[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Action of Dilute Alkali on Tetramethylglucose

BY J. H. SIMONS AND H. C. STRUCK*

The conversion of one sugar into another and the chemistry of sugars in alkaline media are matters of interest to the biologist and the pathologist as well as the chemist. The course of these transformations can never be fixed with exactness, until the underlying fundamental chemistry has been elucidated. Papers by W. Lee Lewis and his co-workers[1] have called attention to the fact that the methylated sugars offer excellent opportunities for testing experimentally the validity of certain of the theories developed to explain the reactivity of the simple sugars. These papers report experiments with methylated hexoses and pentoses. It has been shown that their interconversions are simpler than those of the unmethylated sugars and that the quantity of interfering side reactions is less. With these experiments as a basis it has been shown that the conceptions of Lobry de Bruyn,[2] Wohl and Neuberg,[3] Fischer[4] and Nef[5] do not entirely account for the stability toward alkali and alkaline oxidizing agents of the methylated sugars as contrasted with the unmethylated sugars.

The interconversion of the methylated sugars by means of dilute alkali does not follow exactly the course predictable from the early theories of Nef, and this casts doubt on the application of these theories in their original form to the unmethylated sugars. The theory proposed by Lewis retains the ene-diol of Nef's explanation but considers it formed by a simple tautomeric shift of a hydrogen atom rather than the addition and subtraction of water. In the case of the methylated sugars it was predicted that, due to the lessened mobility of the methyl group, the ketose derivative would not form. This prediction was experimentally fulfilled. An explanation of the mechanism by which the hydrogen atom changes its position in the molecule is not attempted by Lewis. A means is suggested in the present work which may account for this.

Upon treatment of either tetramethylglucose or tetramethylmannose with dilute alkali, it was found that the iodine absorption of the solution steadily rose. Upon acidification the iodine absorption dropped to the original value. There was only negligible loss of sugar to saccharinic acids and gums. As the iodine absorption of the solution rose, the rotation dropped for tetramethylglucose and rose for tetramethylmannose until an equilibrium value of 42 ± 1° was reached. This is the calculated value for the equimolecular mixture of the two substances. After acidification analysis by actual separation and identification showed equal amounts of the two sugars to be present.

Lewis postulated that the high absorption of iodine was due to the presence of the common ene-diol in quantity sufficient to be detected by the iodine titrations. As this quantity must be relatively high after the reaction has proceeded for some time in alkaline solution, the calculation of the quantities of the two epimeric sugars cannot be made, as the rotation of the ene-diol is unknown. In the present work it will be shown that there are a number of substances present in the reacting solution. As their rotations are unknown, these calculations become even more difficult.

In spite of many attempts by these workers as well as the present writers, this common ene-diol has not been isolated and identified.

The absorption of iodine furnishes an analytical means for studying the rate of the reaction; and it was conceived in attempting this work that rate measurements could supply data of considerable value in a study of this reaction. As previous curves showing the course of the reaction were not precise enough to use in quantitative considerations, the technique had to be improved in order to obtain the needed precision. As the fate of the hydroxyl ion was of interest, this was followed by means of a glass electrode. It has been possible from the results obtained to construct an hypothesis of the mechanism of the reaction and to obtain reasonably quantitative correlations be-

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* Present addresses of authors: J. H. S., 6217 N. Talman Ave.; H. C. S., 940 N. Lorel Ave., Chicago, Illinois.

[1] (a) Gustus and Lewis, THIS JOURNAL, **49**, 1512 (1927); (b) Wolfrom and Lewis, *ibid.*, **50**, 837 (1928); (c) Greene and Lewis, *ibid.*, **50**, 2813 (1928); (d) Gross and Lewis, *ibid.*, **53**, 2772 (1931); (e) Neher and Lewis, *ibid.*, **53**, 4411 (1931); (f) Loder and Lewis, *ibid.*, **54**, 1040 (1932).

[2] Lobry de Bruyn and Van Ekenstein, *Rec. trav. chim.*, **14**, 156, 203, 213 (1895); **16**, 162, 259, 262, 278 (1897); **19**, 1 (1900).

[3] Wohl and Neuberg, *Ber.*, **33**, 3099 (1900).

[4] Fischer, *ibid.*, **28**, 1149 (1895).

[5] Nef, *Ann.*, **357**, 214 (1907); **376**, 1 (1910); **403**, 204 (1913).

tween theory and experimental data. This theory has also been applied to the available rotation measurements with fair agreement for the first few hours of the reaction.

Experimental Procedure

The tetramethylglucose used was prepared according to the method of W. N. Haworth[6] as modified by Gustus and Lewis.[1a] The product had the following properties: m. p. 88° (uncorr.), $[\alpha]^{20}_{D}$ +82° (molar concentration), analysis (Zeisel) calculated for $C_6H_8O_2(OCH_3)_4$, OCH_3 = 52.54; found, OCH_3, 51.61, 53.06.

The solutions of calcium and barium hydroxide were prepared by dissolving the c. p. materials in distilled water, allowing the carbonates to settle, and withdrawing the clear supernatant solution with a siphon.

The iodimetric aldose analyses were made by the method of Cajori[7] as modified by Wolfrom and Lewis.[1b] The procedure is as follows. Approximately one-tenth gram of the crystalline substance or an aliquot part of the reaction solution is transferred accurately to a 500-cc. Erlenmeyer flask and mixed with 50 cc. of water. To this solution is added 25.00 cc. of tenth normal iodine solution and 4 cc. of approximately 2.75 normal sodium carbonate solution. The contents are stirred, and the flask is placed in the dark at room temperature for two to two and one-half hours. The solution is then neutralized with 10% sulfuric acid, a few drops of acid being added in excess, and the iodine immediately titrated with 0.05 normal sodium thiosulfate solution. Under these conditions tetramethylglucose and tetramethylmannose quantitatively consume one mole of iodine per mole of sugar.

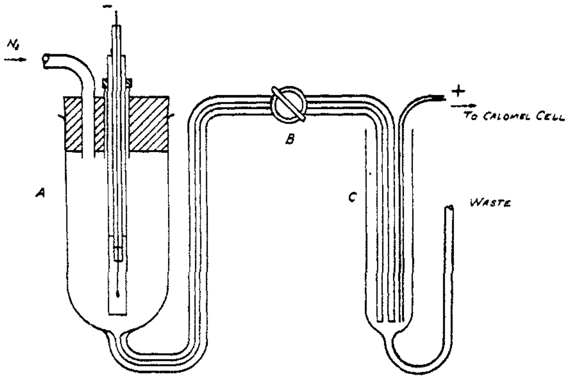

Fig. 1.—Diagram of apparatus.

The reaction between tetramethylglucose and alkali was carried out in the apparatus shown in Fig. 1. The reaction vessel containing the glass electrode was the negative half of a galvanic cell and a saturated calomel cell the positive half. A saturated potassium chloride bridge connected the two half cells. The reaction vessel, A, was approximately 120 by 50 mm. in size. Air was excluded by means of a tightly fitted two-holed rubber stopper. Through one opening nitrogen was passed whenever the vessel was open for sampling or measurement of electromotive force. The nitrogen was drawn from a tank and was freed from carbon dioxide by passing through two scrubbers containing 50% sodium hydroxide solution. A scrubber containing concentrated sulfuric acid followed, and then one with a half molar solution of potassium chloride. The other opening in the stopper was fitted with a glass tube and was kept closed at all times except when samples were withdrawn or electromotive force measurements made. The cell, bridge and saturated calomel half cell were fastened to a board and immersed in an oil thermostat maintained at 35°. The stopcock B was not greased and was closed at all times during the reaction. The solution film made the electrical connection. Potassium chloride solution was saturated at 35° by passing a solution saturated at room temperature through a tube filled with the solid salt in the thermostat before leading it to the calomel cell. The glass electrodes were of the type recommended by MacInnes and Dole.[8] The electromotive force measurements were made on a type K Leeds and Northrup potentiometer and quadrant electrometer and are probably correct to ± 0.0001 volt.

The glass electrodes and calomel cells were checked by observing the electromotive force developed, when an exactly 0.05 molar solution of potassium acid phthalate was placed in the reaction cells. The values never varied more than 0.1 millivolt from the accepted value for this solution. In order to check the use of the set-up with alkaline solutions, the alkali normality of three known solutions was determined. Calcium hydroxide solution saturated at 35° is approximately 0.035 normal as given in the "International Critical Tables." We obtained the value of 0.0329 for this and on its dilution with four-fifths its volume of water we found a normality of 0.01775 against the known value of 0.0177. A sample of a pure alkaline solution was found by titration with standard acid to be 0.0865 *N* and by the glass electrode, 0.0889 *N*.

Before beginning a series of determinations, a sample of the alkali which was to be used was placed in the cell and its concentration determined from the electromotive force. A molar solution of tetramethylglucose was then prepared with this alkali by dissolving exactly 5.900 g. of the sugar in enough of it to make a total volume of 25 cc. It has been found by many tests that it requires very nearly 20.00 cc. of the alkali to do this, so that the alkali concentration of the solution before reaction with the sugar is 20% less than that of the alkali used to make the solution.

Immediately after being prepared the sugar solution was placed in the clean cell, which was then closed. A slight pressure of nitrogen forced the solution through the capillary tube, and the stopcock was closed. A sample of 0.5 cc. was withdrawn by means of a graduated pipet for iodimetric analysis. The electromotive force was determined as soon as possible after the solution was placed in the cell. Subsequent analyses were performed at close intervals during the early hours of the experiment and less often later. The reverse reaction was carried out by means of gaseous hydrogen chloride, which was generated by dropping concentrated sulfuric acid on pure sodium chloride moistened with water and dried by passage through concentrated sulfuric acid. It was introduced into the cell from a gas buret.

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[6] Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

[7] Cajori, *J. Biol. Chem.*, **54**, 617 (1922).

[8] MacInnes and Dole, *Ind. Eng. Chem., Anal. Ed.*, **1**, 57 (1929); This Journal, **52**, 29 (1930); Dole, *ibid.*, **53**, 4260 (1931).

For rotation measurements the fresh solution was immediately sealed in a polariscope tube and kept in an air thermostat at 35° except when being used in the instrument. With the more concentrated alkalies the solutions became red in color after several days, and measurements could not be made using sodium light. Red light obtained by filtering white light through ruby glass was found suitable, and enough measurements made using both sodium and red light to obtain the ratio of the rotation with the two light sources. This ratio enabled the rotations made with red light to be calculated on the sodium light basis.

Results

In Table I are given the results of the iodine absorption experiments, and these are shown graphically in Figs. 2 and 3. The rotation experiments are recorded in Table II. From these the following qualitative points readily may be deduced.

(1) The hydroxyl ion concentration of the alkali used to prepare the solution is reduced to a very small value upon the addition of the sugar. This value will be shown later to be the concentration in equilibrium with the sugar, which acts as a weak acid. After this sudden change, the hydroxyl ion concentration does not change appreciably during the first several days of the reaction and only very slowly thereafter.

(2) In the first hour or two of the reaction the reaction velocity curve levels off and then later rises at a more rapid rate. This portion of the curve will, for convenience, be called the break. It is more pronounced in experiments with the weaker alkali. It appears to occur at about the same level of consumption of tetramethylglucose in the different runs. The time at which it occurs and also the length of the level portion are roughly inversely proportional to the hydroxyl ion concentration. (In experiment I the points after 9.7 hours are not close enough together to show this phenomenon.)

(3) An apparent equilibrium is reached at the end of the reaction with the iodine absorption value approximately 215% independent of the

TABLE I

REACTION OF TETRAMETHYLGLUCOSE IN ALKALINE SOLUTION AT 35°. SOLUTION MOLAR IN TETRAMETHYLGLUCOSE

Expt.	1			2			3	
Alkali	Calcium hydroxide			Calcium hydroxide			Barium hydroxide	
Normality	0.0178			0.0329			0.08897	
Time, hrs.	% apparent aldose −100%	$(OH^-) \times 10^{-3}$	Time, hrs.	% apparent aldose −100%	$(OH^-) \times 10^{-3}$	Time, hrs.	% apparent aldose −100%	$(OH^-) \times 10^{-3}$
0.0	0.0	0.6244	0.0	0.0	1.094	0.0	0.0	3.144
.1	0.9		.02	2.5	1.094	.05	2.8	
.5	1.2		.33	3.9	1.094	.5	3.8	
1.0	1.6		.50	3.9	1.094	1.0	4.4	
1.5	1.9		.75	4.0		1.5	4.5	
2.0	2.2		1.00	4.4	1.094	2.0	5.1	
3.0	3.6		1.33	4.4	1.094	3.0	5.6	
4.0	4.9		1.67	4.9	1.107	4.0	7.9	
5.0	5.4		2.00	5.0	1.103	5.0	10.8	
6.0	6.0		2.33	5.1		6.0	10.5	
7.0	6.3		2.67	5.2		7.0	11.7	
8.0	6.6		3.00	5.3	1.094	8.0	13.0	
9.7	7.0		3.50	5.4		10.0	14.7	
24.7	11.5		4.10	5.7	1.094	24.5	24.4	
48.7	15.9		5.00	7.1	1.086	73.3	50.9	
97.0	45.5^f		23.0	17.0	1.111	121.0	63.0	
121.0	50.9	0.2062	24.0	18.6		169.3	76.5	
192.5	58.7		47.25	24.2		240.7	87.5	
240.3	62.1		52.0	25.8	0.8864	264.7	90.0^a	
264.8	59.6^a	0.0949	73.2	31.0	.9344	315.0	90.2^b	$(H^+) \times 10^{-3}$
278.7	59.5		76.9	31.4		25 days	97.5^j	0.1312
327.0	60.4^b	$(H^+) \times 10^{-3}$	95.3	32.2	.9522	18 hrs.	9.5	1.120
32 days	60.1^c	0.0036	119.0	54.5^f		42.7	8.1	
32 days	60.1^d	0.298	144.0	52.9		66.7	3.2	
1.7	60.1		167.9	56.4		90.7	1.5	
2.7	55.5		215.9	60.9		102.7	0.4	
21.5	45.3		263.7	59.8		151.0	.5	
45.5	42.2		18 days	64.0^g	.0089	175.0	.4	
69.5	37.1		20 days	68.1	.0004			
117.5	37.0^e		23 days	72.8^h	0.77×10^{-8}			
146.5	0.9	6.886	26 days	19.2^i				

TABLE I (*Concluded*)

Expt.... 4			5			6		
Alkali.... Barium hydroxide			Barium hydroxide			Barium hydroxide		
Normality.... 0.1388			0.1620			0.4799		
Time, hrs.	% apparent aldose −100%	$(OH^-) \times 10^{-3}$	Time, hrs.	% apparent aldose −100%	$(OH^-) \times 10^{-3}$	Time, hrs.	% apparent aldose −100%	$(OH^-) \times 10^{-3}$
0.0	0.0	4.848	0.0	0.0	5.945	0.0	0.0	9.307
.1	2.5		.1	2.5	5.999	.05	0.1	
.5	4.2		.3	4.8	5.955	.2	3.2	
1.0	4.9		.5	5.6	5.955	.33	4.7	
1.5	5.7		.75	7.4	5.955	.5	5.6	
2.0	7.1		1.0	9.0	5.955	.75	7.6	
3.0	9.5		1.33	9.7	5.955	1.00	9.4	
4.0	11.5		1.67	9.9		1.33	12.0	
5.0	13.8		2.00	10.3	5.955	1.67	13.0	
6.0	15.9		2.33	10.9		2.0	14.7	
7.0	17.6		2.67	11.2		2.5	16.8	
8.0	19.0		3.00	12.6	5.955	3.0	20.3	
9.5	21.6		3.33	13.1		4.0	24.1	
27.5	42.5		3.67	13.3		5.0	27.2	
77.5	71.6	4.924	4.00	13.8		8.5	29.7	
120.7	88.4		4.5	14.4	5.768	10.1	33.8	
168.9	118.4		5.5	16.7		24.25	64.8	
195.0	114.9		23.5	39.3	5.725	52.0	94.8	
217.0	117.9	2.927	28.1	44.8		72.3	106.0	
313.0	115.2[a]		47.0	86.3		99.2	113.9	
385.0	87.8[b]		72.0	100.8		145.2	113.9	
28 days	87.8[k]	$(H^+) \times 10^{-3}$	96.0	103.4		169.5	114.0	
0.0 hrs.	75.2	1.287	144.0	112.9		245.3	113.7	
72.0	−16.3		167.5	118.8		456.0	88.4	
			191.7	111.7				
			15 days	98.7[l]	3.574			
			17 days	119.8	0.00127			
			20 days	121.1[m]				
			21 days	17.8				
			22 days	11.8				
			23 days	11.7				

[a] Placed in refrigerator at 3 to 6°. [b] Returned to thermostat. [c] 4.77 cc. of hydrogen chloride gas added. Volume corrected to standard conditions. [d] After gas was added. [e] 2.75 cc. of hydrogen chloride gas added. [f] Thermostat control found defective and temperature above 35° for some hours previous. [g] Saturated with carbon dioxide. [h] Acidified with hydrogen chloride gas. [i] Solution exhausted. [j] 22.77 cc. of hydrogen chloride gas added. [k] 33.5 cc. of hydrogen chloride gas added. [l] Carbon dioxide bubbled into solution. [m] Added hydrogen chloride gas.

hydroxyl ion concentration. This was not reached with the first three experiments, probably because of the slowness of these reactions. When this condition is reached, changing the hydroxyl ion concentration of the solution even to the extent of saturating it with carbon dioxide has no or very little effect upon it; but, if the solution has not stood an excessive length of time, making it distinctly acid will reduce the iodine absorption value to 100%. This reverse reaction proceeds at measurable rates, and, if carefully studied, could be used for reaction velocity measurements.

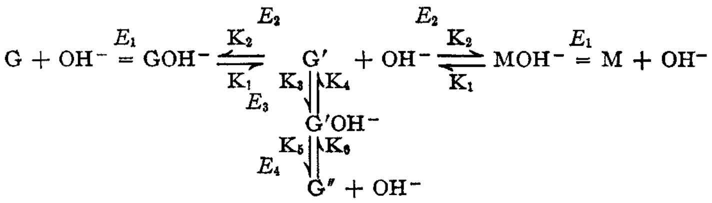
(4) The destruction of the sugar by alkali (side reactions) increases with both time and strength of the alkali, and this apparently reduces the iodine absorption value.

(5) The initial rate of the reaction is roughly proportional to the hydroxyl ion concentration, and the approach toward final equilibrium is more rapid the more concentrated the alkali. Only a small mole fraction of alkali with respect to tetramethylglucose is necessary to cause the reaction to proceed.

(6) The optical rotation of the reacting solution drops with time and more rapidly the more concentrated the alkali. For the more concentrated alkali solutions values lower than that given by an equimolecular mixture of tetramethylglucose and tetramethylmannose are reached. Upon acidification this value then rapidly drops considerably lower and then slowly rises again to about the same value.

Theoretical Treatment

It is necessary to set up a schematic representation of the reactions involved in order to justify the qualitative results listed above and also obtain numerical correlations between the data and mathematical consequences of the theory. After many unsatisfactory attempts the following formulation was found usable



$$\begin{tikzcd}
\mathrm{G} + \mathrm{OH}^- \overset{E_1}{=} \mathrm{GOH}^- \arrow[r, shift right, "\mathrm{K}_1"'] & \mathrm{G}' \arrow[l, shift right, "\overset{E_2}{\mathrm{K}_2}"'] \arrow[d, shift right, "\overset{\mathrm{K}_3}{E_3}"'] + \mathrm{OH}^- \arrow[r, shift left, "\overset{E_2}{\mathrm{K}_2}"] & \mathrm{MOH}^- \overset{E_1}{=} \mathrm{M} + \mathrm{OH}^- \arrow[l, shift left, "\mathrm{K}_1"] \\
 & \mathrm{G}'\mathrm{OH}^- \arrow[u, shift right, "\mathrm{K}_4"'] \arrow[d, shift right, "\overset{\mathrm{K}_5}{E_4}"'] & \\
 & \mathrm{G}'' + \mathrm{OH}^- \arrow[u, shift right, "\mathrm{K}_6"'] &
\end{tikzcd}$$

G and M represent tetramethylglucose and tetramethylmannose, respectively, and GOH^- and MOH^- are the ions formed from these by the instantaneous reaction with alkali. G′ and G″ are unidentified molecular substances and $\mathrm{G'OH}^-$ an unidentified ion. These three substances are, however, the materials responsible for the high iodine values.

This scheme satisfies the qualitative requirements of the results in the following manner: (1) It supplies an intermediate substance G′ giving high

TABLE II

ROTATION CHANGES OF TETRAMETHYLGLUCOSE IN ALKALINE SOLUTION AT 35°; SOLUTION MOLAR IN TETRAMETHYLGLUCOSE

Expt	1[a]		2[a]		3[a]		4[a]	
Alkali	$\mathrm{Ca(OH)_2}$		$\mathrm{Ca(OH)_2}$		$\mathrm{Ba(OH)_2}$		$\mathrm{Ba(OH)_2}$	
Normality	0.03		0.04		0.05		0.06	
(OH^-) calcd.	.001		.00136		.0017		.0021	
	Time, hrs.	$[\alpha]^{20}_{\mathrm{D}}$	Time, hrs.	$[\alpha]^{20}_{\mathrm{D}}$	Time, hrs.	$[\alpha]^{20}_{\mathrm{D}}$	Time, hrs.	$[\alpha]^{20}_{\mathrm{D}}$
	3	+79.2	7	+75.5	0.2	+74.4	1.5	+77.4
	19	69.4	29	61.9	6	71.6	6	71.0
	26	66.3	50	55.0	28	56.4	26.5	52.9
	45	58.6	70	50.9	49	49.9	59	45.3
	118	46.6	96	48.2	69	46.1	76	43.7
	144	44.5	121	46.2	95	42.7	102	42.3
	182	43.6	169	44.2	120	42.0	127	41.5
			192	44.0	169	40.2	176	40.9
			250	43.0	191	40.6	193	41

Expt.	5		6		7		8	
Alkali	$\mathrm{Ba(OH)_2}$		$\mathrm{Ba(OH)_2}$		$\mathrm{Ba(OH)_2}$		$\mathrm{Ba(OH)_2}$	
Normality	0.0811		0.1263		0.1561		0.1956	
(OH^-) calcd.	.00287		.0045		.00564		.0071	
	Time, hrs.	$[\alpha]^{20}_{\mathrm{D}}$	Time, hrs.	$[\alpha]^{20}_{\mathrm{D}}$	Time, hrs.	$[\alpha]^{20}_{\mathrm{D}}$	Time, hrs.	$[\alpha]^{20}_{\mathrm{D}}$
	0.5	+72.60	0.5	+76.77	0.5	+81.02	0.083	+75.95
	2.0	71.50	1.5	74.98	1.5	73.97	4.5	62.55
	3.0	70.00	2.5	72.84	3.5	67.77	8.0	55.91
	4.0	69.40	3.5	70.74	46.5	42.97	23.0	42.55
	5.0	68.44	19.0	51.84	51.25	41.92	29.0	40.93
	6.0	67.44	20.0	51.28	70.5	39.78	47.5	39.17
	6.75	66.76			75.5	39.53	52.0	38.22
	22.0	55.58			94.5	38.72	71.0	36.99
	23.5	54.77			99.5	38.08	77.5	36.84
	27.0	53.18			118.5	37.50	96.0	35.4
	29.0	52.03			123.5	37.32	100.0[c]	34.8
	46.0	46.99			142.5	37.03	143.0	29.1
	48.5	42.82			147.25	36.76	147.5	29.0
	51.0	42.06			165.5	36.24	167.0	28.6
	54.0	42			214.5	35.74	167.7[d]	0.72
					220.0[b]	16.52	168.7	3.54
					239.0	31.5	169.5	13.2
					262.5	31.5	191.5	19.1
							196.0	19.1
							215.0	19.6
							220.0	19.8
							239.0	21.2

[a] Taken from paper of Wolfrom and Lewis.[1b] [b] At 217 hours the solution was diluted with an equal volume of approximately six normal hydrochloric acid. [c] Rotations from this point on were made with red light and converted into the sodium light basis by multiplying by 1.16. [d] Before rotation was taken the solution was diluted with an equal volume of approximately three normal hydrochloric acid.

iodine absorption through which the equilibrium between tetramethylglucose and tetramethylmannose may be established. (2) In the instantaneous equilibrium reaction, E_1, it represents the neutralization of alkali on addition of the sugar. As tetramethylglucose and tetramethylmannose are found in equal quantities after the reaction system has been reversed with acid, the same equilibrium constant must represent the relation between tetramethylmannose and hydroxyl ion. (3) As G′ and G″ are formed with the regeneration of OH^-, the reaction system can proceed without any considerable reduction of hydroxyl-ion concentration of the solution. A small reduction may and should take place depending on the values of the equilibrium constants. A greater reduction probably takes place due to destruction of the sugar by small amounts of side reactions. (4) If the formation of G′ is rapid compared to its transformation into G″, it may approach equilibrium with GOH^- and MOH^- before there is any considerable formation of $G'OH^-$ and G″. This accounts for the leveling off of the curves, and its height should be approximately but not exactly independent of hydroxyl-ion concentration. (5) The initial rate of the reaction as given by the iodine absorption values is proportional to the GOH^- concentration. The reaction must, therefore, begin with this ionic substance. (6) A final equilibrium condition is accounted for with the entire system in equilibrium. This again should be approximately but not exactly independent of hydroxyl-ion concentration, although its rate of approach should be more rapid with more concentrated alkali. Small changes in hydroxyl-ion concentration should have but small effect on this final condition, but the complete reversal of the reactions with strong acid shows that irreversible reactions are not involved, if side reactions are neglected. (7) As the scheme in effect represents two steps catalyzed by hydroxyl ions, *i. e.*, the formation of G′ and of G″, it is seen why only a relatively small amount of hydroxyl ion is needed to effect the transformations. (8) If the optical rotations of G′, $G'OH^-$ and G″ are not greatly different from that of an equimolecular mixture of G and M, then the optical data are not in conflict with this scheme.

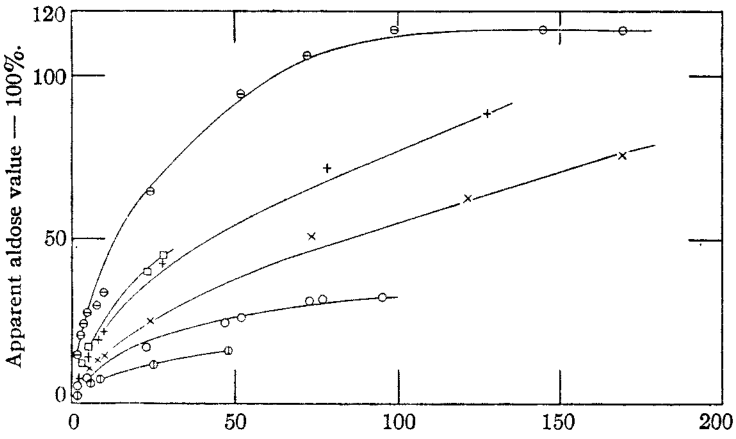


Fig. 2.—Rate of reaction of tetramethylglucose in dilute alkali: Expt. 1, ; Expt. 2, ○; Expt. 3, ×; Expt. 4, +; Expt. 5, □; Expt. 6, ⊖.

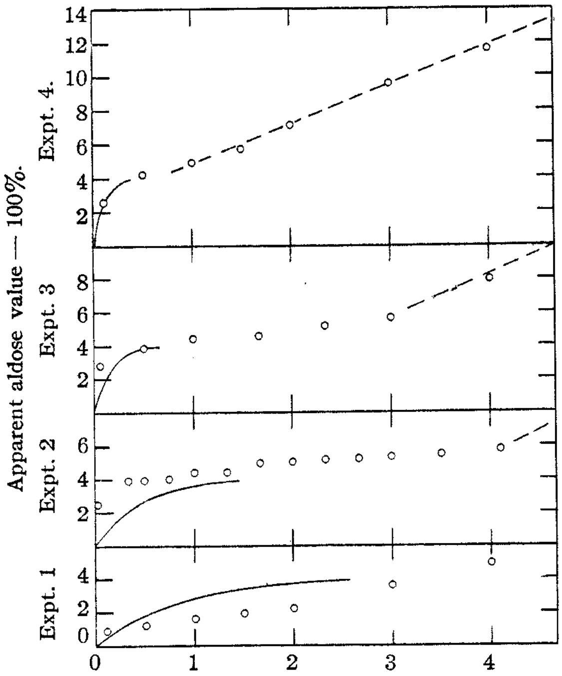


Fig. 3.—Rate of reaction of tetramethylglucose in dilute alkali (first few hours): full line represents theoretical curve; circles, experimental points.

Derivation of Equilibrium Equations.—As the scheme represents a set of equilibria, it is possible to set up equilibrium equations and obtain values for the equilibrium constants. As the hydroxyl ion concentration remains fairly constant for the duration of any one experiment, it will be assumed to be constant. The iodine absorption values are the only experimental data upon which to make the calculations. G and M react with one mole of iodine per mole and it is assumed that GOH^- and MOH^- react with the same amount. This is reasonable, for the apparent aldose value is 100% at the start of the reaction and (the formation of GOH^- and MOH^- is instantaneous) is independent of the alkali concentration. G′, $G'OH^-$, and G″ are assumed to consume three moles of iodine per mole. This is not unreasonable, for there is evidence that the reactions have not gone to completion and apparent aldose values in excess of 200% are obtained. To assume them to have unequal iodine absorption powers increases the mathematical difficulties until they are insurmountable and would not greatly affect the results obtained.[9]

Let B = (G) + (M) + (GOH^-) + (MOH^-)
A = (G′) + $(G'OH^-)$ + (G″)
A + B = total sugar concentration = 1 (our sugar solutions are all 1 molar)
Apparent aldose value for 100% B = 100%
Apparent aldose value for 100% A = 300%
B + 3A = experimental apparent aldose value
2A = experimental apparent aldose value − 100%

$$E_1 = \frac{(GOH^-)}{(G)\,(OH^-)} = \frac{(MOH^-)}{(M)\,(OH^-)} = \frac{(GOH^-) + (MOH^-)}{((G) + (M))(OH^-)} = \frac{(GOH_0^-)}{(G_0)\,(OH^-)} \qquad (1)$$

where (GOH^-_0) and (G_0) are the initial concentrations of GOH^- and G immediately upon making the solution. As $(G_0) + (GOH^-_0) = 1$, (OH^-) is experimentally determined by the glass electrode, and (GOH^-_0) is the reduction of hydrogen ions upon addition of the sugar, E_1 may be readily calculated. In Table III are seen these values with encouraging constancy in the different experiments with the exception of experiment 6, where the high concentration of the alkali may cause a second or third ionization of the sugar to take place. An electrometric titration of the sugar with strong alkali indicates that this does take place. E_1, it should be noted, establishes the primary ionization constant of tetramethylglucose and tetramethylmannose acting as acids.

$$E_2 = (G')(OH^-)/[(GOH^-) + (MOH^-)] \qquad (2)$$

and $E_1E_2 = (G')/[(G) + (M)]$

As the level portion of the rate curve is approximately at an apparent aldose value of 104% (this is closely estimated in treating the rate equations), A = 0.02; and as at this point $(G'OH^-)$ and (G″) may be assumed to be zero, E_2 can be calculated from E_1. This value is given in the table. In making this calculation A is assumed =0.02 for experiment 3 and the theoretical change of this with (OH^-) applied to the calculations for the other experiments.

$$E_3 = (G'OH^-)/(G')(OH^-) \qquad (3)$$

and

$$E_4 = (G'')(OH^-)/(G'OH^-) \qquad (4)$$

Since electrical neutrality is maintained in the solution

$$(GOH^-_0) = ((GOH^-) + (MOH^-)) + (G'OH^-) \qquad (5)$$

As $((GOH^-) + (MOH^-)) = ((G) + (M))E_1(OH^-)$ from eq. 1, and $(G'OH^-) = ((G) + (M))E_1E_2E_3(OH^-)$ from eqs. 1, 2, and 3, and $(G) + (M) = B/[1 + E_1(OH^-)]$ from the definition of B and eq. 1

$$E_3 = \frac{(GOH^-_0)\,(1 + E_1\,(OH^-))}{BE_1E_2\,(OH^-)} - \frac{1}{E_2} \qquad (6)$$

From this equation E_3 is calculated and the results appearing in Table III show fair constancy. An apparent aldose value of 215% at final equilibrium is assumed for all experiments.

Since the total amount of derivatives of tetramethylglucose must remain constant

$$(G_0) = ((G) + (M)) + (G') + (G'') \qquad (7)$$

(G′) and (G″) may be evaluated in terms of ((G) + (M)) from eqs. 1, 2, 3 and 4; then

$$(G_0)(1 + E_1(OH^-)) = B(1 + E_1E_2 + E_1E_2E_3E_4) \qquad (8)$$

E_4 is now calculated from this equation and the results in Table III show remarkable constancy.

The level portion of the curve should vary with

TABLE III

EQUILIBRIUM CONSTANTS

Experiment	1	2	3	4	5	Average
(OH^-) of base[a]	0.0178	0.0329	0.08987	0.1388	0.1620	
(OH^-) of soln. × 1000	.6244	1.094	3.132	4.848	5.945	
(G_0)	.986	0.975	0.932	0.894	0.876	
(GOH^-_0)	.0136	.0252	.0680	.1062	.1237	
E_1	22.12	23.66	23.31	24.51	23.74	23.46
E_2 × 10,000	9.347	9.352	9.346	9.347	9.363	9.351
E_3	1302	1464	1429	1543	1470	1442
E_4 × 100	4.198	4.198	4.198	4.198	4.199	4.198

[a] The increase in volume upon addition of sugar will reduce these figures by 20%.

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(9) Formulas appearing in parentheses represent concentrations.

(OH$^-$) according to the equation (G$'$) = $E_1E_2/[1 + E_1E_2 + E_1(\mathrm{OH}^-)]$ from eqs. 1 and 2, as B in this case = 1 − (G$'$). As the variation in E_1(OH$^-$) is small compared to $1 + E_1E_2$, the level of the curve should not change greatly. In a similar manner it can be shown that the variation of the final condition of equilibrium with (OH$^-$) should satisfy the equation

$$\mathrm{A} = \frac{E_1E_2 + E_1E_2E_3(\mathrm{OH}^-) + E_1E_2E_3E_4}{1 + E_1(\mathrm{OH}^-) + E_1E_2 + E_1E_2E_3(\mathrm{OH}^-) + E_1E_2E_3E_4}$$

and it will be seen that small changes in (OH$^-$) will affect the final conditions of equilibrium but slightly.

Derivation of First Rate Equation.—It was not found practicable to derive rate equations to deal with the entire scheme of the reaction at one time. The problem was separated into two parts and rate equations derived for each part separately. The first part is the approach to the break in the curves and the second the approach to final equilibrium.

For the first derivation we will assume the forward reaction from G$'$ to be negligible. We will consider G and M and also MOH$^-$ and GOH$^-$ together as this will have no effect upon our results. We will then define a, b and h as the concentrations of G, GOH$^-$ and OH$^-$, respectively, at the start of the reaction, as soon as the instantaneous neutralization of the sugar has taken place. We will also define w and x as the amounts of G and GOH$^-$ consumed in time t. From this it is seen that the concentration of GOH$^-$ at time t is $b - x + w$, that of OH$^-$ is $h - w + x$, and that of G$'$ is x. The rate of change of (GOH$^-$) may be expressed by the equation

$$-\frac{\mathrm{d}(b - x + w)}{\mathrm{d}t} = K_1(b - x + w) - K_2hx - \frac{\mathrm{d}w}{\mathrm{d}t} \qquad (9)$$

As we have assumed constant (OH$^-$), $h - w + x = h$, and $w = x$. Integrating the equation we obtain

$$x = \frac{bK_1}{hK_2}(1 - e^{-hK_2t}) \qquad (10)$$

As x is the concentration of G$'$, it is obtainable from the experimental curves for this first portion of the reaction, for here it is equal to A.

When $t = \infty$, eq. 10 becomes $x_2 = bK_1/hK_2$, where x_2 is the equilibrium value of x. Various values of x_2 were tried in the calculation of K_2 and the closest fit came with $x_2 = 0.02$. As b and h are known for the various experiments, the ratio K_2/K_1 is readily calculated and found to be for expt. 1, 1090, expt. 2, 1153, expt. 3, 1086, expt. 4, 1095, expt. 5, 1040, and an average of 1093. Transforming eq. 10 we have

$$K_2 = \frac{2.303}{ht}\log\frac{1}{1 - 0.02x}$$

Selecting points along the curve drawn to fit experiment 3 values of K_2 are found. These are shown in Table IV. Their average is 33.78, whence K_1 = 0.03091. Using these values of K_1 and K_2 theoretical curves for experiments 1, 2, 4 and 5 are calculated and tabulated in Table V. Their agreement with the experiments is shown in Fig. 3.

TABLE IV

RATE CONSTANT K_2 FROM EXPERIMENT 3

Time, minutes	Apparent aldose value	x	K_2
3	101.14	0.0057	35.76
6	101.91	.00955	34.61
9	102.46	.0123	33.94
12	102.88	.0144	33.94
15	103.16	.0158	33.33
20	103.49	.01745	33.03
25	103.70	.0185	33.30
30	103.83	.01915	33.95
35	103.90	.0195	34.15
40	103.92	.0196	31.76
		Average	33.76

TABLE V

CALCULATED RATE CURVES FOR FIRST PART OF REACTION

x (selected)	Apparent aldose value	Calcd. time in minutes, expts. 1	2	3	4
0.0025	100.5	6.3	3.6	0.8	0.7
.005	101.0	13.7	7.9	1.8	1.4
.0075	101.5	22.3	12.7	2.9	2.3
.010	102.0	32.9	18.8	4.2	3.5
.0125	102.5	46.6	25.9	6.0	4.9
.014	102.8	57.2	32.7	7.4	6.0
.015	103.0	65.9	37.6	8.5	6.9
.016	103.2	76.6	45.6	9.9	8.0
.017	103.4	90.3		11.6	9.5
.018	103.6	109.8	62.6	14.1	11.5
.019	103.8	143.3	81.8	18.5	15.1
.020	104.0	∞	∞	∞	∞

Derivation of Second Rate Equation.—The derivation of equations from which K_3 and K_4 may be calculated is accomplished by assuming the reaction forming G$'$ at equilibrium at the start of the subsequent reaction. This gives us a new zero time for the rate equation. We will continue to assume the hydroxyl ion concentration constant. The terms α, β, and γ are defined as the concentrations at zero time of G + M, GOH$^-$ + MOH$^-$, and G$'$, respectively, and w, x, y, and u the amount of G + M, GOH$^-$ + MOH$^-$, G$'$, and G$'$OH$^-$ consumed in time t. From these it is seen that $\alpha - w$, $\beta - x + w$, $\gamma - y + x$, $y - u$, and u are the concentrations of G + M, GOH$^-$ + MOH$^-$, G$'$, G$'$OH$^-$ and G$''$, respectively, at time t. The rate of change of G$'$ with time is given by the equation

$$-\frac{\mathrm{d}(\gamma - y + x)}{\mathrm{d}t} = K_3(\gamma - y + x)h - K_4(y - u) - \frac{\mathrm{d}x}{\mathrm{d}t} \text{ or}$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = K_3h(\gamma - y + x) - K_4(y - u) \qquad (11)$$

We know that $\alpha + \beta + \gamma = 1$, $E_1 = b/ah = \beta/\alpha h = (\beta - x + w)/(\alpha - w)h$, $E_2 = \gamma h/\beta = (\gamma - y + x)h/(\beta - x + w)$. We also know that $(\text{GOH}^-_0) = (\text{GOH}^-) + (\text{MOH}^-) + (\text{G}'\text{OH}^-)$, that is, $\beta = \beta - x + w + y - u$ or $u = w - x + y$. From these equations we can obtain expressions for α, β and γ in terms of E_1, E_2 and h and can also obtain expressions for x and u in terms of y, E_1, E_2 and h. Placing these expressions for γ, x, and u in eq. (11) and integrating, we obtain

$$t = \frac{1}{Z} \ln \frac{K_3\gamma h + yZ}{K_3\gamma h}$$

where

$$Z = \frac{-E_1h(K_4 + K_3E_2)}{1 + E_1h + E_1E_2} \qquad (12)$$

Converting this into a series expansion and transforming the variable x into y, we obtain

$$\frac{-xE_1\left(\frac{K_4}{K_3} + E_2\right)}{(1 + E_1h)\gamma} = tZ + \frac{(tZ)^2}{!2} + \frac{(tZ)^3}{!3} + \cdots \qquad (13)$$

TABLE VI

SECOND RATE EQUATION, CORRELATION WITH EXPERIMENTS

Time in min.	Expt. 1 % apparent aldose −100%			Expt. 2 % apparent aldose −100%			Expt. 3 % apparent aldose −100%			Expt. 4 % apparent aldose −100%		Expt. 5 % apparent aldose −100%	
	Expt.	Calcd.	$K_4 \times 1000$	Expt.	Calcd.	$K_4 \times 1000$	Expt.	Calcd.	$K_4 \times 1000$	Expt.	Calcd.	Expt.	Calcd.
120												9.8	6.4
240												12.0	8.1
330												14.3	9.9
600												17.3	12.3
1200	10.2	7.4	2.+	16.4	9.9	1.059	22.2	20.1	1.245	35.3	28.2	41.9	35.6
1800	12.6	9.4	1.725	20.9	15.3	0.9380	28.4	28.2	1.105	44.5	39.1	56.5	45.8
2400	14.6	11.4	1.535	24.4	16.5	.9220	34.0	35.6	1.030	52.1	48.5	69.2	56.2
3000	16.1	13.4	1.400	26.9	19.7	.8460	39.4	42.5	0.9710	58.4	56.7	79.4	65.6
3600	17.3	15.2	1.270	28.9	22.7	.8100	44.3	49.0	.9200	63.8	64.0	87.4	74.0
4200	18.3	17.0	1.190	30.4	25.6	.7875	48.8	54.3	.9060	68.7	70.2	93.7	81.6
4800	19.1	18.9	1.050	31.4	28.6	.7635	52.5	59.5	.8785	73.1	75.3	99.1	88.1
5400			0.9550	32.0	31.4	.7500	56.0	64.3	.8395	77.3	81.7	103.4	94.5
6000			.8735	32.5	34.1	.7315	59.0	68.4	.8275	81.1	85.7	106.6	100.4
6600						.7095	61.6	71.6	.8165	84.8	90.4	109.3	106.2
7200						.6930	64.2	75.6	.8010	88.0	94.4	110.5	111.6
7800						.6820	66.6	78.0	.8000	91.2	98.4	111.6	116.9
8400						.6750	68.9	81.9	.7930	94.4	102.6	112.7	122.0
9000						.6570	71.3	87.5				113.7	127.0
9600						.6518	73.7	89.4					
10200						.6460	76.2	91.9					

Start of curve taken after the lapse of 200 minutes for expt. 1, 186 min. for expt. 2, 131 min. for expt. 3, 75 min. for expt. 4, and 20 min. for expt. 5. Grand average $K_4 = 0.0009264$.

As $\gamma = E_1E_2/(1 + E_1(h + E_2))$ and $K_3 = E_3K_4$, numerical values of K_4 may be obtained from the experimental data by the use of eq. (13). The values of the equilibrium constants have been determined previously, h is known, and A (obtained from the height of the curve) = (G$'$) + (G$'$OH$^-$) + (G$''$) = $x + \gamma$. Conversely, once the value of K_4 is found, theoretical curves may be calculated. The correlation with the experiments is tabulated in Table VI and a typical example shown graphically in Fig. 4. The agreement is rather remarkable, particularly as the time of the start of this rate curve is but an estimation. The deviation of the theoretical from the experimental curves is in the direction to be expected, for the theory is based on the assumption of constant hydroxyl ion concentration. As the reaction proceeds there is a small amount of destruction of sugar by side reactions, which would make the experimental curves lower than the theoretical. These side reactions also reduce the hydroxyl ion concentration and lower the experimental curve by lowering the rate of the reaction.

When an attempt is made to solve for K_5 and K_6 in a manner similar to that used for K_3 and K_4, it is found that they are infinite. This means that the equilibrium expressed by E_4 is instantaneously reached.

Application to Rotation Curves.—It was not found possible to apply this theory to the rotation curves over their entire course, as a single equation treating the entire series of reactions simultaneously was not obtainable and the rotations of the substances present other than tetramethylglucose and tetramethylmannose are unknown. A correlation can be found, however, for the first few hours, as here the amounts of these other substances are small. We shall follow the scheme used in our first rate derivation. The forward reaction from G′ will be considered negligible. We shall in addition define x' and y as the amounts of MOH⁻ and G′, respectively, consumed in time t, whence $y - x'$ is the concentration of MOH⁻ at time t. The change of (MOH⁻) with time is represented by the equation

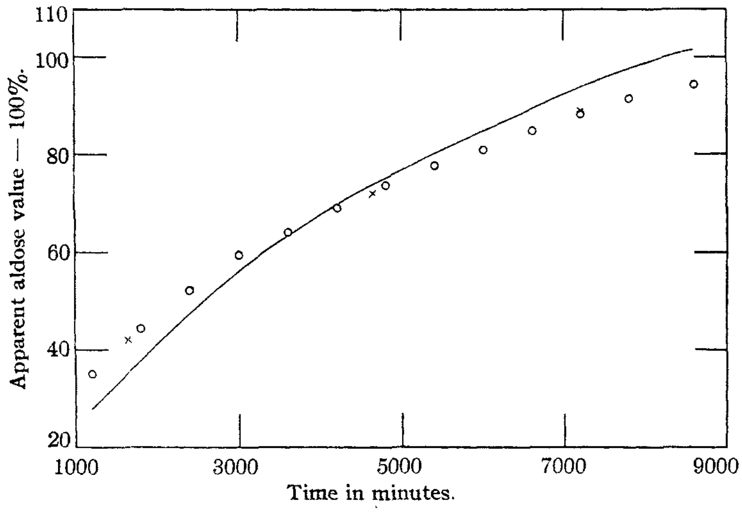


Fig. 4.—Experiment 4: comparison of curve from second rate equation with experiment. Full line represents theoretical curve; circles, points taken from experimental curve; crosses, experimental points.

$$-\frac{\mathrm{d}(y - x')}{\mathrm{d}t} = -\frac{1}{2}K_2h(\mathrm{G}') + K_1(y - x') + \frac{\mathrm{d}x'}{\mathrm{d}t} \quad (14)$$

The fraction ½ appears because the backward reaction from G′ forms equal quantities of GOH⁻ and MOH⁻. (G′) is given from our first rate derivation as

$$\frac{hK_1}{hK_2}(1 - e^{-hK_2t}) \qquad b = \frac{E_1H}{E_1h + 1}$$

As $E_1 = (y - x')/x'h$, $x' = y/(E_1h + 1)$. Eq. (14) simplifies to

$$\frac{\mathrm{d}y}{\mathrm{d}t} + K_1by = {}^1/_2bK_1(1 - e^{-hK_2t}) \quad (15)$$

On integration this becomes

$$2y = 1 + \frac{hK_2}{K_1b - hK_2}e^{-K_1bt} - \frac{K_1b}{K_1b - hK_2}e^{-hK_2t} \quad (16)$$

As $K_1b = 0.02\ hK_2$

$$y = 0.5 - 0.511\ e^{-0{,}02hK_2t} + 0.0102\ e^{-hK_2t} \quad (17)$$

y represents the total concentration of M plus MOH⁻, and MOH⁻ is assumed to have the same rotation as M. Similarly GOH⁻ is assumed to have the same rotation as G. The rotation of G′ is neglected as its concentration is small and its rotation quite likely is not greatly different than a racemic mixture of G and M. The rotation, therefore, is 82.0–79.6 y.

From eq. (17) theoretical curves for the change in rotation for the first few hours may be calculated. The agreement between these and the experimental curves is shown in Fig. 5. The rotation change with time for a solution of tetramethylmannose may be treated in a similar manner.

Discussion

All the equilibrium and rate constants have been calculated which appear in the assumed

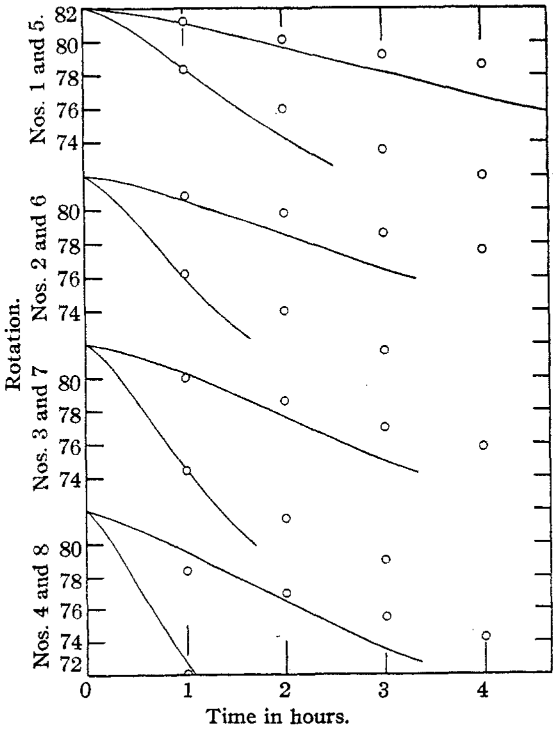


Fig. 5.—Start of rotation curves: full lines represent theoretical curves; points are taken from smooth curve drawn on plot of experimental curves.

scheme of the reaction. As the correlation of these with the experiments seems to be fairly good, the theory is substantiated to this extent.

The formulas used for the molecular species involved, G, M, GOH^-, etc., are treated without prejudice in regard to the structure of these substances; and any structural formulas which would give the required series of reactions would satisfy the theory. For example, the neutralization equation has been written for convenience $G + OH^- = GOH^-$. It could just as well have been written $HG = G^- + H^+$ and should be, if the chemistry involved showed the latter to be more nearly correct.

If this theory is correct there are several consequences of interest.

(1) The reaction of tetramethylglucose and tetramethylmannose with dilute alkali and by analogy probably the similar reactions of other sugars begins with the instantaneous formation of a sugar ion. This ion is the reactant from which subsequent products are formed.

(2) The final substance G″ is formed in at least two steps each catalyzed by hydroxyl ion. The mechanism for these steps is given in the theory as the addition of hydroxyl ion and its subsequent loss in forming a new substance.

(3) There is not one intermediate substance involved, the ene-diol of older theories, but at least two such substances and also at least two ionic forms.

(4) A mechanism for the migration of a hydrogen atom from one place in the molecule to another may be deduced from this theory and in this way explain the previously postulated tautomeric shift.

(5) The theory in no way conflicts with the assumption of an ene-diol. It does, however, enlarge upon the mechanism of its formation.

(6) The reason for the difficulties encountered in the attempted isolation of the intermediate substance or substances is explained, for it is seen that these react with both concentrated acids and bases, and most methods of attempting to obtain them involve the use of these reagents. We are able to suggest methods of obtaining G′ and G″ in a solution in their greatest concentration relatively stable, and with the least amount of other substances present, when a one molar sugar solution is used. The maximum concentration of G′ is 0.02 molar and can be obtained by allowing a molar solution of tetramethylglucose in 0.03 normal alkali to stand for about two hours at room temperature and out of contact with air. The solution should then be treated with acid, until the *p*H is about 7.5 to 7.7, and no further change of hydrogen ion concentration should be made. G″ can be obtained at a concentration of about 0.55 molar by allowing a molar solution of tetramethylglucose in about 0.14 normal alkali to stand for about 160 hours at room temperature or slightly above out of contact with air. At this time the solution should be saturated with carbon dioxide and no further change of the hydrogen ion concentration made.

There are several points in which our theory is not complete, and we are interested in gathering data to clarify them. The theory shows that the two equilibrium conditions should become lower (that is, smaller (G′) and (G″)), as the concentration of the alkali is increased, although this would be beyond the limits of the precision of our measurements. On the other hand, high acidity reforms the original sugars. This suggests that there is a different set of reactions for the acid solutions probably involving hydrogen ions instead of the hydroxyl ions as we have represented. These steps involving hydrogen ion, which we have ignored, may also account for the slowness of approach to the maximum equilibrium value of the curves with the more dilute alkali and the apparent abnormality of the curve with the most dilute alkali.

The concentration of hydroxyl ion during the course of the reaction cannot theoretically remain absolutely constant even ignoring side reactions, as we have assumed it. Unless there is some reduction in this concentration the reactions could not proceed. More critical studies of this might produce results of value.

If the latter portions of the rate curves are extrapolated back ignoring the break, they do not strike the origin or to the left of it but reach the time coördinate at a finite time. This indicates that there is still another step in the series of reactions. The fact that the length of the break is roughly inversely proportional to the hydroxyl ion concentration indicates that this is an additional step catalyzed by hydroxyl ion. Further work using different sugar concentrations, temperatures and other solvents might solve this point. This further indicated step would not vitiate any of the results herein reported.

The deviation of the theoretical rotation curves from the experimental is in the direction to be expected from the assumptions made in deriving the equation. The indication of the probability of additional substances between G′ and MOH⁻ is also brought out here, for in that case the formation of MOH⁻ and M would be slower than given by our theoretical calculations.

The assumption of the consumption of three mols of iodine per mole of the substances G′ and G″ suggests from the possible structural formulas of these substances that one mole of carbon dioxide is formed for each mole oxidized. We propose to make this test.

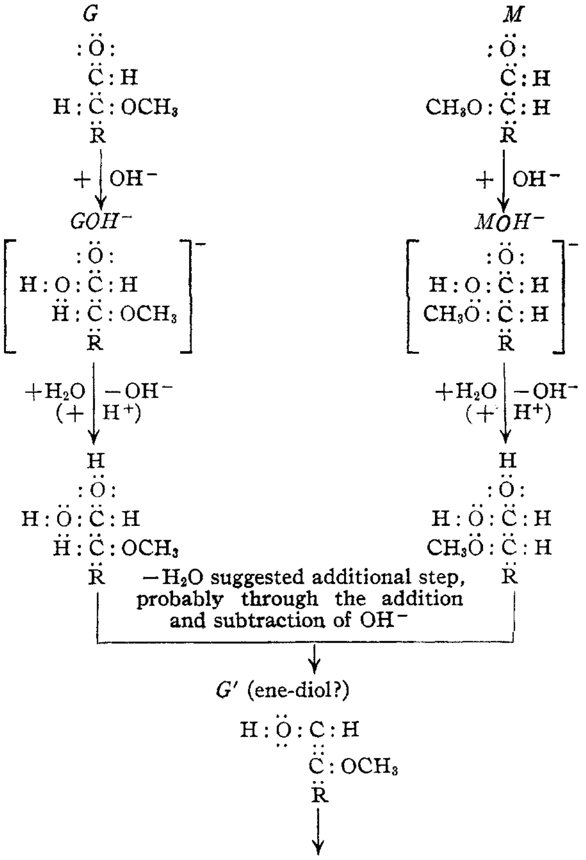
The drop in optical rotation upon the addition of concentrated acid to the reaction mixture at equilibrium suggests the instantaneous formation of a substance of low rotatory power, which then slowly is transformed into tetramethylglucose and tetramethylmannose by some unknown steps. This strengthens our assumption that the reverse reaction with acid proceeds through a different series of reactions than the forward reaction with alkali. This phenomenon may furnish the means of studying the reverse reaction.

We offer the following as an example of how structural formulas may be fitted into our theory.

As this example is only an attempt at an illustration of the application of our theory to structural formulas, we do not present it as even a tentative mechanism. Its truth or falseness does not prejudice the theory.

The method of attack pursued in this work offers possibility of use in other problems, particularly those involving intermediate substances that are difficult or impossible of isolation. Further work may solve some of the perplexing structural problems of sugar chemistry. We have in view doing work along these lines on pentamethylglucose, on dimethylglyceraldehyde, and on other substances.

We wish to express our appreciation for the assistance and encouragement of Dr. W. Lee Lewis, who directed the organic chemical work involved. The problem grew out of some of his researches.



Subsequent reactions to produce a more stable form of G′ as for example the addition of water or formation of a ring.

Summary

A study of the action of dilute alkali upon tetramethylglucose has been made using physical chemical data.

A theory of the mechanism of the reaction has been deduced and applied to the data.

The equilibrium and rate constants employed in this theory have been calculated from the experiments, and fair constancy over a series of experiments has been obtained.

The theory has been also applied to the rate of change of rotation of the sugar solution with time.

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