For large values of the time (*i.e.*, greater than about an hour) equation (15) reduces to

$$\frac{1}{(a-y)^2} = \frac{2k_1}{0.01}\left[t - \frac{1}{k_2}\right] + \frac{1}{a^2} \qquad (16)$$

This equation predicts a linear dependence of $1/(a-y)^2$ on $[t - (1/k_2)]$ with a slope equal to $2k_1/0.01$ and intercept equal to $1/a^2$. The results of plotting (using $k_2 = 0.14$ as previously) the data of section 2 of Table II are shown in Fig. 5. The value of k_1 so calculated is 0.29, in good agreement with the previous value of 0.31. The intercept is $1/a^2$. Deviation occurring at times greater than 16–20 hours is probably due to the side reaction (1), which produces polythionate, becoming important.

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[CONTRIBUTION FROM THE APPLIED PHYSICS LABORATORY, THE JOHNS HOPKINS UNIVERSITY]

The Kinetics of Hexose Formation from Trioses in Alkaline Solution[1]

BY WALTER G. BERL AND CHARLES E. FEAZEL

The kinetics of the formation of D,L-sorbose and D,L-fructose by the condensation of D,L-glyceraldehyde in sodium hydroxide solution have been investigated, using a colorimetric method, based on the anthrone–sulfuric acid reagent, for the quantitative estimation of the hexoses. The reaction was found to follow first-order kinetics at a given concentration of alkali. The effects of variables such as sodium hydroxide concentration, temperature and the initial concentration of glyceraldehyde have been investigated. An increase in rate on the addition of dihydroxyacetone to the reaction was observed, confirming the work of earlier investigators.

The products of the reaction were subjected to paper partition chromatography to demonstrate the virtual absence of triose at the end of the reaction. It is suggested that a branched-chain hexose may be formed as a by-product in the condensation.

The production of hexoses from D,L-glyceraldehyde in alkaline solution was first studied by Emil Fischer[2a] who obtained a mixture of sugars later identified as D,L-fructose and D,L-sorbose by Schmitz.[2b] This work was repeated by Fischer and Baer, using D-glyceraldehyde as a starting material and barium hydroxide solution as the medium.[3] The use of optically active material allowed them to follow the course of the reaction polarimetrically, and to show that the addition of dihydroxyacetone greatly increased the rate of reaction. The interpretation of these data was that in the condensation of glyceraldehyde, the primary step is the conversion of glyceraldehyde to dihydroxyacetone, followed by reaction between the two to form the ketohexoses. The yields of D-fructose and D-sorbose were estimated to be 50 and 44%, respectively, by precipitation and separation of the phenylosazones.

Meyerhof and Schulz[4] studied the condensation of glyceraldehyde with dihydroxyacetone in trisodium phosphate solution and regarded it as coming to a triose–hexose equilibrium containing about 92% hexose.

We have investigated the kinetics of the condensation of D,L-glyceraldehyde in dilute sodium hydroxide solution, using as an analytical tool the anthrone–sulfuric acid colorimetric test for hexoses.[5–9]

(1) This investigation was supported by the Bureau of Ordnance, U. S. Navy, under contract NOrd 7386. Part of this work was submitted by C. E. Feazel as a thesis toward the requirements for the M.S. degree at the University of Maryland, June, 1950.

(2) (a) E. Fischer and J. Tafel, *Ber.*, **20**, 2566, 3384 (1887); (b) E. Schmitz, *ibid.*, **46**, 2327 (1913).

(3) H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **19**, 519 (1936).

(4) O. Meyerhof and W. Schulz, *Biochem. Z.*, **289**, 87 (1936).

(5) R. Dreywood, *Anal. Chem.*, **18**, 499 (1946).

(6) L. Sattler and F. W. Zerban, *Science*, **108**, 207 (1948).

(7) E. E. Morse, *Anal. Chem.*, **19**, 1012 (1947).

(8) D. L. Morris, *Science*, **107**, 254 (1948).

(9) F. J. Viles and L. Silverman, *Anal. Chem.*, **21**, 950 (1949).

Experimental

Materials.—Crystalline D,L-glyceraldehyde (m.p. 142–144°) from Concord Laboratories, and crystalline dihydroxyacetone (m.p. 75–80°) from Bios Laboratories, were used. Anthrone was prepared by the method of "Organic Syntheses,"[10] and dissolved in C.P. concentrated sulfuric acid to give a 0.2% solution. This reagent is unstable and must be used within a few days of its preparation. It also gives better results if allowed to stand at room temperature for at least eight hours before use.

Apparatus.—The kinetic determinations were made in a constant temperature bath, the temperature of which was controlled to within ±0.1°. A Klett–Summerson test-tube photoelectric colorimeter equipped with a Baird Associates 6275 Å. filter[9] was used for the colorimetry. The *p*H was measured with a Leeds and Northrup *p*H meter.

Use of the Anthrone Reagent.—The applicability of Beer's law to the hexose–anthrone color has been demonstrated,[9] and was checked in our work with standard solutions of D-glucose, D-fructose and L-sorbose. The anthrone reagent used in the kinetic work was standardized before each experiment with a solution of D-glucose. In order to convert the colorimeter readings to fructose and sorbose concentration, it was necessary to apply an empirical correction factor, due to the fact that while fructose was found to give the same color intensity as glucose for equal concentrations, the intensity of the sorbose–anthrone color was 76% of the glucose–anthrone color, for the same concentrations. Therefore, assuming that fructose and sorbose were formed in equal amounts during the course of the reaction, the fructose–sorbose equivalent of the colorimeter reading was equal to the glucose equivalent multiplied by 1.13. The presence of glyceraldehyde did not interfere appreciably with the intensity at 6275 Å. of the hexose–anthrone color and could be neglected.

Experimental Procedure.—In carrying out the runs, weighed samples of D,L-glyceraldehyde were dissolved in 80–90 ml. of water in a 100-ml. volumetric flask, which was then immersed in the constant temperature bath. Then a measured volume of 0.1000 *N* carbonate-free sodium hydroxide solution (also at reaction temperature) was added and the contents of the flask diluted to 100.0 ml. A 1.0 ml. sample was removed and diluted to an appropriate volume in a volumetric flask. This latter volume was chosen so that 1.0 ml. of the contents would have a triose content of 40–50 γ. From this flask two 2.0 ml. aliquots were pipetted into Klett–Summerson test-tubes. To each

(10) K. H. Meyer, "Organic Syntheses," Coll. Vol. I, 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1946, p. 60.

tube was added 4.0 ml. of 0.2% anthrone–sulfuric acid reagent from a constant head buret. The tubes were allowed to cool in air to room temperature and the intensity of the color was measured in the colorimeter. This process was repeated at intervals during the determination. After conversion of the colorimeter readings into hexose concentration, a curve such as that in Fig. 1 was obtained for each experiment. Each point represents the average of duplicate readings.

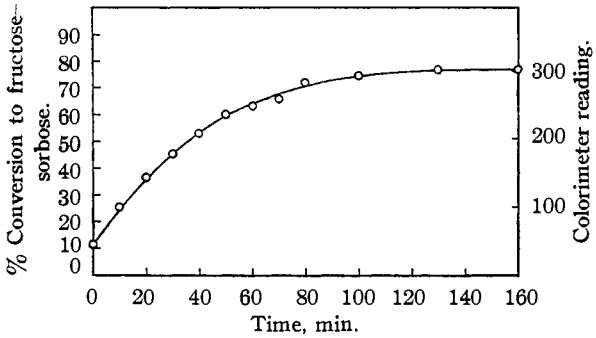


Fig. 1.—Formation of fructose and sorbose from 500 mg. of glyceraldehyde in 100.0 ml. of 0.0100 *N* sodium hydroxide at 20°.

The total error involved in the kinetic determinations was estimated at ±5%, by carrying out the experimental operations on solutions of D-fructose. Duplicate kinetic experiments agreed to within this amount. Solutions of D-fructose in 0.0100 *N* sodium hydroxide showed a decrease of 14% in hexose content after standing 25 hours at 20°. During the condensation reaction, therefore, the effect of alkali on the hexoses produced would not be detectable.

Kinetic Results

In order to calculate rate constants for the condensation of glyceraldehyde, assumptions had to be made about that portion of the triose which was not converted into fructose and sorbose. It was assumed that the disappearance of the glyceraldehyde occurred by several simultaneous and irreversible reactions, all of the same kinetic order. Therefore, the difference between the initial and final colorimeter readings represented the total amount of glyceraldehyde which was converted into fructose and sorbose. It was found that the kinetic data could be fitted satisfactorily into the simple first-order equation for any given experiment. However, the value of the rate constant thus obtained was found to be dependent on both the concentration of hydroxyl ion and the initial concentration of glyceraldehyde, as shown in Tables I and II.

TABLE I

DEPENDENCE OF RATE CONSTANT ON INITIAL GLYCERALDEHYDE CONCENTRATION

Initial concn. of glyceraldehyde[a]	Rate constant, min.$^{-1}$	Final % conversion	Calcd. rate constant, min.$^{-1}$ [b]
75	0.034	55	4.4
125	.032	68	4.8
250	.031	85	6.6
500	.025	77	8.6
750	.019	91	9.0
1000	.015	76	8.8
2000	.0084	76	8.4

[a] mg./100.0 ml. of 0.0100 *N* NaOH at 20°. [b] For unit hydroxyl ion concentration.

TABLE II

DEPENDENCE OF RATE CONSTANT ON SODIUM HYDROXIDE CONCENTRATION

Initial concn. of glyceraldehyde[a]	NaOH, normality	Rate constant, min.$^{-1}$	Final % conversion
500	0.0050	0.012	84
	.0100	.025	77
	.0200	.046	86
1000	.0100	.015	76
	.0200	.029	83

[a] mg./100.0 ml. at 20°.

The variation of rate constant with initial glyceraldehyde concentration may be partially explained by assuming that glyceraldehyde, like other sugars, behaves as a very weak acid, lowering the effective hydroxyl ion concentration of the solution. The true concentration of hydroxyl ion may be calculated by assuming a dissociation constant of 5×10^{-13} for glyceraldehyde,[11] and using the procedure of Glasstone.[12] This leads to the figures shown in the last column of Table I. The calculated rate constant for a unit hydroxide ion concentration is approximately the same for all experiments except those with very low starting concentrations of glyceraldehyde.

The effect of temperature on the rate constant is shown in Table III. The Arrhenius activation energy may be computed from the slope of the plot in Fig. 2 as 24.5 kcal. per mole.

TABLE III

TEMPERATURE DEPENDENCE OF RATE CONSTANT

Initial concn. of glyceraldehyde[a]	Temp., °C.	Final % conversion	Rate constant, min.$^{-1}$
500	0.0	88	0.0013
	14.0	79	.0096
	20.0	77	.025
	25.0	68	.056
1000	20.0	76	.015
	25.0	81	.025

[a] mg./100.0 ml. of 0.0100 *N* sodium hydroxide.

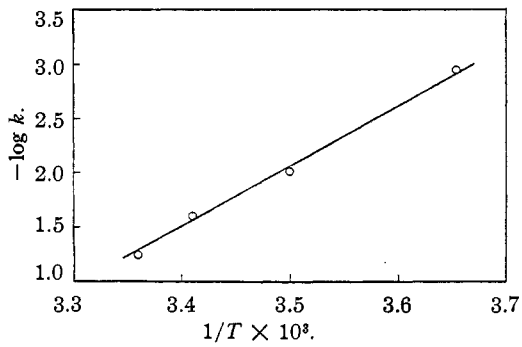


Fig. 2.—Log k *vs.* $1/T$ for glyceraldehyde condensation.

Sodium chloride was added to the reaction to study the primary salt effect, in the hope of obtaining information about the nature of the particles entering into the rate-determining reaction. The rate constants for the condensation of 500 mg. of glyceraldehyde in 100.0 ml. of 0.0100 *N* sodium hydroxide at 20°, containing C.P. sodium chloride in concentrations of 0.0100 and 0.0200 mole per

(11) L. Michaelis and P. Rona, *Biochem. Z.*, **49**, 232 (1913).

(12) S. Glasstone, "Introduction to Electrochemistry," D. Van Nostrand Co., Inc., New York, N. Y., 1942, p. 393.

liter, were 0.024 and 0.024 min.$^{-1}$, respectively. This absence of a salt effect indicates that the rate-determining step involves a neutral molecule.

The effect of adding dihydroxyacetone to the reaction was to increase greatly the rate of hexose formation, and decrease the yield, in agreement with the results of Fischer and Baer. Table IV shows the rate constants for experiments with both trioses, as well as with dihydroxyacetone alone. The reaction in all cases was found to be first order with respect to the total triose concentration.

TABLE IV

EFFECT OF DIHYDROXYACETONE ON RATE CONSTANT

Concentration of reactants[a]				
Glyceraldehyde	Dihydroxyacetone	NaOH normality	Rate constant min.$^{-1}$	Final % conversion
250	250	0.0100	0.047	70
250	250	.0050	.034	57
500	500	.0100	.049	69
0	500	.0100	.012	32

[a] mg./100.0 ml. at 20°.

The Nature of the Reaction Products

Since the total yield of fructose and sorbose amounts to only 75–90% in the condensation of glyceraldehyde, and is even lower when dihydroxyacetone is added to the reaction, a considerable portion of the starting material must have been converted into other products, or else have remained in equilibrium with the hexoses. The virtual absence of glyceraldehyde in the reaction products was shown by paper partition chromatography; other possible by-products such as pyruvaldehyde and lactic acid were also found to be absent.

An additional side reaction is indicated by the recent isolation, in 45% yield, of a branched-chain ketohexose from the alkaline condensation of dihydroxyacetone under conditions similar to those used in this work.[13] The formation of this substance in the condensation of mixtures of the two trioses, or even when glyceraldehyde alone is the starting material, would account for the incomplete conversion of the trioses to straight-chain hexoses. Since 5-hydroxymethylfurfural is presumed to be an intermediate in the anthrone reaction,[6] a branched-chain hexose would not appear in our yield figures.

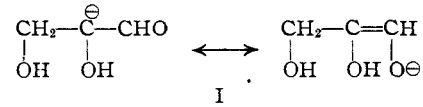
The paper partition chromatography of the reaction products was carried out on strips of Whatman No. 1 filter paper in the usual type of apparatus[14] with *n*-butanol as a solvent, using the solution resulting from the condensation of 2.000 g. of glyceraldehyde in 100.0 ml. of 0.0100 *N* sodium hydroxide, neutralized with dilute hydrochloric acid. Development of the chromatogram with Tollens reagent showed two slightly separated spots, due to fructose and sorbose, plus a third doubtful spot slightly ahead of these. Samples removed from the reaction mixture before completion of the reaction and chromatographed showed a fourth spot well in advance of the hexoses, due to glyceraldehyde. The sensitivity of the chromatographic method toward glyceraldehyde was tested in parallel experiments; it was found that a solution containing one milligram per milliliter could be detected. Therefore, in the above experiment, the glyceraldehyde present at the end of the reaction must have been less than 100 mg. or 5% of the starting concentration. The absence of higher sugars, such as nonoses, formed by the condensation of triose and hexose, was demonstrated by the above experiment, since it was found in similar experiments that D-glucose and D-gluco-L-*gala*-octose[15] could easily be separated.[16] Chromatography of dihydroxyacetone condensates yielded only one ill-defined spot.

The absence of appreciable amounts of pyruvaldehyde in the reaction products was to be expected upon consideration of the work of Evans[17] on the conversion of trioses to pyruvaldehyde. From his data, first-order rate constants of 0.00113 min.$^{-1}$ and 0.00081 min.$^{-1}$ may be calculated for the conversion of glyceraldehyde and dihydroxyacetone, respectively, to pyruvaldehyde in 1.053 *N* sodium hydroxide solution. Making the assumption that these rate constants are directly proportional to the hydroxyl ion concentration, the corresponding rate constants in 0.0100 *N* sodium hydroxide solution would be 1.07×10^{-5} min.$^{-1}$ and 0.77×10^{-5} min.$^{-1}$. These constants are so small compared to those of the condensation reaction that pyruvaldehyde formation must take place to a negligible extent under the conditions of our experiments. In addition, the Ariyama test for pyruvaldehyde[18] was applied to the products of glyceraldehyde condensation; essentially no more color was formed than from fructose itself.

The formation of any appreciable amount of lactic acid during the condensation was ruled out by the fact that the *p*H of the solution changed very little during the reaction (*e.g.*, from 11.6 to 11.4 in four hours for a solution of 500 mg. of glyceraldehyde in 100.0 ml. of 0.0100 *N* sodium hydroxide).

Discussion

The rate-determining step in the condensation of glyceraldehyde to fructose and sorbose must be connected with the formation of dihydroxyacetone, since the addition of the latter to the reaction causes an increase in rate. The primary process, on the other hand, is probably the formation of the anion resonance hybrid I by the action of a hydroxyl ion on the neutral glyceraldehyde molecule.[19]



I

The transformation of this anion into the anion resonance hybrid of dihydroxyacetone II might involve the intermediate formation of either the neutral enediol or a doubly charged anion. An

(13) L. M. Utkin, *Doklady Akad. Nauk S. S. S. R.*, **67**, 301 (1950).

(14) S. M. Partridge, *Nature*, **158**, 270 (1946).

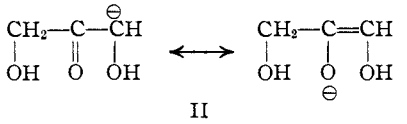
(15) Sample obtained through the kindness of Dr. C. S. Hudson.

(16) E. L. Hirst and J. K. N. Jones, *Discussions Faraday Soc.*, No. 7, 268 (1949).

(17) E. W. L. Evans and W. R. Cornthwaite, THIS JOURNAL, **50**, 486 (1928).

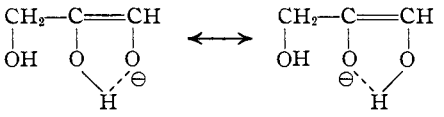
(18) N. Ariyama, *J. Biol. Chem.*, **77**, 359 (1928).

(19) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 231.



II

alternative, less likely, possibility is that both I and II are resonance forms of the same substance due to hydrogen bonding, *e.g.*



If the rate-determining step involves a neutral molecule, as suggested by the primary salt effect data, this step may be the attack of a hydroxyl ion on either the glyceraldehyde molecule or the neutral enediol.

It should be noted that, although the starting materials were dimeric D,L-glyceraldehyde and dihydroxyacetone, the rates of depolymerization should not have to be taken into account. The rate of depolymerization of dimeric dihydroxyacetone has been found to be[20]

$$k = 0.00255 + 4.03 \times 10^{7}[OH^{-}]\text{min.}^{-1}$$

and that of dimeric glycolaldehyde[21]

$$k = 0.0073 + 3.15 \times 10^{6}\,[OH^{-}]\text{min.}^{-1}$$

Furthermore, our experiments showed no difference in reaction rate between freshly prepared solutions of glyceraldehyde and those which had stood at room temperature for two weeks.

Acknowledgments.—We wish to express our appreciation to Dr. G. Forrest Woods, of the University of Maryland, for his interest in this work, and to Mr. M. L. Peller for the preparation of the anthrone.

(20) R. P. Bell and E. C. Baughan, *J. Chem. Soc.*, 1947 (1937).
(21) R. P. Bell and J. P. H. Hirst, *ibid.*, 1777 (1939).

SILVER SPRING, MARYLAND RECEIVED OCTOBER 2, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICS, UNIVERSITY OF MICHIGAN]

Electron Microscopic Observations on the Unit of Length of the Particles of Tobacco Mosaic Virus[1]

BY ROBLEY C. WILLIAMS[2a] AND RUSSELL L. STEERE[2b]

The electron microscope is used to gain evidence of the degree of uniformity of length of the rod-like particles of tobacco mosaic virus. Small droplet patterns, obtained by spraying the virus suspension upon specimen screens, are photographed and the lengths measured of all virus rods in the patterns. Most of the lengths are uniform as measured. The lengths of the few non-uniform rods in each pattern are summed, and the sum divided by an integer. It is found that the division can be performed without remainder in almost all cases, and it is concluded that in the partially purified suspensions examined over 96% of the virus particles exist as either monomers of very uniform length, or as dimers. The length of the monomer of tobacco mosaic virus is found to be 298 ± 1 mμ.

Introduction

In recent years many investigators have examined the distribution in length of the particles of tobacco mosaic virus (TMV) by the aid of the electron microscope. A review of the literature up to the close of 1945 is included in a paper by Sigurgeirsson and Stanley[3] on the distribution in length of the virus particles in unpurified, fresh suspensions and in unpurified, stored suspensions. Since 1945 several papers[4,5] have appeared in which the distribution in length of the TMV particles has been studied as a function of the age of the virus infection, the method of extraction from the plant cells, the *p*H and ionic strength of the extraction and suspending media, and the method of purification of the virus particles.

While the details of the reported observations appear to be somewhat contradictory and confusing, certain generalizations can be made: (1) one type of particle (to be here called the monomer), about 290 mμ in length, is usually found in greatest abundance, with a secondary abundance maximum at twice this most common length; (2) some particles considerably shorter than the monomeric length are always found; (3) any post-extraction treatment of the virus suspensions, such as purification or severe changes in *p*H, causes the distribution curve of lengths to broaden; (4) even for the monomer a fairly broad distribution of lengths is reported, of the order of 100 mμ at the foot of the distribution curve of these particles.

In the investigations concerning the lengths of the TMV particles, as measured on electron micrographs, there is seen a tendency to identify the lengths as measured with those that are presumed to exist in the virus suspensions themselves. It is to be anticipated, however, that in the preparation of electron microscopic specimens the effects of the rapidly varying interfacial surface tensions, which must accompany the drying of a large drop of aqueous material, could radically change the distribution in length of particles as elongated as those of TMV. Such a change might result from the breakage or the aggregation of the particles, or even from multiple events such as aggregation followed by breakage. The net result of these changes *must always be to broaden* the distribution

(1) This research has been supported in large part by a grant from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) (a) The Virus Laboratory, University of California, Berkeley, California; (b) Rockefeller Institute for Medical Research, New York City, New York.

(3) Sigurgeirsson and Stanley, *Phytopath.*, **37**, 20 (1947).

(4) Crook and Sheffield, *Brit. J. Exp. Path.*, **27**, 328 (1946); Oster and Stanley, *ibid.*, **27**, 261 (1946).

(5) Takahashi, Rawlins and collaborators, *Am. J. Bot.*, **33**, 356 (1946); *Phytopath.*, **37**, 73 (1947); **38**, 279 (1948); **39**, 672 (1949); *Am. J. Bot.*, **36**, 642 (1949); *J. Bact.*, **57**, 131 (1949).