may be seen that mannose at 0.25 molar showed marked deviation. This would indicate that the decrease in ring stability caused by high concentration is more pronounced with mannose and becomes apparent at lower values.

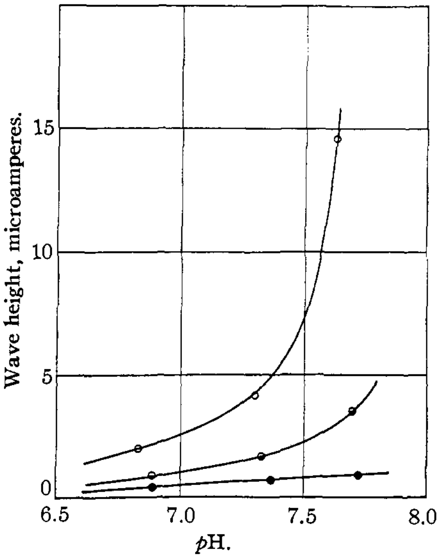


Fig. 7.—D-Mannose, variation of reducible form with pH and concentration: ○, 0.50 M; ◒, 0.25 M; ●, 0.10 M.

The results for galactose reduction (Fig. 8) show that for this sugar even the 0.10 M curve is not linear with pH as found for 0.25 M glucose and 0.10 M mannose. Apparently the stability of the pyranose ring is decreased by the galactose configuration and the concentration and pH effects already noted are of greater influence than with glucose and mannose.

In the pentose series much higher proportions of the reducible form are encountered. Apparently the terminal CH_2OH group in the hexoses exerts a stabilizing influence on the pyranose ring. Comparative data for the pentose reductions are collected in Fig. 9. It will be noted

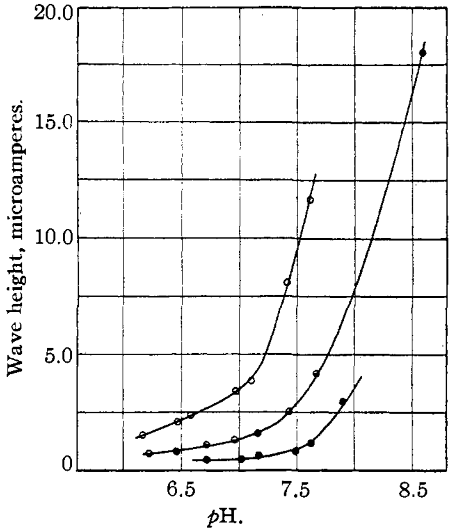


Fig. 8.—*D*-Galactose, variation of reducible form with *p*H and concentration: ○, 0.50 *M*; ⊖, 0.25 *M*; ●, 0.10 *M*.

that none of the pentose sugars shows linear increase in reducible form with *p*H at 0.25 *M*. This also is true at 0.10 *M* although the deviations are not as marked. In this figure a tentative curve is shown for ribose at 0.10 *M* concentration.

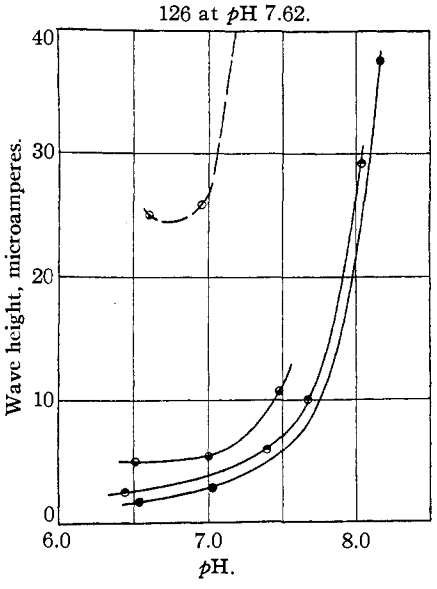


Fig. 9.—Pentoses, variation of reducible form with *p*H in 0.25 *M* solution (ribose at 0.10 *M*): ○, *D*-ribose; ⊖, *D*-lyxose; ⊖, *L*-arabinose; ●, *D*-xylose.

The behavior of ribose as well as that of its configurational homolog in the hexose series, allose, is strikingly different from that of the other sugars investigated. Data for these two sugars are shown together in Fig. 10. Not only do these sugars show many times the amount of reducible form encountered with other hexoses or pentoses but also they show minima in the amounts of the reducible form at points very near the neutral region. It is possible and, indeed, to be expected from mutarotation evidence, that this increase in amount of reducible form in the acid region is the general behavior of all sugars. Possibly the minima in the curves for other aldoses are so flat over an extended range in the neutral region as to be indeterminable under the conditions of this investigation.

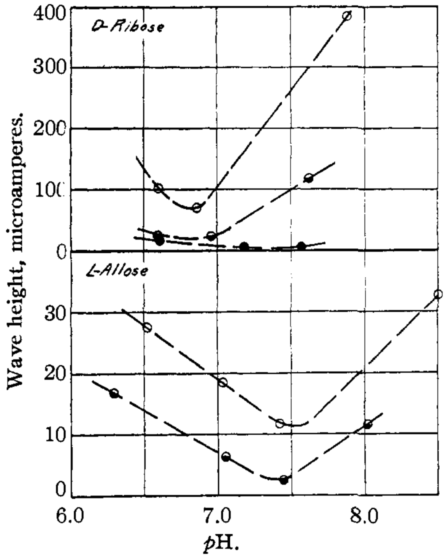


Fig. 10.—*D*-Ribose (above) and *L*-allose (below), variation of reducible form with *p*H and concentration: ○, 0.25 *M*; ⊖, 0.10 *M*; ●, 0.02 *M*.

The surprising difference in amount of reducible form encountered between allose and ribose and other aldoses is apparently a result of the configuration of hydroxyl groups in these sugars.

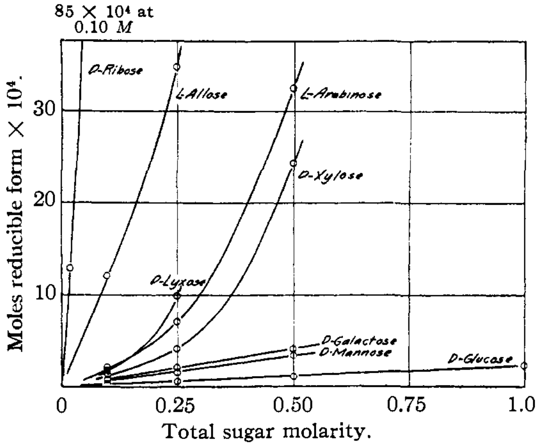


Fig. 11.—Relation between total sugar concentration and amount of reducible form at *p*H 7.00 and 25°.

TABLE I

VARIATION OF REDUCIBLE FORM WITH *p*H AND CONCENTRATION AT 25°

	Percentage reducible form[a]								
	0.10 *M*			0.25 *M*			0.50 *M*		
Sugar	*p*H 6.5	7.0	7.5	*p*H 6.5	7.0	7.5	*p*H 6.5	7.0	7.5
D-Ribose	(10)	(8.5)	(30)						
D-Lyxose	0.15	0.18	0.36	0.31	0.40	(0.72)		(2.5)	
L-Arabinose	.13	.22	.40	.16	.28	.46	0.33	(1.25)	
D-Xylose	.10	.13	.36	.10	.17	.37	.26	0.48	
L-Allose	(2.28)	(1.10)	.51	(2.56)	(1.38)	(.73)		(1.6)	
D-Galactose	0.070	0.085	.14	0.062	0.082	.18	.066	0.082	0.30
D-Mannose	.040	.062	.11	.039	.064	.14	.043	.066	.26
D-Glucose	.012	.022	.040	.016	.024	.044	.016	.024	.072

[a] $\frac{\text{Moles Reducible Form}}{\text{Moles Total Sugar}} \times 100$

It seems likely that ribose is physiologically significant due to this difference.

Variation in amount of reducible form with concentration at *p*H 7.0 is shown for all of the sugars investigated in Fig. 11. The very extensive difference between the behavior of the hexoses and pentoses is apparent. The similar behavior of allose and ribose and their difference from the other aldoses may also be seen. Table I lists the actual values at *p*H 6.5, 7.0 and 7.5. It may be seen that values for the hexoses (exclusive of allose) are all below 0.3%, the limit for spectroscopic detection. The higher values obtained for allose and ribose are correct only with regard to order of magnitude since they are so far removed from the normal values that the validity of the standardization is doubtful.

The Variation in Half-Wave Potentials with *p*H and Concentration.—The variations observed in the half-wave potentials for the reduction of the various aldoses are listed in Table II. Error due to *IR* drop across the cell has not been considered since for the highest waves measured this quantity amounts to only about 0.5 millivolt. It will be noted that half wave potentials for all sugars investigated fall within a narrow range (−1.50 to −1.80 volts) and that there is a pronounced shift in the half-wave potentials with both *p*H and concentration. The displacement appears to be roughly proportional to the height of the observed wave.

Comparison of Amounts of Reducible Form with Lippich's Data for Hydrocyanic Acid Additions.—In Table III values for the per cent. reducible form for the various aldoses in 0.1 *M* solution at *p*H 7.0 are compared with values for the per cent. of *aldehydo* form calculated from the data for "instantaneous" combination with hydrocyanic acid obtained by Lippich.[5] These data were calculated from the amount of hydrocyanic acid which combined with a 5-g. sample of sugar in 0.23 *M* solution, 0.04 *M* in potassium cyanide at 19°, in two seconds reaction time. The figure for mannose is not strictly comparable with those for glucose and galactose since it was determined on a slightly different basis. It may be seen that there is close agreement between the two estimations although from present considerations Lippich's values should be somewhat higher.

The Relation of Reducible Form to Mutarotation Velocity.—The various pentoses and hexoses are arranged in order of decreasing amount of re-

TABLE II

VARIATION IN HALF WAVE POTENTIALS[a] WITH *p*H AND CONCENTRATION

	0.10 *M*			0.25 *M*			0.50 *M*		
Sugar	*p*H 6.5	7.0	7.5	*p*H 6.5	7.0	7.5	*p*H 6.5	7.0	7.5
D-Glucose	−1.58 v.	−1.58	−1.58	−1.58	−1.59	−1.60	−1.68	−1.60	−1.62
D-Galactose		1.59	1.63	1.57	1.60	1.65	1.59	1.62	1.71
D-Mannose		1.55	1.60		1.57	1.63		1.59	1.66
L-Allose	1.67	1.71	1.75	1.71	1.79				
D-Lyxose	1.53	1.55	1.59	1.57	1.60	1.63	1.52[b]	1.54[b]	1.57[b]
D-Xylose	1.50	1.54	1.59	1.50	1.54	1.59	1.56	1.61	1.67
L-Arabinose	1.54	1.58	1.62	1.58	1.61	1.64	1.58	1.64	1.72
D-Ribose	1.79	1.81	1.87	1.79	1.81	1.83			

[a] Referred to the normal calomel electrode. [b] 0.05 molar.

TABLE III

COMPARISON OF FREE ALDEHYDE ESTIMATIONS

Sugar	Cantor and Peniston (0.25 M, pH 7.0 25°), %	Lippich 0.23 M (0.04 N KCN 19°), %
D-Glucose	0.024	0.035
D-Mannose	.064	.062
D-Galactose	.082	.126
L-Allose	(1.38)	
D-Xylose	0.17	
L-Arabinose	.28	
D-Lyxose	.40	
D-Ribose	(8.5) (0.1 M)	

ducible form relative to glucose at 0.25 M and pH 7.0 in Table IV. Values of mutarotation velocity relative to glucose are calculated from the values for $k_1 + k_2$ as tabulated by Hudson[2] for mutarotation of sugars in water at 20°. It is evident that there is substantial agreement between the order of these two phenomena. (The value for the mutarotation velocity of galactose is questionable since Lowry[2] has shown that galactose mutarotation is not representable by a first order mechanism). It seems reasonable to conclude from this agreement that the *aldehydo* form is an intermediate in mutarotation and that its concentration is an important controlling factor.

TABLE IV

RELATION OF REDUCIBLE FORM TO MUTAROTATION VELOCITY

Sugar	Relative free aldehyde at 0.25 M and pH 7.0 (25°)	Relative mutarotation velocity
D-Ribose	354	Anomalous and rapid
D-Lyxose	16.7	10.0
L-Arabinose	11.7	4.8
D-Xylose	7.1	3.2
L-Allose	57.7	Anomalous and rapid
D-Galactose	3.4	(1.6)
D-Mannose	2.7	1.7
D-Glucose	1.0	1.0

The Relation of Reducible Form to Configuration and Structure.—Variations in the amount of reducible form existing in equilibrated sugar solutions apparently arise from such factors as the nature of substituent groups and their relative configurations influencing the stability of the pyranose ring. It has already been shown that the amount of reducible form for hexose sugars is much less than for the configurationally corresponding pentoses and this difference has been attributed to the stabilizing influence of the terminal CH_2OH group. Table V presents the hexose and pentose sugars investigated arranged in order of increasing amount of reducible form. The formation of parallel series with regard to the configurations on the first three asymmetric carbon atoms is clearly evident.

These data offer additional evidence for the relationship between structure and reactivity in the sugar series and it is believed that the polarographic method is a new and valuable tool which should aid in the further clarification of this important problem. It is hoped to continue this investigation by extending the range of variables here considered and by studying other configurational types of sugars and their derivatives.

Experimental Section

Description of Sugar Samples Investigated

***D*-Glucose,** anhydrous, prepared by twice recrystallizing commercial glucose from 70% alcohol.

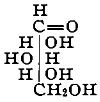
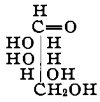
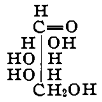
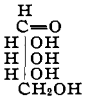
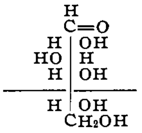
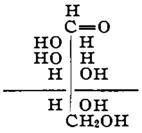
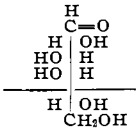
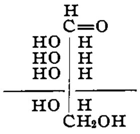
***D*-Mannose,** anhydrous, Pfanstiehl.

***D*-Galactose,** anhydrous, Pfanstiehl; recrystallized from 90% ethanol-water.

***L*-Allose,** anhydrous; recrystallized from 95% ethanol-

TABLE V

RELATION OF REDUCIBLE FORM TO STRUCTURE

				
	D-Xylose	*D*-Lyxose	*L*-Arabinose	*D*-Ribose
% free aldehyde pH 7.0 (0.1 M)	0.13	0.18	0.22	(8.5)
Half-wave potential at pH 7.0 (0.1 M)	−1.54	−1.54	−1.58	−1.81
				
	D-Glucose	*D*-Mannose	*D*-Galactose	*L*-Allose
% free aldehyde pH 7.0 (0.1 M)	0.022	0.062	0.085	(1.10)
Half-wave potential pH 7.0 (0.1 M)	−1.58	−1.55	−1.59	−1.71

water. This sample was kindly presented by Dr. F. L. Humoller of Loyola University, Chicago.

D-**Xylose,** anhydrous, Pfanstiehl.

L-**Arabinose,** anhydrous, Pfanstiehl; recrystallized from 80% ethanol-water.

D-**Lyxose,** anhydrous, Eastman Kodak Co.

D-**Ribose,** anhydrous, Pfanstiehl.

5-Hydroxymethylfurfural used for standardization of wave heights was prepared according to the method of Middendorp[10] and was twice distilled at 0.1 mm. immediately before use, m. p. 30° (uncorr.).

Composition of Solutions for Polarographic Analysis.—Samples of sugars for analysis were prepared by diluting aliquot portions of normal or half normal solutions. Investigations in all cases were conducted within ten hours of preparation. Solutions were buffered with mixtures of 0.1 M potassium dihydrogen phosphate and 0.1 M potassium hydroxide. The buffer concentration for all determinations was 0.02 M in potassium. Lithium chloride, 0.1 N, was used as the "indifferent electrolyte." Thallous chloride was used as a reference for half-wave potentials. Concentrations of thallous ion ranged from 5×10^{-5} to 1×10^{-3} molar, depending on the suitable galvanometer sensitivity for the reduction wave.

Polarographic Procedure.—Determinations were conducted using a Heyrovsky Model 10 Micropolarograph (Sargent) equipped with a photographic recorder and an Ayrton shunt. By means of the Ayrton shunt in the circuit it was possible to vary the ratio of galvanometer current to total cell current from 1:1 to 1:10,000, thus bringing a wide range of concentration of reducible material into the scope of the instrument. For comparative purposes all waves measured in this work have been reduced to wave heights at a sensitivity of 1:20. The galvanometer deflection was calibrated at various ratios of galvanometer current to cell current by means of a standard resistance (10,150 ohms). One millimeter deflection at a sensitivity of 1:20 was equal to 1.09×10^{-7} ampere of cell current. The constants for the capillary throughout the investigation were: $m = 0.00227$ g. of mercury per second and $t = 3.07$ average seconds per drop. All polarograms were obtained using a voltage increment of 0.5 volt per minute. *p*H values listed were determined with the glass electrode.

Calculation of the Polarogram Data.—Half wave potentials reported are all referred to the normal calomel electrode. They were calculated from the known constant value for thallous ion of -0.48 volt and the value of 0.1815 volt per centimeter experimentally determined for the particular instrument by determining the distance between half-wave points for thallium and barium ($E_{1/2} = -1.94$ v.). The Heyrovsky method of tangents was used for the measurement of half wave potentials and wave heights on the polarograms. The accuracy of measurement was within ± 0.5 millimeter $= \pm 0.01$ volt or $\pm 5.5 \times 10^{-8}$ ampere at a sensitivity of 1:20.

Summary

1. It has been shown that, contrary to earlier reports, aldoses are reducible at the dropping mercury cathode.

2. The reduction of aldoses has been attributed to the presence of an *aldehydo* form in the equilibrated solutions and the amount of this form has been estimated under various conditions of *p*H and concentration for 4 hexose and 4 pentose sugars.

3. The equilibrium in sugar solutions has been shown to be extremely mobile and to be displaced readily by changes in *p*H, concentration and temperature.

4. The effect of variation in temperature on the glucose equilibrium has been studied and interpreted in terms of the probable nature of the components involved.

5. The amounts of reducible form of the aldose sugars have been correlated with the rates of mutarotation and the role of an *aldehydo* form as an intermediate in this transformation has been indicated.

6. The amount of reducible form has been shown to have a direct relation to configuration and structure in the sugar series.

ARGO, ILLINOIS

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[10] Middendorp, *Rec. trav. chim.*, **38**, 1 (1919).