Base-Catalyzed Triose Condensations[1]

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Abstract: The aldolization reactions of D-glyceraldehyde and of dihydroxyacetone, catalyzed by inorganic or organic bases, have been shown to yield mixtures of D-fructose, D-sorbose, and DL-dendroketose. Using chemical assays, enzymatic assays, and polarimetric measurements, the previously reported kinetics of the glyceraldehyde aldolization have been substantiated as being first order with respect to the triose and the base. Using dilatometric measurements, the reaction kinetics of the dihydroxyacetone aldolization have been shown to be first order with respect to the triose and the base. The general base catalysis of both reactions has been illustrated in the chemical sense through the use of dilute aqueous solutions of variously substituted pyridines, and in each case the phenomenon of steric hindrance to general base catalysis has been demonstrated. These data are used to compare the two trioses with each other, to compare the trioses with related systems, to reinterpret certain literature inferences, and to set forth an over-all reaction scheme for the triose aldolization system.

A. The Aldolization of D-Glyceraldehyde

One of the important types of reactions occurring in the living cell involves the formation of carbon–carbon bonds *via* aldol-type condensations.[2] A notable example is found in the photosynthesis cycle of plants in which a condensation between glyceraldehyde phosphate and dihydroxyacetone phosphate occurs to form fructose 1,6-diphosphate.[3] As a prelude to the synthesis of polyfunctional catalysts for this system, an investigation of the simple base-catalyzed condensation of the unphosphorylated analogs, *viz.*, dihydroxyacetone and glyceraldehyde, has been undertaken.

The hydroxide ion catalyzed reaction of glyceraldehyde has been the subject of several previous investigations. Earliest among these was the experiment of Fischer and Tafel,[4] in which DL-glyceraldehyde was treated with an alkaline solution to yield a mixture of sugars shown a generation later to be DL-fructose and DL-sorbose.[5] Employing D-glyceraldehyde as the starting material, Fischer and Baer[6] carried out a more carefully controlled condensation and, on the basis of phenylosazone separation and quantitation, stated the mixture to consist of 50% D-fructose and 44% D-sorbose. Concurrently, Meyerhof and Schulz[7] studied the base-catalyzed condensation of glyceraldehyde and dihydroxyacetone and concluded that an equilibrium was established which contained 8% triose and 92% hexose. Data from these several investigations, combined with deuterium incorporation studies[8] and a determination of the reaction kinetics,[9] led to the postulation of a rate-determining conversion of glyceraldehyde to dihydroxyacetone anion followed by a rapid condensation of glyceraldehyde with dihydroxyacetone; the condensation, thus, is an example of a general base catalyzed reaction.

Inasmuch as the design of a polyfunctional catalyst requires a detailed understanding of the mechanism of the reaction to be catalyzed, a reinvestigation of the glyceraldehyde–dihydroxyacetone condensation was initiated in the hope of (a) reconciling discrepancies noted in certain of the earlier studies, (b) discovering catalysts other than hydroxide ion, and (c) gaining further understanding of this reaction in particular and aldol reactions in general.

Composition of Condensation Mixture. Although the hexoses in the reaction mixture from the aldolization of glyceraldehyde appeared, on the basis of the experiments of Fischer and Baer,[6] to be almost entirely fructose and sorbose, the facile interconversion of glyceraldehyde and dihydroxyacetone along with the demonstrated conversion of dihydroxyacetone to dendroketose[10] indicated that the branched-chain hexose might also be formed. Berl and Feazel[9] had already suggested this possibility, and the present work has verified the fact that the base-catalyzed aldolization of D-glyceraldehyde leads to a mixture of D-fructose, D-sorbose, and DL-dendroketose in a ratio depending upon the conditions of the reaction. In a typical condensation, the mixture is comprised of 35–40% D-fructose, 35–40% D-sorbose, and 20–30% DL-dendroketose. Tagatose and psicose, the two other straight-chain hexoses that might also form in the condensation, have been shown by paper chromatographic assay to be present in no more than trace amounts.

Although it had initially been assumed that the equilibrium mixture contained triose,[7,11] paper chromatographic assay indicated that reactions which had proceeded for a sufficiently long time contained too little to detect. The absence, within the limits of sensitivity of the assay methods employed, of trioses as well as nonoses, pyruvaldehyde, and lactic acid in the glyceraldehyde condensation mixtures has also been cited by Berl and Feazel.[9,12]

(1) This work was supported, in part, by Grant No. A-2398 from the National Institutes of Health to whom the authors express their gratitude.

(2) W. J. Rutter, *Enzymes*, **5**, 341 (1961); E. Racker, *ibid.*, **5**, 397, 407 (1961).

(3) M. Calvin and J. A. Bassham, "The Photosynthesis of Carbon Compounds," W. A. Benjamin Co., New York, N. Y., 1962.

(4) E. Fischer and J. Tafel, *Ber.*, **20**, 2566, 3384 (1887).

(5) E. Schmitz, *ibid.*, **46**, 2327 (1913).

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

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

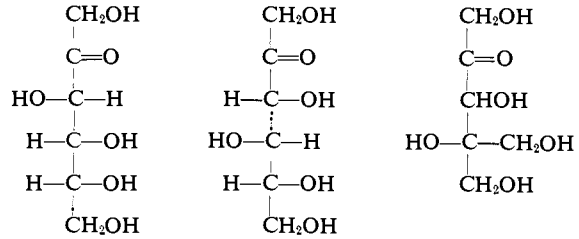
(8) K. F. Bonhoeffer and W. D. Walters, *Z. Physik. Chem.*, **A181**, 441 (1938).

(9) W. G. Berl and C. E. Feazel, *J. Am. Chem. Soc.*, **73**, 2054 (1951).

(10) L. M. Utkin, *Dokl. Akad. Nauk SSSR*, **67**, 301 (1950); *Chem. Abstr.*, **44**, 3910 (1950).

(11) C. D. Gutsche, R. S. Buriks, K. Nowotny, and H. Grassner, *J. Am. Chem. Soc.*, **84**, 3775 (1962).

(12) Upon treatment with base, glyceraldehyde is converted, *inter alia*, to dihydroxyacetone and pyruvaldehyde. To the extent that the



Assay Methods. Of the several colorimetric methods that have been described for the determination of hexoses, the two that were employed in the present investigation were the thiourea–resorcinol procedure[13] and the anthrone procedure.[14] Both of these give reasonably accurate results if control assays are carried through with each set of determinations, but neither one is completely specific for hexoses. In the thiourea–resorcinol test the color values for glyceraldehyde, dihydroxyacetone, and dendroketose relative to fructose are 20, 5, and 18%, respectively; in the anthrone test the values are 95, 78, and 41%. The statement by Berl and Feazel[9] that in the anthrone test as employed in their kinetic investigations "the presence of glyceraldehyde did not interfere appreciably with the intensity at 6275 A" and "a branched-chain hexose would not appear in (the) yield figures" is puzzling; in our experiments both of these materials as well as dihydroxyacetone gave significant color intensities.

If D-glyceraldehyde is used as the starting material, the fructose formed is the naturally occurring D form and, therefore, amenable to enzymatic assay. Through the use of a system of enzymes which catalyze the conversion of fructose → fructose 6-phosphate → glucose 6-phosphate → gluconic acid 6-phosphate,[15] the amount of fructose present was measured by spectrophotometric determination of the amount of nicotinamide-adenine dinucleotide phosphate (NADP) required in the last step of the sequence. Assays on mixtures containing sorbose, glyceraldehyde, dihydroxyacetone, and dendroketose showed that these materials interfered only slightly (in the case of dihydroxyacetone) or not at all (in the case of glyceraldehyde, sorbose, and dendroketose). When carefully executed, the assay is felt to be accurate to ±3% and would appear to give the most reliable estimate of the amount of straight-chain hexose produced in the condensation.

A method for the determination of aldoses, devised by Willstätter and Schudel,[16] involves iodine (hypoiodite) as the oxidizing agent. It is applicable, for instance, to the determination of glyceraldehyde in the presence of fructose and sorbose. In the early phases of this investigation, the Willstätter–Schudel assay was applied to the condensation mixtures, and a value was obtained which was thought to represent unreacted glyceraldehyde. In many instances, the sum of the values of fructose by enzyme assay, sorbose by colorimetric assay, and "glyceraldehyde" by the iodine assay gave a material balance close to 100%. As discussed above, however, the final reaction mixtures are now known to contain little, if any, glyceraldehyde. A brief investigation of the Willstätter–Schudel assay has shown that (a) it is very sensitive to the amount of sample assayed, (b) while fructose and sorbose are not oxidized, dendroketose is oxidized to the extent of 28% and dihydroxyacetone to the extent of 34–65% (depending upon the sample size), and (c) mixtures of glyceraldehyde and dihydroxyacetone undergo oxidation in a capricious fashion. These observations, the explanation for which is obscure, indicate that this method must be used with some caution.

Fructose–Sorbose Ratio. Employing the enzymatic assay for fructose and the thiourea–resorcinol assay for total hexose, the fructose/(sorbose + dendroketose) ratio was obtained for a series of condensations effected with various inorganic bases as shown in Table I. These data are in error to the extent that the sorbose value, *via* the total hexose value, fails to take into account the amount of branched-chain sugar present. However, the results with a given base are replicable, the limits of error in the fructose and total hexose assays are *ca.* ±0.03, and the fact that the ratio shows a small but real dependence on the nature of the cation is thought to be valid. Catalysis of a condensation with a strong base ion-exchange resin resulted in a somewhat higher fructose/(sorbose + dendroketose) ratio which suggests a relationship with the size of the cation. There is, in fact, a rough correlation between the radii of the simple, unhydrated cations and the ratios of fructose/(sorbose + dendroketose).

Table I. Fructose/(Sorbose + Dendroketose) Ratio for Hydroxide Ion Catalyzed Aldolizations of D-Glyceraldehyde

Catalyst	Temp, °C	Time, hr	Fructose/(sorbose + dendroketose) ratio	Cation radius, A
0.05 *N* LiOH	25	2.5	0.89	0.60
0.05 *N* NaOH	25	2.5	1.05	0.95
0.05 *N* KOH	25	2.5	1.21	1.13
0.05 *N* $Sr(OH)_2$	25	2.5	1.11	1.33
0.05 *N* $Ba(OH)_2$	25	2.5	1.17	1.35
Amberlite IRA-400 (OH^-)	25	1.0	1.40	Large

Catalysis by Inorganic Bases. The specific reaction rate constants for hydroxide, carbonate, and bicarbonate ions as base catalysts for the condensation were obtained by carrying out the condensation in carbonate–bicarbonate buffer solutions at three different pH levels. Assuming the reaction to be first order with respect to the glyceraldehyde[8,9] and first order with respect to each of the base species present, the rate expression can be expressed as

$$-d[GA]/dt = [GA](k_{OH^-}[OH^-] + k_{CO_3^{2-}}[CO_3^{2-}] + k_{HCO_3^-}[HCO_3^-])$$

latter takes place, the hexoses $CH_2OHCHOHCHOHCH_2COCHO$ and $CH_3COCHOHCHOHCOCH_2OH$ might be present in the reaction mixture. It has been pointed out by Berl and Feazel,[9] however, that the data of Evans [W. L. Evans and W. R. Cornthwaite, *J. Am. Chem. Soc.*, 50, 486 (1928)] indicate that in dilute sodium hydroxide solution the rate of pyruvaldehyde formation is so slow (compared to the condensation reaction) that its presence can be disregarded.

(13) J. H. Rose, J. H. Epstein, and N. P. Goldstein, *J. Biol. Chem.*, 178, 839 (1949).

(14) F. J. Viles and L. Silverman, *Anal. Chem.*, 21, 950 (1949); for additional references see J. E. Hodge and B. T. Hofreiter in "Methods in Carbohydrate Chemistry," Academic Press Inc., New York, N. Y., 1962, p 389.

(15) We are indebted to our former colleague, Professor R. K. Crane, now at the Department of Physiology, Rutgers University, for his aid in working out this method.

(16) R. Willstätter and G. Schudel, *Ber.*, 51, 780 (1918).

Table II. Rates of Reaction of Inorganic Base Catalyzed Aldolization of D-Glyceraldehydes[a]

pH	[OH], M	[Na+], M	pK_A'	$[CO_3^{2-}]$, M	$[HCO_3^-]$, M	k, M^{-1} sec^{-1} Obsd	k, M^{-1} sec^{-1} Calcd
9.00	1×10^{-5}	0.65	9.698	0.076	0.424	8.55×10^{-5}	8.56×10^{-5}
9.60	4.0×10^{-5}	0.80	9.650	0.232	0.268	1.79×10^{-4}	2.08×10^{-4}
10.10	1.26×10^{-4}	0.95	9.606	0.360	0.140	3.55×10^{-4}	3.61×10^{-4}

[a] Initial [GA] = 0.444 M; [total carbonate + bicarbonate] = 0.500 M; temperature, 30.00 ± 0.03°.

To assess the effective concentrations of bicarbonate and carbonate ions present in a particular reaction mixture, advantage was taken of the observation by Olson and Simonson[17] that activity coefficients of anions can be correlated with the concentration of the cation. A series of buffer solutions was prepared covering the range of sodium ion concentrations used in the kinetic runs, and the apparent dissociation constant (pK_A') was calculated from the relation $pK_A' = pH - \log [CO_3^{2-}]/[HCO_3^-]$. A plot of pK_A' *vs.* total sodium ion concentration showed that pK_A' decreases with increasing $[Na^+]$ in an approximately linear fashion (albeit with some scattering of the points). In a kinetic run the knowledge of the total sodium ion concentration (from the known amounts of sodium carbonate and sodium bicarbonate employed) permitted the pK_A' to be read from the graph, and this along with the measured pH of the solution (differing from that of the buffer in the absence of glyceraldehyde; glyceraldehyde has a pK_A of 12.3[18]) allowed the effective concentrations of carbonate and bicarbonate anions to be calculated. The data for kinetic runs at three pH levels, shown in Table II, yield the following values for the specific reaction rate constants: $k_{OH^-} = 0.86$ M^{-1} sec^{-1}; $k_{CO_3^{2-}} = 6.8 \times 10^{-4}$ M^{-1} sec^{-1}; $k_{HCO_3^-} = 6.0 \times 10^{-5}$ M^{-1} sec^{-1}. Using these specific reaction rate constants, over-all reaction rate constants can be computed for the three runs, and these values are shown in the last column of Table II. While the agreement between observed and calculated over-all specific reaction rate constants is rather good, the absolute magnitudes of the individual specific reaction rate constants must be viewed with some skepticism. The k_{OH^-} value comes out of the solution of the set of simultaneous expressions as the difference between large numbers and probably is accurate to no better than ±0.3 M^{-1} sec^{-1}. The value for $k_{CO_3^{2-}}$ is probably fairly good, but the value for $k_{HCO_3^-}$ appears to be much higher than would be predicted on the basis of the relative basicities of CO_3^{2-} and HCO_3^-.

Catalysis by Organic Bases. Although anhydrous pyridine effects the Lobry de Bruyn–Alberda van Ekenstein conversion of glyceraldehyde to dihydroxyacetone without the incidence of aldol condensation,[19] aqueous pyridine induces the latter reaction as well.[11] Using the enzymatic assay for D-fructose and a modified (see Experimental Section) thiourea–resorcinol assay for sorbose + dendroketose, the extent of condensation was measured for a variety of reaction conditions, as set forth in Table III. On the basis of these data it is concluded that (a) catalysis by hydroxide ion is almost negligible at pH 7, (b) catalysis by buffer ions HPO_4^{2-} and $H_2PO_4^-$ at pH 7 is small but probably real, (c) catalysis by pyridine at pH 7 and 6 is striking and even at pH 5 is demonstrable, and (d) catalysis by pyridine bases is sensitive to the steric environment around the nitrogen.

Table III. Base-Catalyzed Aldolization of D-Glyceraldehyde

Catalyst	pH	Temp, °C	Time, hr	Buffer	Product composition: Fructose	Product composition: Sorbose + dendroketose
OH^-	12.7	25	2	None	41	40
H_2O	6.8–7.0	35	40	None[a,c]	7	6
H_2O	7.0	35	40	HPO_4^{2-}–$H_2PO_4^-$	15	12
4% pyridine	7.0	35	40	B–B·HCl[a]	36	35
4% imidazole	7.0	35	40	B–B·HCl[a]	37	36
4% pyridine	6.0	35	40	B–B·HCl[a]	36	35
4% pyridine	5.0	35	72	B–B·HCl[a]	9	7
2% pyridine	7.4[b]	30	48	None	18	19
2% 2-methylpyridine	7.8[b]	30	48	None	20	22
2% 4-methylpyridine	7.8[b]	30	48	None	30	30
2% 2,4-dimethylpyridine	8.4[b]	30	48	None	32	30
2% 2,6-dimethylpyridine	8.5[b]	30	48	None	8	10
2% 2,4,6-trimethylpyridine	8.8[b]	30	48	None	18	25
2% N-methylimidazole	8.4[b]	30	48	None	31	30
4% glycine	7.0	30	48	None	6	6

[a] Total ionic strength of system = 0.150. [b] Initial pH of reaction mixture. [c] Reaction carried out in closed apparatus equipped with calomel and glass electrodes and a serum cap to allow periodic check and adjustment of pH.

To further delineate the general base catalysis of this condensation and to study in more detail the effect of steric hindrance in the pyridine base, a kinetic analysis was undertaken. By means of polarimetric measurements on D-glyceraldehyde solutions containing the appropriate base, the kinetic data presented in Table IV were obtained. When Brønsted relationships were tested by plotting the log of the specific reaction rates *vs.* the pK_{BH^+} values, families of bases were revealed as illustrated in Figure 1. Those bases such as pyridine, 3-methylpyridine, 4-methylpyridine, and 3,4-dimethylpyridine which carry only hydrogens at the positions adjacent to the nitrogen atom fall near a straight line with $\beta = 0.37$. Those bases such as 2-methylpyridine, 2,4-dimethylpyridine, and 2,5-dimethylpyridine which carry one methyl group adjacent to the nitrogen atom, however, fall near a straight line, displaced downward from the first one and with

(17) A. R. Olson and T. R. Simonson, *J. Chem. Phys.*, **17**, 1167 (1949). We are indebted to Professor J. L. Kurz for pointing out this reference and for instructing us in the application of this method.
(18) L. Michaelis and P. Rona, *Biochem. Z.*, **49**, 232 (1913).
(19) H. O. L. Fischer, C. Taube, and E. Baer, *Ber.*, 60, 479 (1927); see J. C. Speck, Jr., *Advan. Carbohydrate Chem.*, **13**, 63 (1958).

Table IV. Rates of Reaction of Organic Base Catalyzed Aldolization of D-Glyceraldehyde[a]

Catalyst	pK_{BH^+}	Base concn, M	Free base concn at pH 7.0, M	Obsd rate, sec^{-1} [b]	Specific reaction rate, $M^{-1}\ sec^{-1}$
Pyridine	5.17	0.452	0.445	7.72×10^{-6}	1.74×10^{-5}
2-Methylpyridine	5.97	0.410	0.375	7.11	1.90
3-Methylpyridine	5.68	0.414	0.395	11.5	2.91
4-Methylpyridine	6.02	0.414	0.373	13.6	3.65
2,4-Dimethylpyridine	6.63	0.355	0.249	8.09	3.24
2,5-Dimethylpyridine	6.40	0.355	0.284	7.77	2.74
3,4-Dimethylpyridine	6.46	0.355	0.275	20.1	7.32
2,6-Dimethylpyridine	6.72	0.352	0.231	1.39	0.60
2-Ethylpyridine	5.97	0.355	0.324	4.58	1.41
Imidazole	7.03	0.590	0.284	14.9	5.25
N-Methylmorpholine	7.41	0.400	0.102	40.5	39.7
Diazabicyclo[2.2.2]-octane	4.18[c] 8.19[d]	0.360	0.357	68.7	19.2
Pyrrolidine	11.27	0.480	2.58×10^{-6}	3.76	146,000

[a] The concentration of glyceraldehyde was 0.445 M in all cases, the pH was adjusted to 7.0, and the temperature was maintained at 30 ± 0.03°. The specific rate constants were obtained by dividing the observed rates by the molar concentration of free base at pH 7.0. The pK_{BH^+} values are those recorded by A. Albert in "Physical Methods in Heterocyclic Chemistry," Vol. I, A. R. Katrisky, Ed., Academic Press Inc., New York, N. Y., 1963, p 1. [b] Corrected for hydroxide-catalyzed reaction using $k_{OH} = 0.8\ M^{-1}\ sec^{-1}$. [c] pK_{BH^+} for diprotonated amine, *e.g.*, $BH_2^{2+} \rightleftarrows BH^+ + H^+$. [d] pK_{BH^+} for monoprotonated amine.

$\beta = 0.36$. Those bases such as 2,6-dimethylpyridine and 2,4,6-trimethylpyridine which carry two methyl groups adjacent to the nitrogen atom are displaced even further downward. Clearly, as the bulk of the groups in the vicinity of the nitrogen atom of the pyridine ring increases, the compounds become less effective catalysts than their relative basicities toward strong acids would predict.

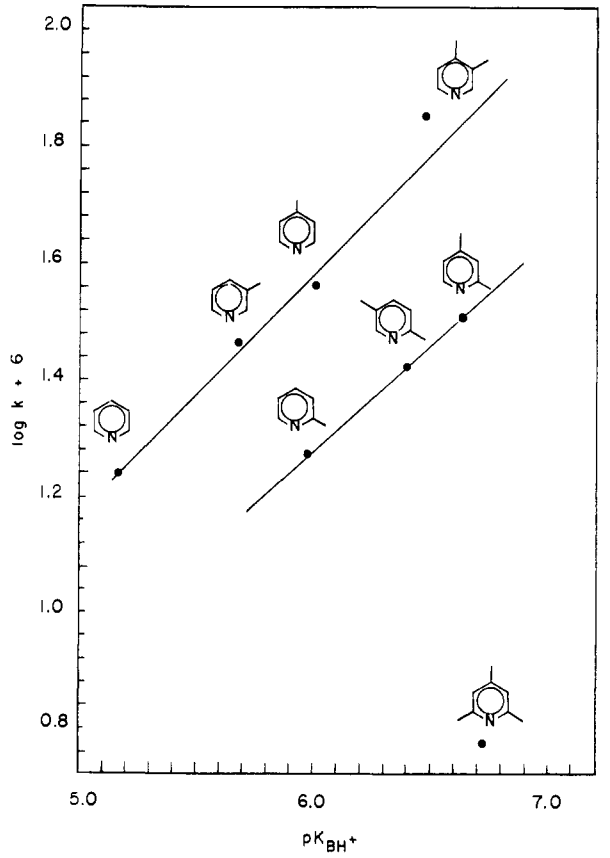


Figure 1. Brønsted plots for pyridine-catalyzed aldolization of D-glyceraldehyde.

In addition to pyridine bases, a few other monoamines were also tested as catalysts for the glyceraldehyde aldolization reaction (see Table IV). Two of these, N-methylmorpholine and imidazole, proved to be comparable in efficacy to the pyridine bases; another two, diazabicyclo[2.2.2]octane and pyrrolidine, proved to be considerably more powerful than the pyridine bases. Whether this is to be ascribed solely to steric effects or whether additional factors (*e.g.*, iminium intermediates in the case of pyrrolidine) might also be operative is not known.

Diamines as Catalysts for the Glyceraldehyde Aldolization Reaction. Several aldol and aldol-type reactions has been shown to be catalyzed by primary and secondary amines[20-25] whose function is thought to be to form an imine or iminium compound, thereby facilitating reaction at the carbonyl carbon and/or increasing the acidity of the α proton. The unexpectedly large specific reaction rate constant for pyrrolidine (see Table III) may be due to nucleophilic catalysis of this type. In the hope of combining nucleophilic enhancement of α-proton lability with intramolecular α-

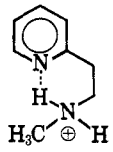
Table V. Relative Rates of Reaction of Diamine-Catalyzed Aldolization of D-Glyceraldehyde

Catalyst[a]	Relative rate
Pyridine	1.00
2-(β-Methylaminoethyl)pyridine	0.11
4-(β-Methylaminoethyl)pyridine	0.80
2-Aminopyridine	1.1

[a] The concentration of glyceraldehyde was 0.445 M, the concentration of base was 40.0 g/l., the pH was adjusted to 7.0, and the temperature was held at 30°

(20) P. Kalnin, *Helv. Chem. Acta*, **11**, 977 (1928); R. Kuhn and S. Ishikawa, *Ber.*, **64**, 2347 (1931).
(21) F. G. Fischer and A. Marschall, *ibid.*, **64**, 2825 (1931).
(22) W. Langenbeck and G. Borth, *ibid.*, **75**, 951 (1942).
(23) T. I. Crowell and D. W. Peck, *J. Am. Chem. Soc.*, **75**, 1075 (1953).
(24) J. A. Gascoigne, W. G. Overend, and M. Stacey, *Chem. Ind.* (London), 402 (1959).
(25) T. A. Spencer and K. K. Schmiegel, *ibid.*, 1765 (1963); T. A. Spencer, H. S. Neel, T. W. Flechtner, and R. A. Zayle, *Tetrahedron Letters*, 3889 (1965).

proton abstraction, several pyridines carrying amine-bearing side chains were tested. It is clear from the results recorded in Table V, however, that these catalysts are no better than pyridine itself. The particularly poor showing of 2-(β-2-methylaminoethyl)pyridine as compared with 4-(β-methylaminoethyl)pyridine and with pyridine may be ascribed to the existence of the intramolecularly hydrogen-bonded structure I,



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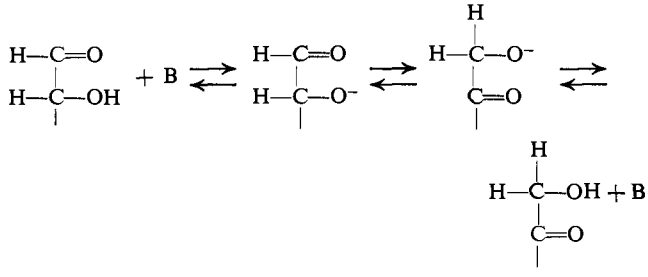
the effect of which would be to reduce the availability of the pyridine nitrogen.

Rate Dependence on Pyridine Concentration. Anhydrous pyridine has been used to effect the conversion of glyceraldehyde to dihydroxyacetone,[19] and the absence of aldolization under these conditions indicates the necessity of water for the condensation process.[26] A study of the amount of hexose formed as a function of the water content of the reaction mixture indicates that this is, in fact, the case. As the per cent of pyridine in water was increased from 1 to 8%, the yield of hexose climbed from 22 to 60%; it remained in the vicinity of 60–68% as the pyridine concentration was incrementally raised from 8 to 60%; it then fell sharply to 8% as the pyridine concentration was increased to 85%. Although pyridine concentrations higher than 85% were not investigated, it seems probable that the extent of hexose formation would continue to fall. The presently accepted mechanism for the Lobry de Bruyn–Alberda van Ekenstein transformation,[19] based on deuterium incorporation studies in the glucose–fructose system,[27] assumes the enolate anion to be an intermediate. Since this is the same species that almost certainly must be involved in the aldol condensation, the failure of the aldol step to occur in anhydrous pyridine is puzzling. Either the pyridine interferes in some fashion with the aldol step *or* a route for the aldose–ketose transformation exists which does not involve the enolate anion. For instance, the process illustrated below represents an alternative which finds



precedents in the Cannizzaro reaction, the benzilic acid rearrangement, and the base-catalyzed rearrangements of α-dicarbonyl compounds.[28] Although the deuterium-exchange data in the glucose–fructose transformation[27] and the tritium exchange data in the triose-phosphate isomerase catalyzed equilibration of glyceraldehyde phosphate and dihydroxyacetone phosphate[29] support the enolate mechanism, both of these systems involving aqueous media. Further work is in progress to elucidate the mechanism of the anhydrous pyridine-catalyzed glyceraldehyde to dihydroxyacetone conversion, for this system represents the simplest possible model for the triose phosphate isomerase enzyme.

B. The Aldolization of Dihydroxyacetone

The aldolization of glyceraldehyde has been shown in part A to yield a mixture of fructose, sorbose, and dendroketose. The straight-chain hexoses are the result of isomerization of a portion of the glyceraldehyde to dihydroxyacetone followed by the mixed aldol condensation between these two trioses; the branched-chain hexose is the product of the self-condensation of dihydroxyacetone. Because the branched-chain product is formed in the glyceraldehyde aldolization reaction and because the rate of ionization of dihydroxyacetone is pertinent information for the design of a polyfunctional catalyst for the mixed aldol condensation, a study of the aldolization reaction of dihydroxyacetone has also been undertaken.

Composition of Condensation Mixture. The base-catalyzed aldolization of glyceraldehyde yields a mixture which is comprised of approximately two parts of fructose + sorbose and one part of dendroketose. The base-catalyzed aldolization of dihydroxyacetone yields a mixture of complementary composition, *i.e.*, approximately two parts of dendroketose and one part of fructose + sorbose. The dihydroxyacetone reaction mixtures, just as those from glyceraldehyde, show no detectable amounts of trioses after sufficiently long treatment with base From reaction mixtures which had progressed for shorther periods, however, it was possible to detect dihydroxyacetone, well separated from the hexoses on paper chromatograms.

Catalysis by Inorganic Bases. The method of choice for following the aldolization reactions of dihydroxyacetone was dilatometry. The volume change accompanying this reaction is considerably greater than that for acetone itself,[30,31] a circumstance which renders this sometimes exacting procedure a particularly suitable one in the present instance. In a typical case a 0.44 *M* aqueous solution of dihydroxyacetone in a dilatometer of 65-ml capacity carrying a 1-mm diameter capillary produced a change in liquid level during the course of a condensation reaction of *ca.* 12 cm.

Using a solution buffered at pH 9.57 with carbonate–bicarbonate, a series of condensations was carried out to test the order of the reaction. When the logarithm of the dihydroxyacetone concentration [*i.e.*, log $(R_t - R_\infty)$, where R represents the dilatometer readings] was plotted against time, a straight line (indicative of a process first order in dihydroxyacetone) was obtained. This relationship was substantiated by plotting the

(26) The Lobry de Bruyn–Alberda van Ekenstein rearrangement is thought to require both a base (*e.g.*, pyridine) and an acid (*e.g.*, water) and to proceed somewhat more rapidly in slightly moist pyridine than in strictly anhydrous pyridine (private communication from Professor J. C. Speck, Michigan State University).

(27) Y. J. Topper and D. Stetten, *J. Biol. Chem.*, **189**, 191 (1951); J. C. Sowden and R. Schaffer, *J. Am. Chem. Soc.*, **74**, 505 (1952).

(28) See M. L. Bender and R. Breslow in "Comprehensive Biochemistry," Vol. 2, Elsevier Publishing Co., Amsterdam, 1962, p 197, for a brief but pertinent discussion.

(29) S. V. Rieder and I. A. Rose, *Federation Proc.*, **15**, 337 (1956).

(30) V. K. LaMer and M. L. Miller, *J. Am. Chem. Soc.*, **57**, 2674 (1935).

(31) F. H. Westheimer and H. Cohen, *ibid.*, **60**, 90 (1938).

Table VI. Dependence of Pseudo-First-Order Rate Constant on Dihydroxyacetone (DHA) Concentration[a]

DHA concn, mole/l.	Pseudo-first-order rate constant, sec^{-1}	% dev from mean
0.700	1.81×10^{-4}	+2.49
0.600	1.76	−0.34
0.500	1.73	−2.04
0.400	1.80	+1.92
0.300	1.73	−2.04

[a] pH 9.57; temperature, 30.00 ± 0.03°.

measurement of the specific reaction rate constants for hydroxide, carbonate, and bicarbonate ions as base catalysts for the condensation. Assuming the reaction to be first order with respect to each of the base species present, the rate for a reaction carried out in a carbonate–bicarbonate buffered solution can be expressed as

$$-\mathrm{d[DHA]}/\mathrm{d}t = [\mathrm{DHA}](k_{\mathrm{OH}^-}[\mathrm{OH}^-] + k_{\mathrm{CO_3}^{2-}}[\mathrm{CO_3}^{2-}] + k_{\mathrm{HCO_3}^-}[\mathrm{HCO_3}^-])$$

Employing the method described in part A, the dihydroxyacetone aldolization was carried out in buffer solutions at three different pH levels to give the results shown in Table VII. From these data the following values for the specific reaction rate constants can be calculated: $k_{\mathrm{OH}^-} = 0.43\ M^{-1}\ sec^{-1}$; $k_{\mathrm{CO_3}^{2-}} = 5.14 \times 10^{-4}\ M^{-1}\ sec^{-1}$; $k_{\mathrm{HCO_3}^-} = 1.27 \times 10^{-4}\ M^{-1}\ sec^{-1}$. Using these specific reaction rate constants, over-all reaction rate constants can be calculated for the three runs, and these data are shown in the right-hand column of Table VII. While the agreement between observed and calculated over-all specific reaction rate constants is excellent, the absolute magnitudes of the individual specific reaction constants must, as in the analogous case of glyceraldehyde, be viewed with skepticism. The k_{OH^-} value comes out of the solution of the set of simultaneous expressions as the dif-

Table VII. Rates of Reaction of Inorganic Base Catalyzed Aldolization of Dihydroxyacetone[a]

pH	$[OH^-]$, M	$[Na^+]$, M	pK_A'	$[CO_3^{2-}]$, M	$[HCO_3^-]$, M	k, $M^{-1}\ sec^{-1}$ Obsd	k, $M^{-1}\ sec^{-1}$ Calcd
8.95	8.92×10^{-6}	0.65	9.698	0.076	0.424	9.69×10^{-5}	9.67×10^{-5}
9.57	3.72×10^{-5}	0.75	9.668	0.252	0.248	1.77×10^{-4}	1.78×10^{-4}
10.20	1.58×10^{-4}	0.95	9.606	0.400	0.100	2.88×10^{-4}	2.86×10^{-4}

[a] Initial [DHA] = 0.444 M; total [carbonate + bicarbonate] = 0.500 M; temperature, 30.00 ± 0.03°.

Table VIII. Rates of Reaction of Organic Base Catalyzed Aldolization of Dihydroxyacetone[a]

Catalyst	pK_{BH^+}	pH	Base concn, M	Free base concn, M	Obsd rate,[b] $sec^{-1} \times 10^6$	Specific reaction rate, $M^{-1}\ sec^{-1}$
Pyridine	5.17	8.18	0.450	0.450	3.44	6.22×10^{-6}
2-Methylpyridine	5.97	8.18	0.400	0.397	4.56	9.83×10^{-6}
3-Methylpyridine	5.68	8.18	0.450	0.448	5.22	10.2×10^{-6}
4-Methylpyridine	6.02	8.18	0.355	0.447	5.92	11.8×10^{-6}
2,3-Dimethylpyridine	6.57	8.18	0.355	0.348	6.03	15.4×10^{-6}
2,4-Dimethylpyridine	6.63	8.18	0.355	0.343	8.01	21.4×10^{-6}
2,5-Dimethylpyridine	6.40	8.10	0.355	0.348	5.76	15.0×10^{-6}
2,6-Dimethylpyridine	6.72	8.18	0.355	0.343	4.75	11.9×10^{-6}
3,4-Dimethylpyridine	6.46	8.18	0.355	0.348	8.36	22.1×10^{-6}
3,5-Dimethylpyridine	6.15	8.10	0.245	0.241	5.92	22.3×10^{-6}
2,4,6-Trimethylpyridine	7.59	8.64	0.275	0.252	11.3	37.4×10^{-6}
N-Methylmorpholine	7.41	8.75	0.355	0.340	4.34	121.0×10^{-6}
Imidazole	7.03	8.38	0.355	0.339	8.82	23.0×10^{-6}
Pyrrolidine	11.27	8.97	0.400	0.00177	48.7	2.47×10^{-2}
Diazobicyclo[2.2.2]octane		8.50	0.355	0.238,[c] 0.117[d]	58.7	
Free base	8.19					2.23×10^{-4} (free base)
		9.00	0.355	0.307,[c] 0.047[d]	74.5	
BH^+	4.18					3.65×10^{-5} (monoprotonated base)

[a] The concentration of dihydroxyacetone was 0.445 M in all cases, and the temperature was maintained at 30 ± 0.03°. The specific rate constants were obtained by dividing the corrected rates by the molar concentration of free base present. The pK_{BH^+} values are those recorded by A. Albert in "Physical Methods in Heterocyclic Chemistry," Vol. I, A. R. Katrisky, Ed., Academic Press Inc., New York, N. Y., 1963, p 1. [b] Corrected for hydroxide-catalyzed reaction using $k_{\mathrm{OH}^-} = 0.4\ M^{-1}\ sec^{-1}$. [c] Concentration of unprotonated base. [d] Concentration of monoprotonated base.

logarithm of the average rate (*i.e.*, $\Delta[\mathrm{DHA}]/\Delta T$) during a specific time interval *vs.* the logarithm of the average concentration of dihydroxyacetone during that interval. The slope of the plot, indicating the order of the reaction with respect to the concentration variable,[32] was 1.04 ± 0.03 for a series of determinations carried out at 30.00 ± 0.01°. Further verification was obtained from a series of determinations, carried out under identical conditions, in which the initial concentration of dihydroxyacetone was varied. The values for the pseudo-first-order rate constant, recorded in Table VI, fluctuated only ±2.5%.

Having established the reaction as first order in dihydroxyacetone, attention was directed to the

(32) A. R. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961.

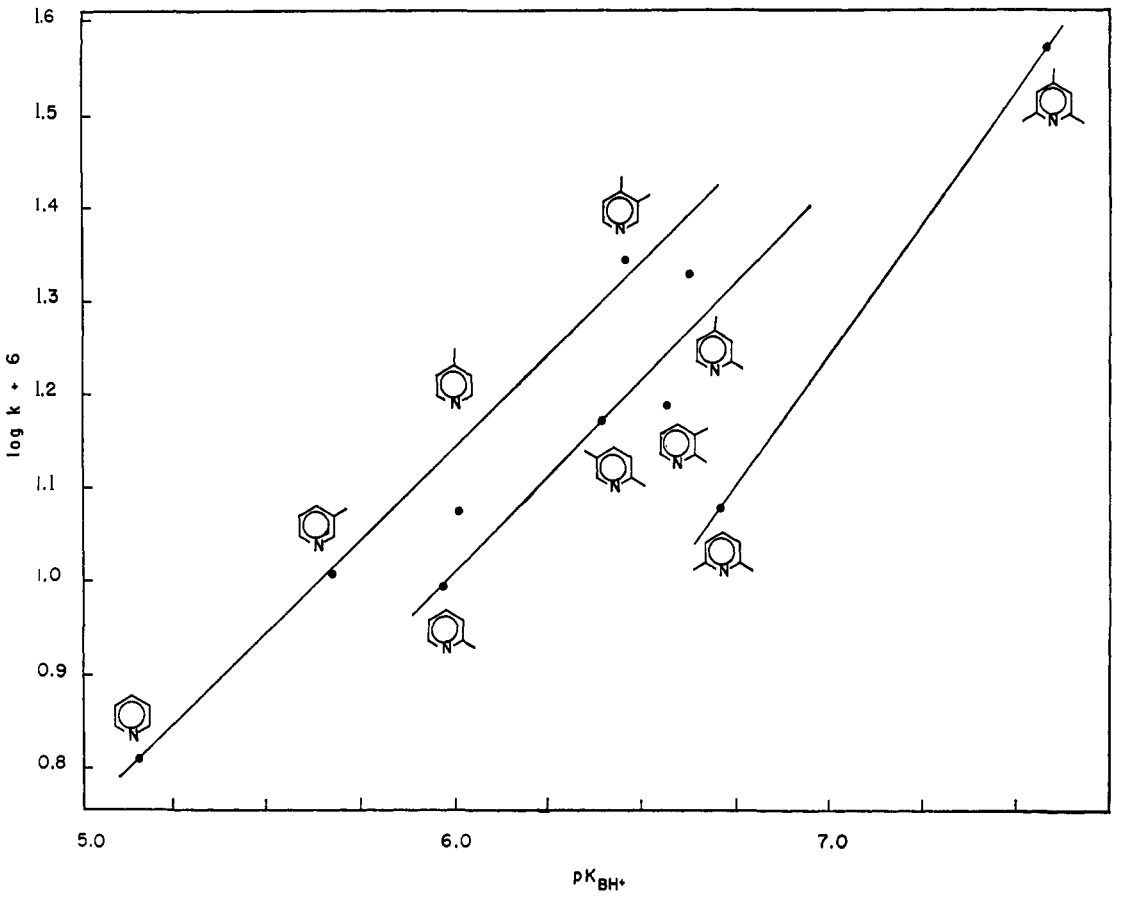


Figure 2. Brønsted plots for pyridine-catalyzed aldolization of dihydroxyacetone.

ference between large numbers and is probably no more accurate than ± 0.3 M^{-1} sec^{-1}. The value for $k_{CO_3^{2-}}$ is probably fairly good, but the value for $k_{HCO_3^-}$ appears to be much higher than would be predicted on the basis of the relative basicities of CO_3^{2-} and HCO_3^-.

Catalysts by Organic Bases. Employing dilatometry to measure the reaction rate, the aldolization of dihydroxyacetone in the presence of various amines was investigated. The kinetic data are shown in Table VIII and the Brønsted plots are illustrated in Figure 2. With the dihydroxyacetone aldolization, just as with the glyceraldehyde aldolization, the pyridine bases can be grouped in families with respect to catalytic efficacy. Those bases which carry only hydrogens at the α positions are most effective, those bases which carry methyl groups at both α positions are least effective, and those bases which carry only one methyl group at the α position fall in between.

C. Comparisons of Glyceraldehyde and Dihydroxyacetone Aldolization Reactions. Discussion and Summary of Results

The data for the aldolization reactions of glyceraldehyde and dihydroxyacetone presented in parts A and B will be discussed with respect to: (a) similarities and differences between the two triose systems, (b) similarities and differences between the triose systems and related systems, (c) the over-all reaction scheme for the glyceraldehyde–dihydroxyacetone system.

Comparison of Inorganic Base Catalyzed Aldolizations. The specific reaction rate constants for the hydroxide ion catalyzed aldolization of glyceraldehyde (*via* polarimetric measurements) and dihydroxyacetone (*via* dilatometric measurements) obtained in the carbonate–bicarbonate buffer system are 0.8 M^{-1} sec^{-1} and 0.4 M^{-1} sec^{-1}, respectively. To check these values under conditions more comparable to those used by Berl and Feazel[9] and also to check the comparability of the data obtained from the two different assay procedures (polarimetry *vs.* dilatometry), the aldolizations of glyceraldehyde and dihydroxyacetone were carried out in 0.02 N sodium hydroxide solution using dilatometric measurements in both instances. From these experiments the pseudo-first-order rate constants were found to be 4.75×10^{-4} sec^{-1} for glyceraldehyde and 3.37×10^{-4} sec^{-1} for dihydroxyacetone. Using the procedure employed by Berl and Feazel[9] for converting these to second-order rate constants, values of 0.5 M^{-1} sec^{-1} for glyceraldehyde and 0.355 M^{-1} sec^{-1} for dihydroxyacetone were obtained. These values are in reasonably good agreement with those from the carbonate–bicarbonate buffer system and with those reported by Berl and Feazel[9] (k_{OH} for glyceraldehyde = 0.14 M^{-1} sec^{-1} at 20° which extrapolates to *ca.* 0.42 M^{-1} sec^{-1} at 30°; k_{OH} for dihydroxyacetone[33] = 0.07 M^{-1} sec^{-1} which, assuming the temperature dependence to be comparable to that for glyceraldehyde, extrapolates to *ca.* 0.21 M^{-1} sec^{-1} at 30°).

(33) The value reported by Berl and Feazel[9] for the aldolization of dihydroxyacetone in 0.0100 N sodium hydroxide is 0.012 min^{-1}. Assuming dihydroxyacetone to have the same dissociation constant as glyceraldehyde, application of the correction which these authors used for the glyceraldehyde data (*cf.* S. Glasstone, "Introduction to Electrochemistry," D. Van Nostrand Co., Inc., New York, N. Y., 1942, p 393) gives a value of 0.07 M^{-1} sec^{-1}.

Table IX. Base-Catalyzed Rates of Ionization of Glyceraldehyde, Dihydroxyacetone, Acetone, and Diethyl Ketone

	Ionization rates, $k \times 10^6\ M^{-1}\ sec^{-1}$					
	Glyceraldehyde (GA)	Dihydroxyacetone (DHA)	Acetone[a] (A)	Diethyl ketone[a] (DEK)	k_{DHA}/k_{GA}[b]	k_A/k_{DEK}
Inorganic bases						
OH^-	8×10^5	4×10^5			0.5	
CO_3^{2-}	7×10^2	5×10^2			0.7	
HCO_3^-	6×10^1	12×10^1			2.0	
Type I pyridines						
Pyridine	17.4	6.22	5.7	2.4	0.357 (2.14)	2.37
3-Methylpyridine	29.1	10.2	13.4	6.7	0.350 (2.10)	2.00
4-Methylpyridine	36.5	11.8	20.4	9.2	0.323 (1.94)	2.22
3,4-Dimethylpyridine	73.2	22.1	38.0	19.8	0.302 (1.83)	1.93
Type II pyridines						
2-Methylpyridine	19.0	9.83	15.2	6.5	0.517 (3.11)	2.34
2,4-Dimethylpyridine	32.4	21.4	44.0	15.5	0.660 (3.97)	2.84
2,5-Dimethylpyridine	27.4	15.0	32.0	11.6	0.547 (3.28)	2.76
Type III pyridines						
2,6-Dimethylpyridine	6.0	11.9	10.2	3.1	1.99 (11.9)	3.29
2,4,6-Trimethylpyridine		37.4	18.5	5.2		3.56

[a] Values taken from J. A. Feather and V. Gold, *J. Chem. Soc.*, 1752 (1965). [b] The values in parentheses are the k_{DHA}/k_{GA} values multiplied by a factor of six to facilitate comparison with k_A/k_{DEK}.

An observation that is difficult to reconcile with the comparative rates of ionization of glyceraldehyde and dihydroxyacetone is the apparent increase in the magnitude of the first-order rate constant that results when mixtures of glyceraldehyde and dihydroxyacetone are used. Fischer and Baer[6] noted an increase in the rate of glyceraldehyde disappearance upon the addition of dihydroxyacetone, and Berl and Feazel[9] measured rate constants for 1:1 mixtures of glyceraldehyde–dihydroxyacetone that were *ca.* two times greater than that for glyceraldehyde alone. It has been suggested[32] that the increased rate for the mixed condensation is due to the greater rate of ionization of dihydroxyacetone compared with glyceraldehyde, and that the decreased rate for the aldolization of dihydroxyacetone is due to a slow condensation step. The present study shows, however, (a) that the rates of ionization of glyceraldehyde and dihydroxyacetone are comparable and that the former may even be the greater, (b) that the aldolization of dihydroxyacetone is a general base catalyzed reaction and that the condensation step, therefore, is not rate limiting. Clearly, further work on this aspect of the triose condensation should be undertaken.

Comparison of Organic Base Catalyzed Aldolizations. Steric effects in general base catalyzed reactions have been noted in a number of instances. Pearson and Williams[34] found that in the base-catalyzed iodination of nitroethane 2,6-dimethylpyridine was *ca.* three times less effective than would be predicted from its pK_{BH^+} value. Studying the same reaction in somewhat greater detail and using 2-nitropropane in addition to nitroethane Lewis and Allen[35] found retardation factors for 2,6-dimethylpyridine as large as 100. Retardation factors ranging from 13 to more than 100 have been detected in various other systems including the hydration of *sym*-dichloracetone,[36] the mutarotation of glucose,[37] the enolization of ketones of the structure CH_3COR,[38,39] and the deuterium-exchange reaction of butyraldehyde.[40] The present results add still other examples of steric effects in general base catalyzed reactions.

Table IX lists the specific reaction rate constants for the ionization of glyceraldehyde, dihydroxyacetone, diethyl ketone, and acetone. The last pair are included on the premise that the steric environments at the carbon adjacent to the carbonyl group are comparable in glyceraldehyde *vs.* diethyl ketone and in dihydroxyacetone *vs.* acetone. There is, in fact, a correspondence between the behavior of the ratio k_{DHA}/k_{GA} and $k_{acetone}/k_{diethyl\ ketone}$ as the nature of the base is changed from type I (pyridines unsubstituted at the α positions) to type II (pyridines substituted with a methyl group at the α position) to type III (pyridines substituted with a methyl group at each of the α positions). As the steric demands of the base increase, steric hindrance in the carbonyl compound becomes more important and the rate ratios (expressed as indicated) increase.

The least hindered bases, those of type I, are more reactive toward glyceraldehyde than toward dihydroxyacetone This may be explained in terms of the relative acidities of the α hydrogens of glyceraldehyde and dihydroxyacetone. In both compounds the carbon α to the carbonyl group carries an OH group and a C=O group. In glyceraldehyde, however, there is also a CH_2OH group attached to this carbon, whereas in dihydroxyacetone the CH_2OH group is separated by an intervening carbonyl group. The greater number of electron-withdrawing groups directly attached to the α carbon of glyceraldehyde should make it the stronger acid.[41] With the introduction of a methyl group in

(34) R. G. Pearson and F. V. Williams, *J. Am. Chem. Soc.*, 75, 3073 (1953); 76, 258 (1954).
(35) E. S. Lewis and J. D. Allen, *ibid.*, 86, 2022 (1964).
(36) R. P. Bell and M. B. Jensen, *Proc. Roy. Soc.* (London), A261, 38 (1961).
(37) F. Covitz and F. H. Westheimer, *J. Am. Chem. Soc.*, 85, 1773 (1963).
(38) J. A. Feather and V. Gold, *Proc. Chem. Soc.*, 306 (1962); *J. Chem. Soc.*, 1752 (1965).
(39) M. L. Bender and A. Williams, *J. Am. Chem. Soc.*, 88, 2502 (1966).
(40) J. Hine, J. G. Houston, and J. H. Jensen, *J. Org. Chem.*, 30, 1184 (1965).
(41) The difference in electron withdrawal at the α carbon in glyceraldehyde and dihydroxyacetone should be comparable to that at the methyl group in ethanol and 1-propanol. The 0.30-ppm downfield shift for the methyl resonance of ethanol from that of 1-propanol in the nmr spectrum gives a measure of the relative electron-withdrawing effects of CH_2OH *vs.* CH_2CH_2OH (N. S. Bhacca, L. F. Johnson, and J. N. Schoolery, "NMR Spectra Catalog," Varian Associates, 1962, spectra 14 and 43).

the α position of the base (type II pyridines), glyceraldehyde loses some of its advantage over dihydroxyacetone, and with the introduction of methyl groups at both of the α positions of the base (type III pyridines), dihydroxyacetone becomes the more rapidly ionizing species. This may be ascribed to the greater steric hindrance in the vicinity of the α proton in glyceraldehyde as compared with dihydroxyacetone, a difference which becomes apparent only when the base becomes sufficiently bulky. Thus, the advantage of a greater inherent acidity possessed by glyceraldehyde and the advantage of a minimal steric hindrance possessed by dihydroxyacetone assume differing degrees of importance depending upon the particular base that is employed as the catalyst. It is interesting to note that, if the specific reaction rate constants for the inorganic ions are taken at face value, hydroxide corresponds approximately to class I pyridines, carbonate to class II pyridines, and bicarbonate to class III pyridines. That all of these bases are more discriminating than pyridine indicates that they are larger than their simple representation would suggest, a fact commensurate with their extensive hydration in aqueous solution.[42]

Comparison of the Aldolizations of Trioses and Their Nonhydroxylated Analogs. While glyceraldehyde and acetaldehyde exhibit the same equilibrium and kinetic behavior upon base-catalyzed aldolization (*i.e.*, both are examples of general base catalyzed reactions), dihydroxyacetone and acetone behave quite differently. Whereas dihydroxyacetone undergoes base-catalyzed condensation to furnish an equilibrium mixture which contains too little of the triose for detection, acetone undergoes base-catalyzed condensation to give only *ca.* 2% of diacetone alcohol. The much larger equilibrium constant in the case of dihydroxyacetone is probably to be ascribed to the greater electrophilic character of its carbonyl carbon; it is generally true that nucleophilic entities add more effectively to electron-depleted carbonyl functions.[43] The greater electrophilicity of the carbonyl groups in dihydroxyacetone is also reflected in the comparative kinetics for the two condensation reactions. Whereas acetone shows a second-order dependence on acetone concentration (the condensation step being slower than the ionization step) dihydroxyacetone shows only a first-order dependence on dihydroxyacetone concentration (the ionization step being rate determining). On the basis of rates of halogenation and rates of deuterium exchange,[32,44] the hydroxide-catalyzed ionization of acetone has been shown to be *ca.* 1000 times faster than the condensation reaction and to have a specific reaction rate constant of 0.25 M^{-1} sec^{-1}. This value is close to k_{OH} of 0.4 M^{-1} sec^{-1} for the ionization of dihydroxyacetone. For the dihydroxyacetone aldolization to show only a first-order dependence on dihydroxyacetone, therefore, demands that the condensation step be considerably greater than 1000 times that for the acetone system, *i.e.*, to be considerably faster than 0.4 M^{-1} sec^{-1}.

(42) E. R. Nightengale, Jr., *J. Phys. Chem.*, **63**, 1381 (1959).

(43) A good discussion of the effect of structure of the carbonyl compound in nucleophilic addition reactions is presented by W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964) (*cf.* p 110).

(44) R. P. Bell and O. M. Lidwell, *Proc. Roy. Soc.* (London), **A176**, 88 (1940).

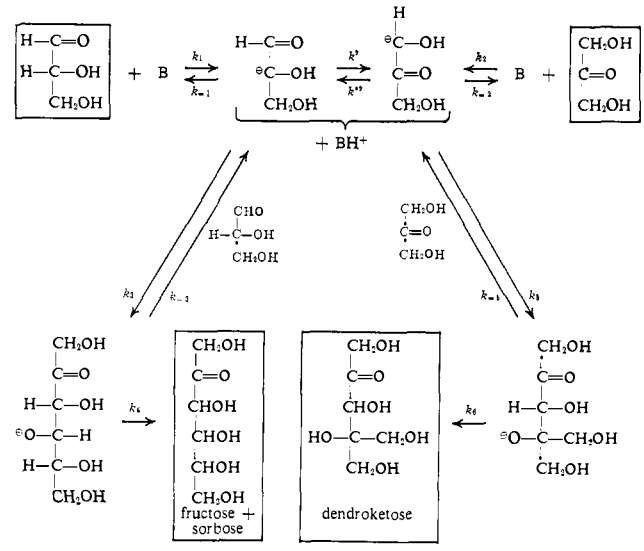


Figure 3. Glyceraldehyde–dihydroxyacetone condensation system.

The correspondence in the ionization rates for acetone and dihydroxyacetone is purely fortuitous. Whereas acetone exists in aqueous solution almost entirely in the keto form, dihydroxyacetone is appreciably hydrated and dimerized. The rate of dedimerization[45] and dehydration[46] is assumed to be fast compared with the rate of ionization and not to be the rate-determining process. However, the true ionization constant for dihydroxyacetone is equal to the measured rate divided by the fraction of free dihydroxyacetone present. This quantity is indicated to be quite small by the fact that the carbonyl absorption band in the infrared spectrum of an aqueous solution of dihydroxyacetone is rather weak. Thus, the true specific reaction rate constant for the ionization of dihydroxyacetone must be considerably greater than 0.4 M^{-1} sec^{-1}. That it would be expected to be greater than that of acetone is reasonable, for the hydroxyl groups exert an inductive electron withdrawal which must increase the acidity of the α hydrogens.

Over-All Reaction Scheme. The family of reactions involved when either glyceraldehyde, dihydroxyacetone, or a mixture of these trioses is treated with base is illustrated in Figure 3. On the basis of the data accumulated from this and previous studies, it is now possible to conclude that (a) regardless of which triose is employed, the active anion is that corresponding to dihydroxyacetone; (b) regardless of which triose is employed, the product consists of a mixture of fructose, sorbose, and dendroketose, although the relative proportions of these hexoses depends upon the starting triose; (c) the rates of ionization of glyceraldehyde and dihydroxyacetone are comparable, but dihydroxyacetone becomes relatively more acidic as the steric demands of the catalyzing base increase; (d) the reac-

(45) R. P. Bell and E. C. Baughan, *J. Chem. Soc.*, 1947 (1937).

(46) Although the hydration of dihydroxyacetone has not been studied, that of *sym*-dichloroacetone, a reasonably close companion, has been found by R. P. Bell and M. B. Jensen, *Proc. Roy. Soc.* (London), **A261**, 38 (1961), to have a $k_{pyridine} = 1.6 \times 10^{-3}$ M^{-1} sec^{-1}. The reverse reaction, dehydration, is of comparable rate (*sym*-dichloroacetone is 45% hydrated at equilibrium) and, thus, over 100 times faster than the pyridine-catalyzed ionization of dihydroxyacetone. The rate of hydration–dehydration of acetaldehyde, also, has been shown to be considerably faster than the rate of ionization [R. P. Bell, M. H. Rand, and K. M. A. Wynn-Jones, *Trans. Faraday Soc.*, **52**, 1093 (1956)].

tion of dihydroxyacetone anion with glyceraldehyde (to form fructose and sorbose) or with dihydroxyacetone (to form dendroketose) is enough faster than the conversion of the trioses to dihydroxyacetone anion ($k_3 >> k_1$, $k_5 >> k_2$) that the over-all kinetics are second-order in each case, *i.e.*, rate = k[base][triose]; (e) the rates of the reactions in the forward direction are enough faster than the rates of reaction in the reverse direction that conversion of triose to hexose is essentially complete[47] ($k_3 >> k_{-3}$, $k_5 >> k_{-5}$); (f) the aldolizations of glyceraldehyde and dihydroxyacetone both fall in the category of general base catalyzed reactions, a fact demonstrable both in the kinetic sense and the chemical sense; (g) on the premise that the equilibrium constant, glyceraldehyde $\rightleftarrows$ dihydroxyacetone, is *ca.* 17,[19] the rate of condensation of dihydroxyacetone anion with glyceraldehyde must be at least ten times greater (on the basis of product composition) than with dihydroxyacetone; however, on the basis of the relative rates of aldolization of acetaldehyde (ionization step rate determining but not a great deal faster than the condensation step[48]) and acetone (ionization step comparable in rate to that of acetaldehyde and *ca.* 1000 times faster than the condensation step[49]), it is probable that the glyceraldehyde condensation rate exceeds that of dihydroxyacetone by several orders of magnitude.

Experimental Section

Assay Procedures. A. Enzymatic Assay for D-Fructose. In a typical condensation 200 mg of D-glyceraldehyde yields *ca.* 150 mg of straight-chain hexoses, approximately half of which is D-fructose. The 100 ml of eluate (see the condensation procedure) thus contains *ca.* 75 mg of fructose. An aliquot of this solution was diluted tenfold, and a 1-ml sample was placed in a 5-ml volumetric flask and treated with 1.5 ml of solution A (see below), 1.5 ml of solution B (see below), and sufficient water (*ca.* 0.5 ml) to bring the total volume to 5.0 ml. After allowing the mixture to stand at room temperature for 30 min, the optical density at 340 mμ was measured. In those condensations where lesser amounts of fructose were formed, the initial dilution was adjusted so that the amount of fructose in the final aliquot was between 0.5 and 0.7 mg/ml. In every set of determinations a blank sample was run along with the test samples, the optical density of the blank being subtracted from that of the test samples. Also in every instance a sample containing a known amount of D-fructose was carried through the same operation as a check on the accuracy of the assay. In a typical assay, fructose gave an optical density corresponding to 0.362 optical density unit/mg of fructose. This value may vary, however, owing to changes in the enzyme solutions, and it is for this reason that it is advisable to carry through a known sample each with set of determinations. Solution A was prepared in the following manner. A solution of 50–100 units of glucose 6-phosphate dehydrogenase (Sigma TypeV) in 10 ml of Tris buffer (see below), a solution of 25,000 units of hexokinase (Sigma Type *III*) in 9 ml of "Tris buffer and 1 ml of EDTA (see below), and a solution of 100 mg of phosphoglucose isomerase (Sigma Chemical Co.) in 10 ml of water were dissolved in 20 ml of EDTA and 50 ml of water to give a total of 100 ml of solution A. Solution B was prepared in the following manner. A solution of 0.090 g of nicotinamide-adenine dinucleotide phosphate (NADP) in 7 ml of water, a solution of 1.65 g of adenosine triphosphate (disodium salt, trihydrate) in 40 ml of water adjusted to pH 7.4 with sodium hydroxide, and 5 ml of a solution of magnesium chloride solution (see below) were combined and diluted to a total volume of 100 ml. A magnesium chloride solution was prepared by dissolving 40.7 g of magnesium chloride hexahydrate in 500 ml of water; the Tris solution was prepared by dissolving 24.42 g of tris(hydroxymethyl)-aminomethane in 500 ml of water and adjusting the pH to 8.0 with 2 *N* hydrochloric acid; and the EDTA solution was prepared by adding 7.2 g of ethylenediaminetetraacetic acid disodium salt and 25 ml of 0.4 *M* magnesium chloride solution to 500 ml of water and adjusting the pH to 8.0 with 0.5 *N* sodium hydroxide.

B. Thiourea-Resorcinol Method for Total Hexoses.[13] The thiourea-resorcinol reagent was prepared by dissolving 100 mg of resorcinol and 250 mg of thiourea in 100 ml of glacial acetic acid. The storage life of this solution is limited, and it should be prepared fresh at least every 2 weeks. An aliquot from the condensation product containing 0.075–0.125 mg (*e.g.*, *ca.* 1 ml of the 100 ml eluate) was added to a 5-ml volumetric flask and treated with 0.5 ml of thiourea-resorcinol solution and 3.5 ml of concentrated hydrochloric acid to bring the total volume to 5.0 ml. The mixture was transferred to a small test tube and heated 50.0 min at 60 ± 0.1°. The sample was then quickly cooled to room temperature, and the optical density at 520 mμ was measured. Since the intensity of the color is strongly dependent on the time and temperature of heating, it is necessary in every set of determinations to include samples of known hexose composition. Relative to fructose, the intensity of the color developed by sorbose and dendroketose is 62 and 19%, respectively, while that from glyceraldehyde and dihydroxyacetone is 20 and 5%, respectively.

C. Thiourea-Resorcinol Method for Sorbose-Dendroketose. Since the sorbose-dendroketose value obtained by the method described above depends upon a separate assay for the amount of fructose present, any errors in that value are reflected and multiplied in the sorbose-dendroketose value. This may be avoided by carrying out the total hexose determination on a sample from which the fructose has been removed (*via* the enzymatic procedure) and in which, as a result, the only hexoses present are sorbose and dendroketose. Thus, after the fructose assay had been completed, 3 ml of the solution used in that assay was placed in a 10-ml volumetric flask and treated with 0.5 ml of thiourea-resorcinol reagent and 6.5 ml of concentrated hydrochloric acid to bring the total volume to 10 ml. The determination was completed as described above, and the optical density value for sorbose was employed in the calculation. This means that the amount of sorbose actually present equals the amount calculated as sorbose-dendroketose minus 0.29[dendroketose]. Lacking an independent measure of the amount of dendroketose, however, that part of the reaction which was not D-fructose was expressed as sorbose-dendroketose using only the sorbose optical density value.

D. Anthrone Method for Hexoses.[14] The anthrone reagent was prepared by dissolving anthrone in concentrated sulfuric acid to make a 0.1% solution. Solutions no older than 3–4 days should be used. A sample containing 0.100–0.200 mg of total hexose was added to 3 ml of anthrone reagent, and the volume was brought to 5.0 ml in a volumetric flask. The solution was allowed to stand at room temperature for 5 min, and the optical density at 625 mμ was then measured. In all cases, control determinations with samples containing known amounts of fructose and sorbose were carried out simultaneously with the assays of the unknowns. Relative to fructose, the intensity of the color developed by sorbose and dendroketose is 104 and 41%, respectively, while that from glyceraldehyde and dihydroxyacetone is 95 and 78%, respectively. The color-forming capacity of the various hexoses and trioses was carefully checked several times, and the absorption spectrum for each of the solutions was measured, confirming the fact of an intense band at 620–625 mμ along with an additional pair of bands at 475 and 535 mμ.

E. Iodine Method for Glyceraldehyde. The procedure described by Willstätter and Schudel[16] was used in the following fashion. A sample, taken in sufficient amount to contain *ca.* 10–15 mg of glyceraldehyde, was diluted to 20 ml of water, treated with 10 ml of 0.1 *N* iodine solution and 15 ml of 0.1 *N* sodium hydroxide solution, and allowed to stand at room temperature for 15 min. The solution was then acidified with 10 ml of 10% sulfuric acid and titrated with 0.1 *N* sodium thiosulfate solution using starch as an indicator. The amount of iodine consumed should be *ca.* 3 ml, each milliliter corresponding to 4.505 mg of glyceraldehyde. For reasons that are not clear, the "apparent" glyceraldehyde value is dependent on the size of the sample used and on the other materials that might be present. While the straight-chain hexoses (*e.g.*, fructose and sorbose) undergo no oxidation under these conditions, dendroketose may to a greater or lesser extent suffer oxidation.

(47) The equilibrium constant for dihydroxyacetone phosphate + glyceraldehyde phosphate $\rightleftarrows$ fructose 1,6-diphosphate is 1.2×10^4 M^{-1}; the equilibrium constant for dihydroxyacetone phosphate + glyceraldehyde $\rightleftarrows$ fructose 1-phosphate is 2×10^4 M^{-1}; the equilibrium constant for dihydroxyacetone + glyceraldehyde $\rightleftarrows$ fructose is not known but would presumably be greater than 2×10^4 M^{-1}.

(48) R. P. Bell and P. T. McTigue, *J. Chem. Soc.*, 2983 (1960).

(49) See ref 32, p 335, for a discussion of this reaction.

Dihydroxyacetone presents a particularly baffling picture. When samples containing 20–70 mg of this triose were subjected to the assay, values ranging from 34 to 65% of the amount corresponding to the oxidation to the corresponding acid were obtained; however, 20 mg of a 1:1 mixture of glyceraldehyde and dihydroxyacetone behaved as though it were entirely glyceraldehyde; a 7:3 mixture of glyceraldehyde and dihydroxyacetone behaved in a comparable fashion, but a 3:7 mixture of glyceraldehyde and dihydroxyacetone gave a value of only 77% (corresponding to 100% oxidation of three parts of glyceraldehyde, 100% oxidation of three parts of dihydroxyacetone, and 40% oxidation of four parts of dihydroxyacetone).

F. NAD Method for Dihydroxyacetone.[50] The NAD reagent was prepared by dissolving 100 mg of nicotinamide–adenine dinucleotide (NAD) in a small amount of water, adding 0.75 ml of 0.1 *N* sodium hydroxide, adjusting the pH to 6.0, and diluting to 5 ml. A Tris solution was prepared by dissolving 36.5 g of tris(hydroxymethyl)aminomethane in 100 ml of water and adjusting the pH to 10.0 with 1 *N* hydrochloric acid. A sample containing *ca.* 0.250 mg of dihydroxyacetone was treated with 0.200 ml of NAD solution and 0.100 ml of Tris solution, diluted to 5 ml, and heated at 60° for 1 hr. The mixture was cooled, and the optical density at 340 mμ was measured. In a typical experiment an optical density of 0.600 was observed. Since this value is dependent on the time and temperature of heating, however, it is necessary to run a sample containing a known amount of dihydroxyacetone along with the unknown. Glyceraldehyde subjected to this assay gives a color 20% as intense as that from dihydroxyacetone, while the hexoses develop no color whatsoever.

G. Paper Chromatographic Assays. Whatman No. 1 paper was used with a developing system consisting of ethyl acetate (eight parts), pyridine (two parts), and water (one part). The papers were developed for 12 hr, and the positions of the sugars were ascertained by means of a periodate–benzidine spray.[51] For quantitative determinations duplicate samples were run side by side. After the development, the paper was cut vertically between the courses of the two samples. One of these strips was treated with the periodate–benzidine spray to locate the positions of the sugars and using this as a guide; the other strip was cut horizontally between the positions of the sugars. These pieces were then separately eluted and quantitatively assayed by means of the anthrone test (see above). Using known amounts of sugars the accuracy of the determination for fructose–sorbose and dendroketose was shown to be ±5%. In the case of dihydroxyacetone the recovery was only 70% (possibly condensation to dendroketose occurs during the development), and when glyceraldehyde is present the resolution of the system is diminished. The R_t values observed were 0.50 for dihydroxyacetone, 0.22 for dendroketose, and 0.12 for fructose–sorbose.

Procedures for Studying Glyceraldehyde Condensations. A. Chemical Assay of Products. A 200-mg sample of D-glyceraldehyde with $[\alpha]_D$ +13.5° (reported[6,52] for optically pure D-glyceraldehyde) was dissolved in *ca.* 3 ml of water, treated with the particular base to be used in the condensation and adjusted to the particular pH level desired, and the total volume was brought to 5.0 ml by the addition of more water. The flask was immersed in a constant-temperature bath and allowed to stand for the desired length of time. The reaction was then terminated by passing the reaction mixture through a 10 × 1 cm column of Amberlite IR-120 resin in the acid form. In those cases where salts had been added to the reaction mixture the effluent from the Amberlite IR-120 column was passed through an Amberlite IR-450 column in the hydroxyl form. The column(s) were washed with water, and the total volume of the effluent was diluted to 100.0 ml. The subsequent assays of the components of the reaction mixture were carried out with aliquot amounts of this solution. The ion-exchange columns were used one time only and were then cleaned and refilled with fresh resin. The spent resins are most conveniently combined and regenerated in larger batches.

Employing this condensation technique and the assay methods described in the previous section, the data recorded in Tables I and II were obtained. For the reaction carried out at pH 7.0 (see 2nd entry of Table II), however, a somewhat more elaborate system was required in order to maintain the pH constant in the absence of any buffering capacity of the solution. In this case the reaction vessel consisted of a bulb of *ca.* 10-ml volume to which was attached two arms to accommodate a glass electrode and a calomel electrode and a third arm capped with a septum to allow the introduction of liquid *via* a syringe. In this vessel was placed the 5 ml of reaction mixture which had been adjusted approximately to pH 7. After the vessel was sealed with the septum cap and the electrodes were in place, the tube was shaken so as to allow the reaction mixture to react with the carbon dioxide in the air in the vessel. The pH was then adjusted to 7.0 by sodium hydroxide solution, introduced through the septum *via* a hypodermic syringe. The assembly was placed in the constant-temperature bath, the pH was checked periodically during the course of the reaction, and acid or base was added as required to bring the pH back to 7.0. At no time after the initial adjustment did the pH differ from 7.0 by more than a few tenths of a pH unit.

B. Polarimetric Determinations. Solutions containing 200 mg of D-glyceraldehyde in *ca.* 4.5 ml of water were treated with the basic catalyst, adjusted to pH 7.0 with 2 *N* hydrochloric acid, diluted to 5.00 ml, and added to a polarimetric tube 20 cm in length. After measuring the initial rotation, the tube was placed in a thermostated bath held at 30 ± 0.01° and it was removed at intervals for subsequent rotation measurements. From the known rotations of D-glyceraldehyde ($[\alpha]$ +13.5°), D-fructose ($[\alpha]$ −92°), D-sorbose ($[\alpha]$ +42.9°), and dendroketose (optically inactive), the observed rotations could be translated into glyceraldehyde concentrations. Plotting the log [glyceraldehyde] *vs.* time gave lines which were straight throughout the entire course of the reactions. Multiplying the slope by 2.303 gave the observed reaction rates, and these were converted to specific reaction rates by dividing by the concentration of free bases present in the reaction mixture at pH 7.0. This value was then corrected for the small amount of hydroxide ion catalyzed reaction accompanying the organic base catalyzed reaction.

Procedure for Studying Dihydroxyacetone Condensations. A 4.000-g (0.444 mole) sample of dihydroxyacetone (Wallerstein Chemical Co.[53]) was dissolved in *ca.* 90 ml of water, the requisite amount of catalyst was added, the pH was adjusted by the addition of dilute HCl (if necessary), the solution was diluted to 100.0 ml, and the pH was again measured. This solution was then added by means of a 50-ml hypodermic syringe to a dilatometer bulb of *ca.* 65-ml capacity to which was attached a capillary tube 1 mm in diameter and 40 cm long. The dilatometer apparatus was placed in a water bath maintained at 30.00 ± 0.03°, and the level of the liquid in the capillary was measured at various time intervals by means of a cathetometer, readable to 0.005 cm. The pH was measured at the conclusion of the run and in all cases was found to have not changed significantly. Replicate runs were carried out in most cases, and deviations of less than ±5% between runs was noted. The log $(R_t - R_\infty)$ *vs.* time plots showed no departure from linearity for the first two-thirds of the reaction in any of the runs, but occasionally small deviations were noted in the last third of the reaction for very slow condensations. These deviations appeared to be random and may probably be ascribed to the difficulty in accurately extrapolating the dilatometer readings to obtain the R_∞ value.

DL-Dendroketose. Following a previously published procedure,[10] dihydroxyacetone was treated with dilute aqueous sodium hydroxide, and the resulting product was converted to O-benzoyl-di-O-isopropylidene-DL-dendroketose, mp 120–121° (lit.[10] mp 121°). A 3.0-g sample of this material was added to 100 ml of 0.1 *N* sulfuric acid and heated at 60° for 6 hr, during which time the reaction mixture became homogeneous. Neutralization with barium carbonate, filtration, and evaporation of the water left 2.1 g of dendroketose as a colorless gum. The homogeneity of this material was shown by paper chromatography on Whatman No. 1 paper with ethyl acetate (three parts), acetic acid (three parts), and water (one part) as the developing system. When a 0.420-g sample of dendroketose in 10 ml of water maintained at pH 10.7 by a carbonate–bicarbonate buffer was heated at 31° for 36 hr, a paper chromatographic examination of the mixture revealed no trace of fructose or dihydroxyacetone.

(50) M. U. Tsao and E. L. Schwartz, *Anal. Biochem.*, **3**, 448 (1962).
(51) J. A. Cifonelli and F. Smith, *Anal. Chem.*, **26**, 1132 (1954).
(52) A. Wohl and Fr. Momber, *Ber.*, **50**, 456 (1917).
(53) Recrystallization of the commercial product did not change the melting point, and paper chromatography indicated the presence of only one substance.