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PYRANOSE-FURANOSE INTERCONVERSIONS WITH REFERENCE TO THE MUTAROTATIONS OF GALACTOSE, LEVULOSE, LACTULOSE, AND TURANOSE[1]

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ABSTRACT

Mutarotation measurements at 20 and 0° C are reported for lactulose and turanose in buffered solutions at pH 4.6 and for galactose, glucose, and levulose at numerous points between pH 1 and 7. The mutarotation of levulose and the rapid mutarotation of galactose are extremely sensitive to the catalytic action of acids and bases and vary with pH in like manner. The mutarotation reactions of levulose, lactulose, and turanose differ fundamentally from the alpha-beta pyranose interconversions and resemble in all respects the rapid mutarotation reactions of sugars which contain the galactose, talose, and idose structures. Since the mutarotation of levulose is like that of the fructofuranose set free from sucrose by the action of invertase, but in the opposite direction, the mutarotation of levulose must consist in a pyranose-furanose interconversion. The conclusion that the mutarotation of levulose results from a pyranose-furanose interconversion leads to the following important deductions: (1) That the mutarotation of lactulose is caused by a furanose-pyranose change and that crystalline lactulose is a furanose; (2) that the mutarotation of Hibbert's 1,3,4-trimethyl-fructofuranose results from a furanose-pyranose change and that the sugar is properly classified as a furanose; (3) that the mutarotation of turanose is due to a pyranose-furanose change and therefore the glucosido group is not united with the fifth or sixth carbon, but in all probability it is united with the third carbon; (4) that the rapid reactions characteristic of the complex mutarotations of galactose and other sugars are pyranose-furanose interconversions and that the complex mutarotations arise from the establishment of an equilibrium between the furanose and the normal alpha and beta pyranoses.

CONTENTS

[1] This paper was read before the Division of Sugar Chemistry and Technology of the American Chemical Society at Dallas, Tex., April 1938.

I. THE SUGARS IN SOLUTION

The determination of the composition of sugar solutions is a problem of great complexity because the sugars in solution readily change from one modification to another. By direct acetylation under different conditions, no less than five isomeric penta-acetates can be obtained for most sugars and by treatment with methyl alcohol containing hydrogen chloride, methyl pyranosides, and methyl furanosides are obtained. For these and other reasons, it seems probable that an equilibrium is established between the open chain and the alpha and beta pyranose and furanose modifications. But undoubtedly the system is more complex and various hydrated, enolic, and ionized forms take part in the equilibrium. The course of the mutarotation reactions, and the solubilities, rates of oxidation, and other properties of the sugars clearly show that the proportions of certain modifications are so small that they cannot be detected by direct methods. Extensive investigations [1, 2, 3] [2] have shown that sugars containing the glucose, mannose, and gulose structures exist in solution almost completely as the normal alpha and beta isomers. Furthermore, the correlation of the optical rotations of the alpha and beta methyl-pyranosides with the alpha and beta normal sugars [4, 5, 6], together with the observation that the alpha and beta normal sugars are oxidized directly to delta lactones [7, 8], shows that the normal sugars are pyranoses and consequently the equilibrium solutions of glucose and similar sugars consist for the most part of the alpha and beta pyranoses. Hence the mutarotation reaction of α- and β-*d*-glucose principally results from the interconversion of the alpha and beta pyranoses [1, 2]. The mutarotation of sugars containing the galactose, talose, and idose structures, on the other hand, consists in a slow reaction, the interconversion of the alpha and beta pyranoses, accompanied by a rapid reaction, the conversion of the normal pyranoses to labile modifications and the interconversion of these labile modifications. Although the latter reaction is more complex, for convenience it can be considered as a single rapid reaction and the equilibrium can be treated as a three-membered system. The mutarotations of α- and β-*d*-glucose [2], α- and β-*d*-mannose [2, 9], β-*d*-allose [10], α-*d*-gulose $CaCl_2.H_2O$ [2, 11], α-*d*-xylose [2, 12], α- and β-*d*-lyxose [2], α-*d*-α-galaheptose [3], β-*d*-α-glucoheptose [3], β-*d*-β-galaheptose [3, 15], α- and β-lactose [2], β-maltose [2], and α- and β-*d*-4-glucosidomannose [16], follow, within experimental error, the equation for a first-order reversible reaction. On the other hand, the mutarotations of α- and β-*d*-galactose [2, 17, 18, 19], α- and β-*d*-talose [2, 20], α-*l*-arabinose [2, 21], β-*l*-arabinose $CaCl_2.4H_2O$ [2, 22], *l*-ribose [2, 23], mannose $CaCl_2.4H_2O$ [2, 24], α- and β-*d*-α-mannoheptose [3], α-*d*-β-guloheptose [3], *d*-β-glucoheptose [25], α-*d*-β-mannoheptose [26], and α-*d*-α-guloheptose [26, 27] are complex and for expression require an equation containing two exponential terms.

The labile constituents in the solutions of galactose and most aldoses are present in such small quantities that, although since the time of Pasteur many mutarotation measurements have been made, the existence of the rapid reaction was not definitely recognized until 1926 [17]. Hydration and the formation of ions occurs very rapidly and can be excluded as possible explanations for the rapid mutarotation

[2] Figures in brackets here and elsewhere in the text correspond to the numbered references at the end of this paper.

reactions. Although enolization occurs in alkaline solutions, it does not take place to an appreciable extent in acid solutions, and hence it cannot be used to explain a reaction which occurs to approximately like degree over a wide pH range. The absorption spectra, reactions, and properties of the sugars in solution demonstrate that the open-chain modifications are present in minute quantities only. Obviously these cannot be the labile substances responsible for the rapid mutarotations. This reasoning by elimination leaves the furanoses to account for the rapid mutarotation reactions. Despite the fact that it has been tacitly assumed that the constituents responsible for the rapid mutarotation are either the free aldehyde or furanose modifications, there has been no proof previous to this paper that the labile substances are either of these.

Several mechanisms for the rapid reactions may be postulated and subjected to mathematical analysis, but this procedure is of questionable value until more information is at hand regarding the substances which take part in the reactions. The rapid reactions may be caused by the interconversion of the alpha and beta furanoses or by a shift in the oxygen ring. In support of the latter hypothesis, it will be recalled that when mannose $CaCl_2.4H_2O$ is dissolved in water a rapid reaction occurs at a rate comparable to the rapid reactions of galactose, talose, and similar sugars and that the freshly dissolved sugar on bromine oxidation [28] yields largely mannonic γ-lactone with only a small quantity of mannonic δ-lactone, but that as the rapid mutarotation reaction proceeds the yield of γ-lactone decreases and the yield of δ-lactone increases. This is evidence that the rapid mutarotation reaction of mannose $CaCl_2.4H_2O$ consists in the change of the furanose to the pyranose modification.

It has been demonstrated by Hudson, Lowry, and others [29] that the mutarotations of sugars which establish equilibrium between two modifications follow the equation for a first-order reversible reaction. But, as shown by Riiber and Minsaas [17], Smith and Lowry [18], and others, the mutarotation of the sugars which establish equilibrium with substantial proportions of three or more modifications requires for expression a complex equation of the type

$$[\alpha]_D = A\ 10^{-m_1 t} + B\ 10^{-m_2 t} + \ldots\ldots + C \qquad (1)$$

The experimental observation that the mutarotations of certain sugars can be expressed by one exponential term, such as might be anticipated for two substances in dynamic equilibrium, while the mutarotations of other sugars require for expression two exponential terms, such as might be anticipated for three substances in dynamic equilibrium, does not necessitate that the mutarotations in the two groups be entirely different. Probably, in the first group, the changes caused by the formation of the labile isomers are much less than the errors of observation. Nor does the application of the equation containing two exponential terms signify that the system contains only three isomers but rather that the proportions of other isomers are small or that the velocity constants are not favorable for the detection of the changes. The mutarotation constant for the slow reaction is designated as m_1, and the constant for the rapid reaction is designated as m_2. The constant, m_1, is essentially the same as the "mutarotation coefficient", k_1+k_2, but m_2 is more or less empirical and represents the rapid reactions that cause the deviations in the mutarota-

tions of many sugars. It is not a true velocity constant but rather a function of the constants representing the rates of formation and alteration of the more labile modifications of the sugar in solution. Comparisons of the two reactions, represented by m_1 and m_2, under various conditions have shown that each reaction has characteristic properties which can be used to determine to which class it belongs. The nature of these distinctive properties will be developed in the following sections.

II. COMPARISONS OF THE MUTAROTATIONS OF LEVULOSE AND GALACTOSE

The relative extent of the two mutarotation reactions differs widely for the various sugars, but shows marked parallelism for sugars of similar structure. Sugars containing the galactose structure give complex mutarotations which reveal the existence of a characteristic rapid reaction. Fischer's projectional formula reveals that the pyranose modification of *d*-fructose [3] or levulose could be structurally related to either *α*-*l*-galactose or to *β*-*d*-allose. Since the optical rotation of levulose is in accord with the *α*-*l*-galactose structure [3, page 513], it is of interest to ascertain whether the mutarotation of levulose is like that of *α*-*l*-galactose. Although the mutarotation of levulose had been investigated extensively [31, 32, 33] we made new measurements to be sure that small deviations had not been overlooked and to obtain comparable data for levulose, galactose, and glucose. The results of these measurements are given in table 11, page 793. Much to our surprise the mutarotation of levulose, even at low temperatures, fails to give any evidence of the complex changes characteristic of the mutarotation of *α*-*d*-galactose. But, as will be shown in the next paragraph, the mutarotation of levulose is remarkably similar to the rapid mutarotation reaction of galactose and differs fundamentally from the alpha-beta pyranose interconversion.

In previous publications we have shown that the equilibrium proportions of the constituents involved in the rapid mutarotation reactions vary with temperature changes [30], whereas the equilibrium proportions of the alpha and beta pyranoses do not vary widely with temperature. It has been known for a long time that the optical rotation of levulose varies with temperature and that when the temperature of a levulose solution is changed a mutarotation occurs. As shown by Hudson's measurements, and confirmed by us, when a solution of levulose is cooled the resulting thermal mutarotation occurs at the same rate as the mutarotation of the freshly dissolved sugar (see table 9, page 791). The direction of the change shows that lowering the temperature shifts the equilibrium towards the more levorotatory modification, namely, the form known in the crystalline state. According to the Le Chatelier-Braun principle the direction of the shift caused by a lowering of the temperature indicates that heat is liberated by the conversion of the less levorotatory modification to the more levorotatory substance (levulose). This is in agreement with the energy measurements of Riiber and Esp [33] who showed that in the reverse reaction (the mutarotation of freshly dissolved levulose) heat is absorbed. These mutarotations and energy changes are similar to those found for galactose. As we noted in a previous publication

[3] The modification of *d*-fructose, which gives an initial specific rotation of −133°, will be called levulose in this paper—it is called *β*-*d*-fructose by Hudson and *α*-*d*-fructose by Isbell.

[2, page 163] when a solution of *d*-galactose is cooled the optical rotation increases rapidly to a maximum and then decreases slightly. The initial increase in optical rotation shows that the equilibrium is shifted from the labile modifications towards the alpha pyranose. Such being the case, the direction of the equilibrium shift is the same for galactose as for levulose, and the energy content of α-*d*-galactopyranose must be less than that of its labile modification. This is in accord with the work of Riiber and Minsaas [17] who reported that the temperature of a freshly prepared solution of α-*d*-galactose falls at first, passes a minimum, and then rises. The initial decrease in temperature which accompanies the rapid mutarotation reaction is evidence of the absorption of heat and corresponds to the decrease in temperature which accompanies the mutarotation of levulose.

The correlation of the rapid mutarotation of α-*d*-galactose and the mutarotation of levulose receives additional confirmation by the changes in volume and refractivity which occur during the respective mutarotation reactions. It was shown by Riiber and Minsaas [17] that a freshly prepared solution of α-*d*-galactose expands during the first 15 minutes at 20° C and then contracts. Since the expansion occurs at approximately the same rate as the rapid mutarotation, in all probability it is caused by the conversion of α-*d*-galactose to the labile modifications. A freshly prepared solution of levulose also expands during the first 15 minutes at 20° C. As shown by Riiber and Esp [33] the rate of expansion agrees with the rate of mutarotation and is clearly due to the same reaction. The striking similarity between the rapid reaction of galactose and the mutarotation reaction of levulose is illustrated further by a comparison of Riiber and Esp's measurements of molecular refraction for freshly prepared levulose solutions with those made by Riiber and Sørensen [36] for galactose. Riiber and Sørensen report that when α-*d*-galactose is dissolved in water the molecular refraction increases with the rapid mutarotation reaction, while Riiber and Esp report that when levulose is dissolved in water the molecular refraction increases at a rate comparable to that of the mutarotation reaction. The comparisons which have been cited bring out the marked similarity of the mutarotation of levulose and the rapid mutarotation of α-*d*-galactose; in the next section it will be shown that the mutarotation of levulose is like the other rapid mutarotation reactions and differs from the normal alpha-beta pyranose interconversion.

III. CLASSIFICATION OF THE MUTAROTATION REACTIONS

The rapid mutarotation reactions have certain characteristics which distinguish them from the normal alpha-beta pyranose interconversions. One of these, the effect of temperature on the equilibrium, has been considered already and found to support the classification of the mutarotation of levulose with the rapid reactions. The effect of temperature on the mutarotation rates, m_1 and m_2, also affords a means of classification. Previous measurements in this laboratory with many sugars have shown that the heats of activation obtained from the temperature coefficients for the rapid reactions involving the labile modifications are less than the heats of activation for the alpha-beta pyranose interconversions. In order to determine whether the heat of activation for the mutarotation of levulose agrees with the heat of activation for the rapid or for the slow reaction, mutarotation

measurements were made at 20 and 0° C under the usual conditions in ordinary distilled water. Check determinations gave velocity constants which differed by more than what appeared to be a reasonable experimental error. Further investigation revealed that the discrepancy was caused by variations in the carbon dioxide content of the water used for dissolving the sugar. This difficulty was overcome by making the measurements in the presence of an acid-base buffer. The results so obtained are reported in table 1.

TABLE 1.—*Effect of temperature on rate of mutarotation*

Sugar	t_1	t_2	Slow change			Rapid change			pH
			$m_1 \times 10^3$ at t_1	$m_1 \times 10^3$ at t_2	Q [a]	$m_2 \times 10^3$ at t_1	$m_2 \times 10^3$ at t_2	Q	
	°C	°C							
α-d-Glucose	20.05	0.10	6.48	0.766	17,000				4.6
α-d-Galactose	20.05	[b].15−(.30)	8.26	.969	17,300	73.6	11.9	14,600	4.6
β-d-Galactose	20.50	.20	8.51	.905	17,600	78.5	12.1	14,700	4.6
Levulose	20.05	.00				59.2	9.03	14,900	4.6
Turanose	20.70	.20				86.1	13.6	14,400	4.6
Lactulose	20.00	.10				43.5	6.04	15,800	4.4

[a] $2.3026 \log \frac{k_1}{k_2} = \frac{Q}{1.9864}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$.

[b] The temperature during the rapid reaction was +0.15°, but during the slow reaction it rose to +0.30° C.

The heats of activation obtained for the mutarotations of levulose (14,900), turanose (14,400), and lactulose (15,800) are in agreement with the heat of activation for the rapid mutarotation reaction of galactose (14,600). These values are in accord with the values of 12,000 to 15,000 previously found for the rapid mutarotation reactions of other sugars and differ from the values 16,000 to 18,000 previously found for the alpha and beta pyranose interconversions.

For a long time it has been known that the rate of the mutarotation of levulose is particularly sensitive to small changes in acidity [31, 32]. To ascertain whether the rapid mutarotation reaction of galactose is likewise particularly sensitive to acid and basic catalysts we conducted a series of measurements on galactose, levulose, and glucose under strictly comparable conditions. The results of these measurements and other work has shown that the rapid mutarotations in general are much more sensitive to acid and basic catalysts than the normal alpha-beta pyranose interconversions. The results obtained by measurements in solutions of different acidity are summarized in tables 2 and 3, while the curves given in figures 1 and 2 illustrate graphically the marked difference in the effect of the catalysts on the two reactions. Notice in figures 1 and 2 that the normal mutarotations of galactose and glucose remain nearly constant from pH 3 to pH 6, while the rates for the mutarotation of levulose and the rapid mutarotation of galactose rise much more rapidly on either side of pH 4. The effect of hydrogen and hydroxyl ions on the velocity constants for glucose at 25° C was found by Hudson [37] to follow an equation of the following type

$$m = A + B[H^+] + C[OH^-] \qquad (2)$$

TABLE 2.—*Effect of acids and bases on the mutarotation constants at 20° C.*

Buffer used	m	pH	$[H^+]$	$[OH^-]$ [a]	Equation calculated by assumption that:
			α-d-Glucose. $m=0.0060+0.18\,[H^+]+16{,}000\,[OH^-]$		
0.001 *M* succinic acid	0.0060	3.70	2.00×10^{-4}	3.47×10^{-11}	$m=A$
o-Nitrophenol buffer 2	.0069	6.91	1.23×10^{-7}	5.62×10^{-8}	$m=A+C[OH^-]$
0.01 *N* HCl	.0079	1.98	1.05×10^{-2}	6.61×10^{-13}	$m=A+B[H^+]$
			α-d-Galactose (slow reaction). $m=0.0081+0.35\,[H^+]+17{,}800\,[OH^-]$		
0.001 *M* KH phthalate	0.0081	4.41	3.89×10^{-5}	1.78×10^{-10}	$m=A$
o-Nitrophenol buffer 2	.0091	6.91	1.23×10^{-7}	5.62×10^{-8}	$m=A+C[OH^-]$
0.01 *N* HCl	.0117	1.99	1.02×10^{-2}	6.76×10^{-13}	$m=A+B[H^+]$
			α-d-Galactose (fast reaction). $m=0.069+9.7\,[H^+]+1{,}370{,}000\,[OH^-]$		
0.001 *M* KH phthalate	0.069	4.41	3.89×10^{-5}	1.78×10^{-10}	$m=A$
o-Nitrophenol buffer 2	.146	6.91	1.23×10^{-7}	5.62×10^{-8}	$m=A+C[OH^-]$
0.01 *N* HCl	.168	1.99	1.02×10^{-2}	6.76×10^{-13}	$m=A+B[H^+]$
			Levulose. $m=0.055+7.3\,[H^+]+1{,}570{,}000\,[OH^-]$		
0.001 *M* KH phthalate	0.055	4.42	3.80×10^{-5}	1.82×10^{-10}	$m=A$
o-Nitrophenol buffer 2	.145	6.92	1.20×10^{-7}	5.75×10^{-8}	$m=A+C[OH^-]$
0.01 *N* HCl	.133	1.97	1.07×10^{-2}	6.46×10^{-13}	$m=A+B[H^+]$

[a] Calculated from the pH measurement by use of the expression, $\log [OH^-]=-14.16+pH$. The value for the ion product of water at 20° C is taken as $10^{-14.16}$ (Int. Crit. Tables **6**, 152 (1929)).

TABLE 3.—*Summary of mutarotation constants obtained in buffered aqueous solutions of different acidity*

pH ±0.05	Solvent [1]	Temperature ±0.05° C	Velocity constant m	m [2] corrected to 20° C	Ratio [3]
		α-d-Glucose			
1.05	0.1 *N* HCl	20.00	0.0230	0.0230	3.57
1.98	0.01 *N* HCl	20.05	.00797	.00793	1.23
2.37	0.004 *N* HCl	20.20	.00705	.00691	1.07
3.03	0.001 *N* HCl	20.30	.00670	.00650	1.01
3.70	0.001 *M* succinic acid buffer 2	20.00	.00598	.00598	0.93
3.80	Approx. 0.1 *M* succinic acid buffer 1	20.00	.00765	.00765	1.19
4.60	KH phthalate buffer 2	20.05	.00648	.00645	1.00
5.57	KH phthalate buffer 3	20.25	.00688	.00671	1.04
5.57	KH phthalate buffer 3	20.20	.00680	.00666	1.03
6.13	KH phthalate buffer 4	20.00	.00679	.00679	1.05
6.45	KH phthalate buffer 5	20.05	.00692	.00689	1.07
6.43	*o*-Nitrophenol buffer 1	20.20	.00672	.00658	1.02
6.91	*o*-Nitrophenol buffer 2	20.25	.00709	.00692	1.07

[1] For the composition of the buffer solutions used, see table 8, page 790.

[2] The Arrhenius equation and the value of Q given in table 1 were used for converting the velocity constants to the standard temperature, 20° C.

[3] Ratio of velocity constant at the pH indicated to that at pH=4.6, the latter measured in 0.005 *N* potassium acid phthalate buffer solution 2.

TABLE 3.—*Summary of mutarotation constants obtained in buffered aqueous solutions of different acidity*—Continued

pH ±0.05	Solvent	Temperature ±0.05° C	Velocity constant m	m corrected to 20° C	Ratio
	Levulose				
1.04	0.1 *N* HCl	20.00	0.710	0.710	12.01
1.97	0.01 *N* HCl	20.05	.134	.133	2.25
2.37	0.004 *N* HCl	20.20	.0893	.0878	1.49
2.99	0.001 *N* HCl	20.00	.0627	.0627	1.06
4.04	KH phthalate buffer 1	20.70	.0602	.0564	0.95
4.42	0.001 *M* KH phthalate buffer 6	20.00	.0548	.0548	.93
4.61	KH phthalate buffer 2	20.35	.0612	.0593	1.00
4.57	KH phthalate buffer 2	20.05	.0592	.0589	1.00
5.54	KH phthalate buffer 3	20.25	.0880	.0861	1.46
5.64	KH phthalate buffer 3	20.20	.0866	.0851	1.44
6.11	KH phthalate buffer 4	20.05	.102	.102	1.73
6.45	KH phthalate buffer 5	20.05	.114	.114	1.93
6.92	*o*-Nitrophenol buffer 2	20.25	.148	.145	2.45
	α-*d*-Galactose (slow reaction)				
1.07	0.1 *N* HCl	20.00	0.0466	0.0466	5.67
1.99	0.01 *N* HCl	20.05	.0118	.0117	1.42
2.36	0.004 *N* HCl	20.20	.00963	.00944	1.15
3.04	0.001 *N* HCl	20.00	.00841	.00841	1.02
4.41	0.001 *M* KH phthalate buffer 6	20.05	.00817	.00813	0.99
4.55	KH phthalate buffer 2	20.05	.00826	.00822	1.00
5.57	KH phthalate buffer 3	20.20	.00849	.00832	1.01
5.7–6.2	$NaHCO_3$ buffer 1	20.00	.00819	.00819	1.00
6.09	KH phthalate buffer 4	20.00	.00851	.00851	1.04
6.45	KH phthalate buffer 5	20.00	.00874	.00874	1.06
6.91	*o*-Nitrophenol buffer 2	20.25	.00936	.00912	1.11
	β-*d*-Galactose (slow reaction)				
4.67	KH phthalate buffer 2	20.50	0.00851	0.00808	-------
	α-*d*-Galactose (rapid reaction)				
1.99	0.01 *N* HCl	20.05	0.169	0.168	2.29
2.36	0.004 *N* HCl	20.20	.130	.128	1.75
3.04	0.001 *N* HCl	20.00	.0846	.0846	1.15
4.55	KH phthalate buffer 2	20.05	.0736	.0733	1.00
5.57	KH phthalate buffer 3	20.20	.0886	.0871	1.19
5.7–6.2	$NaHCO_3$ buffer 1	20.00	.0951	.0951	1.30
6.09	KH phthalate buffer 4	20.05	.110	.110	1.50
6.45	KH phthalate buffer 5	20.05	.133	.132	1.80
6.91	*o*-Nitrophenol buffer 2	20.25	.149	.146	1.99
4.41	0.001 *M* KH phthalate buffer 6	20.05	.0697	.0694	0.95
	β-*d*-Galactose (rapid reaction)				
4.67	KH phthalate buffer 2	20.50	0.0785	0.0752	-------

Reduction of the results reported in table 2 to equations of like form, using the velocity constants in 0.01 *N* HCl for the determination of *B*, and the velocity constants in the presence of the *o*-nitrophenol buffer 2 for the determination of *C*, gives the following expressions:

Glucose at 20° C, $m_1=0.0060+.18\ [H^+]+16{,}000\ [OH^-]$
Galactose at 20° C, $m_1=0.0081+.35\ [H^+]+17{,}800\ [OH^-]$
Galactose at 20° C, $m_2=0.069+9.7\ [H^+]+1{,}370{,}000\ [OH^-]$
Levulose at 20° C, $m_2=0.055+7.3\ [H^+]+1{,}570{,}000\ [OH^-]$

Although these expressions are strictly comparable one to the other, they are arbitrary because they are based on measurements made in buffered solutions containing anions of weak acids. The work of Lowry and Smith [29], Kuhn and Jacob [38], Brönsted and Guggenheim [39], and others shows that the anions of weak acids and the molecules of undissociated strong acids have marked catalytic effects.

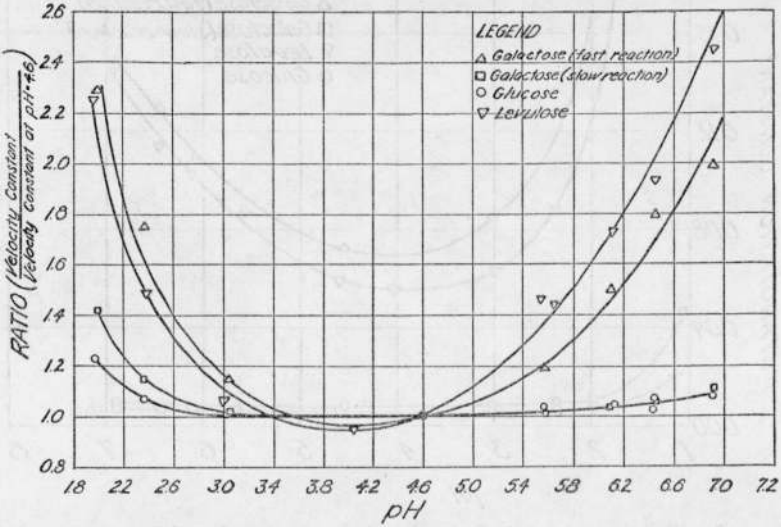


FIGURE 1.—*Variation of mutarotation constants with acidity.*

But on account of the difficulty of maintaining uniform conditions in unbuffered solutions, buffers were used by us even though their use complicates the problem by introducing another variable. A description of the buffers and a summary of the results are given on page 790 of this paper, while in figures 1 and 2 the results are represented in the form of curves. As clearly shown by the numerical equations and the curves of figures 1 and 2, the catalytic effects of the hydrogen and hydroxyl ions are much larger for the fast reactions than for the normal slow reactions. This difference is characteristic and serves to differentiate between the two reactions. The experimental results as expressed by the equations require minimum values at hydrogen-ion concentrations of 2.5×10^{-5}, 3.9×10^{-5}, 1.9×10^{-5}, and 3.2×10^{-5} for the mutarotation constants of glucose, levulose, and the slow and fast reactions of galactose. These correspond to the pH values of 4.61, 4.41, 4.73, and 4.50, respectively. The differences in the minimum points, however, are not sufficient to be characteristic properties.

Aside from showing the similarity in the mutarotation of levulose

to the rapid mutarotation reactions of galactose the results so far obtained emphasize the necessity of closer control of acidity in the measurements of the complex reactions. Because the rate of mutarotation of glucose is nearly constant over relatively wide changes in acidity, carbohydrate chemists have grown careless in the control of pH in mutarotation measurements. For sugars exhibiting rapid mutarotation reactions (ketoses and certain aldoses) this frequently leads to large variations in the results. Thus if the mutarotation measurements are made in freshly boiled water substantially free from carbon dioxide, much higher constants are obtained than if the measurements are made in ordinary water, which always contains carbon dioxide. A few results obtained with different samples of

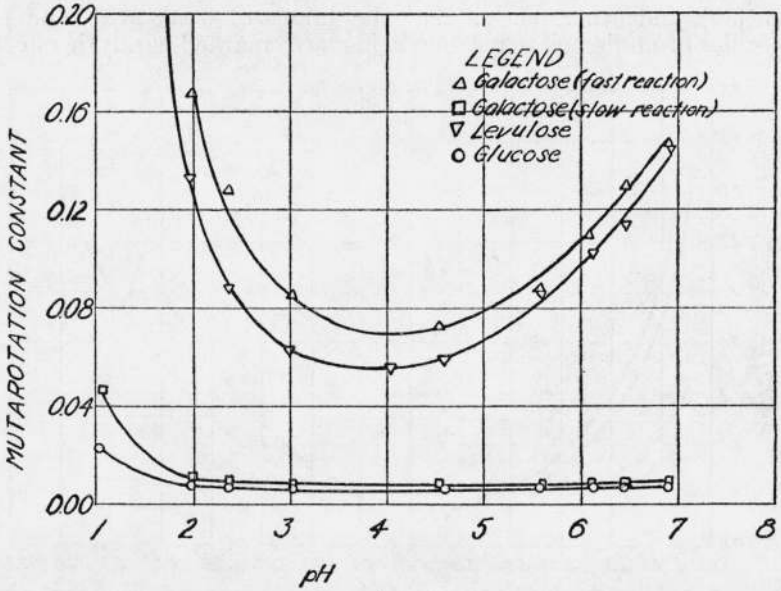


FIGURE 2.—*Relative catalytic effects of hydrogen and hydroxyl ions on rapid and slow mutarotation reactions.*

water are given in table 4. The measurements clearly show that it is essential to describe the solvent in more detail than has been customary in the past.

Buffered solutions have not been widely used for mutarotation measurements because of the catalytic effect of the buffering substances. However, our results verify the previous conclusions of H. Euler and A. Hedelius [40] that 0.001 *M* buffer solutions show a negligible catalytic effect. While such solutions have only a small buffer capacity, the capacity is sufficient for solutions of purified sugars to control the pH within 0.1 to 0.2 unit. This accuracy is great enough to give reproducible and comparable constants when the hydrogen ion concentration is near that for which the minimum rate of mutarotation is observed. Pure potassium acid phthalate is easily obtained (National Bureau of Standards Standard Sample 84), and since 0.001 *N* solutions are readily prepared and have a pH of about 4.4 at 20° C (which is near that for the minimum mutarotation value), we suggest that this solution be used as the solvent when

mutarotation measurements are made on new sugars so that ultimately a comparable set of mutarotation constants will be established for all sugars.

TABLE 4.—*Differences in mutarotation constants obtained in freshly boiled and ordinary distilled water*

Sugar	Distilled water	$m_1 \times 10^3$	$m_2 \times 10^3$	Sugar	Distilled water	$m \times 10^3$
α-*d*-Galactose	Ordinary	8.0	79	Levulose	Ordinary	54
Do	Boiled	8.4	108	Do	Boiled	115
α-*d*-Talose	Ordinary	26.3	126	Turanose	Ordinary	77
Do	Boiled	43.3	248	Do	Boiled	110

IV. EVIDENCE THAT THE MUTAROTATION OF LEVULOSE IS A PYRANOSE-FURANOSE INTERCONVERSION

In the previous sections we have shown that the rapid mutarotation reaction of galactose is like the mutarotation of levulose, and that the rapid mutarotation reactions are different from the alpha-beta pyranose interconversions. In this section, evidence will be presented showing that the levulose mutarotation is a result of a change from the pyranose to the furanose modification and, hence, that the complex character of the mutarotation reactions of galactose and other aldoses is due to a pyranose-furanose change accompanying the normal alpha-beta pyranose interconversion.

Reference has already been made to the mutarotation of mannose $CaCl_2.4H_2O$ and to the evidence which indicates that the rapid mutarotation of this compound is due to a furanose-pyranose interconversion. It has also been shown that the rapid mutarotation of mannose $CaCl_2.4H_2O$ is similar to the rapid mutarotations of galactose and levulose. Even though marked differences in the mutarotation of levulose and the mutarotations of the common aldoses have been recognized for a long time, most authorities have been content to ascribe the differences to the fact that levulose is a ketose. But in 1909 Hudson [41] showed that when sucrose is split by invertase a form of fructose is liberated which mutarotates at the same rate as levulose, but in the opposite direction, and in 1930 he stated [42] that "the measurements furnish clear proof that the initially liberated fructose is indeed a beta form but that it shifts its ring during the mutarotation." This important deduction was somewhat obscured by the fact that Hudson at that time assigned a 2,4 ring to the fructose constituent of sucrose. It is now generally accepted [43] that the fructose constituent of sucrose is a furanose and that normal fructose or levulose is a pyranose. Obviously the mutarotation reported by Hudson is caused by the conversion of the freshly liberated fructofuranose to levulose. In other words, the reaction consists in the partial conversion of the fructofuranose to the fructopyranose. The rate of this reversible reaction is the same as the rate for the mutarotation of levulose.[4] The two phenomena represent different directions of the same reversible reaction and the mutarotation of levulose consists in the partial conversion of a fructopyranose to a fructofuranose. This conclusion leads to some very interesting and important deductions which are discussed in the following sections.

[4] The equality of the mutarotations of levulose and of the fructose set free from sucrose by invertase is confirmed by our calculations of velocity constants as set forth on page 792 of this paper.

V. CHARACTER OF THE COMPLEX MUTAROTATION REACTIONS

Our investigations of the mutarotation reactions have been inspired by the desire to ascertain what modifications of the sugars are present in aqueous solutions and how rapidly the modifications change from one form to another. This problem has been attacked by studying the products resulting from reactions taking place in solution under conditions where the interconversion of the various isomers is slow, and by investigating the rates and mechanisms of the reactions. These studies have revealed that the equilibrium solutions of certain sugars, as for example galactose, arabinose, and talose, contain products other than the normal alpha and beta sugars. The oxidation of mannose $CaCl_2.4H_2O$ led to the inference that these modifications of unknown structure are furanoses, but independent evidence was needed to support this idea. We believe that the correlation of the mutarotation of levulose with the rapid reactions characteristic of the complex mutarotations of certain aldoses, in conjunction with the mutarotation of the fructofuranose set free from sucrose, shows that the rapid mutarotation reactions, with "heats of activation of about 14,000" and characterized by relatively high heats of reaction and by exceptionally marked sensitivity to acid and basic catalysts, are pyranose-furanose interconversions and that the complex mutarotations arise, at least largely, from the establishment of an equilibrium between the furanoses and the normal alpha and beta pyranoses. Presumably the equilibrium involves small concentrations of the open-chain modifications, but on account of the low concentrations these do not materially affect the optical rotations. Further study of the mutarotation of levulose will provide additional information about the pyranose-furanose interconversions and should lead to intimate knowledge of the mechanisms of the reactions and the determination of the minor constituents.

VI. STRUCTURE AND MUTAROTATION OF LACTULOSE

Lactulose, or 4-galactosido-fructose, first prepared by Montgomery and Hudson [44], exhibits a mutarotation reaction which is similar to that of levulose but differs in that it takes place in the opposite direction. If the mutarotation of lactulose be the reverse of the mutarotation of levulose, it should consist in the partial conversion of a furanose to a pyranose. It is therefore of importance to ascertain whether the mutarotation of lactulose is like the mutarotation of levulose except for direction. The similarity of the mutarotations of levulose and lactulose is shown by the following experimental results: (1) The thermal mutarotations given in table 9 reveal that the equilibrium between the various modifications of the two sugars varies with temperature in the same manner. In both cases, lowering the temperature shifts the equilibrium towards the more levorotatory modification; (2) the heats of activation for the two reactions, 14,900 and 15,800, are comparable and differ from the heats of activation for alpha-beta pyranose interconversions; (3) the catalytic effects of acids and bases on the rates for the two reactions are very pronounced, even in slightly acid solutions, and (4) the minimum mutarotation rates, 0.055 and 0.043 at 20° C, are comparable. The changes in optical rotation as well as the changes in volume show that the two reac-

tions, if alike, take place in opposite directions. Thus it appears that the mutarotation of lactulose is analogous to that of levulose but differs in that the less levorotatory modification is the known crystalline product. Since the mutarotation of levulose presumably results from the conversion of the pyranose to the furanose, the mutarotation of lactulose presumably results from the partial conversion of the furanose to pyranose, and in all probability crystalline lactulose is a furanose sugar, namely, 4-galacto-pyranosido-fructofuranose.

VII. STRUCTURE AND MUTAROTATION OF 1,3,4-TRIMETHYL-FRUCTOFURANOSE

In 1931 Hibbert, Tipson, and Brauns [45] reported a crystalline sugar which they designated as 1,3,4-trimethyl-fructofuranose. Since the hydroxyls on carbons 5 and 6 are free, the methylated sugar might crystallize in either the furanose or pyranose modification. From a comparison of the properties of the 1,3,4-trimethyl-fructose with those of 3,4,6-trimethyl-fructose, Hibbert, Tipson, and Brauns concluded that the crystalline sugar was a furanose. This important conclusion receives confirmation by comparison of its mutarotation with the mutarotations of levulose and lactulose. As may be seen from the data of table 5 (taken from the publication of Hibbert, Tipson, and Brauns [45]), 1,3,4-trimethyl-fructofuranose exhibits mutarotation. The optical rotation changes from a less levorotatory to a more levorotatory value at a rate comparable to that of levulose. The mutarotation is analogous to that of lactulose and the reverse of that of levulose. Hence it seems probable that the less levorotatory form of 1,3,4-trimethyl-fructose is the furanose modification and that the crystalline sugar is properly classified as a furanose.

TABLE 5.—*Mutarotation of 1,3,4-trimethyl-fructose* [1]

Time	$[\alpha]_D^{21}$	$m\times10^3$	Time	$[\alpha]_D^{21}$	$m\times10^3$
Minutes			*Minutes*		
4	−23.8	------	30	−51.4	69.0
5	−26.5	43.9	60	−51.85	------
6	−29.2	46.4			
17	−40.7	30.8	Average	----------	[2] 49.2
20	−48.3	56.1			

[1] Data of Hibbert, Tipson, and Brauns [45].
[2] The minimum mutarotation constant for levulose is 55×10^{-3}.

VIII. MUTAROTATIONS OF OTHER FRUCTOSE DERIVATIVES

The validity of the hypothesis that the mutarotation of levulose consists for the most part in a pyranose-furanose interconversion can be tested by the investigation of related sugars in which the formation of either the furanose or pyranose ring is prevented by substitution on the fifth or sixth carbon. Previously Hudson [46] observed that tetraacetyl fructose prepared by him and Brauns [47] does not exhibit mutarotation. The corresponding 1,3,4,5-tetramethyl-fructose was reported by Irvine and Patterson [48] to have a very small mutarotation in which the specific rotation changes from −124.3 to −123.2.

This change [5] is small in comparison with the large change found for the mutarotation of levulose. Probably the mutarotation is caused by a normal alpha-beta interconversion, in which connection it will be recalled that we have shown [49] that sorbose gives a complex mutarotation consisting in a small rapid change accompanied by a small slow change. The small slow change during the mutarotation of sorbose appears to be caused by an alpha-beta pyranose interconversion. This indicates that the alpha-beta interconversion occurs in the pyranose ketose series but that the equilibrium lies far to one side, so that the establishment of equilibrium results in the formation of only small quantities of the beta pyranoses. Unfortunately we did not have 1,3,4,5-tetramethyl-fructose and therefore the determination of the effect of temperature on the mutarotation reaction and the study of other properties which might be used to ascertain whether it gives a rapid mutarotation reaction must remain for future investigation.

TABLE 6.—*Thermal mutarotation of 3,4,6-trimethyl-fructose* [1]

Time	Saccharimeter reading	$m \times 10^3$	Time	Saccharimeter reading	$m \times 10^3$
Minutes	°S		*Minutes*	°S	
0	(7.82)	------	60.77	8.73	7.4
6.23	8.53	------	76.70	8.75	6.8
9.55	8.55	8.2	90.36	8.77	------
14.63	8.57	6.7	150.73	8.81	------
19.55	8.59	6.5	Equilibrium	8.86	------
30.30	8.66	9.0			
47.39	8.70	7.6	Average	----------	7.5

[1] Change observed after cooling a 2.5-percent aqueous solution from 26 to 0.2° C.

This sample was furnished us by the courtesy of R. F. Jackson and Emma McDonald, of this Bureau.

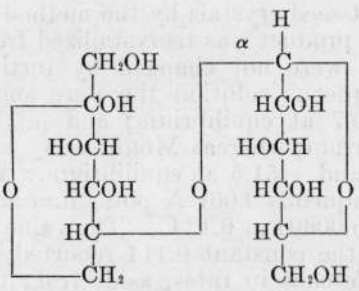
The study of another sugar which cannot form the pyranose ring was expedited by R. F. Jackson and Emma McDonald, of this Bureau, who very kindly provided us with a small quantity of 3,4,6-trimethyl-fructose. Although this sugar is known only in the form of a sirup, the absence, or existence, of the characteristic levulose mutarotation reaction can be ascertained by studying the change in optical rotation which follows a change in temperature. It will be recalled that when a solution of levulose is cooled a mutarotation takes place, which shows that lowering the temperature shifts the equilibrium towards the more levorotatory modification. It is evident a priori that if the same reaction occurs for trimethyl-fructose as occurs for levulose, the mutarotation which follows a decrease in temperature should reveal a change corresponding to the formation of the more levorotatory modification. It may be observed from the data for 3,4,6-trimethyl-fructose given in table 6 that lowering the temperature shifts the equilibrium towards a more dextrorotatory substance. This equilibrium shift is much smaller and in the opposite direction to that found for the thermal mutarotation of levulose and, since it is not probable that methylation would alter the relative energy content of two closely related stereoisomers the reaction does not appear to be the same as the reaction which predominates in the thermal mutarota-

[5] This mutarotation is to be investigated further because a somewhat larger mutarotation is reported to occur in benzene solution.

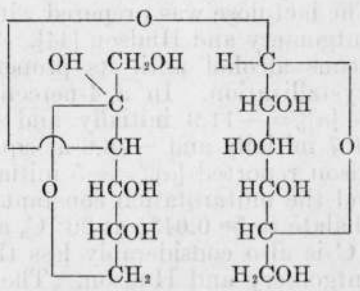
tion of levulose. It is noted, however, that the rate, 0.0075, for the thermal mutarotation of 3,4,6-trimethyl-fructose does not differ widely from the rate, 0.009, for the thermal mutarotation of levulose. The significance of this agreement, if it has any significance, is that the rate of the pyranose-furanose interconversion for levulose does not differ widely from the rate of a reaction which may consist in the interconversion of the alpha and beta furanose modifications of 3,4,6-trimethyl-fructose. The preparation and study of other sugars which cannot form both the pyranose and furanose rings is in progress and will be reported in future publications.

IX. MUTAROTATION AND STRUCTURE OF TURANOSE

The structure of turanose represented by formula I was assigned by Pacsu [50] in 1931, but as he points out in a recent paper [51], this structure does not account for the five octa-acetates which have been prepared. If structure I were correct, turanose would not exhibit a pyranose-furanose mutarotation because the hydroxyl on the fifth carbon would be blocked. The mutarotation of turanose, including the effect of temperature changes on the equilibrium state and on the reaction rates, is like the mutarotation of levulose and like other mutarotations which we have shown to consist in the interconversion of the pyranose and furanose modifications. This is evidence that the hydroxyl groups on both the fifth and sixth carbons in the fructose portion of the turanose molecule are free. Since turanose forms an osazone, the hydroxyl on the first carbon is also free. Hence the glucosidic group must be attached on either carbon 3 or carbon 4. If the glucosidic group were attached to carbon 4, and if these sugars have like rings for the glucosidic group, the osazone of turanose would be identical with the osazone of either cellobiose or maltose. Although the osazones of maltose [52], cellobiose [53], and turanose [54] are known products, they were prepared again in order to be certain that they are distinct substances. We found that the three substances have different optical rotations, melting points, and solubilities, and therefore they must be separate substances, and the glucosidic group of turanose cannot be combined with the fourth carbon. In all probability the structure of turanose corresponds to formula II.



I. Pacsu formula for turanose.



II. Proposed formula for turanose.

The conclusion that the ordinary mutarotation of levulose requires the existence of free hydroxyls on carbons 5 and 6 also explains the lack of appreciable mutarotation of hepta-acetyl turanose [55] without the

postulation of an orthoacid structure. This compound is analogous to tetra-acetyl-fructose and appears to be different from the orthoacids which contain a free hydroxyl. As we have shown previously [20], the latter compounds are very sensitive towards weak alkali, pyridine, and methyl alcohol, whereas hepta-acetyl-turanose was prepared by using silver carbonate and was recrystallized from alcohol without decomposition. Judging from its stability in alcoholic solution, it is probable that hepta-acetyl-turanose is a normal acetate rather than an orthoacid.

X. EXPERIMENTAL PROCEDURE

1. PREPARATION AND PURIFICATION OF THE SUGARS

The sugars used in this investigation were carefully purified and finally recrystallized slowly from water or aqueous alcohol. The *α-d*-galactose and *β-d*-galactose were part of the supply used for the measurements previously reported [2].

The sample of levulose was obtained by several recrystallizations of a sample of pure, white, crystalline levulose originally prepared in the Bureau's levulose plant. The final recrystallization of the levulose sample was made from aqueous alcohol, while the massecuite was kept in motion. In a 4-percent aqueous solution the sugar gave $[\alpha]_D^{20} = -132.2$ initially and -92.4 at equilibrium; and $[\alpha]_D^0 = -132.9$ initially and -103.4 at equilibrium. The mutarotation constants in the presence of 0.005 *N* potassium acid phthalate buffer (pH=4.6) were found to be 0.0592 at 20.05° C and 0.0090 at 0° C.

The turanose was prepared from melezitose by the method of Hudson and Pacsu [56]. Crystallization was induced by seed furnished by D. H. Brauns from his original sample. The product so obtained, after recrystallization from aqueous alcohol, gave in a 4-percent aqueous solution $[\alpha]_D^{20.7} = +27.3$ initially and $+75.8$ at equilibrium; and $[\alpha]_D^{0.2} = +27.8$ initially and $+70.0$ at equilibrium; whereas Hudson and Pacsu reported an initial value of +22 at 22° C and an equilibrium value of +75.3. We found the mutarotation constant to be 0.086 at 20.7° C, and 0.0136 at 0.2° C, in a solution of 0.005 *N* potassium acid phthalate buffer (pH=4.6); whereas Hudson and Pacsu found a constant of 0.097 in an aqueous solution for which the acidity was not reported.

The lactulose was prepared without seed crystals by the method of Montgomery and Hudson [44]. The product was recrystallized from aqueous alcohol until its properties were not changed by further recrystallization. In a 4-percent aqueous solution the pure sugar gave $[\alpha]_D^{20} = -11.9$ initially and -50.7 at equilibrium; and $[\alpha]_D^{0.1} = -10.7$ initially and -56.6 at equilibrium; whereas Montgomery and Hudson reported $[\alpha]_D^{22} = -5$ initially and -51.5 at equilibrium. We found the mutarotation constant in aqueous 0.001 *N* potassium acid phthalate to be 0.0435 at 20° C, and 0.00604 at 0.1° C. The value at 20° C is also considerably less than the constant 0.114 reported by Montgomery and Hudson. The difference in rates, aside from the small difference in temperature, may be due to a difference in acidity, which varies with the carbon dioxide content of the water used in making the measurements.

2. MUTAROTATION MEASUREMENTS

The sample was placed in a dry 100-ml glass-stoppered flask and 50 ml of water or the buffered solution at the desired temperature was added quickly with agitation. Time was measured with a stop watch, starting with the addition of the water. The optical rotations were measured in a 4-dm Schmidt & Haensch water-jacketed tube on a Bates saccharimeter. The conversion factor 0.3462 was used throughout for converting degrees sugar to angular degrees. The readings in degrees sugar are converted to specific rotations by multiplying by the ratio of the equilibrium specific rotation to the observed equilibrium rotation in sugar degrees.

A summary of the mutarotation constants obtained from measurements in solutions containing acids and bases is given in table 3, page 779; in table 7 the measurements on the ketoses are summarized; while the experimental data for a part of the measurements are given in table 11.

The method for calculating the velocity constants is described in detail on page 156 of reference [2]. Briefly, the values of m_1 are calculated from the equation

$$m_1=\frac{1}{t_2-t_1}\log\frac{r_1-r_\infty}{r_2-r_\infty} \tag{3}$$

using values for t_1, t_2, r_1, and r_2, obtained after the virtual completion of the fast reaction. The values of m_2 for the rapid reactions are calculated from the equation

$$m_2=\frac{1}{t_2-t_1}\log\frac{d_1}{d_2} \tag{4}$$

in which d_1 and d_2 represent the differences between the observed rotations at various times and those obtained by extrapolation of the long period back to the same time. For levulose, lactulose, and turanose, however, the slow reaction is absent or negligibly small, and therefore the values for m_2 are calculated from the usual mutarotation equation. All velocity constants are given in common logarithms.

The buffered solutions were prepared from ordinary distilled water, saturated with carbon dioxide air, by adding the reagents indicated in table 8. The acidity of each solution was determined by using a glass-electrode pH meter. Measurements of pH were made before adding the sugar and after the mutarotation was complete. Since the anions of the acids used as buffers have marked catalytic effects, the velocity constants for the sugars in buffered solutions are larger than those which would be obtained in the absence of these buffers. Our results indicate that *o*-nitrophenol has a relatively small catalytic effect and consequently the catalytic coefficients for the hydroxyl ion were calculated from the measurements in which this buffer was used. The equations thus obtained are empirical and only approximate the results obtained from measurements at points of intermediate acidity. Probably the differences are due to the catalytic action of the various buffers.

Even though we had measured the mutarotation of α-*d*- and β-*d*-galactose previously, new measurements were made in buffered solutions because our experience with levulose indicated that in non-buffered solutions differences in acidity frequently cause variations in the constants which are far greater than the errors of the observations. In the buffered solutions like values for m_1 and for m_2 were obtained

from alpha and beta galactose. The agreement of the constants as obtained from the two sugars is far too close to be merely a matter of chance and therefore it is pertinent to the interpretation of the reactions.

The mutarotation measurements with levulose $CaCl_2.3H_2O$ reported in table 11 were made in order to ascertain whether previous investigators [57] might have overlooked a rapid initial mutarotation. The mutarotation was found to be like the mutarotation of levulose and hence the sugar constituent of levulose $CaCl_2.3H_2O$ is the normal form of levulose.

TABLE 7.—*Summary of optical rotatory measurements for ketose sugars*

Concentration	Temperature	Initial	Equilibrium	Molecular rotation	Solvent for mutarotation	pH at 20° C	$[\alpha]_D$ "t" minutes after sugar is dissolved	Ref.
				LEVULOSE				
g/100 ml	°C	$[\alpha]_D$	$[\alpha]_D$	$[M]$				
4.0	20.05	−132.2	−92.4	−23,810	0.005 *N* KH phthalate buffer 2.	4.6	$-39.8\times10^{-.0592t}-92.4$	[59]
4.0	20.00	-------	-------	--------	0.001 *N* KH phthalate buffer 6.	4.4	$-39.8\times10^{-.0548t}-92.4$	[33]
3.9	0.00	−132.9	−103.4	−23,930	0.005 *N* KH phthalate buffer 2.	4.6	$-29.5\times10^{-.00903t}-103.4$	[31]
				TURANOSE				
4.1	20.70	+27.3	+75.8	+9,340	0.005 *N* KH phthalate buffer 2.	4.6	$-48.5\times10^{-.0861t}+75.8$	[56]
3.9	0.20	+27.8	+70.0	+9,510	do	4.6	$-42.2\times10^{-.0136t}+70.0$	[56]
				LACTULOSE				
3.9	20.00	−11.9	−50.7	−4,070	0.001 *N* KH phthalate buffer 6.	4.4	$+38.8\times10^{-.0435t}-50.7$	[44]
3.8	0.10	−10.7	−56.6	−3,660	do	4.4	$+45.9\times10^{-.00604t}-56.6$	[44]
				l-SORBOSE [1]				
11.3	20.0	−44.3	−43.4	−7,980	Distilled water	------	$-0.86\times10^{-.040t}+0.59\times10^{-.25t}-43.4$	[49]
11.6	0.4	−43.9	−43.3	−7,910	do	------	$-0.55\times10^{-.0054t}+0.30\times10^{-.030t}-43.3$	[49]

[1] The measurements for *l*-sorbose were reported in a previous publication [49].

TABLE 8.—*Compositions of the buffered solutions*

Buffer	Method of preparation	pH at 20° C	Total molarity of buffer
KH phthalate buffer 1	6.0 ml of 0.1007 *N* HCl was added to 50 ml of 0.1000 *N* KH phthalate and solution diluted to 1 liter.	4.0	0.005
KH phthalate buffer 2	8.0 ml of 0.1064 *N* NaOH was added to 50 ml of 0.1000 *N* KH phthalate and solution diluted to 1 liter.	4.6	.005
KH phthalate buffer 3	34.6 ml of 0.1064 *N* NaOH was added to 500 ml of 0.0100 *N* KH phthalate and solution diluted to 1 liter.	5.6	.005
KH phthalate buffer 4	40.0 ml of 0.1064 *N* NaOH was added to 500 ml of 0.0100 *N* KH phthalate and solution diluted to 1 liter.	6.1	.005
KH phthalate buffer 5	45.4 ml of 0.1064 *N* NaOH was added to 500 ml of 0.0100 *N* KH phthalate and solution diluted to 1 liter.	6.45	.005
KH phthalate buffer 6	0.204 g of KH phthalate was dissolved in 1 liter	4.4	.001
o-Nitrophenol buffer 1	12.0 ml of 0.1064 *N* NaOH was added to 1 liter of solution saturated [1] at 20° C.	6.4	.01
o-Nitrophenol buffer 2	30.0 ml of 0.1064 *N* NaOH was added to 1 liter of solution saturated [1] at 20° C.	6.9	.01
Succinic acid buffer 1	315 ml of 0.1064 *N* NaOH was added to 1 liter of 0.1000 *M* succinic acid solution.	3.8	.08
Succinic acid buffer 2	0.118 g of succinic acid dissolved in 1 liter	3.7	.001

[1] This approximately saturated solution contains 1.50 g of *o*-nitrophenol per liter of solution. The total molarity of the *o*-nitrophenol in the buffer solutions is approximately 0.01 *M*. The greater solubility of *p*-nitrophenol may make *p*-nitrophenol preferable for the preparation of buffer solutions in the range pH=6 to 8.5.

3. THERMAL MUTAROTATIONS

An 8- to 10-percent sugar solution in a 4-dm water-jacketed silver tube was allowed to reach equilibrium at the upper temperature (25 to 30° C) and its optical rotation was then read. The water was drained from the jacket and a mixture of water and alcohol cooled to 0° C was pumped through the jacket. Time was measured from the moment when the cold circulating liquid was turned on. When the temperature became constant (about 5 minutes after cooling started) at approximately 0° C, saccharimeter readings were made and the velocity constants calculated in the usual manner. The thermal mutarotation of 3,4,6-trimethyl-fructose is reported on page 786, while the thermal mutarotation of *d*-galactose is reported on page 190 of reference [2]. The thermal mutarotations of levulose, lactulose, and turanose are reported in table 9.

TABLE 9.—*Thermal mutarotations of levulose, lactulose, and turanose*

Time	Saccharimeter reading	$m \times 10^3$	Time	Saccharimeter reading	$m \times 10^3$
		Levulose [1] at 0.10° C			
Minutes	°S		*Minutes*	°S	
0	(−82.69)		50.30	−92.32	9.11
6.35	−86.20		59.94	−93.04	9.07
10.17	−86.98	9.08	75.36	−93.93	9.00
15.06	−87.94	9.37	90.83	−94.58	8.95
20.06	−88.79	9.32	134.56	−95.62	8.87
25.00	−89.54	9.28	24 hr	−96.36	
30.23	−90.26	9.28			
39.88	−91.36	9.18	Average		9.14
		Lactulose [2] at 0.15° C			
0	(−55.84)		50.62	−61.24	6.71
7.94	−56.62		60.80	−61.93	6.65
12.10	−57.18	6.30	75.43	−62.73	6.55
16.60	−57.82	6.72	91.41	−63.43	6.47
20.58	−58.33	6.76	120.20	−64.32	6.32
25.12	−58.87	6.77	167.38	−65.18	6.13
32.19	−59.64	6.79	20 hr	−66.19	
39.92	−60.39	6.80			
			Average		6.58
		Turanose [3] at 0.15° C			
0	(89.96)		30.70	+83.97	12.5
6.38	+87.23		40.72	+83.16	12.5
7.70	+86.97	13.4	50.98	+82.56	12.4
9.95	+86.59	12.6	72.08	+81.74	12.3
15.12	+85.76	12.7	90.50	+81.35	12.2
19.92	+85.15	12.4	22 hr	+80.74	
25.43	+84.52	12.3			
			Average		12.5

[1] Change observed after cooling an 8-percent aqueous solution buffered with 0.001 *N* potassium acid phthalate from 25 to 0.10° C. The reading at zero time was made at 25° C.

[2] Change observed after cooling a 10-percent solution buffered with 0.001 *N* potassium acid phthalate from 27 to 0.15° C.

[3] Change observed after cooling a 10-percent aqueous solution buffered with 0.001 *N* potassium acid phthalate at pH 4.4 from 26 to 0.15° C.

4. MUTAROTATION AFTER INVERSION OF SUCROSE

The method developed for analyzing the complex mutarotations is especially useful for studying the mutarotations which follow the splitting of sugars by enzymes. Thus when sucrose is split by invertase the mutarotation of the glucose set free can be considered to represent the slow reaction, m_1, while the mutarotation of the fructofuranose can be considered to represent the fast reaction, m_2. The value of m_1 is obtained from the observed rotations after the rapid reaction is complete by application of the usual formula, and the value of m_2 is obtained from the equation

$$m_2=\frac{1}{t_2-t_1}\log\frac{d_1}{d_2},$$

where d_1 and d_2 represent the differences between the extrapolated slow mutarotation and the observed rotations. As may be observed from the results reported in table 10, application of these equations to the data of Purves and Hudson [58] gives very satisfactory values for the mutarotation constants of the glucose and fructose constituents. The velocity constant for the mutarotation of the fructose component (0.0530) at pH 4.5 buffered with sodium acetate is in agreement with that (0.0548) which we obtained for the reverse reaction in a solution at pH 4.4 buffered with potassium acid phthalate and at the same temperature. The constant for α-*d*-glucose (0.00632) is also in excellent agreement with that (0.00648) found for the mutarotation of the freshly dissolved α-*d*-glucose in aqueous solution at pH 4.6.

TABLE 10.—*Mutarotation of fructofuranose and α-d-glucose liberated from sucrose by invertase* [a]

Fructose constituent				Glucose constituent		
Time	Purves and Hudson reading	Deviation	$m_1\times10^3$	Time	Purves and Hudson reading	$m_1\times10^3$
Minutes	°V	°V		*Minutes*	°V	
3.3 [b]	19.95	16.21	----------	30.0	−0.81	--------
4.1	18.36	14.80	(49.4)	35.0	−1.79	--------
5.0	16.63	13.27	51.1	40.0	−2.64	--------
6.2	14.68	11.57	50.5	50.0	−3.94	6.64
7.0	13.41	10.48	51.2	60.3	−4.99	6.33
8.0	12.01	9.29	51.4	80.5	−6.69	6.25
9.1	10.46	7.96	53.3	190.0	−10.57	5.79
10.0	9.33	7.02	54.2	29 hr	−11.81	--------
11.0	8.43	6.32	53.1			
12.0	7.41	5.50	54.0	Average	----------	6.25
13.0	6.46	4.75	55.0			
15.1	4.96	3.65	54.9			
17.0	3.86	2.90	54.6			
Average	----------	----------	53.0			

[a] Calculations based on data taken from the paper of Purves and Hudson, J. Am. Chem. Soc. **56**, 706 (1934). The temperature was 20° C and the pH was 4.5.

[b] Although Purves and Hudson give earlier data, the calculations were begun at 3.3 minutes, at which time we considered the inversion to be practically complete. The slow reaction is calculated from the reading at 40 minutes.

XI. EXPERIMENTAL DATA FOR MUTAROTATION MEASUREMENTS

The experimental data for the mutarotation measurements conducted in buffered solutions at approximately pH 4.6 are recorded in table 11. These measurements are representative of those used for determining the constants given in table 3. In order to conserve space the optical-rotation measurements at other acidities are not given in detail.

TABLE 11.—*Experimental data for the mutarotation measurements in buffered solutions*

LEVULOSE

3.9 g per 100 ml at 0.00° C Read in a 4-dm tube (pH=4.61 at 20° C) KH phthalate buffer 2			4.0 g per 100 ml at 20.05° C Read in a 4-dm tube (pH=4.57 at 20° C) KH phthalate buffer 2		
Time	Observed reading	$m\times10^3$	Time	Observed reading	$m\times10^3$
Minutes	°S		*Minutes*	°S	
2.39	−59.22		1.45	−57.81	
4.10	−58.81	(8.38)	2.52	−55.71	60.7
10.10	−57.32	9.18	4.49	−52.65	59.7
21.46	−55.04	9.15	7.61	−49.24	59.0
30.53	−53.60	9.09	9.93	−47.47	59.0
40.39	−52.29	9.09	12.96	−45.87	58.9
50.02	−51.27	9.05	15.99	−44.81	58.8
60.45	−50.37	9.02	19.87	−43.96	58.6
76.73	−49.34	8.91	170	−42.70	
89.80	−48.70	8.89			
105.57	−48.11	8.91	Average		59.2
22hr	−46.59				
Average		9.03			

LEVULOSE $CaCl_2.3H_2O$

7.9 g per ml at 20.55° C Read in a 4-dm tube (pH=3.88 at 20° C) KH phthalate buffer 2					
Time	Observed reading	$m\times10^3$	Time	Observed reading	$m\times10^3$
Minutes	°S		*Minutes*	°S	
2.10	−74.01		10.60	−61.50	67.6
2.91	−72.17	(61.2)	12.76	−60.17	68.0
3.69	−70.43	64.4	15.96	−58.91	67.9
4.59	−68.63	66.1	19 hr	−56.96	
5.41	−67.23	66.5			
6.75	−65.28	67.0	Average		66.9
8.71	−63.08	67.3			

TABLE 11.—*Experimental data for the mutarotation measurements in buffered solutions*—Continued

LACTULOSE

3.8 g per 100 ml at 0.1° C Read in a 4-dm tube (pH=4.42 at 20° C) 0.001 *N* KH phthalate solution 6			3.9 g per 100 ml at 20.0° C Read in a 4-dm tube (pH=4.40 at 20° C) 0.001 *N* KH phthalate solution 6		
Time	Observed reading	$m\times10^3$	Time	Observed reading	$m\times10^3$
Minutes	°S		*Minutes*	°S	
2.36	−5.35		1.56	−7.95	
3.19	−5.59	(6.49)	2.41	−9.19	(44.1)
5.64	−6.22	6.05	3.77	−10.88	42.7
11.31	−7.66	6.13	4.98	−12.27	43.1
19.88	−9.60	6.11	7.69	−14.84	43.6
30.12	−11.63	6.10	9.47	−16.14	43.4
39.97	−13.30	6.06	12.12	−17.74	43.5
49.73	−14.75	6.05	14.95	−19.07	43.9
60.06	−16.10	6.05	17.6	−20.03	44.1
74.86	−17.70	6.03	22.05	−21.05	43.8
90.05	−19.03	6.01	19 hr	−22.95	
105.33	−20.12	6.00	Average		43.5
122.33	−21.09	5.99			
174.08	−22.96	5.95			
23 hr	−24.81				
Average		6.04			

TURANOSE

3.9 g per 100 ml at 0.2° C Read in a 4-dm tube (pH=4.61 at 20° C) KH phthalate buffer 2			4.1 g per 100 ml at 20.70° C Read in a 4-m tube (pH=4.61 at 20° C) KH phthalate buffer 2		
Time	Observed reading	$m\times10^3$	Time	Observed reading	$m\times10^3$
Minutes	°S		*Minutes*	°S	
4.03	+14.80		1.65	+19.13	
5.08	+15.39	(14.8)	3.29	+23.69	86.2
10.25	+17.83	13.9	4.67	+26.54	86.4
14.95	+19.70	13.7	6.45	+29.21	86.2
20.90	+21.69	13.6	8.60	+31.42	86.4
26.62	+23.30	13.5	11.47	+33.20	86.1
33.80	+24.96	13.5	14.39	+34.19	85.1
40.58	+26.24	13.5	19.20	+35.00	(84.5)
50.35	+27.63	13.5	20 hr	+35.54	
60.65	+28.73	13.5	Average		86.1
75.02	+29.76	13.5			
90.33	+30.46	13.5			
19 hr	+31.62				
Average		13.6			

TABLE 11.—*Experimental data for the mutarotation measurements in buffered solutions*—Continued

α-*d*-GLUCOSE

3.9 g per 100 ml at 0.10° C Read in a 4-dm tube (pH=4.61 at 20° C) KH phthalate buffer 2			3.9 g per 100 ml at 20.05° C Read in a 4-dm tube (pH=4.60 at 20° C) KH phthalate buffer 2		
Time	Observed reading	$m_1 \times 10^3$	Time	Observed reading	$m_1 \times 10^3$
Minutes	°S	(k_1+k_2)	*Minutes*	°S	(k_1+k_2)
2.82	+49.75		1.55	+49.84	
5.02	+49.65	0.764	10.07	+46.72	6.56
9.93	+49.41	.806	20.09	+43.56	6.52
16.45	+49.10	.809	29.97	+40.91	6.48
22.08	+48.85	.796	45.25	+37.47	6.47
30.17	+48.52	.771	59.95	+34.84	6.46
44.95	+47.90	.762	75.32	+32.61	6.47
60.13	+47.30	.752	90.21	+30.91	6.46
94.68	+45.95	.749	121.20	+28.38	6.44
116.77	+45.13	.747	18 hr	+24.00	
178.95	+42.94	.751	Average		6.48
227.17	+41.44	.747			
270.92	+40.13	.750			
362.00	+37.76	.750			
48 hr	+23.80				
Average		0.766			

α-*d*-GALACTOSE

3.9 g per 100 ml at 0.15 to 0.30° C Read in a 4-dm tube (pH=4.62 at 20° C) KH phthalate buffer 2					4.0 g per 100 ml at 20.05° C Read in a 4-dm tube (pH=4.55 at 20° C) KH phthalate buffer 2				
Time	Observed reading	$m_1 \times 10^3$	Deviation	$m_2 \times 10^3$	Time	Observed reading	$m_1 \times 10^3$	Deviation	$m_2 \times 10^3$
Minutes	°S		°S		*Minutes*	°S		°S	
3.62	+68.91		1.31		2.10	+66.52		1.84	
9.58	+68.33		1.11	12.1	3.10	+65.72		1.58	(66.2)
15.21	+67.83		0.98	10.9	4.98	+64.29		1.11	76.2
21.66	+67.26		.82	11.3	7.37	+62.76		0.76	72.9
32.87	+66.37		.63	10.9	9.93	+61.28		.50	72.3
42.28	+65.60		.43	12.5	12.08	+60.18		.37	69.8
51.91	+64.96		.36	11.6	14.14	+59.14		.22	76.6
60.91	+64.29		.21	13.9	30.20	+53.05			
118.30	+60.96				39.94	+50.28	8.22		
147.47	+59.55	0.953			49.93	+47.91	8.25		
205.30	+56.93	.973			60.08	+45.93	8.24		
255.21	+54.92	.979			75.08	+43.60	8.27		
316.84	+52.81	.970			89.97	+41.88	8.26		
359.77	+51.47	.971			121.43	+39.51	8.25		
394.81	+50.49	.968			150.09	+38.26	8.31		
48 hr	+38.21				19 hr	+36.60			
Average		0.969		11.9	Average		8.26		73.6

TABLE 11.—*Experimental data for the mutarotation measurements in buffered solutions*—Continued

β-*d*-GALACTOSE

3.7 g per 100 ml at 0.20° C Read in a 4-dm tube (pH=4.61 at 20° C) KH phthalate buffer 2					4.0 g per 100 ml at 20.50° C Read in a 4-dm tube (pH=4.67 at 20° C) KH phthalate buffer 2				
Time	Observed reading	$m_1 \times 10^3$	Deviation	$m_2 \times 10^3$	Time	Observed reading	$m_1 \times 10^3$	Deviation	$m_2 \times 10^3$
Minutes	°S		°S		*Minutes*	°S		°S	
2.27	+23.95		0.89		1.43	+24.38		1.68	
9.53	+24.01		.75	10.2	2.47	+24.35		1.37	(85.2)
14.88	+24.05		.65	10.8	3.19	+24.39		1.22	78.9
20.89	+24.07		.52	12.5	3.95	+24.43		1.06	79.4
30.17	+24.20		.41	12.1	4.77	+24.49		0.91	79.7
41.05	+24.34		.27	13.4	6.12	+24.65		.73	77.2
50.02	+24.49		.20	13.6	8.34	+24.96		.50	76.2
61.41	+24.70		(.13)	(14.1)	10.31	+25.25		.33	79.6
120.52	+25.90				12.67	+25.67		.22	78.5
154.25	+26.60	0.918			30.03	+28.67			
179.77	+27.09	.912			39.97	+30.05	8.33		
212.91	+27.66	.893			49.98	+31.23	8.46		
259.32	+28.43	.895			60.20	+32.22	8.52		
319.15	+29.35	.906			75.09	+33.35	8.56		
48 hr	+36.07				90.02	+34.17	8.52		
					124.78	+35.39	8.55		
Average		0.905		12.1	155.91	+35.97	8.64		
					19 hr	+36.62			
					Average		8.51		78.5

XII. GENERAL SUMMARY

The changes in optical rotation, molecular volume, molecular refraction, and energy content which occur after dissolving levulose and α-*d*-galactose in water clearly show that the mutarotation of levulose is caused by the same kind of reaction as causes the rapid mutarotation of α-*d*-galactose. The similarity of the two reactions and their resemblance to the rapid mutarotation reactions of other sugars are confirmed by the following observations: (1) Alterations in temperature cause large changes in the equilibrium proportions of the labile substances responsible for both the mutarotation of levulose and for the rapid mutarotation reactions; (2) the heat of activation for the reaction representing the mutarotation of levulose is comparable with the heat of activation for the rapid mutarotation reactions, and (3) the constants for the mutarotations of levulose and the rapid mutarotation reactions are both extremely sensitive to the catalytic action of acids and bases and vary with pH in like manner.

Attention is directed to the observation of Hudson that the mutarotation of the fructose liberated from sucrose by invertase occurs at the same rate as the mutarotation of levulose, but in the opposite direction. Since the fructose constituent of sucrose is a furanose and levulose is a pyranose, the mutarotation of fructose set free by invertase must be caused by the conversion of part of the freshly liberated fructofuranose to levulose. The mutarotation of levulose, which is the reverse, must be caused by the conversion of part of the freshly dissolved sugar to the fructofuranose. This conclusion leads to the following deductions: (1) That the mutarotation of lactulose is caused by a furanose-pyranose change and that cyrstalline lactulose is a furanose; (2) that the mutarotation of Hibbert's 1, 3, 4-trimethyl-

fructofuranose results from a furanose-pyranose change and that the sugar is properly classified as a furanose; (3) that the mutarotation of turanose results from a pyranose-furanose change and therefore the glucosidic group is not united with the fifth or sixth carbon but in all probability it is united with the third carbon; and (4) that the rapid reactions characteristic of the complex mutarotations of galactose and other sugars are pyranose-furanose interconversions, and the complex mutarotations arise from the establishment of an equilibrium between the furanoses and the normal α- and β-pyranoses.

The changes which occur during the thermal mutarotation of levulose, lactulose, and turanose are alike for the three sugars and correspond to the changes found in the equilibrium proportions of the labile constituent of galactose under like conditions. The application of "thermal mutarotations" for investigating the mutarotation reactions of sugars which are available only as sirups is illustrated by a study of the "thermal mutarotation" of 2,3,6-trimethyl-fructose.

Velocity constants determined in buffered solutions covering the range between pH 1 to 7 are reported for the mutarotations of glucose, galactose, and levulose. For the rapid reactions, the catalytic effects of hydrogen and hydroxyl ions are much greater than those found for the normal alpha-beta pyranose interconversions. The high sensitivity of the rapid mutarotation reactions to variations in acidity accounts for some of the discrepancies in the mutarotation constants obtained by using distilled water containing variable quantities of carbon dioxide. Hence for accurate measurements the acidity must be controlled. The mutarotation constants found for α-*d*-galactose and β-*d*-galactose in buffered solutions were the same within the experimental error. For lactulose, the mutarotation constant in 0.001 *N* potassium acid phthalate at pH 4.4 at 20° C was found to be 0.0435 with $[\alpha]_D^{20} = -11.9$ initially, and -50.7 at equilibrium; at 0.1° C the mutarotation constant was found to be 0.00604 with $[\alpha]_D^{0.1} = -10.7$ initially and -56.6 at equilibrium. For turanose, the mutarotation constant in a 4-percent aqueous solution buffered at pH 4.6 with 0.005 *N* potassium acid phthalate buffer at 20.7° C was found to be 0.086 with $[\alpha]_D^{20.7} = +27.3$ initially, and $+75.8$ at equilibrium; at 0.2° C the mutarotation constant was found to be 0.0136 with $[\alpha]_D^{0.2} = +27.8$ initially, and $+70.0$ at equilibrium. Mutarotation measurements with levulose in a 4-percent aqueous solution in the presence of 0.005 *N* potassium acid phthalate buffer at pH=4.6 gave $[\alpha]_D^{20} = -132.2$ initially, and -92.4 at equilibrium; and $[\alpha]_D^{0} = -132.9$ initially, and -103.4 at equilibrium, with mutarotation constants of 0.0592 and 0.0090 at 20 and 0° C. The mutarotation of fructose $CaCl_2.3H_2O$ shows that this compound contains the normal modification of levulose.

XIII. REFERENCES

[1] H. S. Isbell and W. W. Pigman, BS J. Research **10,** 337 (1933) RP534.
[2] H. S. Isbell and W. W. Pigman, J. Research NBS **18,** 141 (1937) RP969.
[3] H. S. Isbell, J. Research NBS **18,** 505 (1937) RP990.
[4] L. J. Simon, Compt. rend. **132,** 487 (1901).
[5] H. S. Isbell, BS J. Research **3,** 1041 (1929) RP128.
[6] K. Freudenberg and R. Kuhn, Ber. deut. chem. Ges. **64,** 722 (1931).
[7] H. S. Isbell and C. S. Hudson, BS J. Research **8,** 327 (1932) RP418.
[8] H. S. Isbell, BS J. Research **8,** 615 (1932) RP441.
[9] C. N. Riiber and J. Minsaas, Ber. deut. chem. Ges. **60,** 2402 (1927).

[10] F. P. Phelps and F. J. Bates, J. Am. Chem. Soc. **56,** 1250 (1934).
[11] H. S. Isbell, BS J. Research **5,** 741 (1930) RP226.
[12] C. N. Riiber and O. Bjerkli, Kgl. Norske Videnskab Selskabs, Skrifter, No. 5 (1936).
[13] R. M. Hann, Alice T. Merrill, and C. S. Hudson, J. Am. Chem. Soc. **57,** 2100 (1935).
[14] C. S. Hudson and E. Yanovsky, J. Am. Chem. Soc. **39,** 1037 (1917).
[15] R. M. Hann and C. S. Hudson, J. Am. Chem. Soc. **59,** 550 (1937).
[16] H. S. Isbell, BS J. Research **5,** 1179 (1930) RP253; **7,** 1115 (1931) RP392.
[17] C. N. Riiber and J. Minsaas, Ber. deut. chem. Ges. **59,** 2266 (1926).
[18] G. F. Smith and T. M. Lowry, J. Chem. Soc. **1928,** 666.
[19] N. R. Sørensen, Kgl. Norske Videnskab Selskabs, Skrifter No. 2 (1937).
[20] W. W. Pigman and H. S. Isbell, J. Research NBS **19,** 189 (1937) RP1021.
[21] C. N. Riiber and N. A. Sørensen, Kgl. Norske Videnskab Selskabs, Skrifter, No. 7 (1933).
[22] E. M. Montgomery and C. S. Hudson, J. Am. Chem. Soc. **56,** 2074 (1934).
[23] F. P. Phelps, H. S. Isbell, and W. W. Pigman, J. Am. Chem. Soc. **56,** 747 (1934).
[24] J. K. Dale, BS J. Research **3,** 459 (1929) RP106.
[27] H. S. Isbell, J. Am. Chem. Soc. **56,** 2789 (1934).
[26] H. S. Isbell, J. Research NBS **20,** 97 (1938) RP1069.
[27] H. S. Isbell, J. Research NBS **19,** 639 (1937) RP1052.
[28] H. S. Isbell, J. Am. Chem. Soc. **55,** 2166 (1933).
[29] T. M. Lowry and G. F. Smith, Rapports sur les Hydrates de Carbone, 10th Conference of the International Union of Chemistry (Liége, p. 79, 1930).
[30] H. S. Isbell and W. W. Pigman, J. Research NBS **16,** 553 (1936) RP892.
[31] J. M. Nelson and F. M. Beegle, J. Am. Chem. Soc. **41,** 559 (1919).
[32] C. S. Hudson, J. Am. Chem. Soc. **30,** 1564 (1908).
[33] C. N. Riiber and V. Esp, Ber. deut. chem. Ges. **58,** 737 (1925).
[34] H. W. Wiley, J. Am. Chem. Soc. **18,** 81 (1896).
[35] C. S. Hudson, J. Am. Chem. Soc. **31,** 66 (1909).
[36] C. N. Riiber and N. A. Sørensen, Norges Tekniske Høiskole, Avhandlinger Til 25 Ars Jubileet (1935).
[37] C. S. Hudson, J. Am. Chem. Soc. **29,** 1571 (1907).
[38] R. Kuhn and P. Jacob, Z. physikal. Chem. **113,** 389 (1924).
[39] J. N. Brönsted and E. A. Guggenheim, J. Am. Chem. Soc. **49,** 2554 (1927).
[40] H. Euler and A. Hedelius, Biochem. Z. **107,** 150 (1920).
[41] C. S. Hudson, J. Am. Chem. Soc. **31,** 655 (1909).
[42] C. S. Hudson, J. Am. Chem. Soc. **52,** 1716 (1930).
[43] W. N. Haworth, The Constitution of Sugars p. 68 (Edward Arnold & Co., London, 1929).
[44] Edna M. Montgomery and C. S. Hudson, J. Am. Chem. Soc. **52,** 2101 (1930).
[45] H. Hibbert, R. S. Tipson, and F. Brauns, Canadian J. Research **4,** 235 (1931).
[46] C. S. Hudson, Sci. Pap. BS **21,** 365 (1926) S533.
[47] C. S. Hudson and D. H. Brauns, J. Am. Chem. Soc. **37,** 2736 (1915).
[48] J. C. Irvine and J. Patterson, J. Chem. Soc. London **121,** 2696 (1922); see also C. Anderson, W. Charlton, W. Haworth, and V. Nicholson, J. Chem. Soc. 1337 (1929).
[49] W. W. Pigman and H. S. Isbell, J. Research NBS **19,** 443 (1937) RP1035.
[50] E. Pacsu, J. Am. Chem. Soc. **53,** 3099 (1931).
[51] E. Pacsu and F. B. Cramer, J. Am. Chem. Soc. **59,** 1061 (1937).
[52] E. Fischer, Ber. deut. chem. Ges. **17,** 583 (1884).
[53] G. Zemplen, Z. Csuros, and Z. Bruckner, Ber. deut. chem. Ges. **61,** 927 (1928).
[54] E. Fischer, Ber. deut. chem. Ges. **27,** 2488 (1894).
[55] E. Pacsu, J. Am. Chem. Soc. **55,** 2451 (1933).
[56] C. S. Hudson and E. Pacsu, J. Am. Chem. Soc. **52,** 2519 (1930).
[57] A. B. Smith and B. Tollens, Ber. deut. chem. Ges. **33,** 1277 (1900).
[58] C. B. Purves and C. S. Hudson, J. Am. Chem. Soc. **56,** 706 (1934).
[59] R. F. Jackson and J. A. Mathews, BS J. Research **8,** 403 (1932) RP426.

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